Response to the Subject Editor's Comments

Overall Response: Many thanks for finding two extraordinary reviewers for this manuscript. We have learned so much from their comments, which are informative and right to the point. In our hearts, we are grateful.

In responding to their extensive comments and also to yours, numerous changes have been made to the original manuscript. Major changes are summarized as follows:

- The new approach has been tested with100 new simulated A/Ci curves and the test results are shown in Figure 5 and 6 (new figures).
- The connection of the FvCB model with the change-point model is discussed. The differences and similarities with typical change-point models studied by the statistics community are also discussed.
- The identification of true independent parameters is now described for all three limitation states. This allows us to explain more clearly what parameters or parameter expressions can be estimated and under what conditions, if the fitting is done correctly and if the dataset is adequate. A theoretical parameter resolvability table is added (Table 2, new).
- A proof for the relative order (position) of the three limitation states along the Cc (Ci) axis is given. This order is used to reduce the number of limitation state combinations that must be tested in fitting an A/Ci curve. From this proof, an inequality relationship among Vcmax, J and TPU is also established.
- We removed the material related to the asymptotic estimation of TPU from this present manuscript because the manuscript is getting too long. We will report the asymptotic approach in a separate paper. However for your and the reviewers' reference, our response still includes a defense of this approach.

1. Comment: I am very interested in the manuscript, as are the reviewers. Both reviewers have identified issues they feel need to be addressed. Some points relate to quoting other treatments more carefully.

Response: We have revised the relevant text to give a more sensitive discussion of existing methods. In particular, we have removed the word 'blindly' from a sentence that one reviewer found particularly objectionable.

2. Comment: Referee 2 raises particular concern about estimation of TPU (as the limitation it may impose). Given that RuBP regeneration is itself curving towards a saturating value at high [CO2], it is difficult to see that the present treatment could always find the TPU limitation unless it is expressed as a change of shape beyond the change from Rubisco to regeneration limitation. How does the experimenter know that the TPU limitation is not much greater than the asymptote caused by J?

Response: We need to give more detailed, clear explanation on the rationale of the asymptotic approach. More tests are also needed. But we are running out space so we decided to cut this part off entirely. We will write a separate paper on this issue and we

will keep your comment in mind. But here we offer the following response to your comment.

In our original manuscript, our presentation of the asymptotic approach for estimating TPU gave readers the impression that the approach was a perfect alternative to having TPU-limited points in the dataset. This certainly was not our intention. It would be much better to have TPU-limited points in the dataset. The following assumptions are needed in order for the approach to be valid:

- The three limitation states exist simultaneously (not necessarily in the dataset, but for the leaf sampled under the measurement condition).
- The TPU-limited photosynthesis is described by the reduced form: Ap= 3Tp-Rd (that is, alpha=0).
- The A/Ci curve contains points not too far away from the saturating level.

The approach is used only if the dataset contains no TPU-limited points (EDO is able to detect whether or not points of a particular limitation state occur in the dataset).

We need to clarify that the asymptote used in the estimation of TPU is the asymptote exhibited by the whole A/Ci curve, not "the asymptote of RuBP limited photosynthesis" as stated in the comment by Reviewer #2. This is important because the asymptote of the RuBP-limited photosynthesis is A=J/4-Rd while the asymptote of the whole A/Ci curve is A=3Tp-Rd.

Thus the key is whether the asymptote of the whole A/Ci curve can be estimated with a reasonable accuracy. If it can, the asymptotically estimated TPU is biologically meaningful.

We argue that the asymptote of the whole A/Ci curve can be estimated as long as the A/Ci curve contains points not too far away from the saturating level. The three biochemical states described by the FvCB model are not independent. They are correlated. If there is nothing else that relates them to each other, they are at least from the same leaf. No A/Ci curves vary erratically. That means, if we have information about two of the three states, we ought to be able to make some inference about the third one. In fact, in the revised manuscript, we proved that as long as the three limitation states exist, Vcmax, J and Tp have to obey the following inequality:

$4Vcmax > J > 12\ Tp$.

Thus, if we know any of the three parameters, we can automatically set a range for other two parameters. Of course, we should not take the condition "as long as the three limitation states exist" lightly. If one decides to measure an A/Ci curve at low PAR, then the RuBP regeneration might be the only state that exists and the asymptotic approach would not be valid for such a dataset.

The Reviewer #2 himself/herself commented that "the differences between TPU limited and RuBP limited photosynthesis are usually minor except under extreme conditions".

(See Comment 3 in Reviewer 2.) This comment is relevant to our argument here. If you have some RuBP limited points with a range of Ci values, you can pretty much determine the magnitude of the rates of TPU-limited photosynthesis (that is, the TPU-limited photosynthesis cannot be off very much from the RuBP limited photosynthesis).

Thus we strongly feel that the asymptotic approach does have merit. The asymptotically estimated TPU is biologically meaningful as long as the A/Ci curve contains points not too far away from the saturating level. Of course, this is not to say that researchers should now forget about covering TPU-limited points in their measurement.

3. Comment: As an aside to the paragraph before Eq (1), I note that dePury & Farquhar (PC&E 1997 20, 537-557 -their Eq 25) showed a related result - the light intensity at which A becomes I-saturated.

Response: Thanks for this information. We are planning a separate manuscript on how the new fitting approach affects the estimated Jmax-Vcmax relationship. The current manuscript focuses on methodology and a detail discussion of the relationship is beyond its scope.

4. Comment: Page 5. I note half way down the page a reference to dark respiration. This should refer to 'day' respiration, as it may or may not be closely related to the rate in the dark.

Response: Change made.

5. Comment: At the bottom of the same page, there is reference to the FvCB model. The most widely used version is the limiting case of perfect coupling, but other solutions were given. So this just emphasizes the point made by the reviewer that it would be useful to describe the model succinctly first so that the reader knows which is being referred to.

Response: Suggestion adopted.

6. Comment: On page 10 I had difficulty understanding the discussion after Eq (12). In my text Eq (11) appears to refer to both forms of the solution for Ap. If we take the simple case of alpha = 0, the second case becomes if Ci< gamma* +A/gi, ie if Cc<gamma*. And net photosynthesis must always be negative then. Does the label Eq (11) refer only to the first case? This and the discussion there need to be clarified.

Response: This is related to a point we discussed through our email exchanges when the manuscript was first submitted. The current formulation for the TPU-limited photosynthesis is biologically unrealistic if Cc <= (1+3alpha)gamma* because under such a condition, Wp is negative with the formulation Wp = 3TpCc/(Cc-(1+3alpha)). If Wp is allowed in the comparison via min{Wc, Wj, Wp), Wp will always be selected. Also note that when Cc=(1+3alpha)gamma*, Wp is undefined. To avoid this unrealistic situation, we only compare Wc and Wj to calculate A when Cc <= (1+3alpha)gamma*. When alpha is zero, the second case is not needed because it is identical with the

restriction placed on TPU; but when alpha is not zero, the second case is needed. So Eq (11) is valid for both forms of Ap except that the TPU limitation is not considered when Cc<(1+3alpha)gamma*. In the revised manuscript, this condition is stated explicitly.

7. Comment: As mentioned above I find it hard to see how we can obtain TPU from the asymptote expressed as Eq (17) or any other without more info.

Response: See Response to Comment 2.

8. Comment: At the top of page 29, do those [CO2] refer to ambient values?
Response: Ambient CO2 values (CO2 values inside the leaf chamber).

9. Comment: It is perhaps good for me and the reviewers that the ideas are so fully explained, but the manuscript will need to be shorter before it could be published. So page 31 could perhaps be cut back, for example. And Table 2.

I look forward to reading another submission with these and the reviewers' comments addressed.

Response: We tried hard to identify and cut nonessential materials. We removed the text related to the asymptotic estimation of TPU. We also cut back the text in the Conclusion section. We considered cutting Table 3 (originally Table 2). But Reviewer #1 thought that table was quite helpful. We hope you find that this manuscript contains sufficient merits to justify its length.

Response to the comments by Reviewer#1

1. The overall comments: The methods presented in this manuscript may constitute a very significant advance in the estimation of the parameters of the FvCB model of carbon assimilation, and could be the definitive solution to a longstanding problem. They might be of even broader importance, in the study of change-point models in general. However, the description of these methods, despite its length, lacks information essential to allow readers to repeat the work presented. Because the purpose of the manuscript is to novel methodology, giving all necessary information to repeat the work is even more critical than it would be in reporting experiments or observations. In addition, comparisons with other extant methods are very inadequate, and no proof or supporting references from statistical sources are offered.

Given the potential value of this work, and the very real possibility that the authors have achieved a remarkable breakthrough, they should be strongly encouraged to submit a new manuscript that would meet the following two requirements: 1) the manuscript should provide all the information sufficient for readers to apply the proposed method and verify the authors' results independently, and 2) proofs, or references to published descriptions of the same estimation method for change-point regression should be provided. In the following pages, comments that detail those requirements are clearly marked. Other comments must be addressed, but those are required for acceptance. Comparisons with extant methods would add to the interest of the manuscript, but would have to be designed much more carefully than the current version, if they are not to detract from the main arguments.

Response: It has been a delightful and great learning experience to read and respond to these constructive and thought-provoking comments. We admire this reviewer for his/her willingness to give so much to a manuscript she/he is reviewing. As a result of following the suggestions made in this review, several new analyses, including a more detailed proof of parameter resolvability and 100 new simulations, have been done and the manuscript has been revised substantially. Details about what changes have been made will be given in this response.

We did not realize there is a connection to the broad area of change-point problem and estimation until we read this review. In hindsight, we should have and it could have saved us a lot of time. Since 2005, we have been testing the 'simultaneous estimation' approach or the 'transition auto-identifying' approach as discussed in Dubias et al. (2007) and Yin et al. (2009) (we started before these papers were published). We realized early this approach does not work but we did not clearly understand why until recently. Hudson (1966), Hinkley (1969), and Lerman (1980) actually told us why this apparently logical approach does not work! If it works, the estimation of a change-point model would be just like the estimation of any other model and this special statistics field would not exist. In the revision, these historical papers were cited and the uniqueness of the FvCB model as a change-point model was discussed. Without this reviewer, we would be still blind to this important connection. So thank you!

2. Comment: With apologies to the authors when the following comments cover matters that are already well known to them.

As recognized in the manuscript, parameterization of the FvCB model is accomplished by statistical estimation through fitting of the model to gas exchange data obtained for that express purpose. As a regression model, FvCB belongs to the general class of change-point models (other names: piecewise, switching, segmented, multiphase, breakpoint model, etc…). These models are nonlinear simply because different domains of the X space are modeled by different functions. Additionally, FvCB is nonlinear in a second way, through its sub-models being nonlinear. They can be linearized, but not in a common X space. Continuous change-point models are formed by segments that share join points, and may or may not have a continuous first derivative. FvCB is continuous with discontinuous first derivative.

Response: We agree with this characterization of the FvCB model. As we mentioned earlier, we did not realize there is such a broad connection here. Thanks for broadening our perspective.

We also would like to add that it appears the FvCB model has some unique features that are uncommon in change-point models typically studied by the statistics community. For example, the FvCB model has both submodel-specific parameters (e.g. Vcmax, Jmax, Tp) and common parameters that are shared across the submodels (e.g. Rd, gamma*, gi) while in typical change-point models all parameters are submodel-specific. In the revised manuscript, we also discussed a couple of other unique features of the FvCB model as a change-point model. In any case, it seems the FvCB model has a form that is more challenging than a typical change-point model. In discussing this issue in the manuscript, we are clearly stealing ideas from this reviewer. But by discussing it openly, we hope readers would not be as blind as we were to this important connection.

Thanks to this reviewer, we now realize some of the difficulties in fitting the FvCB model are actually commonly encountered in fitting change-point models and they exist because the FvCB model is a change-point model. In the revised manuscript, these difficulties are now presented as consequences of the FvCB model being a change-point model.

3. Comment: In the very easiest case of regression, the case of linear models (linear in the parameters, not necessarily ‘straight-line’) n-1 is the maximum number of parameters that are estimable from data that comprise n points. At that maximum, the quality of those estimates would be poor, both inferentially speaking, and many argue, predictively speaking. As nonlinearity (in the parameters) is introduced, the ratio of estimable parameters to data points goes down very quickly.

Response: We agree with these statements completely.

4. Comment: Following a very mild re-parameterization by the authors (replacing Kc and KO with a single parameter), the model uses eight parameters. Stating, as the manuscript does, that those eight parameters can be reliably estimated from 10 gas exchange points is thus an extraordinary claim. It may not necessarily require extraordinary proof, but it does require more proof than is currently provided, or

reference to a proof in the statistical literature. Since the mid nineteensixties, several solutions to the estimation of change-point models have been proposed (see bibliography by Khodadadi and Beheshti, 2008, Collection of Biostatistics Research Archive, Berkeley Electronic Press). If the authors are in fact applying one of those solutions, which is difficult to ascertain from the current manuscript, they must refer to it explicitly. The exposition would be greatly strengthened by also citing other published applications of the same solution, regardless of scientific field. If their solution is altogether new, they should probably first publish it in an applied statistics journal.

If the method does fulfill its claim, there is a good chance that the key reason is the use of segment-wise error sums of squares for the cost function. The rest of the procedure are important refinements and additions, but a much expanded discussion of segment-wise error sums of squares vs. overall error sums of squares should be added, and probably placed before the rest of the exposition of the new method.

The manuscript indicates that the method has limitations, and suggests that it is quite sensitive to some of the characteristics data (where sampling density should be greatest, for example). Every method has limitations, and the manuscript would be much strengthened by a clearer description these, and by a more precise discussion of what can be expected when data do not meet the required standards.

Response: There is no question that our new method or any other method can only be as good as the data. We showed an inadequate example in one of our 100 new simulated A/Ci curves. In that example, there are 3 Rubisco-limited, 2 RuBP-limited but 10 TPU-limited points. Our new method was not able to estimate the true parameters for this simulated curve. The reason was that when alpha does not equal zero, the model for the TPU is overparameterized and the re-parameterized system with truly independent parameters is ill-conditioned and extremely nonlinear with a product of four parameters. Thus it is very difficult to estimate parameters for an A/Ci curve that contains mostly TPU points, although in real cases, it is rare to have a dataset dominated by TPU points. Also the real A/Ci examples used in the study were not obtained before we had a clear understanding of what constitutes an informative A/Ci dataset. Consequently their curvature regions were not sampled densely and in fitting these curves, we had to fix the values of Γ* and Rd in order to uniquely resolve other parameters. So we believe our new approach solves the FvCB structural problems for parameter estimations but it does not and cannot solve data problems.

In the revised manuscript, we gave a more detailed description on what parameters or parameter expressions can be uniquely resolved under different scenarios of limitation states (see the new Table 2) assuming the dataset is adequately sampled. We proved that when the dataset is adequately sampled, all eight parameters could be uniquely determined. In particular, we emphasized the importance of having a sufficient number of RuBP regeneration-limited points and points in the curvature region. As the number of data points decreases or as the quality of the measurements deteriorates, the number of parameters estimable decreases and their uncertainties increase. We apply several measures to control the quality of estimated parameter values for a given A/Ci dataset. However, these measures were not stated as clearly as desired and some critical references were not given in the original manuscript. This has been changed in the revised manuscript.

We realized the enumeration aspect of the Exhaustive Dual Optimization method (that is, list all possible limitation state combinations for an A/Ci dataset) is similar to the standard treatment in fitting change-point models. We pointed this out in the revised manuscript.

There are several elements that are necessary for successful A/Ci curve analysis, including the re-parameterization of Kc and Ko parameters. But the most important one in our view is the nested structure of the optimization (the parameter optimization is nested within the limitation state optimization). The limitation state optimization is the higher-level optimization and is essential for obtaining biologically meaningful parameters. Once the limitation state optimization is solved, the parameter optimization comes naturally.

A common problem with previous A/Ci fitting methods is that they focus on the parameter optimization, leaving out the more important, higher-level limitation state optimization. From the 101 simulated A/Ci curves we know if we don't get the limitation state combination right, we don't get the parameters right. It is important to note that there is no need for extra information additional to that for parameter estimation in order to conduct the limitation state optimization. This information is already contained in the A/Ci measurements. Therefore, previous methods underuse the information available in an A/Ci dataset while ours uses it to a fuller extent.

The formulation of the cost function is also important as the reviewer noted. It pools together information contained in different limitation states, which minimizes the influence of the overparameterization problem in the Rubisco- and TPU-limited states and increases the likelihood of parameters being successfully estimated from a limited number of data points. Note that this is possible only because there are common parameters across the limitation states. If it were not so, it would not make a difference. There is a theorem in Hudson (1966) that says the overall estimates of the parameters based on the joint cost function are equivalent to local least square estimates when each segment is optimized separately. That theorem applies to the case when there are no common parameters shared across different submodels. So it does not apply to the FvCB model. We discussed the implications of the cost function in the revised manuscript.

5. Comment: "Measures of goodness of fit based on regression residuals often are not sufficient to tell which fitting method is superior because it is easy to overfit an A/Ci curve." Expand this statement into two or three sentences to make it understandable. 'overfitting' is not understood the same way by everyone. Explain what you mean by it.

Response: Since different people may understand the word 'overfitting' differently as the reviewer suggests, we decide not to use this word here. This sentence and the few sentences after it have been revised.

6. Comment: Introduction is generally clear and well explained, but assumes understanding of the model as detailed in the next section. Could the description of the model be moved before the discussion on p.4?

Response: Suggestion followed.

7. Comment: What do you mean by 'prescribed parameters'? Define here, and use consistent terminology thereafter.

Response: The definition of 'prescribed parameters' is provided in the revised manuscript. Prescribed parameters are defined as 'parameters whose values are assigned a priori by assuming the kinetic properties of Rubisco are conserved across species and existing values from *in vivo* or *in vitro* measurements in the literature can be used.'

8. Comment: You make it sound a bit as if the proportionality of Jmax and Vcmax were a consequence of fitting method. Isn't it more a property of the model itself?

Response: There is nothing in the FvCB model that says Jmax and Vcmax must be proportional to each other although it is generally believed that the evolution of photosynthesis may have very well resulted in a balanced relationship between Jmax and Vcmax. But that is not the point we were trying to make here. What we were trying to demonstrate is that in conventional methods, the Jmax-Vcmax relationship is defined by the fitting methods, not by the data they are trying to fit. We showed that if the limitation domains are set in advance, Jmax and Vcmax cannot both be independent and free parameters even they appear so in conventional fitting methods. One can be calculated from the other based on where the researcher decides to separate the limitation domains. We have revised the text to make sure this message is conveyed.

9. Comment: "without any useful constraint from measurements" This expression is very odd. What constitutes a 'useful' constraint? How does a constraint come from measurements? Reword and expand.

Response: This is related to Comment No. 8. The relevant text has been revised with the message expressed in the Response to Comment No. 8.

10. Comment: "The approach of Manter & Kerrigan (2004) is an improvement over the conventional, fixed Ci_CJ approach." In what way?

Response: The sentence has been revised into:

"The approach of Manter & Kerrigan (2004) is an improvement over the conventional, fixed Ci_CJ approach in that it tried to bring some objectivity into the determination of Ci_CJ."

11. Comment. "But in practice, measurements have errors, data points may be few" This is central to the whole problem of estimation, not a practical aside.

"the two sides of an A/Ci curve may not have the same constraining power for gi." Explain or reword.

"gi might not converge at all" Improve expression. A parameter cannot converge. An algorithm or a process does.

Response: Our original text in this area was confusing. It has been revised.

12. Comment: “Cost function” On first occurrence, provide alternate names: objective function, energy function,…

Response: Suggestion followed.

13. Comment: “This unique model structure creates discontinuity in the calculated A/Ci relationship and causes the cost function to be of no minimum or otherwise to be of shapes that are not suitable for optimization when the direct, simultaneous approach is used.” Explain what you mean by ‘discontinuity’, announce that more details will be given later.

Response: Suggestion followed and this sentence has been revised.

14. Comment: Discussion of inconsistent points: Here and elsewhere in the manuscript, the authors appear to lose sight of the difference between the first-principles perspective on the model, and the estimation perspective, or between the theoretical view, and the observational. The difference being the presence of random error ε (scatter). From the estimation perspective, every observed point is a unique, independent realization of a model that includes random error ε (scatter). There is an argument to be made that the inconsistency is no more than a consequence of random error. The inconsistency is not an anomaly, and thus does not need to be corrected. That said, if what the methods used to counteract this inconsistency turn out to produce better parameter estimates, then the authors should try to find out why.

Response: Our use of the term ‘inconsistent points’ is probably not helping. We do not mean the data are inconsistent but rather the fitting is inconsistent so that it leads to some points switching their limitation types before and after the fitting. Apparently it is quite common in fitting change-point models. Such fitting is called ‘inadmissible’ in change-point literatures. In the revised manuscript, we tried to make this clear.

The issue concerns points in the A/Ci dataset that turn out to be in different limitation states from those states that have been used in the estimation of the model parameters in the first place. These points occur in the neighborhood of the limitation state transitions (note that if a point is right at the limitation state transition, this point would be co-limited by two limitation states). They exist because the estimated parameters are not accurate enough to produce the correct behaviors with respect to the relationship between A and Ci (Cc) around the limitation state transitions. Random error, inadequate sampling, and inappropriate methods of fitting can all lead to inaccurately estimated parameters and therefore can all lead to the existence of ‘inconsistent points’. The inconsistency is one in logic and an indication that the parameters are not estimated accurately and therefore should be corrected.

As described in the manuscript, we use an iterative procedure to correct this problem if the problem occurs (it does not occur for every A/Ci dataset) or by introducing co-limited points if swinging points exist.

15. Comment: "the TPU-limited state is often the state missing from an A/Ci curve" Do you mean missing from the data?

Response: Yes, thanks, we do mean 'missing from the data'. Sentence revised.

16. Comment. Required: Please add Yin et al. (2009), PC&E to the citations, and include it in introduction and comparisons.

Response: Yin et al (2009) was added to the citations. For fitting A/Ci curves, Yin et al (2009) actually uses the simultaneous estimation method so it is included in the comparison. We also discussed the ideas of using variable light levels for parameter estimation as proposed in Yin et al. (2009).

17. Comment: The claim that TPU can be estimated without any observations in the TPU region sounds quite strange, and rather alarming. What is described in that later section of the manuscript is in fact interesting, insightful, and very useful, but expressing it in this way makes it sound like the authors propose to fit a model to inexistent data.

Response: Since the manuscript is getting too long and the idea of asymptotically estimating TPU is not closely tied to the main ideas of the manuscript, we decided to drop this part and write a separate paper on it.

18. Comment: Required: if an online software application is going to be provided, the code should be made available, for example as supplemental material. It need not be the literal underlying code in the programming language used in the online application, but what language or software is used must be stated, and every programming step and algorithm called on must be described. Whether the programs can be considered proprietary is obviously outside of this review, but this reviewer and three colleagues (a very small sample, admittedly) would feel very uncomfortable submitting their data for analysis to such a 'black box' program.

Response: We would be happy to provide this reviewer the complete set of source codes upon request. The program, which is written in Fortran, is a hybrid of gradient- and nongradient-based approaches. Its core elements were all taken from existing open sources (netlib.org, Numerical Recipes). In the revised manuscript, a new paragraph is added to explain how the gradient and nongradient approaches are assembled together. We also described a few 'tricks' that we have found very useful.

Any user can go to the website leafweb.ornl.gov to test the performance of the program with their own actual or simulated A/Ci curves.

19. Comment: The FvCB model with CO2 internal transfer conductance

Clear, brief, excellent description of the model. The oddity of seemingly two transitions between Ac and Aj in the customary formulation has been raised before.

Response: Thanks. Following your suggestion made earlier, this section is now moved ahead of the discussion on existing fitting methods.

20. Comment: Sources of difficulty in accurately fitting for parameters in the FvCB model
"The number of model parameters almost equals the number of data points in a typical A/Ci curve and therefore the accuracy of the estimated parameters can be easily affected by measurement noise and bias even though the precision of the estimation may be misleadingly high." Please expand this. The wording is far too imprecise. What exactly is meant by "measurement noise and bias"? It sounds trivial: of course, error (ε) affects the accuracy of the estimates, and of course more points are always better. Bias is a different matter altogether. What needs to be explained is how and why this model would be more sensitive to these phenomena than other models?

Response: we deleted these initial sentences which conveyed well-known points and were distractive to the main theme of the section.

21. Multicollinearity: The first paragraph of this section does not define parameter multicollinearity. It lists some of the consequences of multicollinearity, but without defining it. The paragraph should start with a good definition, then move on to consequences. On a small note, the manuscript makes it seem as if multicollinearity was particular to nonlinear models. This needs to be corrected.

More importantly, what is described in this section is not so much collinearity as overparameterization, mixed with illustrations of the inherent problems in estimating gi , which indeed, as stated in the manuscript, do not stem from collinearity. This section does point out some difficulties, and collinearity is an issue with the model, but this section needs to be rewritten to detangle the various issues addressed.

Some misunderstanding of collinearity may be involved, as suggested throughout this section, and by statements such as this one, on p. 32: "Parameter multicollinearity is carefully checked and avoided." . Collinearity is or is not present, and can be more or less severe; it can be remedied by reparameterization, but it cannot be 'avoided'.

Response: We struggled with several terms from the literature, including multicollinearity, parameter correlation, and overparameterization. (One of our colleagues suggested the words equifinality and multifinality to us. But they seem to be too exotic to be used here.) Our initial reluctance to use the word 'overparameterization' was due to the concern that its use seems to indicate there is some deficiency in the model itself, which is certainly not the case here. What we are trying to show is that when each segment is fit separately, a unique set of parameters will not be obtained no matter how many data points an A/Ci dataset has because the FvCB model is overparameterized for two of the three limitation states. Thus, in rethinking about this issue, 'overparameterization' is a more appropriate term. Thanks for pointing this out.

Note that overparameterization exists in the Rubisco- and TPU-limited states, but not in RuBP regeneration-limited states. So when the three states are fit for separately as in some existing methods, the problem of overparameterization cannot be avoided. But when the three states are fit jointly as is done in the study, the problem could be avoided because there are common parameters shared across the three states and the RuBP limitation does not suffer from the overparameterization problem.

22. Comment: If-Then conditions and SEM
Again, some interesting insights, but also some serious problems. Dubois et al., and Miao et al. may both combine grid searching and simultaneous estimation of two sub-models, but they are in direct contradiction with each other regarding the parameters that can be estimated. The plots of the objective function (Fig 2) are very useful, but they are misleading for two reasons: first, the shapes would change with the number of parameters being estimated, and second, they would be different for other data. The lack of global minimum could be come from too many parameters being included, or from these particular data.

The issue of flat areas in the cost function is real, but it has little to do with the conditional structure of the model. Flat areas in the cost functions are common in nonlinear models, and they do make estimation vulnerable to the choice of starting values for the optimization. But as long as a global minimum does exist in the cost function, and good staring values are supplied, such models can be optimized without difficulty. In both Dubois et al. and Miao et al., finding good starting values is accomplished through grid searching. What is shown here is that the five dimension cost function (four dimensions for four parameters, and one for SSE, the 'response') (four parameters estimated) does not have a global minimum, with these data only. Using fewer parameters would likely not eliminate some flat areas in parts of the domain of the cost function, but it there is nothing here to tell us that it would not have a global minimum for most data, and thus be estimable.

Later on in the manuscript, the structure of the cost function turns out to be pivotal. The discussion in this section should probably be about the cost function itself, yet the manuscript never states the exact expression used for the cost function in SEM.

Response: Again we have to thank this reviewer for pointing out to us that the FvCB model belongs to the category of change-point models. This allows us to see more clearly why SEM is not the approach for dealing with the FvCB model. SEM essentially treats the FvCB model as no different from the more common, 'non-change-point' models in which the same mathematical equations and the same set of parameters apply across the whole variation range(s) of the independent variable(s).

The shape of the cost function is vital for accurate parameter estimation even when one is sure that a global minimum exists. If we have only one or two parameters to estimate, we know with what shapes even a powerful optimization algorithm CANNOT GUARANTEE the finding of the minimum and with what shape a competent algorithm CAN GUARANTEE the finding of the minimum. If the cost function has a shape that is twisted, rugged, convoluted, or crooked, the finding of the minimum cannot be guaranteed. Ironically, if the shape of the cost function is too simple – so simple that it has only a tiny dimple in a vast plain – the finding of the minimum cannot be guaranteed

either. For these shapes, dense grid search can help and may even lead to the minimum SOMETIMES but it can never guarantee the finding of the minimum. However, we know if the cost function is bowl-shaped, the finding of the minimum is guaranteed.

For the estimation with more than two parameters, we cannot even imagine how complicated (or how simple) the shape of a cost function can be due to our confinement to the 3-d space. But if it is difficult to estimate one or two parameters with a wrong cost functional shape, we can imagine how more difficult it can be to estimate more than two parameters if the cost function does not have the right shape.

Thus it is no surprise that Dubois et al and Miao et al are in direct contradiction with each other regarding what parameters can be estimated even though they both combine SEM with grid searching. Because SEM treats change-point models as no different from other models (that is, it uses the If-Then conditions directly within the iterative process), the cost function does not have the right shape. Grid searching can help but cannot quarantine the finding of the minimum – the results depend on chance – how many grids you use and where the grid minimum is located. This is shown clearly in our new 100 simulated examples: for some curves, SEM retrieved the parameters correctly, but for most of them, it failed.

It is true that flat areas in the cost function could be caused by too many parameters or too few data points. But we show flat areas can also be caused by improper fitting methods because of the unique structures of the FvCB model. Once a limitation-state specific parameter moves outside the range in which the corresponding biochemical state is limiting, that parameter has no influence on the cost function so the cost function has to be flat with respect to this parameter.

Another crucial point we missed but realized after reading the literature on change-point models is that if the join points are allowed to VARY FREELY WITHIN the iterative optimization process, the shape of the cost function will be improper even when all segments are described by a simple linear model (one can infer this from Figure 1 in Lerman 1980 and Figure 2 in Hudson 1966). When the segments are described by nonlinear models, even simple ones, the shape of the cost function can become horrific to any statistician in the world (one can infer this from Figure 2b in Lerman 1980).

It is true that the shape of a cost function depends on the number of parameters being estimated and the dataset used. But what we are trying to demonstrate is that for the FvCB model, the shape of the cost function also depends on the fitting method. Later in the manuscript, it is shown under our new approach, for the same dataset and for the same set of parameters, the cost function has the right shape and the correct parameters are retrieved.

It is true that a single global minimum may exist with SEM. But if the global minimum is in a tiny dimple or an extreme shallow depression in a vast plain, to the computer with rounding errors and limited machine precisions, the global minimum might as well not exist. Our example (Figure 2) shows this is the case for SEM.

More importantly, the cost function under SEM in general has no single global minimum. This can be seen from the following general argument. Suppose an A/Ci dataset contains 10 Rubisco-limited points and 2 RuBP-limited points. Under SEM, the minimum of the cost function for the correct fit of 10 Rubisco-limited points and 2 RuBP-limited points may not be smaller than the numerous incorrect fits for 11 Rubisco-limited points and 1 RuBP point because the one RuBP point could be fit for perfectly.

Further, the minimum of the cost function for the correct fit of 10 Rubisco-limited points and 2 RuBP-limited points may not be smaller than for the numerous fits with 9 Rubisco-limited points, 1 RuBP regeneration-limited point and 2 TPU-limited points before the 1 RuBP point and 2 TPU points could be fit for perfectly. Therefore, the cost function under SEM which allows points to change their limitation states freely must have a narrow valley at the bottom that is absolutely flat. For such a cost function, no minimization technique can work because a single global minimum is undefined.

Thus we emphasize how the limitation states (or the join points) are determined is critically important - inside the iterative optimization or outside the iterative optimization, which as our example shows has a huge influence on the shape of the cost function. SEM does it inside, resulting improper shapes; the new approach does it outside, resulting in the right shape for the same dataset and same set of parameters to be estimated.

As in estimation of regular models, how many parameters are being estimated and how many data are available are important questions. But it is also important to realize how the join points are being determined has a crucial influence on the shape of the cost function and thus on whether or not the parameters can be successfully estimated.

23. Comment: Comparison between methods
The concept of comparing the ability of different methods to recover known parameters that were used in generating synthetic data is very useful, and is often used to characterize the strength and limitations of a method, or as practical proof that it works. A comparison of this type would greatly enhance the manuscript, and would go a long way toward convincing both technical and non-technical readers. However, the design and scale of the current comparison are severely inadequate. A single A/Ci curve is no more than a small illustration. Calling it a simulation, and suggesting that it constitutes proof, significantly undermine the credibility of the manuscript. In addition, the manuscript states that the data do not include error. This restrict the utility of the exercise even more. Fitting a single A/Ci curve, and one that does not include error, can be a useful check that no major problem exists in the program used for fitting, but it should not be given more importance than that.

Response: In the revised manuscript, we generated 100 new synthetic A/Ci curves with parameters randomly generated by a random number generator. We tested both the new approach and SEM with these 100 curves. The new approach missed only one while SEM missed most of them. The one curve that the new approach missed was an inadequately sampled one: majority of the points were TPU-limited points (10) with only 2 RuBP points and 3 Rubisco points. The TPU state (when alpha is not zero) is overparameterized, extreme nonlinear, and the reparameterized system is ill-conditioned. So it would be extremely difficult to fit for a dataset that consists of mostly TPU points.

It is a good idea to include errors in the simulated A/Ci dataset. But this would have to be carefully planed with realistic error terms added to both A and Ci. Error covariance between the two variables also needs to be considered. Thus we would have to expand the volume of the manuscript substantially in order to do this adequately, which may be a distraction from the main ideas of the manuscript. In any case, for the present manuscript, the purpose of using artificial A/Ci curves is to see if the fitting methods can retrieve the

parameters correctly. If we include error terms in the simulated A/Ci datasets, we would not be able to attribute clearly the failure of a retrieval to proper causes: the failure could be due to improper setup of error terms or due to incompetent fitting methods.

24. Comment: There are several issues with Figure 1:
- if gi was estimated, the data should be shown with both Ci and Cc values, or at least with Cc.
The fitted line should show the fit to A/Cc values. Does this graph show Cc, or Ci? (same comment for all A/Ci or c? graphs in the manuscript).

Response: We could show with either Ci or Cc as the abscissa but not both in the same plot because there is no uniform correspondence between Ci and Cc across the x axis. We choose to use Ci because in some existing methods, the selection of limitation domains is based on Ci and we want to show how much can be off with such selection. Of course, we could use Ci for some plots and Cc for others, but that would be confusing. In order to keep consistent through the paper, we use Ci for all plots

- There is no true R2 for nonlinear regression. How was pseudo-R2 computed?

Response. Yes, there is no true R2 for nonlinear regression, even more so for change-point models. Nevertheless, some authors use the pseudo-R2 to indicate a good fit for nonlinear regression. As explained in the manuscript, we show the value of pseudo-R2 here, just to show how misleading it can be. In the revised text, it is also indicated it is a pseudo R2. It is computed in the same way as in the linear model.

- Drawing a line between residuals is unusual. In this case, it highlights what the authors call ‘oscillation’, but the meaning of the oscillation is far from clear. Other features of these residuals are more prominent.

Response: the word ‘oscillation’ is probably misplaced in our text as it indicates the presence of some sort of ‘period’ or regularity, which is certainly not the point we are trying to make. What we are trying to express is the fluctuations hidden by the apparent good fit in the plot a. In the revision, we use ‘fluctuation’ instead of ‘oscillation’.

- More problematic is that, judging by the points that belong to each of the three phases, this A/Ci curve is hardly typical. In fact, it is quite different from the real ones the manuscript presents in other figures.

Response: We now include 100 new examples. We did not try to pick up a particular type. In fact, the patterns of A/Ci curves vary greatly from one to another and it would be difficult to tell what a typical A/Ci curve is. We could easily use another example to demonstrate the same point we are trying to make through Figure 1.

25. Comment: The results of the author’s method presented in Table 1 are the single most important reason for considering this method. They are also hugely frustrating, because the method is later only partially revealed, and because this is only one data set.

The accuracy of the recovery of the parameters is of course astounding, but again, because this is a single data set, and in the absence of error, it may not be very meaningful.

Response: Again, we now use 100 new examples.

26. Comment: Including simulation results would greatly strengthen the manuscript. The authors should refer to statistical literature on simulation in this context.
Here are condensed guidelines:
Synthetic observations would be generated for a matrix of parameter values formed by setting each of the eight parameter at intervals within the range where each can reasonably be expected to be found, from a biological standpoint. For example, chose 4 biologically plausible values of each parameter, at the extremes and at the center of their respective plausible range, and generate the 48 combinations of parameter values. Some of those 48 combinations could probably be discarded, because they would not be plausible as combinations. An error structure must then be defined; the simplest being normally distributed, independent error, with a single distribution for the three phases, and constant variance. However, it would be reasonable to suppose that a separate error distribution is associated with each limitation state, and although error-free predictors are assumed, some small error in X is highly likely in real data. Likewise, some small amount of serial correlation in points recorded after each other at different levels of CO2 is very likely in real data. The mean and variance of the error, or errors, would have to be determined, and then for each of those 48 combinations, several hundreds n-point curves would be generated, each with random error added to each point. The model would then be fitted to every curve, using each method.

Only on the basis of that many runs could the behavior of the various methods be characterized. The question to be answered is: under what conditions does a method perform best (How many points are needed? How many parameters can really be recovered? What influence on recovery does ε have? Do separate errors for each sub-model make a difference? What range of parameter values are hardest to recover? How sensitive is a method to correlation in the error? etc…). If the only objective is to demonstrate that a single method works reliably, fewer combination of parameters values could be used, but not fewer than several hundred curves for each of those combinations.

Valid simulation is clearly not a small undertaking, but it would be very persuasive. If the authors wish to use synthetic data to compare methods, the relevant questions should be addressed. The most relevant question for all methods, is of course how well they perform in recovering the known parameter values, and how various changes in the data, particularly the error, affect performance. The relevant claim in Sharkey et al. (2007) is that a priori determination of the joinpoints is preferable. For Dubois et al., it is that, based only on 10 to 15 gas exchange points, only three parameters can be estimated; for Miao et al., that gi and TPU can be estimated too; for Yin et al., that adding fluorescence data leads to better performance. Those comparisons are much more meaningful than SEM vs. EDO vs. a priori joinpoints. As an aside, including Yin et al. in the comparison would add significantly to the simulation burden, because of the added data.

Response: We agree with the reviewer on every word said in this comment. We are grateful for these superb suggestions. Building upon the 101 synthetic A/Ci curves already presented in this paper, we intend to follow through these suggestions.

We also suggest that such work can be done most convincingly when different groups advocating different methods are actively players and when the simulated curves are produced by an independent group. The rationale is that some methods require assigning limitation domains in advance and all methods require initial guess on the values of the parameters to be estimated. Setting these initial inputs is not entirely objective and thus it is best done when the 'optimizer' has no knowledge about the values of the parameters of the simulated A/Ci curves. In this way, no group is accused of being the player, referee and judge at the same time.

To facilitate the community involvement in this very important effort, we are creating an online tool at leafweb.ornl.gov with which simulated A/Ci curves can be created by any user. The user could then use these curves to test our new method at the same website or to test his/her own methods.

27. Comment: Prediction discontinuity. Change-point models with discontinuous first derivative can present difficulties to optimization algorithms with some data, and incidentally, the Nelder-Meade simplex algorithm is recommended when such a problem occurs. It is not clear at all, however, that the discontinuity if the first derivative has any connection with the problem the manuscript labels "prediction discontinuity". The problem with the method of Sharkey et al. (2007) is fairly well illustrated, but it does not in itself contribute to the discussion of discontinuity. It is possible that final segment estimates in the method of Sharkey et al. do not always match a priori choices, but that is not necessarily a bad thing.

Response: We now realize the problem discussed here is what referred to as 'inadmissible fit' in the change-point literature. For the FvCB model that uses Vc = min{Wc, Wj, Wp}, the model would have to be smooth to any order of derivatives at the change-points (not just first derivative) in order not to have this problem. Because there is a contradiction in the logic, it is better this problem is dealt with when it arises.

28. Comment: Guideline for informative A/Ci curve measurements
The arguments for these guidelines, and the guidelines themselves are clearly laid out, and appear reasonable. It seems that equally convincing arguments could be made for different guidelines, though. Here again, synthetic data would be able to address the issue much more definitively.
Required: How robust is the method to departures from these guidelines? What are the consequences of such departures?

Response: We agree with the reviewer on that synthetic data could be used to establish the best practice for sampling A/Ci curves. But such issue can be more fully explored in a separate paper.

Clearly, we cannot get more than what is in the dataset. Among the 100 additional simulated A/Ci data curves, there was one curve that was inadequately sampled. It violated the guideline proposed here. EDO could not retrieve the correct parameters for this curve. Also the real A/Ci examples used in the manuscript did not follow the guideline (they were obtained before we had a clear understanding of what constitutes an informative A/Ci dataset). Consequently the fitting of these real dataset showed evidence of overparameterization due to data insufficiency (that is, second derivatives of some parameters near zero or some parameters stop at bounds) and we had to fix K_{co} and Γ^* in order to resolve other parameters.

We believe the measurement guideline we proposed has a theoretical underpinning and when it is followed, the chance of getting the biologically meaningful parameters is greatly increased.

29. Comment: Guideline for reliable A/Ci curve fitting
"a new numerical scheme" meaning unclear.
"the only solution" this seems rather rash. Enumeration of all possible two and three subset combinations, with constrained sequence, is simple and appealing, but calling it the only solution may not be prudent.

"Without any prior knowledge…" this paragraph is superfluous, and could be stated much more simply in a single sentence. As it is, it is distracting. The accompanying table (Table 2) is very helpful, and largely sufficient.

First paragraph is confusing. "Multicollinearity can be minimized by pooling together information contained in data points of different limitation states because there are shared parameters across limitation states." What other choice do we have?? And how does this reduce collinearity?

"for a limitation state combination that consists of multiple limitation states, all limitation states should be fit simultaneously through the same cost function."
Required: Explain what "a limitation state combination that consists of multiple limitation states" means? Explain "should be fit simultaneously through the same cost function." The manuscript never really reveals how this is achieved.

Collinearity diagnostics: must provide citations for the partial derivatives approach. This does not resemble accepted collinearity diagnostics methods. It does, however, resemble minimum detection techniques. The two issues (multicollinearity and minima) appear tangled throughout the manuscript. The authors could argue that they are in fact connected, but if the argument is made, it is made in a very confusing way.

If guidelines are going to be applied for determining what parameters need to be fixed, they should be defined clearly and unambiguously. Here, the reader is told that some parameters may have to be fixed, but exactly which, and when is not expressed directly and simply.

Response:
"a new numerical scheme" is changed into "a numerical scheme that specifically addresses the FvCB structural uniqueness'.

"the only solution" is changed into "a solution".

The paragraph started with "Without any prior knowledge' is cut back to a single sentence.

As noted earlier, the phrase 'parameter multicollinearity' is replaced with the word 'overparameterization', following this reviewer's suggestion. In the revised manuscript, the reparameterized system with true independent parameters is given for all three limitation states (System of Equations 13, 16, 17). This allows us to point out that when the three systems are joined together (that is, the information in all three limitation states is pooled together), the overall system is overdetermined, meaning all unknown parameters can be uniquely optimized. Such pooling together would not be necessary if one has other independent means to estimate some of the parameters. But if the only information one has is A/Ci curves, we don't see other choices.

"for a limitation state combination that consists of multiple limitation states, all limitation states should be fit simultaneously through the same cost function" is changed to "for a limitation state combination that consists of multiple limitation states (that is, the first 4 scenarios in Table 2), limitation states should not be fit separately, instead all limitation states should be fit jointly through the same overall cost function". A new Table 2 is added.

The issues of overparameterization and minimum are indeed connected. We can attest this from our own experience. We presented strategies to deal with overparameterization due to FvCB structural factors. But overparameterization due to insufficient data can only be dealt with passively, that is through post-optimization checks. But too much detail on this would be a distraction. We give Tarantola (2005) as a general reference.

The mathematical rationale for which parameters could be estimated and which should be fixed is now given. A new Table 2 addresses this issue specifically.

30. Comment: The Exhaustive Dual Optimization (EDO) approach
Required: has this method been described before? Some features are reminiscent of Hudson (1966) Hinkley (1969), and Lerman (1980) but it is difficult to ascertain whether it is identical to any of them. If this method has been described before, provide a citation. The FvCB model has its own unique properties, but the citations above, and others, describe general solutions that are suitable for broad classes of models.

The use of novel acronyms (PO, LSCO, EDO) seems gratuitous. This reviewer finds them distracting (sample size=1, admittedly).

“PO is the optimal determination of the FvCB model parameters for a given limitation state combination. For each of all possible limitation state combinations, a separate PO is done.”
Required: How? This is one of the most important missing element in the description of the method. The sentence following the one quoted here does not provide the required information.

The detailed explanation of the steps is very useful (consider a chart), and most steps seem straightforward and well thought out, but essential elements are missing.
Required: Specifically, on p. 24, heading c: is the manuscript saying that sub-models are fitted separately, or together? And how are the parameters common to all sub-models (Γ*, Rd, gi) estimated? What data are they estimated from, and what optimization algorithm is used? The optimization algorithm must be stated. It is unlikely to make much difference in results, but readers need to know whether commonly accepted algorithms are suitable, or whether only some algorithms, or a special algorithm, are required.

Required: The most notable aspect of the cost function is that it uses segment-wise error sums of squares. This is not a small matter, and should be discussed at some length. The visualizations of it in Fig. 5 are extremely striking, and some explanation of why using segment-wise error sums of squares should result in such ideal shapes needs to be presented. Also, the shapes with respect to Γ* and gi must be added to this figure.

Much more detail should be supplied regarding programming, maybe as supplementary material.

Finally, if the method is indeed unique to this model, and has not been described before, then some theory for inference should be presented. Confidence intervals on the parameter estimates are a minimum requirement. It would be difficult to accept estimates without any inferential information, and calling them reliable without it seems reckless.

Response:
It seems the treatment of enumeration is similar to the standard treatment in classical change-point literature. This connection is now pointed out in the revised manuscript.

Our purpose of using acronyms is for convenience and for saving space as they appear numerous times in the text.

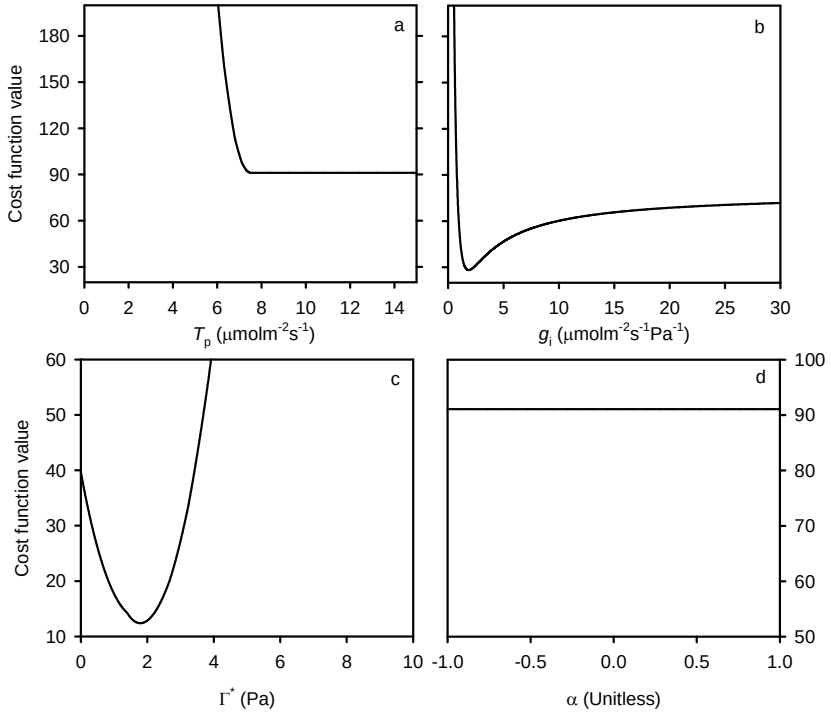
The minimization algorithm is now described.

Heading c is now changed with more detailed information. With the new addition of Table 2 and the explanation of the minimization algorithm, hopefully we have now given enough information.

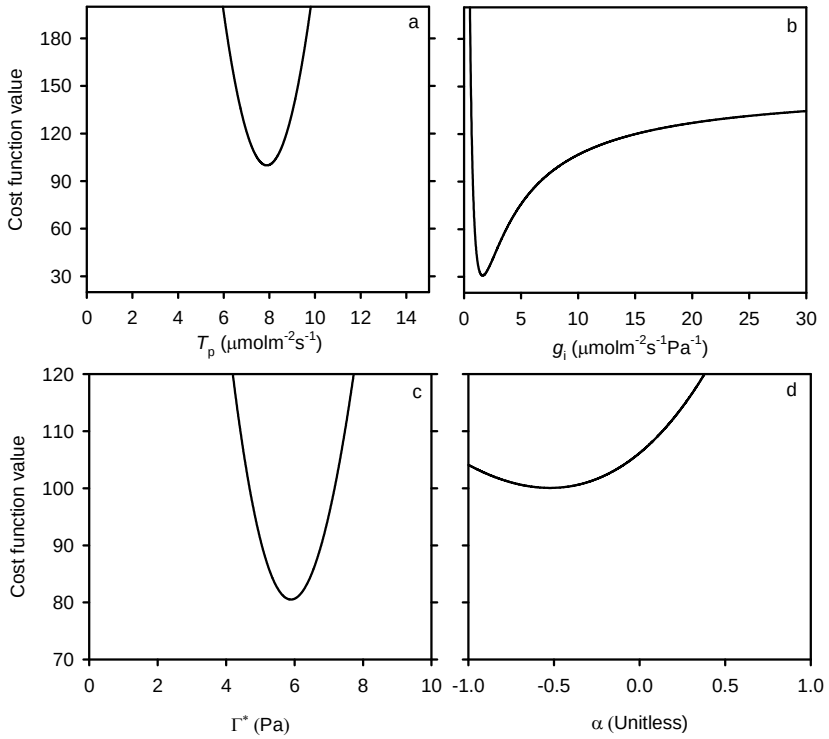
The cost function is now discussed in more details. In particular, we explained why the cost function under EDO behaves nicely. The visualizations of the cost function are for

illustration purpose only. We cannot really image what the overall shape of a cost function looks like in the multidimensional space and besides, each time you cut through a different plane, you get a different shape. Since the manuscript is getting too long and since we have already compared the estimated parameters with the true parameters for 100 synthetic A/Ci curves, we thought plots for a few parameters would serve the purpose. However, we include plots for the rest parameters here for the interest of this reviewer.

Cost function of SEM for the simulated A/Ci curve of Figure 1a in the manuscript



Cost function of EDO for the simulation A/Ci curve of Figure 1a in the manuscript. Note that the minima shown in the plots are not at the true parameters because the plane that cuts through the space of the cost function does not pass through the true minimum of the cost function.



Establishing theoretical confidence intervals for a change-point model as complicated as the FvCB model is not straightforward. A possible way forward is to use a massive number of Monte Carlo simulations with error terms introduced. But this is beyond the scope of this current paper. In the current paper, we relied on rigorous mathematical reasoning, synthetic A/Ci curves, actual A/Ci curves of multiple species, and independent chlorophyll fluorescence measurements to test the new approach.

30. Comment: The asymptotic approach to estimating the TPU rate without the TPU limitation
The observation that TPU parameters can be approximated even in the absence of observations in the TPU-limited range appears novel, and is interesting and important. The manuscript should take care to avoid the appearance that a model is being fitted to inexistent data, which sounds odd, when in fact the approximation presented here seems reasonable.

Response: Thanks. A crucial point that we failed to convey is that a real A/Ci dataset is not some assemblage of random data. In order to form a curve, the three limitation states have to be related. How these states are related to each other is independent information.

We need to give more detailed, clear explanation on the rationale of the asymptotic approach. More tests are also needed. But we are running out space so we decided to cut this part off entirely. When we write a separate paper on this issue, we will keep your comment in mind.

31. Comment: Testing the new approach with simulation
Refer to comments under 'comparison between methods'
Testing the new approach with actual measurements
Interesting illustration. Liked the idea of using concurrent fluorescence measurements to ascertain the location of the change-point. Please add a table to each plot, with the number of phases found in the data, and the parameter estimates.

Response: The parameters estimated are listed. The phases are indicated by different symbols in the plots.

Thanks for the literature provided.

Response to Comments by Reviewer #2

1. Comment: This manuscript adds to a growing list of A/Ci (Cc) analysis programs that are inundating the literature. As with previous, and likely additional, papers, there are clear strengths and weaknesses. Many of the assumptions of the current paper are questionable and in some cases incorrect. Some of the advice neglects known issues with A/Ci analysis, and some of the information with which the authors 'enlighten' the readers is already presented elsewhere in the literature. The mathematical logic of this paper is strong, but the in many cases the math contradicts the biology. The actual program looks like it does an excellent job of fitting curves, but the authors trade "short-comings" of previous attempts with "short-comings" of their own.

Response: We appreciate the critical comments made by this reviewer and are grateful for the citations provided. We note that this reviewer did not criticize the main component of this manuscript, that is, the Exhaustive Dual Optimization approach to fitting A/Ci curves. We hope EDO is not just another addition 'to a growing list of A/Ci (Cc) analysis programs that are inundating the literature' but represents 'the definitive solution to a longstanding problem'. (See comment made by Reviewer #1).

EDO was developed after countless trials, failures and frustrations in an effort that started in 2004 (the year we started to take A/Ci measurements at the Missouri Ozark AmeriFlux site). At first, we focused on testing or developing new optimization algorithms that were faster and more efficient. But these algorithms even failed to retrieve the parameters for simulated A/Ci curves. In the end, we realized we needed to have a thorough understanding of why it was so difficult to fit A/Ci curves. This led us to conduct a structural analysis of the FvCB model from a parameter estimation point of view. This analysis revealed the sources of difficulty. Once these sources of difficulty were identified, we were able to hit the idea of EDO fairly quickly.

Because of all the frustrations of these years, we probably got a little bit carried away with the apparent success of EDO. After reading this reviewer's criticism, we re-read our manuscript and felt we could have treated previous methods in the literature more sensitively. In particular, we felt the word 'blindly' in the paragraph that immediately followed the discussion on previous methods, was used inappropriately. In the revision, this word was removed.

In the following, the specific criticisms are addressed.

2. Comment: On page 4, the authors mention flaws with previous analysis tools. One of them focuses on Sharkey et al. (2007) suggesting exclusion of data between 20 and 30 Pa. This is incorrect – on their workbook they mention 'as a first approximation', exclude this data. This allows for some understanding of where the limitations exist and thus allow for further analysis based on this knowledge. If you are going to be critical of other techniques, then you should ensure that you understand the previous techniques.

Response: We apologize if we misunderstood Sharkey et al. (2007). We were trying to give a brief description of the previous techniques and didn't intend to treat the exclusion of data between 20 and 30 Pa as flawed. In the revision, the following words are used to describe the technique of Sharkey et al (2007):

"Sharkey et al. (2007) suggested that exploratory fitting is conducted first with data between 20 and 30 Pa excluded in order to gain some understanding on the distribution of limitation states and further analysis could then proceed with the aid of this understanding."

3. **Comment:** On page 7 near the top, strong emphasis is given about the need to model TPU for ecosystem models. While this technically is correct, I think the authors need to consider that the differences between TPU limited and RuBP limited photosynthesis are usually minor except under extreme conditions. So, incorrectly estimating RuBP limited photosynthesis as TPU, or vice versa, would result in errors that are well within the errors of even the best ecosystem model. Perhaps this is, then, overstated.

   Response: Ignoring TPU-limited photosynthesis or using inappropriate TPU parameters introduces systematic errors in ecosystem models. These errors might be tiny at short time scales, their integration over time grows as the time scale concerned increases. Ecosystem models are often applied at time scales from decades to hundreds of years. At these time scales, the integration of biases due to inappropriate treatments in TPU-limited photosynthesis could be very significant.

4. Comment: On page 16, the authors mention problems with 'blindly fitting' and A/Ci curve. Again, most previous A/Ci analyses do not suggest blind A/Ci analysis. Rather, there are first attempts for estimating where the limitation occur but this does not mean that analyses are conducted 'blindly'.

Response: Again we regret the use of the word 'blindly' in this context even though it did not refer to any specific technique. This word is removed from the revised manuscript.

5. Comment: On the guidelines for A/Ci analyses, there are three main criteria that are established by these authors. All three are worded as being unique and as correcting 'dogma', but all three of these are either wrong or well known and previously discussed. First, the sentence on page 17 that states, "This suggestion is a deviation from the conventional…" is just wrong. The research community knows that a large number of data points, the more the better, should be taken over all limitations of interest. The second point regarding measurements at the low points is also blatantly incorrect. von Caemmerer et al., 1994 realized that the initial portion of the A/Ci curve it too linear to estimate accurately Vc,max. Thus they used transgenic tobacco with only 10% Rubisco to estimate these parameters. Since then, it was realized that accurate curve fitting requires data points over a wide range of Ci concentrations. The third point can potentially be disastrous for a researcher. Research by Cheeseman (1991) suggests that

heterogeneity associated with stomatal patchiness or other processes might exist across a leaf. If A/Ci measurements are made in a leaf exhibiting some degree of heterogeneity in underlying photosynthetic machinery or in stomatal conductance, then it is the measurements around the transition from one limitation to the next that will be the most prone to error (Chen, Zhu & Long, 2008).

Response: Many thanks for pointing out the papers we missed. It is true that "a large number of data points, the more the better, should be taken over all limitations of interest". In reality, you can only take a very limited number of data points before the state of the leaf is changed (or the leaf is 'overworked'). Our intention in discussing the guideline for A/Ci measurements is to look for a strategy that can get the maximum amount of information out of a limited number of data points.

The full sentence that the reviewer criticized being 'just wrong' reads: "This suggestion is a deviation from the conventional emphasis on the Rubisco-limited state by the research community". We are glad that the community already realizes the importance of other limitation states for getting right the Rubisco-limited state parameters in A/Ci curve analyses and therefore we are glad we were wrong on this one. This sentence is now deleted from the text.

Thanks for pointing out von Caemmerer et al (1994). We really should have attributed credit to this paper. This is now corrected.

We are somewhat surprised by the strong opposition of this reviewer to our suggestion of utilizing transitional points in constraining A/Ci parameter estimations. ('The third point can potentially be disastrous for a researcher'.) We were aware of the potential influence of leaf heterogeneity on transitional points in A/Ci analysis. But both Cheeseman (1991) and Chen, Zhu and Long (2008) seemed to downplay this issue. In Cheeseman (1991), it states 'even when conductance varies with a standard deviation of twice its mean value, the effects on the curve are minor'. In Zhen, Zhu and Long (2008), one can find the following words: 'we conclude that error caused by heterogeneity in the estimation of these parameters *(A/Ci parameters)* and error resulting in turn from their use in crop production and Earth system models will be small'. (Italics are ours.). Because our approach has the advantage of being able to treat transitional points as co-limited, we thought that the disproportional amount of information contained in these points might outweigh the uncertainty due to the potential leaf heterogeneity. Nevertheless, we realize there is no clear cut on this issue and since it is not possible to know where these transitional points are located during the measuring process anyway, the discussion on transitional points is eliminated from the section "Guideline for informative A/Ci curve measurements" in the revised manuscript. This also helps shorten the manuscript.

6. Comment: On Page 26, the authors suggest that TPU limitation can be estimated as the asymptote of RuBP limited photosynthesis. This is absolutely incorrect. Just because equations can be used to establish an estimate of where TPU limited photosynthesis might occur does not mean that this is biologically meaningful. This would result in

researchers having the opportunity to present completely made-up data for TPU and is dangerous.

Response: We need to give more detailed, clear explanation on the rationale of the asymptotic approach. More tests are also needed. But we are running out space so we decided to cut this part off entirely. We will write a separate paper on this issue and we will keep your comment in mind. But here we offer the following response to your comment.

In our original manuscript, our presentation of the asymptotic approach for estimating TPU gave readers the impression that the approach was a perfect alternative to having TPU-limited points in the dataset. This certainly was not our intention. It would be much better to have TPU-limited points in the dataset. The following assumptions are needed in order for the approach to be valid:

- The three limitation states exist simultaneously (not necessarily in the dataset, but for the leaf sampled under the measurement condition).
- The TPU-limited photosynthesis is described by the reduced form: Ap= 3Tp-Rd (that is, alpha=0).
- The A/Ci curve contains points not too far away from the saturating level.

The approach is used only if the dataset contains no TPU-limited points (EDO is able to detect whether or not points of a particular limitation state occur in the dataset).

We need to clarify that the asymptote used in the estimation of TPU is the asymptote exhibited by the whole A/Ci curve, not "the asymptote of RuBP limited photosynthesis" as stated in the comment by Reviewer #2. This is important because the asymptote of the RuBP-limited photosynthesis is A=J/4-Rd while the asymptote of the whole A/Ci curve is A=3Tp-Rd.

Thus the key is whether the asymptote of the whole A/Ci curve can be estimated with a reasonable accuracy. If it can, the asymptotically estimated TPU is biologically meaningful.

We argue that the asymptote of the whole A/Ci curve can be estimated as long as the A/Ci curve contains points not too far away from the saturating level. The three biochemical states described by the FvCB model are not independent. They are correlated. If there is nothing else that relates them to each other, they are at least from the same leaf. No A/Ci curves vary erratically. That means, if we have information about two of the three states, we ought to be able to make some inference about the third one. In fact, in the revised manuscript, we proved that as long as the three limitation states exist, Vcmax, J and Tp have to obey the following inequality:

$4Vcmax > J > 12\ Tp$.

Thus, if we know any of the three parameters, we can automatically set a range for other two parameters. Of course, we should not take the condition "as long as the three

limitation states exist" lightly. If one decides to measure an A/Ci curve at low PAR, then the RuBP regeneration might be the only state that exists and the asymptotic approach would not be valid for such a dataset.

The Reviewer #2 himself/herself commented that "the differences between TPU limited and RuBP limited photosynthesis are usually minor except under extreme conditions". (See Comment 3 in Reviewer 2.) This comment is relevant to our argument here. If you have some RuBP limited points with a range of Ci values, you can pretty much determine the magnitude of the rates of TPU-limited photosynthesis (that is, the TPU-limited photosynthesis cannot be off very much from the RuBP limited photosynthesis).

Thus we strongly feel that the asymptotic approach does have merit. The asymptotically estimated TPU is biologically meaningful as long as the A/Ci curve contains points not too far away from the saturating level. Of course, this is not to say that researchers should now forget about covering TPU-limited points in their measurement.