

Interactive comment on “Inferring the effects of sink strength on plant carbon balance processes from experimental measurements” by Mahmud et al.

## Response to Referee #2

### Overview

The paper by Mahmud et al. presents a data assimilation exercise where data from a manipulative experiment on small trees had been ‘assimilated’ by a carbon balance model (CBM). The manipulation aimed to reduce root sink strength by constraining growth space of the root systems with varying pot sizes (5 – 30 L, in 5 L steps, 35L, and a ‘free’ treatment where trees were grown without limitation). On these trees biomass pools (structural biomass of leaves, wood and roots, non-structural carbohydrates, NSC, in leaves) were measured with different frequencies and used to constrain the CBM which simulated GPP based on parameters derived from punctual measurements of assimilation and respiration. Model runs with different structures (with/without a NSC ‘storage’ pool) were performed to test how important such a carbon buffer is for CBM simulations. The set of parameters of a best suited model (parametrized with three sink strength classes) was discussed with respect to plant carbon (allocation) dynamics in response to sink limitation. In addition, an attribution analysis was performed which aimed to provide information of the underlying mechanisms responsible for changes in biomass from sink limitation. The authors highlight the need for including a ‘storage’ component in vegetation models and the usefulness of their approach for further investigations to ‘develop appropriate representations of sink-limited growth in terrestrial biosphere models’.

We appreciate the reviewer’s comments and careful reading of our manuscript.

### General comments

This is a very nice project as it combines experimental manipulations with a data assimilation procedure. During the last years I have been running several experiments to manipulate the plant carbon balance. I have been thinking repeatedly about such a data assimilation approach to learn more about plant carbon dynamics and the underlying mechanisms. This study here does exactly this and I applaud the authors for making this progress.

We appreciate the reviewer’s positive comments, and are hopeful that he will find our approach useful in future. To aid uptake of our approach, we have made all code freely available as a Git repository:

[https://github.com/kashifmahmud/DA\\_Sink\\_limited\\_experiment](https://github.com/kashifmahmud/DA_Sink_limited_experiment)

That being said, I think that the interpretation of the data and the general presentation of the manuscript can be improved to increase its impact. For example, one of the main findings of the study, i.e. the importance of a storage component for (more) realistic simulations of plant functioning, is a strawman. Plants do have a storage component and of course models that specifically include carbon storage will be more realistic, in particular in situation where NSC may accumulate due to sink limitations.

Here we must respectfully disagree with the reviewer. Although in reality plants do have a storage component, it is not necessarily the case that including such a storage

component in the model will lead to model improvements. Making a model more and more complex to “better represent reality” is unwarranted in many situations – the idea of a model is to abstract reality, not reproduce it. Hence, it is actually quite important to test whether or not adding the storage component improves the performance of the model enough to justify the additional complexity. To address this comment, we will add a sentence to the introduction to explain why we feel it necessary to test the “strawman” null hypothesis.

In my opinion, the merits of the study are not the particular findings but rather the documentation of the potential of the data assimilation approach. The findings have to be taken with caution as the constraints from measurements are simply not sufficient to allow deeper insights into plant functioning. For example, measurements of assimilation and respiration have been done twice only and the photosynthetic parameters derived from these two measurements were used to estimate GPP over the whole season. How robust are these parameters for that purpose? Similarly, leaves were sampled every second week for NSC measurements and the structural biomass of stem and roots was determined only for the fourth months or at the end of the experiment, respectively. Given these limitations, how relevant are your inferences, for example, that sink limitation has led to reductions in photosynthetic rates or enhanced respiratory losses?

We are grateful that the reviewer recognises the potential of the DA approach to provide insight into C balance processes. However, we must also respectfully disagree with his comment that the measurements “are simply not sufficient to allow deeper insights into plant functioning”. Although some measurements we’d like to have had are missing – notably root and stem NSC, growth respiration, turnover, and root exudation – we believe that this particular experiment is sufficiently well-constrained to draw the inferences that we have.

The reviewer mentions photosynthesis. Although we only used two sets of A-Ci data to estimate the parameters, net leaf photosynthesis was measured fortnightly throughout the experiment (Campany et al. 2017) and we can be confident from the lack of trend over time that these measurements are robust and representative. We will add information on this to the methods.

The height, diameter and leaf area were measured fortnightly throughout the experiment, and root biomass is also constrained at the end. As we note in the discussion, there is some question over whether our “Y” value represents growth respiration or other carbon losses from the plant, such as root exudation, but we have reasonable confidence, from the combination of measurements available, in our inference that the C loss term is increased with sink limitation.

No experiment can perfectly quantify the C balance – there will always be data gaps. We are enthusiastic to see our approach applied to other experiments, such as those described by the reviewer, but there are likely also to be gaps in these datasets to constrain the key C balance processes. Fortunately, the DA approach does not need to

have all of the data streams from each of the C stocks or fluxes, and can estimate some missing C stocks from other measurements, although of course the precision of model predictions increases with data availability. We already covered the impact of missing data streams at some length in the discussion; we will refine this discussion to more directly address the reviewer's comment.

Additionally, NSC were measured in leaves only and their distribution among plant organs estimated with fixed parameters. For a study that specifically aims to highlight the role of NSC storage in plant functional processes, this is a critical shortcoming. Within the experimental period, there may have been substantial shifts in the NSC distribution across organs and this could have a substantial impact on the simulated carbon dynamics.

The sink-limited container experiment only measured foliage NSC, and therefore to estimate the partitioning of the non-structural C among different organs, we used data from a different experiment on similar-sized seedlings of a related species (*Eucalyptus globulus*) (Duan et al. 2013). In that experiment, root, stem and wood NSC were measured repeatedly over two months and there was no statistically significant difference in the NSC distribution. We thus believe this is a justifiable assumption. We will add this information to the discussion, and acknowledge this as an uncertainty.

That being said, I think you should rather discuss the approach, its potential but also its limitations. One aspect, for example, is how well a study on seedlings can 'develop appropriate representations of sink-limited growth in terrestrial biosphere models'. Such models usually simulate mature trees, not seedlings. We have recently published a paper addressing this particular topic: how to make use of seedling studies for inferences on mature trees and modeling of vegetation dynamics (see reference from EEB below). Instead of too many inferences I would like to see a critical evaluation of your method, including an assessment of what data are needed to get better constraints for the model. I have done experiments with small trees in growth chambers where GPP and several components of the carbon balance have been assessed continuously or at high temporal resolution. Applying sink limitation (I used source limitation but also drought, which is also a form of sink limitation) in such an experimental setting would allow making much more robust inferences than with the data set used here. Hence, my suggestion is to move away from the current focus of interpreting plant functional responses and instead concentrate on presenting the approach as a promising avenue for how to gain insights into plant functioning. I hope my comments can help increasing the paper's impact.

We will add a discussion of the implications of this study for larger plants, drawing on the review paper mentioned by the reviewer. We already have a section where we discuss potential and limitations of this approach and also identify the data needed to better constrain the model framework in article 4.2 (line 567-580). We will refer to the papers suggested by the reviewer as examples of experiments where this approach could be useful. However, we are reluctant to remove our focus on drawing inferences from the experimental study here. As described above, we believe that our inferences are valid - to the extent that we already describe in the Discussion - and help to

demonstrate the utility of the approach. Hence, we respectfully suggest that we should continue to do both: interpret the responses and present the approach.

Specific comments:

Abstract: Please add what species you have been working with.

Will be added.

L 20: processes affected by growth? That doesn't make sense.

The clause read "processes affected by growth under sink limitation". We will shorten to "processes affected by sink limitation"

L 21: What do you mean by 'component processes'?

We meant various C cycle processes contributing to growth e.g. photosynthesis, respiratory losses, utilization of NSC, allocation pattern, turnover rates as mentioned in the following sentence. There is not space in the abstract to further expand on this; we will elaborate at line 82 in the Introduction.

L27-29: Not much content in this sentence.

We will restructure the sentence.

Introduction in general: The structure of several paragraphs is not ideal and reduces the logical flow. For example, on L 55 you start a paragraph by asking how to include source and sink limitations in models but then you move to NSC storage in models. I understand that storage allows buffering discrepancies in source and sink activity but this is not strictly related to the limitations. A more logical flow would be to say that there is ongoing discussion about realistic implementations of NSC in vegetation models and that, because of their multiple roles in plant functioning, such an implementation also provides a buffer against discrepancies in source and sink activity.

We will reorganize the paragraph with the addition of referee's observation on ongoing discussion about the realistic implementations of NSC in vegetation models.

L74-76: Quantify growth by manipulating rooting volume? That also does not make sense.

We will omit "to quantify growth" from this sentence.

L 88: Very good point!

We are not quite sure which point the reviewer is referring to here. However, as it is positive, no revision is needed.

L90-97: See also paper by Klein T, Hoch G. 2015. Tree carbon allocation dynamics determined using a carbon mass balance approach. New Phytologist 205, 147-159.

We will add a reference to this paper as it's very relevant.

L 105-123: The presentation of the hypothesis is very awkward. Could you present this please in a more accessible and appealing way? This is not a funding proposal but a text

intended for keeping readers keen on reading on. Please rephrase and restructure to make this a flowing text.

The presentation of hypotheses is evidently a matter of taste. We felt that, given we had several different hypotheses to test, it made sense to lay them all out clearly and concisely in this way. We ask for the editor's advice here: we can turn this part into regular paragraphs if he feels it will improve the communication of our ideas.

L137-141: Relocate further up to L 129 (after Australia).

Will be relocated.

L 142: I suggest presenting the data first, then the model.

We will swap the subsection 2.2 and 2.3 to follow reviewer's comment.

Table 1: Why is there no hypothesis for simulation set C?

Simulation set C aimed to quantify the effect of changes individual parameters on overall seedling growth, and that is why this section does not come with any hypothesis.

Results: The results are presented in a very uncommon form. The text repeats the hypotheses (not useful) and reads more like a discussion than a presentation of results. I suggest adapting a more formal style so the reader knows to differentiate between results and interpretation of results.

Again, we refer to the editor for advice. It is true that for many papers, it is appropriate to present the results without reference to the hypotheses, and only return to the hypotheses in the discussion. However, here we felt it was necessary to address the hypotheses directly in the results to better guide the reader through the outcomes of the different model analyses. For example, consider section 3.1, where we present the BIC values for different simulation sets. We could here reduce the paragraph to just lines 299-303:

"Table 3 (Simulation Set A) shows the results for model fits with the optimal grouping strategy (three treatment groups). BIC values were consistently lower for the model including the storage pool; the improvement in model fit is most noticeable for the containerized seedlings."

and move the sentences describing how the hypothesis is tested to the discussion. Our personal feeling is that this makes this result paragraph fairly impenetrable. However, we are happy to take the editor's decision on this.

L 329: You mean Fig. 2. Please correct figure numbering for the following figures also.

We have checked the Figure numbers on the pdf version from BGD library and believe that all figure numbers are correct.

Figure 1 (actually Fig. 2): Add title to each panel (leaf, wood, root, NSC).

We will add the titles to each panel.

L 352: Shouldn't this section be presented before the modeling outcome? Parameters first, then the modeled pools?

When writing the manuscript, we experimented with both orders of presentation. We found this order of presentation to be more logical. Showing the data first allows the reader to see how the DA works in terms of data fit, before moving on to examine the model parameters.

L 413: Is this a sensitivity analysis?

No, it's something a bit different from a sensitivity analysis, which is why we call it an attribution analysis. A sensitivity analysis aims to quantify the sensitivity of model outputs to a change in one or more input parameters. What we are doing is trying to work out (attribute) how much of the observed change in growth is due to the observed change in each parameter.

L 418: This belongs into the methods section.

We have already mentioned the procedure in methods; this sentence was intended to remind the readers that the analysis attributes the change in biomass from the smallest container to free seedling. We will omit this sentence.

L 422: And this should go into the figure caption.

Will be moved to figure caption.

Table 4: Most of this information has been reported in Fig. 4 already.

According to the response to reviewer 1, we will modify Table 4 to present the effect of the parameters changing one at a time resetting the previous parameter to the baseline value, which will illustrate the contribution of each individual parameter separately, along with the interaction among parameters.

L 460-465: The emphasis here is on inferences on processes that are poorly constrained. See my general comments.

The beauty of the DA approach is that it is possible to make inferences about processes that are individually poorly constrained, but contribute to overall growth, and hence can be constrained against total growth. Here, as mentioned above, we have 15 coefficients to determine against 44 mean data values, each of which is determined by 7 replicates. This total amount of data enables us to fully constrain the model. We will add this explanation to the discussion.

L 466-467 (and at beginning of other paragraphs): Please avoid restating the hypotheses.

Again, this is a matter of taste, and we will defer to the editor. The reviewer's point is that we have already stated the hypotheses in the introduction, so there is no need to re-state them here. Our preference is to re-state them, otherwise the reader will need to

keep flipping back to the introduction to check which one “H2” was again, and we think that could be annoying.

#### Discussion in general:

I'd relocate the focus to discuss the potential of the approach and move away from interpreting the model outcome with respect to plant functioning. The discussion is somewhat lengthy and verbose. Please be more concise and to the point.

As explained above, we prefer to keep both emphases in the discussion. We will go through the discussion and attempt to reduce the length and verbosity.

A few suggestions from my own work which are based on whole-plant assessments of the C balance:

Hartmann H, Adams HD, Hammond WM, Hoch G, Landhäusser SM, Wiley E, Zaehle S. 2018. Identifying differences in carbohydrate dynamics of seedlings and mature trees to improve carbon allocation in models for trees and forests. *Environmental and Experimental Botany*.

Hartmann H, McDowell NG, Trumbore S. 2015. Allocation to carbon storage pools in Norway spruce saplings under drought and low CO<sub>2</sub>. *Tree Physiology* 35, 243-252.

Hartmann H, Trumbore S. 2016. Understanding the roles of nonstructural carbohydrates in forest trees – from what we can measure to what we want to know. *New Phytologist* 211, 386-403.

Huang J, Hammerbacher A, Forkelova L, Hartmann H. 2017. Release of resource constraints allows greater carbon allocation to secondary metabolites and storage in winter wheat. *Plant Cell Environ* 40, 672-685.

We have read through the suggested papers and will cite appropriately where relevant.

#### References:

Campany, C.E., Medlyn, B.E. and Duursma, R.A. (2017) Reduced growth due to belowground sink limitation is not fully explained by reduced photosynthesis. *Tree Physiol* 37(8), 1042-1054.

Duan, H., Amthor, J.S., Duursma, R.A., O'Grady, A.P., Choat, B. and Tissue, D.T. (2013) Carbon dynamics of eucalypt seedlings exposed to progressive drought in elevated [CO<sub>2</sub>] and elevated temperature. *Tree Physiology* 33(8), 779-792.