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

Response to Referee #1

#### **Overall Review**

The manuscript uses a data-assimilation technique to combine an essential but well thought model of carbon balance and plant growth with observations of photosynthesis, maintenance respiration, changes in biomass and NSC obtained in seedlings of Eucalyptos tereticornis planted in containers of different volumes and freely in the soil. The original experiment of Campany et al 2017 reproduced indeed different degrees of sink limitations. The dataassimilation technique allows the authors to infer time dynamics of model parameters (e.g., allocation fractions) and to quantify the relative importance of different processes in downregulating plant-growth under sink limitations. The article shows that the reduction of photosynthesis rate due to sink limitation is not sufficient to explain the reduction of plant growth since other adjustments in NSC utilization, allocation dynamics, and modified respiration and leaf turnover rates are playing an important role. The inclusion of a NSC storage pool and the capability to account for sink limitations emerge as key model components if results of the experiment are to be reproduced. While the path toward modeling plant growth under sink-limitations in mature ecosystems and under various environmental conditions remain long, this contribution is surely an important advancement in the right direction. The article is very well written and presented and most important it is very novel with comparison to the existing literature. As far as I know, it is the first time evidence of carbon sink-limitations is presented so markedly and modeled in a realistic context. In summary, I am very positive concerning the content and conclusions of the article. I think the manuscript is making a very important contribution to the field and I sincerely congratulate the authors for this nice piece of work. In the following, I just have some minor comment that can be helpful to improve further the presentation of this work, especially the comments: P.16 Line 352-358 and P.19. Line 416.

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

### Minor comments

P.2 Line 42. The reference "Bonan 2008" is not present in the reference list and if the authors refer to the article "Forests and climate change: forcings, feedbacks, and the climate benefits of forests. Science 2008, 320:1444–1449." I do not think it would be an appropriate reference here. I would rather search for something more related to "plant-growth and forest-growth modeling" rather than something related to Earth System Models.

We had intended to cite Bonan's book "Ecological Climatology" but in line with the reviewer's suggestion we have now chosen something more process-based about the modelling of forest growth: a review by Mäkelä et al. (2000).

P.2 Line 53. I would suggest to add also Paul and Foyer 2001, very relevant here.Paul MJ, Foyer CH. 2001. Sink regulation of photosynthesis. Journal of Experimental Botany 52: 1383–1400.

The reference will be added.

#### P.3. Line 67. It must be "Fatichi et al 2014".

This was a typo, will be corrected.

P.5. Line 146. Maybe this is not a case that has been encountered in this article. However, how does the model work when NPP is negative and therefore maintenance respiration is larger than carbon assimilation? Is maintenance respiration generally subtracted by the non-structural storage or is it done for each of the tissue separately?

We will modify the C balance model to clarify this situation of having negative NPP. The arrow representing the total maintenance respiration,  $R_{m,tot}$  will be relocated to connect with the non-structural C pool (C<sub>n</sub>). So, the total daily inputs of GPP will directly enter into C<sub>n</sub> pool and daily  $R_{m,tot}$  will be then subtracted from this pool before utilizing the reminder for growth.

P.5 Line 171. The relative amount of NSC in the roots appear to be a very small number, 9% of the total, while generally one would expect a significant amount of non-structural C-storage in roots especially for seedlings and grasses. Do you have any explanation for this?

There are not a lot of data on NSC of Eucalyptus seedling roots. We used data from an experiment with a related species, *Eucalyptus globulus*, grown in small pots until four months of age (Duan et al. 2013). There's no obvious reason why these plants would have exceptionally small root NSC contents. We have access to observations from *Eucalyptus parramattensis* saplings in another experiment, which show a higher root NSC fraction, but those plants were considerably bigger and older (~17 months). It is possible that these very fast-growing Eucalypts do not start to accumulate root reserves until they are well-established. We will add this discussion in the manuscript to explain the low root NSC.

P.8. Line 202-206. How are you dealing with the heterogeneity in photosynthetic properties among leaves and among plants? Were they significant? I know that you wrote in Line 211, that you use the mean for each treatment; is this the mean of how many replicates? Did you average the photosynthetic and stomatal model parameters (Vcmax,  $g1, \ldots$ ) or the A-Ci and stomatal conductance values?

Measurements of photosynthesis were made fortnightly throughout the experiment on one fully expanded leaf per plant (Campany et al. 2017). A-Ci curves were also measured twice during the experiment. Treatment effects on photosynthesis were detected immediately on newly produced (fully expanded) leaves and we did not observe variation over time in photosynthetic rates. Hence, the photosynthesis parameters were assumed not to change over time, but were specific for each treatment. This was also queried by reviewer 2. We will add text to clarify this point.

P.9 Line 222-223. If I am reading correctly there are 18 (3x6) coefficient to determine for each treatment, maybe this can be written explicitly to compare with number of

measurements (44 points) in line 231-233. This allows some redundancy even in the case of separating each container size.

The total counts of parameters and measurements will be added to the manuscript to show the actual number of redundancy. There are in fact 5 parameters to determine for each treatment group (as root allocation,  $a_r = 1 - a_f - a_w$ ), so a total of 15 (3x5) coefficients to determine for each treatment group, compared with total 44 data measurements available for each treatment.

# P. 9. Line 243-247. Please explain better this part of the data assimilation methodology, as it is not very clear to me.

We will elaborate on this section to provide more in depth idea of the DA algorithm.

### P. 14. Line 319. There are not "bold values" in Table 3. Probably a formatting issue. Yes, it was a formatting issue, and will be corrected.

P.16 Line 352-358. The lowest utilization rate in seedling in small containers would theoretically lead to an accumulation of NSC, at least in relative terms, which is something we do not see in Figure 2 and Figure 4. The explanation for such non-intuitive results is only provided in the discussion (Line 505-510) and justified as a temporal effect, where NSC first accumulates in seedling in small containers but then they are depleted by the higher respiration costs and leaf turnover rates. I think it would be quite interesting to see in Figure 2, NSC (Cn,f) reported as fraction of total C mass in foliage (Ct,f), e.g., Cn,f/Ct,f. This would serve the double purpose of explaining such a different dynamic in the use of NSC as the season progress and will provide the percentage of NSC in leaves that can be compared with other studies. This will likely highlight a higher concentration in seedling in small containers at the beginning of the season but a lower concentration at the end (as in Figure 4).

We will replace the NSC total plot with an NSC concentration plot in Figure 2, and move the NSC total plot to supplementary material.

P. 18. Line 401-403. The authors for some reason never refer to the concept of Carbon Use Efficiency (CUE), but I suggest it would be useful here to explain the results. Substantially what they are saying is that CUE = (1 - (Rm,tot+Rg)/GPP) is higher in free seedling and it is reduced by sink limitations. Maybe, a figure showing the temporal evolution of CUE for the various treatments would be also an interesting piece of information.

Carbon use efficiency (CUE), calculated as seedling biomass per unit total photosynthesis, was shown in Campany et al. (2017) and we had tried to avoid duplicating results that were already published. However, we will add numbers to this part of the results. The temporal evolution of CUE for various treatment groups is also interesting and will be added to the supplementary material.

P.19. Line 416. I am not sure if parameters were changed one at a time resetting the previous parameter to the original value or if effects are added up (which seems more the case from the presentation of results). If parameters are really changed "one at a time", this does not allow to reproduce all the interactions among parameters and can inflate the role of certain

parameters. Therefore the total effect of Table 4 (54.8 gC plant-1) does not necessarily correspond with the real total effect, which is not reported for comparison. If the parameters are switched on sequentially then you will obtain the total effect but the importance of certain parameters will not be clearly separated, since it will depend on the adopted sequence of switchers. For instance, the role of "k" would be likely smaller when the interplay with the other parameters is considered. Now, I am not asking to running simulations with interactions among parameters since they would be an extremely high number (going factorial) and they will not add much to the overall discussion on the model results. However, this simplification and the specific method needs to be stated explicitly and the difference in the total effect between the simulations changing parameters "one at a time" and the total observed effect needs to be mentioned, since it can provide an idea of the importance of the interaction among parameters.

The reviewer makes a good point. We suggest that we keep the attribution analysis shown in Figure 5 where the effects of various parameters are sequentially added up to get the total effect over the duration of the experiment, but that we modify Table 4 to present the effect of the parameters changing one at a time, resetting the previous parameter to the baseline value. This would fulfil both purposes, eventually showing the total biomass changes and the contribution of each individual parameter separately, along with the interaction among parameters. All these will be adjusted with explicit text to clarify the section.

P.20 Line 445-451. Table 4 and Figure 5. Following my previous comment, I wonder if it is not better to show the effect of each parameter independently rather than the sum of the effects of the parameters on the final biomass. I think it would be better to show the effect of each parameter by itself on the baseline rather than what is shown now. In any case, a clearer explanation of what is shown would be necessary.

According to the previous response, 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.

P. 23. Line 471. I think with regards to the importance of the storage pool in models, Fatichi et al 2016 would fit well here. Fatichi S., C. Pappas, V. Y. Ivanov (2016). Modelling plantwater interactions: an ecohydrological overview from the cell to the global scale. WIREs Water, 3(3), 327-368, doi: 10.1002/wat2.1125

The reference will be added.

P.25. Line 550-552. Another way to say the same concept is that CUE is higher in free seedlings.

Yes, indeed. We will mention CUE while discussing the C utilization rate.

P. 26. Line 586. Karst et al 2016 is not in the reference list.

The reference will be added.

P. 27. Line 605-610. I agree with the authors, but there is still an important challenge of dealing with sink limitations in ecosystems encompassing tall-trees and heterogeneous vegetation types and for which observations for data-assimilation may not be available.

We agree with the reviewer that there are challenges to apply this approach at ecosystem scale due to data availability. However, the main focus of our paper is to build up a foundational step towards understand plant functioning rather than solving ecosystem problems. Moreover, one of the important features of DA is that it does not need all the data streams from every individual C stocks and fluxes. There are several successful examples of DA being applied to forest growth, albeit without a focus on storage (e.g. Bloom et al. (2016), Van Oijen (2008), Williams et al. (2005)). Since this was also a question raised by the second reviewer, we will add a short discussion on the potential for applying DA to investigate storage at ecosystem scale.

# P. 29. Line 675-677. I thank the authors for referencing to my work, but this article is completely irrelevant for the current paper and indeed is just quoted by mistake.

This was a typo, and will be removed.

References:

Bloom, A.A., Exbrayat, J.-F., van der Velde, I.R., Feng, L. and Williams, M. (2016) The decadal state of the terrestrial carbon cycle: Global retrievals of terrestrial carbon allocation, pools, and residence times. Proceedings of the National Academy of Sciences 113(5), 1285-1290.

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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 [CO2] and elevated temperature. Tree Physiology 33(8), 779-792.

Mäkelä, A., Landsberg, J., Ek, A.R., Burk, T.E., Ter-Mikaelian, M., Ågren, G.I., Oliver, C.D. and Puttonen, P. (2000) Process-based models for forest ecosystem management: current state of the art and challenges for practical implementation. Tree Physiology 20(5-6), 289-298.

Van Oijen, M. (2008) Bayesian Calibration (BC) and Bayesian Model Comparison (BMC) of Process-Based Models: Theory, Implementation and Guidelines.

Williams, M., Schwarz, P.A., Law, B.E., Irvine, J. and Kurpius, M.R. (2005) An improved analysis of forest carbon dynamics using data assimilation. Global Change Biology 11(1), 89-105.