**Response to referees**

NPH-MS-2019-30786 Non-structural carbon ages and transit times provide insights in carbon allocation dynamics of mature trees  
by Herrera, David; Muhr, Jan; Hartmann, Henrik; Römermann, Christine; Trumbore, Susan; Sierra, Carlos  
  
Dear editor   
  
Thank you for sending this manuscripts to review by three different referees with a very high understanding of the subject. Their comments and concerns expressed helped us to improve the quality of the manuscript and to better communicate the results of our work. Below, we provide answers to each point raised by the reviewers. We hope that this helps to clarify previous issues about our results and their interpretation.

**Comments from referee 1:**

*This is a nice modelling study which could provide a framework for future researchers interested in simulating the NSC dynamics in tree species, using two previously published models, one form Klein and Koch (2015) and the ACGCA. I also like that the analysis uses R packages freely available in CRAN. While there are language issues throughout, they do not significantly interfere with the clarity of the message and would be easily fixed in revisions. I question certain results and their generality, the treatment of uncertainty, and have issues with some discussion results, as well as the somewhat limited conceptual scope of the introduction/discussion. In particular, ages from the ACGCA are much too old (particularly for leaves) and complete exhaustion of NSC by age class in Fig. 4 is unrealistic. The literature cited is also a bit sparse (only ~29 papers cited I think?), and the paper could benefit from more numerous and explicit connections with the large and diverse literature on NSC dynamics in trees (ie, not just limited to radiocarbon studies)*

We thank the reviewer for recognizing the importance of this manuscript and pointing out parts that can be improved. We will address each point individually in the specific comments below, also pointing out that the results the reviewer finds unrealistic in Fig. 4 are most likely due to a misinterpretation of the figure rather than conceptual problems with the approach.

**Answers to major concerns from referee 1:**

***1)*** *There is little discussion of uncertainty of the method for learning about NSC dynamics. These models are simplifications and are parameterized with fixed parameters not estimated from any data here (and, particularly for the Klein and Hoch model), only parameterized for a few trees at a single site. This strongly limits the generality of these results. I recognize the authors perform a relatively robust sensitivity analysis, but it is hard to really link these to the results, as uncertainty shown in Fig. 8 is not included on the other figures. It would greatly strengthen the conclusions if results figures included uncertainty estimates (ie error bars etc.). These could be obtained from the sensitivity analysis. The authors at least could show alternative trajectories under different sets of assumptions about fluxes among pools.*

We agree that our sensitivity analysis did not reflect clearly the uncertainty associated with the models’ parameters and therefore we included an uncertainty analysis based on Monte Carlo simulations as we describe below.

“Materials and methods

To evaluate how the uncertainty in the models’ parameters affects the mean age and the mean transit time of the species evaluated, a Monte Carlo Simulation (MCS) analysis was performed. This method involves repeated model realizations of a random selection of parameter values within the assumed parameter space (Parkinson & Young, 1998). We used only the most influential parameters in the models output to propagate the uncertainty. The variability associated with each parameter has been derived from Klein & Hoch (2015) for P. halepensis and Ogle & Pacala (2009) for A. rubrum and P. taeda. We assumed that these parameters come from independent Gaussian distributions. This assumption of independence is potentially limiting given that the MCS analysis would yield different results if there were covariance between the parameters. However, the degree of association between parameters is unknown to us, but if better information on their correlation would be available, this uncertainty could be re-estimated.

Results

The uncertainty in the mean ages and in the mean transit time associated with the uncertainty in the most influential cycling rates, described above, was smaller than the differences between the mean values of each species. In general, A. rubrum had higher uncertainties than P. taeda and P. halepensis (Fig. S1). Some very exceptional high mean ages of the NSC are expected in very rare combinations of parameter values at the very limit of their distributions (Fig. S1).”

We provide a supplementary figure (Fig. S1) where we show the uncertainty in the mean age and mean transit time per tree species and per trees’ carbon pool associated with the uncertainty in the most influential parameters. We provide the uncertainty associated with the mean values of the mean age and mean transit time in the text for the entire tree system and in the Table 3 for particular carbon compartments. We did not include this uncertainty in the figures of the manuscript because they are density distribution and it is unrealistic to add error bars to density distributions. An alternative to this would be to plot all the density distributions of each model realization resulted from the MCS, but it would not contribute to the final message and would make the figures extremely difficult to interpret. As far as the uncertainty in the model output is smaller than the expected differences in the mean age and transit times between tree species adding this extra density distributions for each species do not add extra information to the story.

***2)*** *Perhaps some of the above may lead to unrealistically high ages in the ACGCA, which is my major concern here. Mean age of sapwood NSC in Acer rubrum for example is about twice the reported values in Carbone et al. 2013. Even the stump sprouts in that study only had mean NSC ages of 17 years, this is still less than the mean age of stem NSC reported here (21 years). Another example: Mean NSC ages of 3.5 and 5 years in foliage. This is simply inconsistent with reality. Developing leaves become rapidly carbon autonomous, and the notion that deciduous trees are importing large amount of NSC into leaves each year, much less that this carbon has a mean age of 3 years (and thus includes much older carbon) is dubious. These ages suggest there are major limitations in trying to use these models in this way, and in my mind cast doubt on the other results.*

We included in the manuscript extra information that we hope clarifies these points. This comparison between modeled and measured mean ages is very important to highlight because it improves our understanding of model predictions and what NSC measurements actually tell us. We believe the model predictions we obtained are not as unrealistic as the reviewer points out. In particular:

1. The uncertainty analysis that was made as described above did not lead to unrealistic high ages in the ACGCA model, except for some very few cases when the most influential parameter values where at the limit of their distribution. Within one standard deviation, the mean age of the NSC pool may rise up to 13 years for *A. rubrum* (species with highest uncertainties) and up to 4 and 2 years for *P. taeda* and *P. halepensis*, respectively (with about 0.3 years of standard deviation). The pool for the NSC stored in the stem (SSNSC) had the larger proportion of old carbon respect to the rest organ specific pools, with a mean age for *A. rubrum* as 21.9 ± 5.3 years, for *P. taeda* as 14 ± 1.6 years, and for *P. halepensis* as 1.5 ± 0.58. These values are high in comparison with what has been reported previously, but plausible and in agreement with what is expected for the differences between tree species with different storage traits, different functional types, and life conditions.
2. The discrepancies between our estimates and the ones reported in Carbone et al (2013) are not as high as noted if we take into consideration that the modeled and measured pools do not correspond exactly among each other. Carbone et al (2013) measured the age of the total soluble NSC in the stem. This means that this age (10 years) correspond to the mean age of the NSC in the stem, fast and slow pool together. In our models we consider the NSC in the sapwood separated in two pools (fast pool and slow pool). The mean age of the fast pool is 2.56 year and the mean age of the slow pool is 21.9 years, if we combine the fast pools and the slow pool of the NSC in the stem-wood we would have something more comparable to the mean age reported by Carbone et al (2013). We included these calculations in the manuscript in Table 4. In fact, the mean age of the stem-wood NSC in *A. rubrum* that is estimated from our model is 9.97 years. In addition, the parameters used in our model were derived from a literature review carried out by Ogle and Pacala (2009) who gathered information on the parameters of *A. rubrum* and *P. taeda* for the ACGCA model. The source of information ranged across different environments and included alternative species when information for the particular species was not available. This may lead to potentially different parameters in comparison with particular trees from a specific forest type.
3. The mean age of the stump sprouts should not be compared with the mean age of the NSC stored in the stem. The 14C ages of the stem sprouts from the stumps partially reflect the transit time of the NCS in the stem, because the transit time is the age of the carbon that is being allocated to growth and respiration from the NSC system. That is why it should not be compared with the mean age of the NSC in the stem reported in this study, but rather with the transit time after the disturbance. The estimated NSC transit time after the disturbance depends on the amount of the NSC that has been consumed, for *A. rubrum* it reached 17 years at around 60% of consumption.
4. For the NSC ages of the foliage, it should also be considered the combined mean age of the fast and slow pools. 3 and 5 year old NSC in the foliage is restricted to the FSNSC (storage or slow pool in the foliage), and Fig. 3 shows that the age density of FSNSC is quite flat and small. The measurements of the age of the NSC in the foliage do not differentiate between fast and slow pools (FANSC, FSNSC and the proportion of E allocated to foliage). In our estimations: i) The active NSC pool or fast pools in leaves (FANSC) has a mean age of 2.5 years old for *A. rubrum* and 2.1 for *P. taeda*; ii) the storage pools in leaves FSNSC have a mean age of 3.5 years old for *A. rubrum* and 5.22 for *P .taeda*; and iii) a combination of all the pools that are in the leaves (FANSC, FSNSC and E) yield a mean age for NSC in foliage of 1.98 years for *A. rubrum* and 1.91 year for *P. taeda*.

***3)*** *Perhaps I am misinterpreting Figure 4, but it appears that as time progresses, trees exclusively exhaust young NSC and move into progressively older NSC. This is not coherent with understanding of NSC mixing processes in the sapwood. Trees mix young NSC into sapwood, quite deeply. The major driver of respiratory demand is of course the cambium. That trees are remobilizing young NSC, and young NSC only, from all sapwood depths concurrently and respiring it in increasing order of age is not realistic. As far as I understand, Muhr et al. 2018 suggest that the mechanism for reverse chronological mobilization is an emergent property of the heterogeneous spatial distribution of old NSC in the sapwood (that is, oldest NSC is deepest, and thus is respired last, as deep sapwood NSC is remobilized last). My understanding is also that they showed no evidence for this last in, first out, reverse chronological remobilization occurring at the cellular level. And of course this would be inconsistent with the results of Richardson 2015 inferring rapid and continuous interconversion between soluble (ie sugars) and insoluble (ie starches) fractions in the sapwood from nearly identical ages of these two fractions.*

With due respect, the reviewer is indeed misinterpreting the results presented in Fig. 4. The figure shows the age density distribution of the NSC in the entire tree of the three species investigated after subsequent years of a disturbance with no new inputs. The black line (0 years after disturbance) reflects the density of ages of NSC that each tree has in healthy non-limited conditions. The colored lines represent how those distributions would change after the indicated years of holding the trees under zero assimilation. We chose zero inputs because it is easier to distinguish how trees would use the remaining carbon without the noise of new carbon entering the system as it is intended with the girdling experiments used to understand how trees use their stored carbon. The figure does not show that trees consume exclusively carbon of certain age. After X years of disturbance, there is no carbon younger than X because carbon inside of the tree ages along with the tree and there is no new carbon assimilation. For example, after 3 years with zero inputs of new carbon, there cannot be carbon that is two or one year old.

We completely agree that trees mix young carbon deep in the wood. Indeed each of our NSC pool has a well mixture of carbon of all ages, with different abundances (Fig. 3). There is an asymmetric mixture of carbon of different ages in all pools, but this mixture changes from pool to pool depending on its dynamics. In Figure 4, we show the general NSC age distribution per tree and how it changes after the disturbance. Here, it can be noticed that the abundance of young carbon is higher for healthy mature trees, and it decreases exponentially for older ages. After the disturbance, these distributions are the ones that provide the carbon for sustaining the tree (the initial conditions). Therefore, as far as each carbon compartment is well mixed the more abundant young carbon will be used in higher proportion than the less abundant old carbon, making the distribution closer to uniformity after trees consume around 80 or 90% of the storage. Here we offer an alternative probabilistic explanation not necessarily consistent with the ‘first in-last out’ hypothesis or the ‘reverse chronological mobilization of the stored NSC’. Our probabilistic approach suggests that young carbon is used with higher probability simply because it is more abundant than older carbon that has a lower probability of being used.

***4)*** *There is another problem with Figure 4 however, and that is that trees respire NSC pools of certain ages to essentially zero (line 434 “trees consume between 80 and 90% of available carbon”). Near total exhaustion of NSC is not consistent with any experimental work on trees, and in fact is a central argument against carbon starvation as a lone driver of mortality in the tree mortality literature. In a meta-analysis, Adams et al. 2017 (Nature ecology and evolution) showed 40-50% is high, and no studies found exhaustion exceeding 60%. Recent experiments using complete carbon restriction (Wiley et al. 2019 tree physiology) also show that remobilization/respiration of certain NSC pools (ie sugar alcohols) may not actually be possible.*

Again, there is a problem of misinterpretation of the results presented in Figure 4. We made changes in the text to present this point more clearly in the manuscript. Figure 4 does not show that trees completely exhaust young carbon reserves. Zero carbon of young ages after disturbance is simply the effect of the aging of the carbon remaining in the tree after the simulation started and no additions of new carbon.

In the manuscript, line 434 refers to Fig. 6 and not to Fig. 4 as interpreted by the reviewer. In Fig. 6, we show how the mean transit time (the age of the carbon leaving the system, which is carbon being used in respiration and growth) changes over time due to disturbances. After the first 10 years of simulation (during steady state) we applied the disturbance, which consisted in ceasing all the new fixation of carbon for all the subsequent years. The mean transit time started increasing in relation to the consumption of the NSC. In this figure, the exponential increase in the mean NSC transit time is due to the asymmetry of the age distribution of the carbon stored in the NSC pools, the young carbon decreases faster due to its abundance and therefore the mean transit time increased almost exponentially. When the distribution gets flat and almost uniform, the mean transit time increases linearly with a slope of about 1. This is due to the fact that the proportion of carbon of all ages is equal and the increase of the transit time reflects just the aging of the carbon remaining in the tree. We noted that the age distribution of NSC usually flattened when trees consumed between 80 and 90% of the carbon for the three species. We did not intend to represent or estimate the point of mortality, but rather how the transit time would change under disturbance if the trees would have access to all the reserves continuously over time. An advantage of our approach is that we can predict the age of the carbon being used in metabolism and growth at any level of consumption, so it is interesting to evaluate how the mean transit time would look like if trees would consume between 80 and 90% of the reserves. This is the point where the NSC age distribution stops changing. We introduced in the manuscript the estimation of the mean transit time with more realistic consumption values of 50 to 60% to make our results more comparable with the measurements reported in the literature.

We also would like to note that with our modelling framework, we can describe how the mean age of the NSC changes over time under specific conditions that can be simulated in any compartmental model of carbon allocation that has been validated. Explicit representations of mortality and how this is related to the consumption of NSC is beyond the scope of this manuscript.

*I am not sure how many of the subsequent results emerge from those displayed in Fig. 4, but my guess is that the results in both Figs. 5 and 6 come out of the results in this figure. As such, I believe a major revision of the modelling and simulation framework may be required to rectify these issues.*

Fig. 5 and 6 are independent but complementary to the results shown in the Fig. 4. In particular, Fig. 5 shows density distributions of the transit time, which is the NSC that is allocated to respiration and growth before the disturbance (0 years after disturbance) and some years after the disturbance. Figure 6 shows the mean transit time and its changes when the disturbance happened. As mentioned before, the reviewer misinterpreted Fig. 4 and therefore misinterpreted the results presented in Figs. 5 and 6.

***5)*** *In general, parts of the manuscript are under referenced, particularly in the introduction and discussion. In particular the first paragraph of the introduction continually references Hartmann and Trumbore 2016. Background should be diversified as this continues to be a rapidly evolving field of study which is not well described simply by citing a 3-4 year old review and the classic radiocarbon papers. Furthermore, there is a huge body of work on NSC dynamics in trees from concentration measurements as well as pulse-chase labelling studies that is not invoked anywhere in the manuscript.*

We appreciate the reviewer for making us conscious of the under referenced parts of the manuscript and all the reference suggestions given in the comments. We added new references accordingly in the manuscript.

**Answers to specific comments from referee 1:**

*INTRODUCTION*

*Line 48: Please give examples of ecological scenarios where this is important. Carbon starvation is rarely the primary driver of tree mortality. Should probably note the other predictors of stress tolerance (ie traits: hydraulics, shade tolerance, pest defense etc.). Trugman ecology letters 2018 is a good example of the integration of these factors.*

This is an important point that we included in the manuscript as follows:

Carbon limitation may happen because droughts, physical damage, pest, diseases, and floods, which are becoming more frequent due to climatic changes (IPCC 2016, Klein and Hartmann 2018). Mortality associated with these stressful conditions (vor Arx 2017, Breda 2006, Brando et al 2019) may cause biodiversity loss (Allen et al 2010), economic losses (Carciner et al 2011) and long-term modifications to the global carbon cycle (McDowel et al 2018, pugh et al 2019.

*Line 57: also defense and osmoticum. Perhaps see the new paper from Erin Wiley, Landhausser and others (Tree Physiology, 2019) showing that not all stored sugars are available for remobilization under severe carbon stress, and thus, that osmotic control is a separate “sink.” Also relevant here is Huang et al. 2019 “Eyes on the future…” (New Phytologist).*

Included in line 65

*Line 59: “…NSC is allocated in organ specific compartments…” -- Great to see this noted, but then never again is the concept of non-uniform distribution of storage across tree organs brought up in the introduction.*

Probably it was buried in the second paragraph, but we tried to make it clearer along the introduction by changing tissue specific compartments by organ specific compartments.

*Line 62-63: Richardson et al. showed that sugars and starch are of the same age, suggesting rapid exchange between these pools, thus the parenthetical suggestion here that sugars are the active component is incorrect or at least overly simplistic.*

We agree and we eliminated the simplistic suggestion that sugars are the active pool and starch the slow cycling pool. We noted that for the *P. halepensis* model the parameters were estimated under this assumption and that is probably why we do not see differences between fast and slow carbon pools in this model. For the ACGCA however, we do not have the association between chemical composition and cycling speed for each pool.

*Line 70-71. Confusing, do you mean that older NSC is mixed inwards (if so, not sure this is in Trumbore 2015)? Or that inward mixing results in older NSC being found in deeper sapwood. Perhaps clarify.*

We add the next lines: The mixture of NSC inward in the wood, along rays, is older with respect to the mixture of NSC outward in the wood because the proximity to the carbon source -phloem.

*Line 74: “NSC dynamics” ◊ perhaps “The history of remobilization, translocation, and mixing…”*

We believe that NSC dynamics gather the history of remobilization, translocation and mixing, so we decided to leave the NS dynamics to have a more general concept and save space.

*Line 91 “(< 1 year old). However it can increase”. Also perhaps specify you are talking about the mean NSC pool age here.*

We are talking about the age of the carbon that is respired or allocated to growth, which is the transit time.

*Line 91. These papers are from a single forest type, and may not be broadly representative. I’d suggest to note the system (Eastern US temperate forest).*

We included the citation of Mühr et al 2018 to make the citation more representative.

*Line 98-99: “To date, we lack information about how fast assimilated carbon is used” ◊ There are many 13c pulse-chase studies, and even a few at the tree level, including information of respiratory efflux. Here is a review: “Pulse-labelling trees to study carbon allocation dynamics…” Epron et al. 2012 Tree Physiology.  
“and for how long these reserves remain available” ◊ Carbone et al. 2013*

We changed the entire sentence by something more specific as follows:

“To date, we lack systematic understanding about how different are the NSC age distributions between tree organs and tree species, and about the differences in the use of the NSC reserves under outstanding carbon source-sink limitations in species with different phenological types. To answer these questions, it is important to have reliable estimates of NSC age and transit time distributions.”

*METHODS*

*Line 221: zero assimilation ◊ Evidence that this is a realistic assumption? Or justification?*

We used zero assimilation to avoid the noise of new assimilates incorporation in the system after the disturbance and to resemble what it is intended in girdling experiments.

*Line 222: more equivalent to a reflush.*

We did not understand this comment.

*Line 237: package name is misspelled.*

This was corrected.

*Line 252: How is the magnitude of dpi selected? How is the maximum magnitude of dpi selected? What is the difference between these two quantities? How long are the simulation trajectories? How many trajectories is “several?”*

We clarified these issues in the new version of the manuscript. Specific answers to these questions are:

1. dpi is resulted by a random selection of the parameters between the 150 levels assigned to the parameter space.
2. The maximum magnitude of dpi is given by the limits assigned to the parameters.
3. Dpi is the fraction that each parameter changed with respect to the previous value. The maximum magnitude of dpi is how much it would be able to change.
4. Each trajectory has *k* parameters +1 simulations, where each parameter changes once in each trajectory.
5. We estimated 100 trajectories resulting in r\**k*+1 simulations.

*Line 253: I think it would be useful here to describe how this differs or is similar to a sensitivity analysis, and/or what can be uniquely learned from or what is the strength of this “Elementary Effect” approach?*

This approach allows us to identify the most influential parameters on the model output with significantly fewer simulations than more standard quantitative sensitivity analyses, and it is efficient identifying how nonlinear interactions among the parameters influence the model output.

*Line 256: most “sensitive”?*

This was corrected in the manuscript.

*RESULTS  
Line 265: An explanation (or reminder) of the difference between mean age and mean transit time, and why they can differ from each other, would be useful here.*

We included the interpretation of mean age and mean transit time within this sentence.

*Line 282-285: In general, the mean ages reported seem old. However, the mean ages of NSC in the leaves are definitely too old, as NSC is not imported into leaves for storage, nor stored for multiple years therein. Discussed in major comment 2.*

We answered in the response to major comment 2 and included in the discussion of the manuscript.

*Line 292: delete “the”*

This was corrected.

*Line 293: Very unclear. Suggest “95% of the NSC was less than 42 years old and NSC respired or allocated to growth did not exceed 2.9 years.” Also not clear where to find these results on the figures.*

The suggestion, which makes the statement clearer was included in the manuscript. This was calculated from the distributions presented in Fig. 3.

*Line 305: missing an apostrophe.*

This was checked.

*Line 339: “lesser”*

This was corrected.

***Table 2.*** *It would be better to split this table into 2 or at least label which parameters were associated with which model. As it stands I have to look at the parameter symbol, then look in both models to see where it is present. Certain parameters are not defined (Ls, which I assume is defined the same way as LSs, but still not very clear).*

LSs and Ls are the same, it was an unfortunate typo in Fig. 2. Table 2 has all the parameters of the three models listed and ordered by abbreviation, description, and parameter value. The names of the species correspond to the model where the parameter was used: *P. halepensis*, *A. rubrum* and *P. taeda*. We made this clear in the table description.

*Fig. 8. Why not provide R2 or correlations and p value of the fit as well as fitting method? Otherwise difficult to evaluate the importance of these relationships.*

The scatterplots in Fig. 8 are used to show how the model output is affected by numerical changes in the most influential parameters and the red line is provided to illustrate the trend in the sensitivity. The quantification of this trend is given by the two estimated elementary effects μ and σ. Provide R2 would be redundant with those sensitivity measurements and not relevant for the non-linear relationships. Each point in the scatterplot is a model realization with a different combination of parameters, so fitting a line to this “estimates” is pointless given that each point is a result of a model run so we know what is the exact combination of parameters used for each point.

*DISCUSSION  
Line 406-408: Yizhao et al. 2015 – These results are not directly comparable here as they reflect ecosystems, not single trees.*

That was a misplaced reference. The reference was deleted from this statement.

*Line 411-417. You have no evidence for this conclusion, that is, to ignore the importance of spatial heterogeneity of NSC age within tree tissues.*

We are proposing a plausible explanation for commonly observed use of young carbon in metabolism and growth over the stored old carbon based on our NSC distribution estimates and starvation simulations. In our analysis, we show that the distributions of NSC ages in the trees are more abundant in young carbon than in old carbon and therefore, probabilistically, young carbon has more chances to be used in metabolism and growth than old carbon. This is supported by the simulations of carbon starvation where it was observed that the young carbon is consumed in higher proportion than the old carbon until the distribution of NSC ages gets uniform.

The heterogeneity of the NSC age in the tree tissue is represented by the compartmental division between fast and slow cycling pools, and is noted in lines 516-517 of the manuscript. Nevertheless, in each carbon pool the NSC is well mixed.

*Line 433-435. 90% NSC consumption is not consistent with any experimental evidence.*

We are using this value as a threshold to describe the change in the increasing trend of the transit time in our simulations. We are aware that such a large reduction in NSC has not been observed in any empirical study yet, but our simulations can help to interpret results under the assumption that NSC depletion can reach such large values.

*Line 464. Which rates? If you are suggesting changes in allocation, a good example might be Hagedorn et al 2016 Nature plants "Recovery of trees from drought depends on belowground sink control”.*

Yes we are suggesting changes in the allocation. We included the reference in the manuscript and made the point clear.

*Line 467. I think you are talking about specifically about your model, this should be made clear.*

We are talking in general about how we describe the allocation. When we see the allocation as a compartmental model, fluxes and transfer coefficients may differ. In practice, we can measure fluxes and estimate transfer coefficients, and we think this is important to understand when and how allocation or sink strength changes in trees.

*Line 473-478. Long sentence. Split up and reference specific parameters to make this easier to confirm/follow.*

We include this suggestion in the manuscript.

*Line 478: “We now know” change to “Our model suggests”*

The suggestion was included in the manuscript.

*Line 499-502. Perhaps some references to studies showing these dynamics?   
“Climatically controlled reproduction drives interannual growth variability in a temperate tree species” Hacket-Pain 2018 Ecology Letters.*

We included some extra references such as: Epron et al 2011, Hacket-Pain 2018, Epron et al 2012, Huang et al 2018.

**Comments from referee 2:**

*This manuscript presents a study on the estimation of nonstructural carbohydrates age and transit time distributions. The work is of great importance as we lack understanding of carbon allocation traits in trees as well as their responses to environmental change. The authors found that NSC age and transit time distributions were quite different with tree species, and NSC age would increase under limited carbon input, using two carbon allocation models on three tree species. They also evaluated the sensitivity of NSC age and transit time to change of carbon fluxes and found the fluxes in wood and root mattered a lot in influencing NSC age and transit time.   
It is well written and well structured. I only have two minor concerns with the current version of the manuscript.*

We thank the reviewer for the positive opinion and we address the two minor concerns described below.

**Answers to major concerns from referee 2:**

***1)*** *Model comparison. The authors used one model proposed by Klein and Hoch (2015) for Pinus halepensis and another model from Ogle and Pacala (2009) for the other two species. We know that these two compartmental models have similar structures, however, we are not informed if these two models will originally lead to significant differences of estimation. The authors did not give us sufficient evidence of the estimated differences in carbon allocation traits in trees resulted from tree species or models. In other words, can it be possible to use one model to do the estimation on all three tree species?*

This is a very interesting point that we have discussed for a while. Unfortunately, we do not have the means to evaluate assertively the uncertainty related to the model structure. Nevertheless we estimated the mean NSC age and transit time for *P. halepensis* with the ACGCA model structure. We basically increased all the parameters of *P. taeda* to values similar to the reported for *P. halepensis*. The results were quite similar to the ones obtained with the model proposed by Klein and Hoch 2015 (Table 1). A possible big difference between the two models is the assumption behind the separation between fast pool and slow pool. The model proposed by Klein and Hoch 2015 was parameterized with the assumption that sugars are the fast pool and starch are the slow pool, instead the ACGCA model does not consider any conversion between sugars in its estimates.

Table 1 Mean age of the NSC in each NSC pool of P. halepensis trees modeled following the structure established from the ACGCA model but using the similar parameter values to the ones reported in Klein and Hoch 2015.

|  |  |
| --- | --- |
| Pools name | Mean age |
| E | 0.418 |
| FLNSC | 0.698 |
| RLNSC | 0.578 |
| BRLNSC | 1.333 |
| FSNSC | 1.333 |
| FSNSC | 1.198 |
| RSNSC | 1.398 |
| SSNSC | 2.247 |

One of the reasons we included different models in our approach, in addition to the possibility to analyze the NSC dynamics in species with different life conditions, is to show that the applicability of our analysis is not bound to a particular model and can be applied to any compartmental carbon allocation model to study the NSC dynamics.

***2)*** *Steady state. The authors stated that it was a condition when carbon uptake was nearly balanced by respiration and litterfall of mature healthy trees. Does that mean the state is steady when a tree is healthy? How to define a tree is in a steady state or out of a steady state?*

Steady state as considered here implies no directional changes in the carbon mass of the pools, which means that the tree should not be growing or shrinking in terms of mass. We assumed that a healthy mature tree is consistent with this working definition of steady state because it does not show large mass increments as a young growing tree, or continuous losses of mass such as a dying tree. For *P. halepensis* these properties were reported by Klein and Hoch 2015 in their studied trees. For the species of the ACGCA model, we ran the model for 700 time steps to assure that the trees enter in the steady state condition where none of the fluxes and tree mass changed over time. Our simulations of carbon limitation served to contrast the steady state assumption versus a non- steady state condition where the tree starts consuming its NSC and carbon mass starts to diminish.

**Answer to specific comments from referee 2:**

***1)*** *Materials and methods. I suggest you give more information on why you chose these three species in this part. What are their functional types and their living environments?*

The species were chosen because there were models available in the literature that report all equations and parameter values required for the simulations of our analysis. Furthermore, as stated in the manuscript, those species allowed us to compare between contrasting life conditions: between evergreen and deciduous trees in temperate regions, and between mesic growing conditions for temperate species and extreme limiting growing conditions as for *P. halepensis* in Yatir forest.

***2)*** *Fig. 7, all fluxes listed in Table. 2 were included in the analysis?*

Yes, all the fluxes that we listed in the Table 2 where included in Fig. 7. The fluxes that do not have significant influence on the model output pile up in the bottom left part of the plot with the lowest sensitivity indices, and it makes it difficult to differentiate them.

**Comments from referee 3:**

*This is a very well written manuscript that address an important knowledge gap that is clearly summarized in lines 98-101. The authors applied a modelling approach to answer three interrelated questions and show how trees with different storage traits respond differently to carbon starvations. As this is a modeling research, I do have some major concerns that apply to basic modeling standards that need to be addressed for proper interpretation and evaluation of the results.*

We again thanks the positive evaluation and address the major concerns below.

**Answer to major concerns from referee 3:**  
  
***1)*** *The manuscript present three important interrelated questions, but these questions should be associated with hypotheses. How models are expected to respond to these questions? Which model type (architecture) will be more closely appropriate (or related) to the expected hypothesis? Then the discussion should address why the models performed as expected or not as this are important issues to better understand model architecture, development and interpretation of the results.*

To make the hypothesis explicit, we included the following lines: “We expect these compartmental models, which consider organ specific and compound specific carbon pools, to allow us to estimate differences in the NSC age distributions of trees with different life strategies, and to evidence with them different storage traits. We also expect that by estimating the changes of the NSC transit time during a severe carbon limitation condition, we can describe the age structure of the carbon available for sustaining the tree’s metabolism and growth and estimate how long can take for the trees to exhaust their reserves depending on the NSC consumption level.”

***2)*** *The model results do not include uncertainty estimates. This is a modeling exercise so reporting uncertainty is required to evaluate model outputs. If uncertainty is larger than expected mean model differences, then the presented interpretation (based on mean model output without uncertainties) is incomplete or could be potentially wrong. I am not stating that the results are incorrect but without reporting model uncertainty I cannot provide a review about the results of this study. Several authors have large expertise in modeling and are aware of the importance (and difficulty) of reporting uncertainty in this type of exercises.*

We estimated the uncertainty on the model output from the uncertainty of the model parameters. This analysis was reported in the manuscript and we observed that the uncertainty is not bigger than the expected differences between species.

***3)*** *The model code should be made available in a public repository for transparency. Also include model inputs for reproducibility as the code could be very useful to other researchers.*

We are fully committed to transparency and reproducibility and therefore the code will be made available in a Git repository. Additionally, in the paper we are providing the model structure Fig. 1 and Fig. 2, and all the parameters of those models in Table 1. With this information and the function from the packages used from R (SoilR, FME, sensitivity) and from python (CompartmentalSystems) the exact same analysis can be reproduced.

***4)*** *The title should reflect that this is a modelling approach. By reading it I expected that it was an experimental approach.*

We consider that it is important to make it clear that this is a modeling approach, but we think that the title already reflects it since carbon ages and transit times are model diagnostics that cannot be measured directly from observations alone. Besides, it becomes clear that this is a modeling approach very fast in the summary and in the introduction.

***5)*** *The results section has several statements that represent interpretation of the results that should be moved to the discussion section.*

We cleaned up the results as much as possible.

***6)*** *There should be an explicit section about limitations for this study. The discussion section has several sections where the authors discuss limitations, but they are scattered and somehow buried in the text. A clear section about limitations will be useful for proper interpretation of the results and avoid over interpretation.*

We defined a section of conclusions and limitation in the last paragraph. We also left some other limitations in the discussion because we consider that they would be better understood if they are closer to the direct result that can be affected.