Authors' response to reviewer' comments on the manuscript bg-2019-87 "*Tree size and age induced stem carbon content variations cause an uncertainty in forest carbon stock estimation*" by Suhui Ma et al.

To the editor:

Dear Dr. Akihiko Ito,

Thank you very much for the constructive comments and suggestions from you and the two reviewers. These comments can be summarized as three major points: (1) explaining the effects of sampling and measuring methods on the results, (2) adding the uncertainty analyses of different sources in forest C stock estimations, and (3) adding the relationships between stem C concentration of different life forms and tree size (age). We have carefully addressed these comments and revised our manuscript accordingly. Please find our point-to-point responses to these comments as attached at the bottom of this letter. We also attach our updated manuscript with the "track changes".

We are looking forward to receiving your decision.

Best wishes,

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To Anonymous Referee #1:

Ma et al compiled a meta-database of carbon concentrations of different tree organs and analyzed that database for (1) correlations among the carbon concentration of the different organs, (2) relationships between carbon concentrations and tree diameter and age, as well as (3) the error coming from using either a fixed carbon concentration of 50% or the observed carbon concentration of the stem wood when calculating the carbon content of a tree. Although the study contains some interesting elements, the analyses and discussion lack the depth expected from a paper in Biogeosciences.

[Reply] Thanks for your comments. In general, forest biomass C stock is estimated by multiplying total forest biomass by a corresponding biomass C conversion factor, i.e., C concentration (Ma et al., 2018). However, how stem C concentration varies with organs and tree size, and how these variations affect the estimations of forest C stocks remains unclear. For these reasons, we have built a global dataset of age-specific tree organ C concentrations to address aforementioned issues. To further deepen our analyses and discussion as you pointed out, we have revised our manuscript as follows: (1) the effects of measurement errors on the relationships were quantified using a linear mixed model [Lines: 84-87], (2) the sampling methods of tree organs in the field were described in great detail [Lines: 69-71], (3) A homogeneous C concentration along tree height was assumed when using C concentration of stem at 1.3 m to the whole stem [Lines: 89-91], (4) one hypothesis were added into the discussion section to explain the variations of stem C concentration with tree size and age [Lines: 165-167], (5) the effects of these variations on forest C stock estimations were revised [Lines: 176-179], and (6) the uncertainties derived from the variation in C concentration were quantified and discussed [Lines: 186-189]. Thus, we believe that the analyses and discussion in our paper have been improved greatly.

[**Comment 1**] The observations of carbon concentration are treated as if they come without a measurement error. This is of course not the case. Which methods were used to determine carbon content? What is their precision in the range of the observations? Did the laboratories that performed the analyses run a quality control and quality assurance program? If so, what was the reproducibility of their control samples? If not, what is the typical measurement error of the different methods? Do the results hold when measurement errors are accounted for?

[**Reply 1**] Thanks for your comments. In our dataset, two methods were used to measure C concentration: the potassium dichromate oxidation method and the dry combustion method (Table 1), with a measurement error of 2.0–4.0% and 0.3%, respectively. Each C concentration data was the mean value of more than 3 replicates. To evaluate the effects of measurement errors as you pointed out, an error term (the measurement error) was added into the linear mixed-effects model (LMM) in our study as a fixed factor (Tables 2–3). The results of these models showed that the measurement errors had no significant effects on the relationships (Tables 2–3). Therefore, the relationships can hold when the measurement errors were taken into account.

We have added the Table 1-3 into the supplementary information and revised the method section of the manuscript accordingly [Lines 84-87: Two methods (the potassium dichromate oxidation method (47.9% of the sample) and the dry combustion method (52.1%)) were used to measure C concentration in our dataset. Even though the measurement errors were added to the above mentioned models, they showed no significant effects on the relationships (Tables S2-S4). Thus, the measurement errors were not conducted in other analyses.].

Table 1. Measurement methods of C concentration and their errors in the dataset.

Sample size	Percentage	Measurement method	Replicate	Measurement error	Reference
276	47.9%	Potassium dichromate oxidation method	≥3	±2.0%– ±4.0%	Lu, 1997
300	52.1%	Dry combustion method (elemental analyzer)	≥3	±0.3%	Department of chemistry and analytical chemistry, 1982

Table 2. The effect of measurement errors on the relationship between stem C concentration and tree age.

	Value	SE	df	t value	*p value*
Intercept	49.496	0.907	99	54.532	*<0.001*
Age	0.017	0.006	99	2.652	*0.01*
Error term	0.014	0.407	28	0.035	***0.972***

Table 3. The effect of measurement methods on the relationship between stem C concentration and tree size.

	Value	SE	df	t value	*p value*
Intercept	50.406	1.043	70	48.325	*<0.001*
DBH	0.045	0.015	70	2.881	*0.005*
Error term	-0.162	0.452	20	-0.357	***0.724***

[**Comment 2**] The heterogeneity of the organs itself is neither accounted for nor mentioned. How much does the carbon concentration varies within a single stem? And how much variation is there between different individuals of the same species in a single stand? Do the results hold when these heterogeneities are accounted for?

[**Reply 2**] Thanks. The heterogeneities of C concentration in organs in woody plants have been explored in our previous study (Ma et al., 2018), in which the standard deviation (variation or dispersion) of C concentration in reproductive organs, roots, leaves and stem at the global scale were 4.1%, 3.9%, 3.8% and 3.3%, respectively. However, in this study, the heterogeneities of the organs (roots and leaves) were not accounted for and had a minor effect on the results. Because there was a low heterogeneity within each organ in our dataset due to the same sampling methods which have been described in detail in **Reply 3**.

Within a stem, C concentration varies along tree height (Bert and Danjon, 2006), which has been described in detail in **Reply 5**. Stem C concentration also varies with wood components. C concentration in heartwood could be 5.4% higher than that in sapwood (Herrero de Aza et al., 2011). In this study, each sample of stem was sampled at tree height of 1.3 m, which eliminated the variation along the height. As you suggested, the variation of stem C concentration between different individuals of the same species in a single stand was examined (Fig. 1), which ranged from 0.2 % of *Populus davidiana* to 5.3% of *Cyclobalanopsis glauca*. However, our results showed that the stem C concentration (at 1.3 m) of the same species in a single stand increased with tree age and size in most studies (Fig. S1). Therefore, this can validate the robustness of our results.

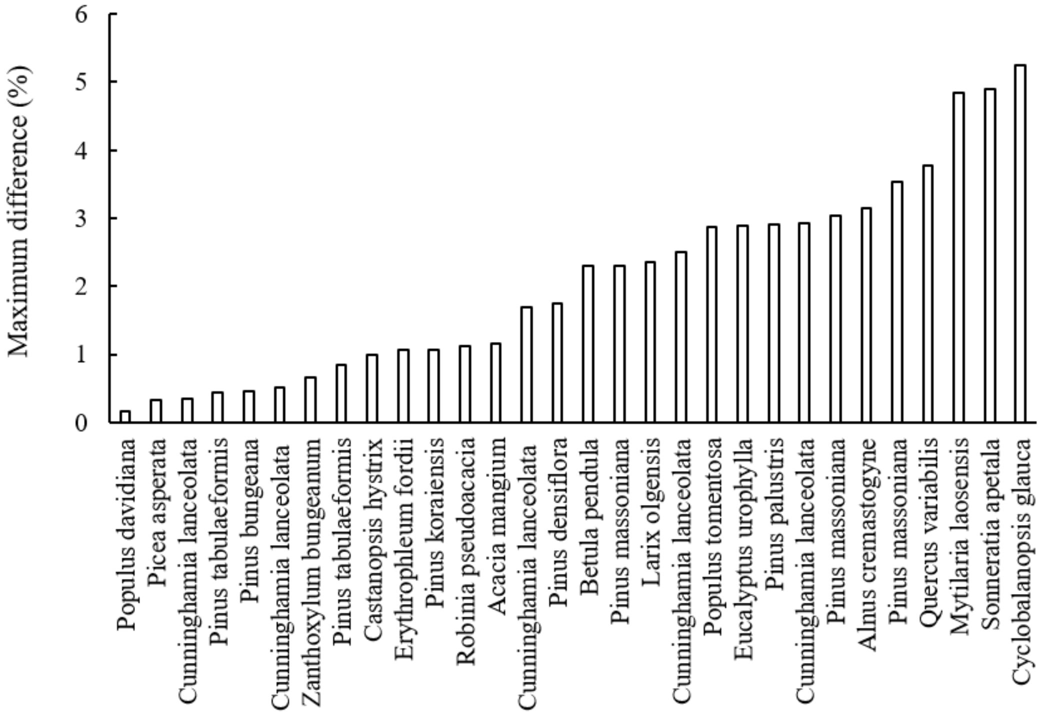

Fig. 1 Maximum differences of stem C concentration of different age individuals of the same species in a single stand.

[**Comment 3**] Where were the samples taken? Which biomes and species are represented in the database? How were the samples taken? Which roots were samples; fine or coarse? Which leaves were sampled; sun or shade, top or bottom of the canopy? If there is heterogeneity with in canopy, stem or root system the sample location doesn't matter but if there is heterogeneity it should be confirmed that samples from different studies can be jointly analyzed.

[**Reply 3**] Thanks. The samples were taken from four countries, including China (86.1%), Estonia (2.6%), Korea (9.5%) and USA (1.7%). The biomes were forests, including tropical (3.5%), subtropical (22.7%) and temperate forests (73.6%). The data was collected from literatures in which the sampling methods had been described in detail. (1) Root samples were from the coarse roots. (2) Leaf samples were the top sun leaves in canopy. (3) Stem samples were from the stem at tree height of 1.3 m. These sentences were added in the manuscript as you suggested [Lines: 69-71]. Thus, the samples from different literatures in our study can be jointly analyzed.

[**Comment 4**] The values reported in table 1 may be significantly different but the standard deviation suggests that the differences are marginal. From this respect I think it would be more honest to report the results in terms of "tendency for lower/higher C concentrations" than in terms of "different" carbon concentrations. A statistical significant difference should not be confused with a meaningful difference.

[**Reply 4**] Thanks for your insightful comment. We revised it accordingly [Lines 109-110: The reproductive organ and stem had the tendency of higher C concentrations than the bark, branch and root.].

[**Comment 5**] Given the absence of a benchmark – in this case a tree for which the carbon concentration was determined in its entirety rather than through sampling – the error estimates are not validated and thus also based on a set of assumptions (i.e., homogeneous radial carbon density within a stem and along the height). These assumptions should be made explicit and it should be discussed how likely they are. In other words, how sure are you that the single observation of carbon concentration of, for example the stem, represents the whole stem? How many samples of carbon concentration does one needs to take from a single stem to obtain the good estimate of its true value?

[**Reply 5**] Thanks for your comments. Following your suggestion, we calculated the errors of using C concentration in stem at different tree heights to the whole tree based on data from Bert and Danjon (2006). We found that using stem C concentration at 1.3 m to represent that of the whole stem could introduce an error of 1.6% while that at relative tree height of 20% or 30% could produce a minimum error (Table 4). This indicates that the C concentration at relative height of 20% and 30% can appropriately represent that of the whole stem. However, it is always convenient to take a sample of stem at 1.3 m while difficult at a higher height, especially for big trees, in an actual application in forests.
As you recommended, we have made an explicit assumption in the manuscript [Lines: 89-91: "Because using C concentration in stem at 1.3 m to the whole stem might only produce a marginal error of 1.6% (Bert and Danjon, 2006), we assumed a homogeneous stem C concentration along tree height when using C concentration in stem at 1.3 m to estimate forest C stocks."].

Table 4. The errors of using stem C concentration at relative tree height (100% = 21.4 m) to that of the whole stem (53.6%) (Bert and Danjon, 2006).

Relative height (%)	0	0.6 (1.3 m)	10	20	30	40	50	60	70	80	90	100
C concentration (%)	55.1	54.7	54.4	53.9	53.4	52.9	52.7	52.5	52.2	51.7	51.0	50.4
Error (%)	2.3	1.6	1.3	0.4	-0.4	-1.1	-1.5	-1.8	-2.3	-3.0	-4.2	-5.1

[**Comment 6**] It is fine to focus a study on a single (very) detailed aspect of the C cycle, in this case the impact of using an exact carbon concentration but in the discussion the scope should be broadened again. The abstract and discussion should report this finding within a comprehensive uncertainty analysis. What are the sources of uncertainty when determining the C concentration of a tree and forest. What are the expected errors of each error/uncertainty source? Such a framework would enable the authors to conclude that determining the carbon concentration is (very) important or not important at all given the overall uncertainty. Given my understanding of our current ability to quantify the carbon content of a tree, allometric relationships and wood density would rank much higher than carbon concentration. When studying the carbon content of a forest, uncertainties from processing remote sensing images and below ground carbon are likely to overrule the uncertainties from the carbon concentration of different plant organs. If the authors can use formal uncertainty analysis to demonstrate that my understanding is wrong, the study could be of interest to the readership of Biogeosciences.

[**Reply 6**] Thanks your insight comments. Following your suggestion, we have conducted the uncertainty analyses (Tables 5–6). Compared with the uncertainties from allometric relationships and wood density, using 50% to the C concentration in organs of different size/age of individuals and of different life forms also introduced large estimation errors (see Table 5). This suggests that we should be cautious about using allometric relationships, wood density and C concentration in forest C stock estimations (Nabuurs et al., 2008). Specifically, the uncertainties induced by variations of C concentration decreased in following order: life form, organ, tree size/age (stem), species, biome and measurement method (Tables 5–6). It was clear that using 50% to stem of age-specific individuals or to the whole tree and employing C concentration in stem to other organs or to the whole tree could produce considerable errors (-9.7–11.1%, -8.6–13.7%, -8.6–25.6% and -2.5−5.9%, respectively), which were the main sources of uncertainties when applying C concentration in the estimations of forest C stocks. (Table 6), which were added into lines 16-18 in abstract in the manuscript as you suggested. This indicates that we have no reason to neglect these uncertainties from C concentration even if the uncertainties from remote sensing and belowground C are likely to be great. We have revised the discussion section of the manuscripts as you suggested [Lines 186-190: "Compared with the uncertainties from measurement methods and from variations of C concentration among species, life forms as well as biomes, the uncertainties from the variations of tree C concentration among organs and stem C concentration among tree size were considerable and should also be taken into consideration in forest C stock estimations (Table S8)."].

In this study, we not only have quantified on the uncertainties from variations in C concentration, but also have examined the relationships between stem C concentration and tree size and age, which are important in biogeochemistry and stoichiometry. Firstly, the size- and age-dependent changes in stem C concentration reflect that C allocation in trees varies at different growth stages. This also implies that, compared with small trees, large trees contribute more to forest C stock due to a greater biomass and a higher C concentration simultaneously. This may be an alternative mechanism of C sequestration in old growth forests (Luyssaert et al., 2008). Secondly, the relationships between stem C concentration, tree size and age highlighted that using a single stem C concentration or 50% to trees in forests at the different developmental or successional stages may overestimate the forest C stocks, especially for regions (such as Asia and tropical region) where plantations and young forests cover large areas (Lewis et al., 2019). We have added these sentences into discussion in the manuscript [Lines: 176-179 & 198-201].

Table 5. The uncertainties of different sources in forest C stock estimations.

Source		Error (%)	Reference
C concentration	Measurement method	0.3 and 2.0–4.0	Department of chemistry and analytical chemistry, 1982; Lu, 1997
	Organ	-11.1–-6.5	Ma et al., 2018
	Organ	-9.7–11.1 (-7.9–25.6)	This study
	Size/Age (Stem)	-2.5–5.9 (-8.6–13.7)	This study
	Species	-6.7–7.2	Zhang et al., 2009
	Life form	-30.9–1.0	Ma et al., 2018
	Biome	-4.8	Martin et al., 2018
Wood density		20.8–28.1	Castaño-Santamaría et al., 2012
Allometric relationships		-15.0–14.0	Van Breugel et al., 2011

Table 6. The uncertainties of using different C concentration in forest C stock estimations.

	5%	95%
50% to stem C concentration	-9.7	11.1
mean stem C concentration to stem C concentration in specific age	-3.4	3.4
stem C concentration to reproductive organ C concentration	-6.1	13.0
stem C concentration to leaf C concentration	-7.9	11.3
stem C concentration to branch C concentration	-5.0	7.8
stem C concentration to bark C concentration	-8.6	25.6
stem C concentration to root C concentration	-4.3	15.2
50% to WMCC	-8.6	13.7
stem C concentration to WMCC	-2.5	5.9

[**Comment 7**] In plantation forest and more natural even-aged forest, diameter and age are strongly related to each other. Diameter increases with increasing tree growth. If carbon concentration increases for one, it could be expected to increase for the other as well. Along the same lines, carbon content is likely to increase with increasing tree height, increasing basal area and increasing wood volume.

[**Reply 7**] Thanks. According to your suggestions, the relationships between stem C concentration and tree height and basal area (at tree height of 1.3 m) were analyzed using the LMMs. The results showed that the stem C concentration increased with tree height (p<0.001;

Fig. 2) and basal area (p=0.014; Fig. 3), respectively. However, the relationship between stem C concentration and wood volume was not conducted due to lack of the data.

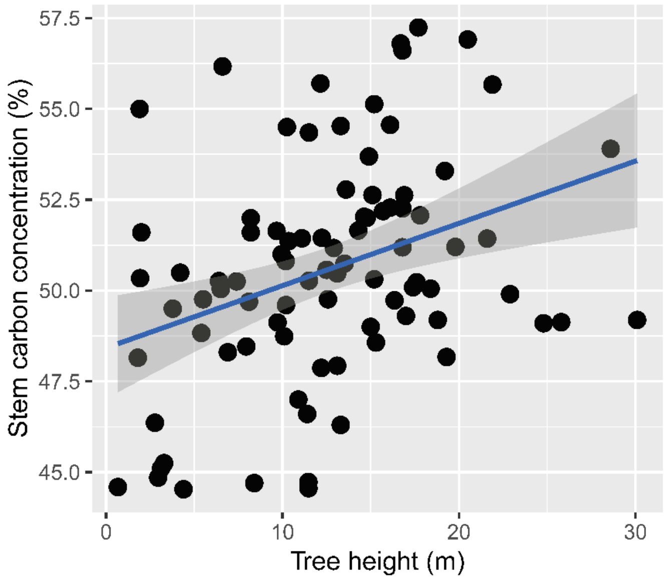

Fig. 2 Stem C concentration increased with tree height.

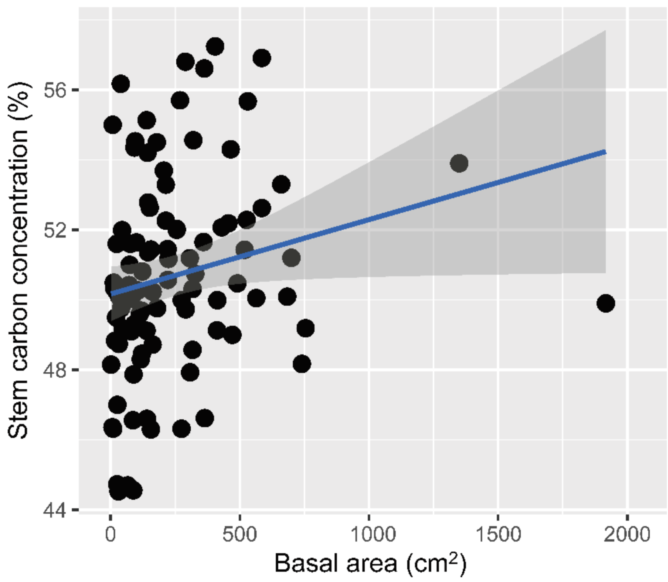

Fig. 3 Stem C concentration increased with basal area.

[**Comment 8**] The discussion is superficial and fails to shed a new light on the results. Some related studies are listed but the discussion does a poor job in presenting a couple of (competing) hypothesis as for why carbon concentration increases with increasing diameter? The sapwood/heartwood hypothesis could easily be tested by back of the envelope calculations.

[**Reply 8**] Thanks for the comments. We added a possible hypothesis to explain the size-dependent changes in stem C concentration: "Lines 165-167: The changes in chemical compounds reflect different strategies and adaptations during tree growth. Larger trees increase the formation of tension wood in response to reinforced wind- or crown-loading (Martin and Thomas, 2013), which may drive the changes in tree growth processes in this study.".

[**Comment 9**] Check the difference meaning (in biogeochemistry) between "content" and "concentration" and use accordingly.

[**Reply 9**] Thanks. The ***content*** and ***concentration*** is a common misconception when denoting the same thing. Both the C content (g C per g dry biomass, %) (Thomas and Martin, 2012; Ma et al., 2018) and the C concentration (g C per g dry biomass, %) (Zhang et al., 2009; Martin et al., 2018) were widely used to convert biomass to C in forest C stock estimations. Additionally, the C concentration (mg C per g dry biomass, mg/g) was widely used to calculate the elemental ratios, such as C/N, C/P and so on. The tree size- and age-dependent changes in stem C concentration are not only important to forest C stock estimations in stand, but also can improve our understanding of plant C concentration variations in stoichiometry. Therefore, we have changed the **content** to **concentration** throughout the manuscript.

To Anonymous Referee #2:

[Comment] This paper addresses the question to which extent variation in carbon (C) content of plant organs affects the precision of C-estimates at aggregated levels when only a mean value is used for all plant organs. Because forests globally play a key role in the C-cycle and thus affect atmospheric CO2 and global warming trends, even small errors at the scale of individual plant organs and plants can scale up to very large total amounts of C. The comprehensive analysis of 576 trees belonging to 24 species comes to the conclusion that the estimates could be 2.5% too small to 6% too large. The authors should perhaps discuss in more detail if they consider this a sufficiently large error to be of concern for the accuracy of global C estimates and their consequences for predictions of global warming or other trends.

[**Reply**] Thanks for your insightful suggestions. Using accurate tree C concentration can decrease the uncertainty in forest C stock and C flux estimations. We estimated the tree C concentration (the weight average C concentration) based on organ biomass proportions in our dataset. Our results showed that using the canonical value of 50% could produce an error of -8.6–13.7% in forest C stock estimations. This uncertainty of global forest C stocks was equivalent to a variation of 31.3–49.7 Pg C (Pan et al., 2011), which was greater than the vegetation C pool of the continental US (15 Pg C) (Dixon et al., 1994).

Due to the great contribution of stem to tree biomass, the variation in stem C concentration and subsequent consequence in forest C stocks have been investigated by recent studies (Gao et al., 2016; Martin et al., 2018). However, these studies ignored the C concentrations in other organs and variations of stem C concentration among different size of individuals. Our results showed that there were significant differences and complicated correlations between C concentration of stem and other organs. Using stem C concentration as tree C concentration (WMCC) could introduce an error of -2.5–5.9% in forest C stocks (Table S7). This error of global forest C stocks could lead to a variation of -9.0–21.3 Pg C (Pan et al., 2011), which was greater than the vegetation C pool of Europe (9 Pg C) (Dixon et al., 1994). Therefore, the variations in tree C concentration among organs and individuals can produce a considerably large error, which should be a taken into account for the accuracy of global forest C estimates and subsequently predictions of global warming or other trends. Following your suggestions, we have added these sentences in the manuscript [Lines: 189-194].

[Comment 1] In their analysis the authors focus on differences in C concentration between plant organs and trees of different size and age; they eliminate site and species effects as random terms in mixed models. For the fixed terms they use type-III sum of squares (SS). I suggest that they may want to look at species as fixed effects as well to discuss differences among species with regard to C-allocation to different organs. This could make for a much richer analysis. Alternatively, they could also test specific fixed-term contrasts among groups of species, e.g. deciduous vs. evergreen or soft- vs. hard-wood species and keep species identity within these groups as random term. Generally, type-III SS have gone a bit out of fashion because they lead to difficult hypotheses if explanatory variables are correlated. It can happen that a set of explanatory variables (here tree size and age) together has a very high type-I SS yet if one adds up their type-III SS the sum is very small. I would prefer different sequences of type-I SS, perhaps followed by a hierarchical partitioning analysis. A problem of type-III analysis is that its result will differ from those shown in Fig. 1-3.

[**Reply 1**] Thanks for your insightful comments. As you suggested, we evaluated the possible effects of organ, age, site and species on the tree C concentration with type-I ANOVA (Table S1). And then the type-I SS was also used in the mixed model in Figs. 1–3. We have replaced the previous Table S1 (with Type III) with a new one (with Type I) in the supplementary material and revised the data analysis [Lines 77-80: for discrepancy of sample size among organs, possible effects of organ, age, site and species on tree C concentration were tested with Type I analysis of variances (ANOVA) in *car* package. The results showed that organ, age, site and species had significant effects on C concentration (Table S1). Then the variation of C concentration with organ was analyzed by using the ANOVA, with multiple comparisons made by Duncan's New Multiple Range test with the *Duncan.test* function (Table 1).].

Additionally, following your suggestion, we evaluated the relationships between stem C concentration among four life forms and tree age and DBH with the LMM (Table 7). We have added Table 7 into the supplement and revised the manuscript [Lines 86-88: The relationships between stem C concentration and tree size among life forms (evergreen vs. deciduous and broadleaves vs. coniferous) were also analysed using the linear mixed-effects models. Lines 119-121: Among different life forms, the stem C concentration also showed positive trends and increased significantly with the DBH and age of evergreen and coniferous trees (Table S6). Lines 155-157: These trends in evergreen and coniferous trees indicated that the variations of stem C concentration with tree size were not necessarily related to tree life forms

(Table S6).].

Table 7. Stem C concentration of different life forms varied with tree DBH and age.

Life forms	DBH			Age		
	Slope	N	p	Slope	N	p
Evergreen	0.073	80	0.002	0.027	100	***0.007***
Deciduous	0.025	15	0.680	0.024	32	0.212
Broadleaves	0.060	31	0.195	0.010	60	0.510
Coniferous	0.068	64	0.005	0.033	72	***0.004***

[Comment 2] This manuscript needs linguistic corrections before it can be put online. Whereas the general grammar and sentence structure are fine, there are many cases where a plural "s" should be added or where an article "the" or "a" is missing.

[**Reply 2**] Thanks. We have polished the manuscript writing with colleagues' help.

[Comment 3] Also, for the title I would suggest putting "variations" in front of "stem": "Tree size- and age-induced variations in stem-carbon content cause uncertainties in forest carbon-stock estimations"

[**Reply 3**] Thanks. We have revised the title as you suggested.

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