

Dear Prof. Aninda Mazumdar and reviewers,

We would like to express our appreciation for your constructive comments. We have incorporated consequent changes in the revised manuscript (accompanying document) based on your comments. In the letter below, we cover point by point the comments and refer to the specific places in the text where we have revised or added information accordingly. To facilitate the evaluation of the responses, original review comments are copied first (in black) followed by our **responses (in blue and in a different font) with revised passages of the text (in italics)**. Line numbers of the revised/added passages of the text correspond to the annotated version of the attached revised manuscript. Every new reference introduced here and which was not mentioned in the original manuscript is listed at the end of this point-by-point response document

The review comments have pointed out a few issues and pertinent observations that required to be addressed. Specifically, main changes concern:

- 1.) Based on the comments from reviewer #1, we have added the results of the sensitivity analysis we performed to demonstrate that no significant changes in the behaviour of our mixing models can be observed using different plant  $\delta^{13}\text{C}$  endmembers (roots, leaves, branches, live, senescent, and surface litter). Hence, validating our choice to use average values of the above- and belowground plant  $\delta^{13}\text{C}$  endmembers in generating our mixing models.
- 2.) Based on the comment from reviewer #2, the meaning of the terms 'young' and 'old' mangroves used extensively in the manuscript has been clarified to avoid misleading the readers on the actual age of the studied mangrove sites.

Given the above changes and the rest of the alterations specified in the point-by-point revision below, we believe that this review process has led to an improved manuscript.

We are grateful for the time you have devoted to our paper. We thank you for your consideration and look forward to hearing from you.

On behalf of the co-authors,

Sincerely,



Rey Harvey Suello

## REVIEWER #1

This is an interesting study highlighting the importance of allochthonous organic carbon contributions to mangrove soil carbon stocks. I recommend its publication in this journal. The study could be greatly improved if the authors provided a sensitivity analysis for their mixing-model calculations. Specifically, the degree to which  $\delta^{13}\text{C}$  signatures vary between different plant materials remains unclear. In case there is large variability, the authors should use different plant  $^{13}\text{C}$  endmembers and demonstrate how estimates of allochthonous OC contributions change accordingly. This is needed because the authors do not know the relative contributions of different plant materials to the SOC pool. More specific comments are provided in the pdf file attached.

**RESPONSE:** We thank you for your positive and constructive review. We have made a series of changes in the manuscript based on your comments and suggestions. To address your main comment, we fully agree on the importance of conducting a sensitivity analysis for our model calculations. Based on our results, indeed, the  $\delta^{13}\text{C}$  signatures of different plant materials varied but not significantly (we provide these data as supplementary information, Table S8). Nevertheless, we still performed a one-factor-at-a-time (OAT) sensitivity analysis using different plant  $\delta^{13}\text{C}$  endmembers (roots, leaves, branches, live, senescent, and surface litter) and observed no significant changes in the behavior of our mixing models after linear regression (we provide the results of our sensitivity analysis as supplementary information, Figures S4, S5, S6 and S7). For this reason, we decided to show results in the main paper obtained when using the averages of our vegetation data as input parameter values for our model. Based on this major comment, we added the following passage in the results section 3.5 (lines 324-327): *"To account for the variability of the  $\delta^{13}\text{C}$  values of the different above- and belowground plant materials (roots, leaves, branches, live, senescent, and surface litter; see supporting Table S8) used as vegetation end-member, a one-factor-at-a-time (OAT) sensitivity analysis was performed and no significant changes in the mixing model results after linear regression was observed (Figure S4, S5, S6 and S7)."*

We provide the itemized responses below for your specific comments in the pdf file with line numbers to indicate their location in the revised manuscript.

- (1) Line 65: Are any of these studies providing a comparison with salt marshes, seagrasses and peatlands? I doubt that mangroves have much higher C stocks than other peat-forming ecosystems. Please provide data for these other ecosystems to support this claim.

**RESPONSE:** Yes, the referenced studies provide such comparisons, and we confirm that they demonstrate higher organic carbon stocks in mangroves as compared to salt marshes, seagrasses and peatlands.

- (2) Line 79-82: Many mangroves also form peats. Peat formation does not rely on sedimentation.

**RESPONSE:** Agreed. We specified peat-formation as one of the main carbon preservation mechanisms of mangroves' autochthonous organic materials. We added this to lines 75-77 where we explain the autochthonous contribution of SOC by local vegetation: *"[...]and peat formation (Ezcurra et al., 2016), and this constitutes the autochthonous SOC in mangrove sediments."*

- (3) Line 131: How about calling it a case study?

**RESPONSE:** We added the text, in italics (lines 130-131): *"...along an estuarine land-to-sea gradient and between old and young mangrove forest sites, for a specific case study in the Guayas Delta, Ecuador."* We note that this is indeed a case study, but we discuss and interpret the results against

the international literature, so that the conclusions from our study are of interest to the international scientific community, and not only of case-specific interest.

(4) Line 333-335: Did you compare results based on belowground vs. aboveground biomass  $\delta^{13}\text{C}$ ? compare: Mueller et al. 2019 (Marine Ecology Progress Series) providing allochthonous SOC estimates based on different plant  $\delta^{13}\text{C}$  endmembers.

**RESPONSE:** The estimated contributions of the autochthonous carbon input were calculated using the averages of below- and aboveground biomass. The main goal of the estimation is to present the relative contribution of the 2 main types of sources of organic carbon namely the allochthonous and autochthonous sources. We show how this may vary when only one type of autochthonous input (above- or belowground biomass) is considered in the calculation (supplementary information, Figure S8). In this respect, as already mentioned before in response to a similar comment, we performed a model sensitivity analysis using different plant  $\delta^{13}\text{C}$  endmembers (roots, leaves, branches, live, senescent, and surface litter) and observed no significant changes in the behavior of our mixing models after linear regression (supplementary information, Figures S4, S5, S6 and S7). Therefore, this sensitivity analysis shows that there are no considerable differences in the mixing model results when using above- or below-ground average values for the plant  $\delta^{13}\text{C}$  endmembers.

(5) Line 443-445: There is no evidence for this because you did not measure organic matter preservation, decomposition or carbon sequestration (i.e. SOC stocks \* accretion rate). You may want to emphasize that low SOC stocks do not imply low SOC sequestration rates in fast accreting systems.

**RESPONSE:** Thank you for this comment. We agree with the point you are making. Indeed, decomposition and carbon sequestration rates are direct evidence of preservation of organic carbon. Since these weren't included in our study, the statement made should be viewed as a suggestion on the possible limited preservation of autochthonously-derived carbon based on the estimation of relative contributions of both allochthonous and autochthonous carbon in the sampled mangrove sediments. We indeed realise that we did not explicitly mention that sediment accretion and SOC accumulation rates were not measured in this study. Hence, we clarified this in the revised manuscript (lines 508-509): “[...] sediment accretion and SOC accumulation rates, which were not measured in this study, are imperative [...]”

Further, we agree on your suggestion that low SOC stocks do not imply low SOC sequestration rates in fast accreting systems, and we believe we have also made this point in our discussion section 4.4 (lines 496-508) where we highlighted that mangrove sites with lower SOC stocks but higher sediment accretion could still have high SOC accumulation rates.

(6) Line 471-476: Bioturbation also aerates the soils and may lead to low preservation of autochthonous organic matter. This could be discussed above

**RESPONSE:** Thank you for this helpful suggestion. We have added this additional potential explanation for the lower preservation of autochthonous OC in section 4.2 (lines 465-467): *In addition, we could visually observe that our study site is heavily bioturbated by red crabs (*Ucides occidentalis*) which may aerate the soil and could potentially lead to low preservation of autochthonous organic matter.*

(7) Line 496-499: I cannot quite follow this statement. First, you did not measure C sequestration rates. It is for instance possible, that young sites sequester more autochthonous OC than old sites as they accrete much faster vertically. Second, even if young sites sequestered less autochthonous OC, they would eventually become old sites.

**RESPONSE:** We agree on your points listed and we believe we were able to cover that in the subsequent statement (lines 499-503).

(8) Line 498-501: I suggest to completely rewrite the Implications section and give directions for future research based on knowledge gaps.

**RESPONSE:** We have partly rewritten this section, by indeed giving more explicit proper direction to further research on extending our assessment of SOC stocks and sources, towards SOC accumulation rates, and that this is lacking so far because there are no data on sediment accretion rates. Hence, we clarified that proper quantification of sediment accretion rates, and combining this with SOC concentrations, is an essential next step towards understanding the environmental variations in SOC sequestration rates in the studied system (lines 508-509).

**REVIEWER #2**

The objective of the paper was to quantify and identify first-order controls of sediment organic carbon stocks and sources along an estuarine land-to-sea gradient and between old and young mangrove forest sites in the Guayas Delta, Ecuador. The paper is well drafted and supported by quality data set; however I suggest checking the language of the paper to avoid grammatical errors.

**RESPONSE:** Thanks a lot for the positive review. We have carefully copyread the paper and corrected for eventual grammatical errors. We provide the itemized responses below for the specific comments with reference to line numbers to indicate revisions in the revised manuscript.

- (1) -In line 76 Kristensen, 2008 and in list in line 567 Kristensen, E., Bouillon, S., Dittmar, T., & Marchand, C. (2008); -In line 111 Hansen et al., 2016 and in the list in line 552 Hansen, K., Butzeck, C., Eschenbach, A., Gröngröft, A., Jensen, K., & Pfeiffer, E. M. (2017); -In line 111 and 113 Craft et al., 2007 and in the list in line 539 Craft, C. (2007); -In line 128 Marchand et al., 2017 and in the list in line 588 Marchand, C. (2017); -In line 398 /399 Kristensen et al., 2008 and the list in line 565 Kristensen, E. (2007); -In line 471 Nam et al., 2015 and in reference list in line 599 Nam, M. V. (2015)

**RESPONSE:** Many thanks for the keen eye! All corrections have been adapted except for the citation of Kristensen et al. (2008). Lines 417-418 of the revised manuscript correctly refer to the cited paper which is also listed as a reference (line 592 of the revised manuscript).

- (2) The references in line 127/ 128 Nelleman et al., 2008 is cited but not listed. Please add to the References

**RESPONSE:** The missing reference has been added to the revised manuscript (line 625-626): *“Nelleman, C., Corcoran, E., Duarte, C. M., Valdés, L., De Young, C., Fonseca, L., & Grimsditch, G. (2009). Blue carbon. A Rapid Response Assessment. United Nations Environment Programme, GRID-Arendal, 78.”*

- (3) Following references are listed, but, I could not find them in the text. Please cross check.  
Cruz-Orozco, R. (1974), Twilley, R. R., Chen, R. H., & Hargis, T. (1992).

**RESPONSE:** Thank you! The abovementioned references have been removed in the revised manuscript.

- (4) In line 526, ‘&’ is missing between authors. In lines 563, 593, 630, 633, 647 ‘and’ is used instead of ‘&’.

**RESPONSE:** Thank you for the observation! The citation format has been checked and the use of ampersand, ‘&’, has been made consistent for the entire reference list of the revised manuscript.

### **REVIEWER #3**

This study deals with the within system variability and sources of SOC in mangroves of Guayas delta, Ecuador, concerning forest age (young vs old) and position (i.e., distance from sea and salinity gradient-downstream, Intermediate and upstream). At the outset I must say that study is quite simple with basic data set for a single estuarine-marine system. However, within this data, authors have tried to extract information which is not readily available for the mangrove ecosystem. For the journal like Biogeosciences, I would have definitely liked to see some more information with additional rigour, for example, the information vis-à-vis age of the sediments, particularly where authors are trying to make distinction between old and new mangroves. Having said that, given the scope of the study and data generated, authors have done a nice job. I have some observations which is of moderate in nature.

**RESPONSE:** We appreciate your positive and constructive review. Concerning the provision of sediment age profiles for the sampled sediment cores, we have tried to perform several radiometric dating methods (based on Pb-210 and Cs-137) but could not extract usable information on sediment age profiles, as we could visually see that our study sites are heavily bioturbated by mangrove red crabs, which probably reworked the vertical sediment profile. The distinction between old and young mangrove sites, as mentioned in the manuscript, was done through remote-sensing analysis, showing that the old mangrove sites already existed as mature mangrove forests on satellite pictures going back to 1984, while young mangrove sites first appeared in 1993, 2006 and 2004 respectively on the different sites. We have provided more data on this in our supplementary information (Table S9).

We provided the itemized responses below for the specific comments with line numbers to indicate their location in the revised manuscript.

- (1) The objectives of the study are clear and followed standard protocols to achieve the objectives. The categorization of sites based on historical LANDSAT satellite images is also acceptable. However, the sites classified as young, witnessed colonization in the last three decades (after 1993). So, the younger sites are not actually young and are established forests now; they are just relatively younger than other sites. I request the authors to address this point in detail for clarity.

**RESPONSE:** Thank you for this remark. Indeed, we categorized the sites based on the analysis of historical LANDSAT images (lines 165-167, Table S9). We agree that technically, our 'young' sites are not young, but rather established forests. Hence we explicitly clarified this in the revised manuscript (lines 167-169): "*Although the selected young sites can't be biologically categorized as young, for the purpose of this study, we will refer to these sites as 'young' as they are still significantly younger than their older counterparts.*" The terms 'young' and 'old' are therefore retained to refer to the age gradient of our study sites.

- (2) The authors discussed the factors responsible for variation in SOC stock and sources between young and old sites and among the position. However, from the data, it is apparent that there are significant differences in SOC stock and content between marine and estuarine mangroves. So, I request the authors to address the point in the discussion. Further, I suggest the authors discuss the contrasting behavior of upstream old and young sites concerning sites of intermediate and downstream location.

**RESPONSE:** Thank you for this comment. We have discussed these notable differences in SOC stocks and content between marine and estuarine mangroves in lines 414-418. With regards to the notable difference in the results obtained in the Upstream (young and old) sites versus the Intermediate and Downstream locations, this may be due to faster development of SOC stocks in the upstream young sites up to levels that are similar to the upstream old sites, as compared to

the development of lower SOC stocks in the young sites in contrast to the old sites in the intermediate and downstream zones. This hypothesis seems to be in line with the difference in the plant species present in the sites. As written in the manuscript (Lines 166-169), there is a strong dominance of *Rhizophora samoensis* (known as a later successional species) in the old sites and *Avicennia germinans* (known as an earlier successional species) in the young sites of the intermediate and downstream sites (with higher salinity), whereas both the young and old sites of the upstream zone (with lower salinity) show a mixture of both mangrove species (*Rhizophora* and *Avicennia*) and an understory of freshwater plant species. This indicates that young and old sites in the upstream zone are in a similar vegetation successional stage, and therefore may have reached similar SOC stock levels, while in the intermediate and downstream zones, the young sites are in an earlier successional stage, with lower SOC levels, as compared to the nearby old sites. To further address this contrasting behavior between sites, we added the following statement in the discussion section (lines 400-411):

*"We observed comparable SOC stocks and contents at the young and old sites of the Upstream zone, as opposed to lower SOC stocks in young sites compared to old sites of the Intermediate and Downstream zones. This may be to some extent attributed to differences in the successional stages of the vegetation in these different sites: in the intermediate and downstream zones (with higher salinity), the young sites are dominated by smaller trees of *Avicennia germinans* (known as an earlier successional species) and the old sites are grown by high stands of *Rhizophora samoensis* (known as a later successional species); while in the upstream zone (with lower salinity), the vegetation was similar on old and young sites, with mixtures of both *Avicennia* and *Rhizophora* and an understory of freshwater marsh species. Hence this may suggest that young and old sites in the upstream zone are in a similar vegetation successional stage, and therefore may have reached similar SOC stock levels; while in the intermediate and downstream zones, the young sites are in an earlier successional stage, where the SOC has reached yet lower levels as compared to the nearby old sites."*

(3) If I am not wrong, from the figure it is apparent the younger sites are on eastern banks and old sites are on western banks, but the GPS points given in Table S1 provide the opposite information. Is it possible that the GPS points are misplaced in Table S1, check!

**RESPONSE:** Thank you for the keen eye! Indeed, the coordinates in Table S1 are incorrectly ordered. We have made necessary corrections in the revised version of our supplementary information (Table S1).

(4) In the discussion, authors state that the in younger marsh sedimentation is higher than the older marsh and extended the analogy to the studied mangrove system. As the younger mangrove grew on the tidal mudflat (I believe), Am I right in assuming that the sedimentation rate would have been similar with or without mangrove. What role the mangrove played in increasing the sedimentation rate?

**RESPONSE:** Thank you for this question. We hypothesize that sedimentation rates are not similar with or without mangroves. The presence of mangroves, in general (young or old), increases sedimentation rates as compared to bare mudflats by effectively reducing hydrodynamics from waves and currents with their complex vegetation structures (particularly their root systems) and

thereby promoting the settling of suspended sediments from the water column during tidal inundations of the mangrove forest. Yet we expect that the sediments samples on young and old mangrove sites have experienced different sedimentation rates, and we expect these differences are mainly due to their positions relative to the tidal frame. Older mangroves have already accumulated more sediments than younger mangroves and therefore we expect the older mangroves have obtained already a higher surface elevation within the intertidal zone as compared to the younger mangroves. Consequently, we expect that the younger mangroves, with lower elevation, are experiencing more tidal inundation, and therefore higher suspended sediment inputs and higher sediment accretion rates, as compared to the older mangroves. This hypothesis is discussed in the manuscript and is proposed as a potential explanation for the lower SOC concentrations and stocks in younger mangroves as compared to older ones, as a consequence of a dilution effect of the SOC by higher rates of mineral sediment accretion on the younger sites (lines 356-389).

(5) By comparing the sediment cores from younger and older mangrove systems, are not you comparing two different time periods? For example, sediment at 50 cm depth at younger mangrove may represent much younger time period than at older mangrove. While making an interpretation, particularly related to relative contribution of allochthonous and autochthonous carbon contribution at a particular time period, how do you reconcile this fact. I think authors should think this through.

**RESPONSE:** Thank you for this comment. We have indeed thought this through. We acknowledge the possible differences in sediment age and sediment accretion rates in the young and old sites. As we discussed in the manuscript (lines 356-377), we expect that sediment accretion rates on the young sites have been larger than on the old sites. This means that it may be expected that the sampled sediment columns are indeed deposited over shorter, more recent time periods in the young sites as compared to the old sites. We proposed this as one hypothesis for the differences in OC% and  $\delta^{13}\text{C}$  between young and old sites. We also made a point in the discussion that we lack actual data on sediment accretion rates and therefore SOC accretion rates (lines 498-501).

(6) The authors state that the sediment  $\delta^{13}\text{C}$  values of the sediment cores are 6-10‰ higher relative to the average vegetation of the sites. If I am seeing it correctly the total variation in  $\delta^{13}\text{C}$  across all reservoirs are in the range of -32 to -24 per mil. Where is the difference of 10 per mil?

**RESPONSE:** Thank you for this observation. You are correct. The 10 per mil difference considers the raw dataset and not the average  $\delta^{13}\text{C}$  of the vegetation samples. We clarified this by deleting the word 'average' on line 295 of the revised manuscript and adding a reference to a table in the supplementary information showing the raw dataset for the vegetation samples.

(7) Also, authors state that  $\delta^{13}\text{C}$  values of the older sites (at the Intermediate, Downstream and Marine sites) are more negative than the younger sites. I am not sure if this is unequivocal. Pl. check.

**RESPONSE:** We refer to Table S2 in our supplementary information for the specific average  $\delta^{13}\text{C}$  values. Based on the data in this table, it can be seen that the statement in question is indeed correct.

References:

Ezcurra, P., Ezcurra, E., Garcillán, P. P., Costa, M. T., & Aburto-Oropeza, O. (2016). Coastal landforms and accumulation of mangrove peat increase carbon sequestration and storage. *Proceedings of the National Academy of Sciences*, 113(16), 4404-4409.

Nellemann, C., Corcoran, E., Duarte, C. M., Valdés, L., De Young, C., Fonseca, L., & Grimsditch, G. (2009). Blue carbon. A Rapid Response Assessment. United Nations Environment Programme, GRID-Arendal, 78.