

Revised version of Van de Broek et al. (2016), *The importance of an estuarine salinity gradient on soil organic carbon stocks of tidal marshes*, Biogeosciences Discuss., doi: 10.5194/bg-2016-285

This document contains 1) point-by-point answers on the comments by referee #1, 2) point-by-point answers on the comments by referee #2 and 3) the revised manuscript and supplementary information in track-changes.

5 *Note 1:* the line numbers in the answers to the referee comments refer to the line numbers in the discussion paper.

Note 2: if possible, we propose to change the title of the manuscript to ‘Controls on soil organic carbon stocks in tidal marshes along an estuarine salinity gradient’.

Note 3: we added data on bulk density depth profiles to the supplementary data.

10 Point-by-point answers on the comments by anonymous referee #1

1. *General comments: The paper is well-written and generally well structured. It addresses an important gap in the field of carbon cycling, namely of measurements in brackish and freshwater marshes. The authors address various aspects clearly and draw attention to the problems associated with different sampling depths. In addition, they indicate what a future sea level rise may entail for the carbon storage dynamics within the Scheldt estuary.*

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We thank the first anonymous referee for the comprehensive comments on our manuscript. These definitely improve the quality of our manuscript substantially. Below we provide answers to all comments.

2. *There are some aspects which need clarification and one main concern of mine is that samples were collected in different seasons. Depth profiles were collected in November whilst aboveground biomass was not collected until August. No mention of this is made in the discussion and I certainly believe that this needs to be addressed and justified.*

20

We agree with the reviewer that this point needs further clarification and should be discussed more extensively. Soil samples were collected at the beginning of this study, in November 2014. However, in order to calculate the total annual biomass produced at the locations where soil samples were collected the maximum annually produced biomass had to be collected. As it has been shown that the timing of peak standing biomass on tidal marsh in western Europe generally occurs in August (Groenendijk, 1984; De Leeuw et al., 1990), vegetation samples were not collected until August 2015. This is now clearly stated in the manuscript. This comment is further answered under question number 18.

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3. *I also miss more discussion on the effect the very different types of vegetation may have on the carbon dynamics of the different marshes. This certainly influences stable isotope signatures and carbon mineralization rates. More comments are found in the specific comments regarding this.*

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This issue is addressed under question number 18.

Specific comments

4. P3 L3f: *Why did the authors limit themselves to the incorporation of in situ produced belowground biomass? Aboveground biomass also produces substantial amounts of litter and can also be buried.*

Here we summarized the most important factors controlling the increase in elevation of tidal marsh platforms.
5 The comments that aboveground biomass can also contribute to elevation changes after burial is certainly valid, and the sentence is adapted to also include aboveground biomass: ‘...and incorporation of in situ produced biomass (both above- and belowground) on the other hand’.

5. P4 L18-20: *the use of PSU/practical salinity unit is discouraged, nowadays salinity as written here would be unitless i.e. the authors should write: “: : salt or polyhaline zone (salinity >18), brackish or mesohaline zone (salinity 5–18) and freshwater/oligohaline zone (salinity 0–5): :”*
10

The PSU notation is removed from the manuscript.

6. P5 L4: *How were these samples stored during their transport i.e. were the 0.03 sections thus homogenized?*

The undisturbed soil cores were divided into 0.03m sections in the field. Every sample was stored in a minigrip bag and transported to the lab: soil samples from the different replicate cores (or from the same core) were
15 never homogenized. This is added to the manuscript: ‘The cores were divided into 0.03 m sections and every soil sample was stored in a reclosable bag in the field before transport to the lab.’.

7. P5 section 2.2: *why were depth profiles collected in November 2014 and aboveground biomass not until the end of August? How do the authors justify using data from such different seasons?!*

For the reply on this point we refer to the answers on comments 2 and 18.

20 8. P5 Section 2.3: *just make one paragraph for easier reading and change title to “Soil and biomass analysis”*

This is adjusted in the manuscript.

9. P5 L18: *What do the authors mean with split? This also needs clarification because now it sounds like only one of the five replicates was analysed. Is this the case, or are you describing what was done to each one of the five replicates? Please clarify.*

25 Indeed, only 1 out of 5 replicates of aboveground biomass was analyzed for C content and C isotopes. This is because every aboveground biomass sample was taken at a 0.5*0.5m area, thus representing the variability in biomass characteristics in an area where biomass is homogenous. The ‘splitting’ refers to the fact that all the biomass collected in this 0.5*0.5m surface was shredded and repeatedly divided into equal parts until only a small fraction was left. This subsample was analyzed in 3 replicates (and the average was calculated and
30 reported). This is clarified in the manuscript: ‘The total aboveground biomass of one of the replicates collected on a 0.25 m² surface area was shredded and repeatedly divided into equal parts until only a small portion was left. This was further grinded...’.

10. P5 L25ff: *The authors sectioned the cores into 0.03 m sections, so, when they say one sample every 0.09m, do they mean it is the sample at 0.06-0.09, or 0.09-0.12 and so forth? The same question applies to when they say every 0.18 m. Maybe rather say ...For the other two replicate profiles every third sample was analysed (i.e. 0.06-0.09, 0.15-0.18, :...:, 0.69-0.72m) to a depth of 0.72m. Thereafter, a sample was analysed every 0.18 m.”*
35

We thank the referee for pointing to this confusing formulation, this is clarified in the manuscript: 'At every location one soil profile was analysed in detail (every other depth sample, i.e. 0 - 0.03, 0.06 – 0.09m, ...). For all three replicate profiles every third sample was analysed (i.e. 0 – 0.03, 0.09 – 0.12, ...) down to a depth of 0.72m. Thereafter, samples were analysed every 0.18 m.'

5 *11. P5 L29: what linear interpolation technique was used to do this?*

In order to obtain organic carbon percentages for all depth intervals, the average organic carbon percentage at the depths at which three replicate samples were analysed (i.e. 0 – 0.03, 0.09-0.12m, ...) was linearly interpolated. This way, we obtained continuous depth profiles of organic carbon percentage. The same method was used to obtain continuous depth profiles of bulk density. Both were necessary to calculate the total OC stocks at the study sites. This is clarified in the manuscript: 'Continuous depth profiles of OC percentage for layers of 0.01m were obtained using the average OC percentage at the depths at which three replicates were analysed (i.e. every 0.09 m). The OC percentages at these depths were linearly interpolated to obtain OC percentages for intermediate layers. Continuous depth profiles for bulk density were obtained in an identical way.'

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15 *12. P6 L6: Was only a check for normality done? Please also mention (and I hope the authors did!) that homogeneity of variance was also checked.*

Both a check for normality (Anderson-Daling test) and homogeneity of variance (Levene's test of equal variances) were performed. This is now mentioned in the manuscript in section 2.4 (Data Analysis).

20 *Please also specify what statistical were done since you mention differences in Figure 4? And please specify which level of probability was used (e.g. "with a level of significance of $p < 0.05$)."*

The level of probability we used was 0.05, this is added to the manuscript (section 2.4). In section 2.4 we state that we checked for differences in mean biomass production rates using a one-way anova test after checking for normality with an Anderson-Darling test in Matlab. This is now complemented with the fact that we checked for homogeneity of variance using a Levene's test.

25 *13. P6 section 3.1: - also include here that detailed results for the grain size (not texture) are in the supplementary information*

This is included in the manuscript: '...with a silt loam grain size (detailed grain size data is provided in the Supplementary Information).'

14. Section 3.2:

30 - *Figure S1 is not maximum annual biomass but as is noted in the figure caption as total biomass. This is a difference so please clarify.*

- *Even if the belowground data was not statistically analysed and no clear patterns are observed, I would have liked to see some comments on what we see i.e. that at the fresh low biomass is clearly very high, that for most sites we see very low values.*

35 - *An explanation is needed here for figure 4 and the letters apparently showing differences. These need to be explained.*

- 'Maximum' biomass is replaced by 'total' biomass in the manuscript

- The differences in belowground biomass production between the sites is now briefly discussed in section 3.2
- In section 3.2 (Results – Vegetation biomass production) we added the meaning of the different letters in figure 4, together with an interpretation of the different letters.

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15. P.7 section 3.4:

- Depth profiles of cumulative OC stock per 0.01 m layer are shown: ... Where does this 0.01 m sectioning come from? The authors make no mention of this in the methods. There you can only find 0.03 m sections or 0.1 and 0.2 m sections. Please clarify what I have missed.

10 - Please be more consistent with the terminology. Within this one paragraph the authors begin by using SOC but then use only OC later

- The subdivision into layers of 0.01m depth was done using linear interpolation based on the depths with known OC%. This was done to graphically show how the total SOC stock up to a certain depth varies between the different sites. It is added to the material and methods section (2.4) that the linear interpolation was done for layers with a thickness of 0.01m. To section 3.4 it is added that the linear interpolation is described in section 2.4.
- The terminology of (S)OC was checked throughout the paper and adjusted where necessary. When it is not clear whether organic carbon in soils or in e.g. vegetation or deposited sediments is referred to, SOC was used. However, if from the context it is clear that we refer to organic carbon in soils, OC is was used.

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16. P7 L 11f: $\delta^{13}\text{C}$ signal of standing vegetation is closely related to the $\delta^{13}\text{C}$ signal of SOC in the topsoil layer. How is this conclusion reached? I presume with standing vegetation you mean the aboveground biomass? I would not agree with this from what I see in figure 6.

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We agree with the reviewer that the conclusion that the $\delta^{13}\text{C}$ signal of topsoil sediments is closely related to the $\delta^{13}\text{C}$ signal of the vegetation is drawn too easily and should be discussed in more detail. Therefore, we adapted this sentence to provide a better overview of the observed relationships between topsoil and vegetation $\delta^{13}\text{C}$ signals: '...SOC in the topsoil layer is similar to the $\delta^{13}\text{C}$ signal of standing vegetation. However, close inspection shows that some differences in the $\delta^{13}\text{C}$ signal between vegetation and topsoil can be observed. At the high freshwater marsh the topsoil $\delta^{13}\text{C}$ signal is higher than the signal for both above- and belowground vegetation, while at the low freshwater marsh the topsoil $\delta^{13}\text{C}$ signal is lower than the above- and belowground vegetation signal. At both the low and high brackish marshes, the topsoil $\delta^{13}\text{C}$ is very similar to the $\delta^{13}\text{C}$ signal of roots, while it is about 1‰ lower compared to the $\delta^{13}\text{C}$ signal of aboveground vegetation. At the high saltmarsh, the topsoil $\delta^{13}\text{C}$ signal has a value in between the $\delta^{13}\text{C}$ signals of above- and belowground vegetation, while at the low saltmarsh the topsoil $\delta^{13}\text{C}$ signal is significantly lower compared to the signal of both above- and belowground vegetation.'

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17. P7 L18: "However, the differences reported in previous studies are almost always much smaller than the differences we find. This may to some extent be related to differences in environmental conditions, but differences in sampling procedures also matter." I agree that the authors want to address the problem of inconsistent sampling depth but I do not think that you can dismiss all the other reasons why there are such differences with this one sentence. The estuaries listed in Table 4 are all very different in terms of their geology, morphology, inputs, outputs, etc. and I would like to see some more discussion of this. One of the aims of this

paper was to determine OC stocks along a salinity gradient of a temperate estuary and its main controls and I think this has to be addressed more thoroughly. Since the authors do actually discuss some of these factors in section 4.3, I would suggest that section 4.3 follows directly to 4.2 (or is combined) because the authors here try and further explain the observed patterns in SOC stocks which is a more natural progression from what is initiated in section 4.1. I would also bring the issue of different sampling depths then as a separate header and not as the first paragraph of the discussion. This is an aspect but not the most important one.

In relation to this it is unclear in line 20 whether the authors refer to differences from this study or from the other studies. This needs to be clarified.

- We prefer not to merge sections 4.2 and 4.3, since in section 4.2 the observed patterns are discussed and interpreted, while in section 4.3 explanations for the observed patterns are discussed. We believe the manuscript would become less clear if the two sections would be merged.
- We agree with the reviewer that the issue of the effect of different sampling procedures should be discussed in a separate paragraph. To improve the structure of the manuscript we will start the discussion with the current section 4.2 (Observed patterns in SOC storage), followed by the section on the controlling factors (current section 4.3 – Explanations for the observed patterns in soil organic carbon stocks). The current section 4.1 (Soil organic carbon stocks along the estuary) will be discussed after this.
- The fact that differences in characteristics between the reported estuaries (e.g. environmental conditions and morphology) will have an effect on the reported SOC stocks in the cited studies is now briefly discusses in the manuscript. We will not discuss this in much detail, as this is not the goal of this study. However, based on the brief discussion the reader is aware of the fact that not only the effect of sampling procedure controls the reported OC stocks.
- In line 20 it is now indicated that these differences refer to the tidal marshes from other studies.

18. Section 4.3.2: I miss a more thorough discussion on the fact that you have very different vegetation types. I presume no $\delta^{13}\text{C}$ values are known for the different plants themselves?

- We do have $\delta^{13}\text{C}$ for the different vegetation types (table S1). We agree with the reviewer that the discussion about the effect of vegetation types on the observed SOC stocks is limited. However, we do not have data to isolate the effect of different vegetation types on the observed SOC stocks along the estuary. Therefore, we complemented the discussion about the effect of vegetation (section 4.3.2) with observations that we made on the low and high portion of the same marsh. Based on this, the effect of vegetation on SOC stocks at the different marshes is now discussed in the manuscript:
 - Freshwater marsh: Although both the low and high marsh are characterized by different vegetation types (*P. australis* and *Salix* forest resp.) and annual biomass production is significantly different (much higher at the low marsh), depth profiles of OC% are remarkably similar. In addition, SOC stocks in the top 0.6m of the soil profile are higher on the high marsh. This shows that the impact of local vegetation on SOC stocks is limited at the freshwater marshes.
 - Brackish marsh: Both the low and high brackish marshes have the same vegetation type (*Elymus athericus*) and rates of annual biomass production are similar. However, both topsoil OC% (about 4% higher at the high marsh) and SOC stocks up to 0.6m depth (much higher for the high

marsh) are significantly different. This again indicates that another factor besides local vegetation controls the size of the SOC stocks at these locations.

- Saltmarsh: At the low marsh *Spartina anglica* is present. It has been shown before that *Spartina* vegetation is very labile and contributes little to the total SOC pool (Boschker et al., 1999; Bouillon and Boschker, 2006; Middelburg et al., 1997). At the high saltmarsh the C4 *Spartina* vegetation has been replaced by a community of C3 species. The OC concentrations in the top decimeters at the high saltmarsh is also higher compared to the low saltmarsh. This indicates that at these locations C3 vegetation species do contribute to the size of the SOC stocks.

- These observations indicate that local biomass production is probably not the dominant factor controlling SOC stocks along the estuary. It may however control local SOC stocks, as is the case on the saltmarshes.

I also struggle with the fact that biomass was only measured in August, whilst all other measurements were taken in November. The influence of weather and climate conditions and subsequently river flow on affecting stable isotope signatures should not be underestimated (e.g. Zetsche et al. 2011, dx.doi.org/10.1016/j.csr.2011.02.006).

We agree with the reviewer that the timing of soil sample collection can have an effect on the $\delta^{13}\text{C}$ signal of the top sediments, as shown by Zetsche et al. (2011). It should be noted that in Zetsche et al. (2011) only the top 0.01 m of sediments on a sandflat were analyzed, which are highly dynamic and characterized by both deposition and erosion. Zetsche et al. (2011) show that the intra-annual variations in the relative contribution of terrestrial-derived and marine C lead to changes in the $\delta^{13}\text{C}$ signal of the top 0.01 m sediments, which is not unexpected in such an environment.

Our study concerns tidal marsh sediments and here only deposition occurs. Our aim was to use the $\delta^{13}\text{C}$ signal of the whole soil profile, combined with the $\delta^{13}\text{C}$ signal from different inputs (allochthonous C and vegetation), to construct hypotheses on the origin of SOC in the studied tidal marshes. Therefore, the timing of soil sample collection will only be of minor importance, as the $\delta^{13}\text{C}$ signal at depth is an integration of the complete annual cycle of $\delta^{13}\text{C}$ variations over the past decades. We do agree that the $\delta^{13}\text{C}$ signal of the very top layer of the profiles we analysed may be affected by the same processes as those described by Zetsche et al. (2011). However, the variation of the contributions of terrestrial/marine/autochthonous C will not affect the deeper sediment layers. As our interpretations are based on the variation of the $\delta^{13}\text{C}$ signals over the whole profile we do not expect that this intra-annual variation to have a strong effect on our results and interpretations. We included this point in the discussion of the manuscript.

I would suggest the authors also look at a recent similar study by Hansen et al. 2016 (DOI 10.1007/s11368-016-1500-8) and see how their results of the importance of salinity can be reconciled in this study also for section 4.3.1.

We thank the reviewer for pointing to the recent study by Hansen et al. (2016). They also clearly show a decrease in tidal marsh SOC stocks with increasing salinity in another western European estuary (Elbe, Germany). We included the result from their study into our manuscript:

- We included their measurements of SOC stocks in tidal marshes in different salinity zones in Table 4.

- Hansen et al. (2016) was cited in the introduction among other studies reporting on estuarine SOC stocks and biomass production along an estuarine salinity gradient.
- We discussed the results of Hansen et al. (2016) in section 4.3.3, where we put forward arguments in order to explain the observed pattern in SOC stock along the estuary.

5 19. P8 L7f: *There is no relationship. Did you analyse this statistically? If so please provide test results here, or at least indicate (data not shown).*

Based on the fact that no relation was detected ($R^2 = 0.004$) it was chosen not to show the correlation. “($R^2 = 0.004$, Data not shown)” was included in the manuscript. If the reader wishes she/he can reconstruct the correlation analysis based on the data given in Table 3 and Table S2.

10 20. P8 L19f: *Elymus is considered an invasive species. Do you think it is invading here and will remain as the dominant vegetation type here? How will this affect influence SOC stocks in the future as conditions favour this plant?*

15 Van der Pluijm and De Jong (2008, in Dutch) indeed show that at least since 1980 this species occupies about 55-60% of the total marsh area at the studied brackish marsh, although the area it occupies did not increase significantly between 1980 and 2004. It is however not invasive on all marshes of the brackish portion of the estuary, as nearby marshes are occupied dominantly with e.g. *Phragmites australis*. Therefore it is difficult to predict whether or not this species will invade other marshes in the future, e.g. due to changing environmental conditions or sea level rise.

20 Based on our data we cannot assess how this species will influence SOC stocks after invasion, as we only have data for brackish marshes under *Elymus* vegetation. We have knowledge of only 1 study that assessed the effect of establishment of *Elymus athericus* on SOC stocks by Valery et al. (2004), who show that over a period of 10 years after establishment of *Elymus*, no significant changes in sediment C concentrations were found. However, as they showed that *Elymus* litter contained significantly more lignin compared to the former vegetation, the tidal marsh changed from a source to a sink of C due to the low mineralization rates of *Elymus* litter. Based on
25 the results from Valery et al. (2004), we do not expect changes in the SOC stock after *Elymus* establishment on a short timescale (10 years), however, increasing SOC stocks can be expected as relatively resistant *Elymus* litter will be incorporated in the marsh sediments.

Figures

30 21. *Personally I would prefer it if the authors used the blue colours always for the saltmarshes (since closest to the blue ocean) and the green colour for the freshwater marshes (closest to land) in the figures. This is more intuitive to the reader.*

This is a good suggestion which will increase the readability of the figure, this is adjusted in the manuscript.

22. *Figure 1: Please increase the font size of the country names in the inset. FYI: A black and white version of the map will not depict the light grey areas.*

35 The font size of the country names is increased (and repositioned, as they appear to have shifted).

23. *Figure 2: Brackish water marsh not just Brackish marsh*

We prefer to keep the term 'brackish marsh' throughout the manuscript and also in the figures. This term is also used in other studies (e.g. Hansen et al. (2016), Callaway et al. (2012), Dausse et al. (2012))

24. Figure 3: All species names should be italicized. Figure caption: At several marshes the former tidal sandflat was reached, whilst at two other locations the marsh sediments extended below the maximum sampling depth of 1.4 m. The vegetation history is based on Temmerman et al. (2003) and information from the $\delta^{13}\text{C}$ profiles of this study, in combination with information from Boschker et al. (1999) and Middelburg et al. (1997). Mix denotes a mixed vegetation which included the following species.... A '?' indicates that no clear identification was possible.

10 The species names are italicized. We added the species types that 'Mix' denotes. We also included that a '?' after a vegetation species denotes that the presence of this species is hypothesized while a '?' at a dashed line denotes that there is uncertainty concerning the exact depth of the vegetation transition.

It is not possible to say only shallow marshes because the sandflat is also reached at the high saltmarsh and I presume only freshwater and brackish water high went beyond 1.4 m? Also specify what mix stands for. The figure has to be understandable on its own.

15 We changed the sentence 'At shallow marshes the former tidal sandflat was reached, at other locations the marsh sediments extended below the maximum sampling depth of 1.4 m.' into: 'At locations where the sandflat was reached this is indicated, at the other locations the marsh sediments extended below 1.4m depth.' Also, a sandflat layer will be added below the low freshwater marsh.

20 25. Figure 4: the inset is very distracting. Please remove. Instead you can insert a break on the y-scale to allow the details to be seen more easily for the belowground biomass. Adjust the figure caption i.e. remove "(the inset... biomass)". Also add the y-axis legend i.e. Biomass production ($\text{g dry weight m}^{-2} \text{ yr}^{-1}$). Replicas should be replicates. The letters to indicate significant differences are confusing. It has to be explained in the figure caption what the different letters stand for. No mention of these are made in the main text which also has to be addressed!

25 The inset is removed and the caption is changed accordingly. We chose not to insert breaks in the y-axis since this increases the figure's height. However, the figure still shows the pattern in root biomass at the different sites, and the reader can access the exact root biomass data in table S2. Furthermore, we will add a y-label. We also explained the different letters in the caption and in the main text.

26. Figure 5: Error bars for specific depths represent the standard deviation.

30 This is changed in the caption.

27. Figure 6: aboveground (circles)... Error bars represent the standard deviation.

These changes are made in the caption

28. Figure 7: write out OC once as organic carbon in the figure caption.

This is adapted

Tables

29. Comments like A, B, C etc. should be added as footnotes. They are footnotes and should not be in the main caption text.

This is changed in all figures containing comments (A, B, ...)

- 5 30. Table 1: please change around C and D (better to have A, B, C in the same line and then D at the bottom for the mixed vegetation. Please also italicize all species names in the footnote D (previously footnote C). Regarding footnote C (previously D): What is texture? It is not texture but grain size that was measured in this study. Why is this called maximum marsh sediment depth? I would rather simply write "Maximum sampling depth". The tidal sandflat that is reached most likely is deeper but probably caused problems with the sampling device? Sand is not easy to sample.

The letters C and D are changed, and species names italicized. In the caption, 'texture' will be changed to 'grain size'.

- 15 We named this 'maximum marsh sediment depth' because at this depth there was a transition from the silt/clay marsh sediments to the sandy former mudflat sediments. At the locations where we cored down to the sandy layer we were always able to collect at least the upper 10cm of sand (deeper sandy sediments were indeed difficult to sample). These sandy layers were also analyzed for grain size and OC content, which also allowed us to delineate the marsh/mudflat boundary based on these depth profiles. We prefer to keep the term 'maximum marsh sediment depth' because this informs the reader on the thickness of the marsh sediments at the sample locations. We explained this better in section 3.1 (Results – Soil characteristics).

- 20 31. Table 2: Keep footnotes C and D and make them A and B. Add to figure caption: "Bulk density values are averages for the upper meter of soil, whilst soil pH and electrical conductivity were measured in the topsoil only.

This is changed in table 2.

- 25 32. Table 3: Increase the space between the line termed saltwater and the next line for 'up to 0.6 m depth' to make this clearer for the reader. Figure caption: Total organic carbon (OC) stock (kg... deviations calculated for the full vertical sampling profiles (depths used for the calculations are given in brackets), and the upper 0.6 m.

The line spacing is increased, and the caption changed according to the comments

33. Table 4: make this into a horizontal table and thus more readable. Perhaps place the location then as a separate column next to the estuary name.

- 30 We changed the table to a horizontal layout, and will add an additional column for the location of the estuary if this does not makes table too wide. In addition, the OC stocks as measured by Hansen et al. (2016) were added to this table.

Supplemental data

34. I would welcome that the excel sheets provided in the supplemental data are at least referred to in the paper.

The excel tables are now referred to in the paper: the texture data is referred to in section 3.1 (Results – Soil characteristics), the OC, CN and $\delta^{13}\text{C}$ data in section 3.3 (Result – Soil organic carbon depth profiles).

35. *Figure S1: see my comments on Figure 4. Please also remove the inset here.*

Figure S1 is adapted in the same way as Figure 4.

- 5 36. *Figure S2: why is there now mention of a depth interval of 0.01m? This is never mentioned previously in this study, only slicing at 0.03 m and 0.1 +0.2 m intervals is ever mentioned. Please explain.*

The 0.01 m depth intervals are based on interpolation, we refer to our answer on comment 15, where we explain why and how this was done.

- 10 37. *Table S3: Please italicize all species names. Replace Oosterschelde with Eastern Scheldt and Westerschelde with Western Scheldt.*

The species names are italicized, and Oosterschelde and Westerschelde put in English.

- 15 38. *Table S2: Figure caption: Average values (\pm SD) for aboveground, belowground (maximum root depth is given in brackets (m)) and total biomass, biomass production, organic carbon and nitrogen concentration (%), C:N ratio as well as the $\delta^{13}\text{C}$ signal (‰ for vegetation at the study sites. Remove footnote A, footnote B: write here in full as a footnote the species. In table: Adjust either DW or dry weight, now have both. Also write species names in full. If you miss space you can shorten Freshwater to Fresh, etc. and add to caption "...at the study sites (freshwater, brackish water and saltwater marshes)."*

- 20 The figure caption is adjusted based on the suggestion of the reviewer. We added a footnote B where the species at the high saltmarsh are listed. We consistently changed 'dry weight' into 'DW' in the table, and species names are now written in full.

Technical corrections

We greatly thank the reviewer for the detailed technical comments that will contribute greatly to the quality of the manuscript. The comments that are listed below without an answer are changed in the manuscript. Answers to technical comments that require explanation are given below as well.

- 25 P2 L14: *downstream of the maximum...*
P3 L2: *replace extratropical with temperate. Extratropical is not normally used in this context.*

- 30 We chose the term 'extratropical', since tidal marshes also occur in other climate zones. Therefore, we propose to change this sentence to 'These are vegetated intertidal areas located along coastlines and estuaries of sub-Arctic to tropical climates, although they occur mostly in temperate zones, and are among the most productive ecosystems on Earth'.

P3 L7: *equilibrium with the local*

P3 L8: *remove 'in particular'*

P3 L16-17: *remove spacing and merge into one paragraph.*

P3 L22: *tidal marshes, for which no data is available, is the*

P3 L23-24: remove separation into paragraphs. These three reasons are all one aspect and should be together in one paragraph.

P3 L25: ...(Craft, 2007). A sharp increase in salinity...

P3 L29: ...2010). In addition, the OC input in tidal marsh...

5 *P3 L32: data not date*

P4 L5: remove space and form one paragraph.

P4 L8: ...stocks in tidal marsh soils. The aims...

P5 L6f: ...0.5m depth, and then in 0.2 increments down to the maximum depth of 1.4m.

P5 L17 and L23: replace weighted with weighed. Samples were placed on a scale, hence they were weighed.

10 *Weighted is used in a different context.*

P5 L19: ...using the Elemental Analyser...

P5 section 2.4: remove line spacing and form one paragraph.

P5 L26: ...analysed to a depth of 0.72m. Below this depth, samples were analysed every 0.18 m.

P6 L5: remove "is"

15 *P6 L17: willow trees were*

P6 L18: what is meant by woody parts, this is not a correct term!

With 'woody parts' we meant the standing vegetation of willow trees. We changed this sentence into: '..., while standing willow vegetation could not be collected...'.
.

P6 L19: deduced from other studies

20 *P6 L23: showed and decreased i.e. past tense.*

We prefer to keep the results section in the present tense.

P6 L26: do not write just in the top of the profile, be more specific, e.g. "...OC concentration in the upper 0.2 m." Or whichever depth it is...

P7 L2: to the low marshes

25 *P7 L11: this is the first time a 'C4 Spartina site' is mentioned, please refer to this differently to make it clearer for the reader.*

This sentence is changed to: 'For all sites except the low saltmarsh, which is characterised with *Spartina anglica* vegetation (C4 type), the $\delta^{13}\text{C}$ signal...'.
.

P8 L3: observations

30 *P8 L3: remove spacing and merge into one paragraph*

P8 L13: deeper down along the profile, both variables

P8 L21: from the decomposition: : : likely, as shifts in ... decomposition are generally in the order of...

P8 L25: On the high saltmarsh: : : with depth also occurs.

P8 L26: ... characterised by a mixture of...

35 *P8 L28: ...marsh growth *Spartina anglica* was also present at this ...*

P9 L 22: remove spacing and merge into one paragraph

P9 L23: that determines

P10 L31: remove spacing, merge into one paragraph

Section 5: merge all into one paragraph.

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Point-by-point answers on the comments by referee #2 (M. Schwartz)

1. The authors have presented a comprehensive assessment of both depositional and preservation factors influencing the accumulation of soil/sedimentary organic carbon across an estuarine salinity gradient. Their analysis of contributions from changes in surface vegetation type (e.g., C3 vs. C4 plants) and geochemical influence of OC decomposition rates at different salinity regimes provides a useful framework for assessing how forecast sea level rise could affect organic carbon storage in estuaries experiencing saltwater intrusion. Their examination of spatial variability in both OC supply and decomposition rates is robust and spans the estuarine salinity gradient.

We greatly thank dr. Schwartz for reviewing our manuscript and for his constructive comments.

2. Notable absent is data for (or an estimate of) sediment accretion rates at each of the three estuarine zones sampled.

This data is available in Temmerman et al. (2004, figure 8) and is added to section 2.1 (Study sites). For the period 1955 – 2002, the following average annual sediment accumulation rates are reported:

- Saltmarsh: about 0.75 and 0.5 cm yr⁻¹ for low and high marshes resp.
- Brackish marsh: about 1 – 2 and 0.5 – 1 cm yr⁻¹ for low and high marshes resp.
- Freshwater marsh: about 1 - 2 and 1 cm yr⁻¹ for low and high marshes resp.

3. How will sea level rise and saltwater intrusion affect the location of the estuarine turbidity maximum and resulting allochthonous OC deposition?

In section 4.4 (Discussion – Implications of sea level rise for estuarine soil organic carbon stocks) we state the maximum turbidity zone is predicted to shift more inland as a consequence of sea level rise. This will indeed effect which portion of the estuary receives a significant input of allochthonous (terrestrial) organic carbon, as this will also shift more upstream. We added to this section that as a consequence of the upstream migration of the maximum turbidity zone, terrestrial organic matter can travel less far downstream in the estuary. As a consequence, tidal marshes which are now located at the downstream end of the MTZ will receive less stable terrestrial OC in the future, which will decrease their potential to sequester OC, as in addition also the sedimentation rates will decrease as a result of the shifting location of the MTZ.

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The importance of an estuarine salinity gradient Controls on soil organic carbon stocks in of tidal marshes along an estuarine salinity gradient

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Abstract

Tidal marshes are sedimentary environments ~~that and~~ are among the most productive ecosystems on Earth. As a consequence ~~tidal marshes, and vegetated coastal ecosystems in general, they~~ have the potential to reduce atmospheric greenhouse gas concentrations ~~_as they efficiently by~~ sequestering soil organic carbon (SOC). In the past decades, most research on SOC storage in marsh environments has focused on salt marshes, leaving carbon dynamics in brackish- and freshwater marshes largely understudied and neglecting the diversity among tidal marshes. ~~Moreover, most existing studies underestimate total organic carbon (OC) stocks due to shallow soil sampling, which also influences reported patterns in OC storage along estuaries.~~ We therefore conducted an extensive sampling campaign to quantify and characterize SOC stock in marshes along a salinity gradient in the Scheldt estuary (Belgium and The Netherlands). We find that SOC stocks vary significantly along the salinity gradient of a temperate estuary (Scheldt estuary, Belgium and The Netherlands), from 46 kg OC m⁻² in freshwater marshes to 10 kg OC m⁻² in saltmarshes. ~~Moreover, Our data also show that most existing studies underestimate total organic carbon (OC) stocks due to shallow soil sampling; this, which also influences reported patterns in OC storage along estuaries.~~ In all tidal marsh sediments the SOC concentration ~~has a~~ is more or less constant ~~downward value~~ from a certain depth ~~below the surface~~ downward. However, this concentration decreases with increasing salinity, indicating that the amount of stable SOC decreases from the upper estuary towards the coast. Although the net primary production of macrophytes differs along the estuary, our data suggest that ~~these~~ differences in OC storage are caused mainly by variations in suspended sediment concentration and stable particulate OC (POC) content in the water along the estuary. The fraction of terrestrial suspended sediments and POC that is transported downstream of the maximum turbidity zone is very limited, contributing to smaller amounts of long term OC sequestration in brackish- and saltmarsh sediments. In addition, high rates of sediment deposition on freshwater tidal marshes in the maximum turbidity zone promote efficient burial of OC in these marsh sediments.

Keywords

Tidal marshes, estuarine salinity gradient, soil organic carbon, organic carbon preservation

25 1 Introduction

As a consequence of increasing atmospheric greenhouse gas concentrations and the recognition that soils have the potential to store vast amounts of organic carbon (OC), there is a large interest in the OC storage potential of soils in different ecosystems (Duarte et al., 2013; Govers et al., 2013; Scharlemann et al., 2014). Although coastal vegetated habitats occupy only 0.2 % of the ocean surface, it has been estimated that they account for approximately 50 % of carbon burial in marine sediments, referred to as blue carbon (Donato et al., 2011; Duarte et al., 2013; Mcleod et al., 2011; Nelleman et al., 2009). It has recently been shown that the OC sequestration potential of these ecosystems will depend to a large extent on future climatic changes and sea level rise (Cherry et al., 2009; Kirwan and Blum, 2011; Kirwan and Mudd, 2012; Weston et al.,

2011). Moreover, changing carbon storage in these ecosystems can potentially cause important feedbacks to atmospheric concentrations of carbon dioxide (CO₂) and methane (CH₄) (Duarte et al., 2013; Pendleton et al., 2012; Poffenbarger et al., 2011). Constraining the amount of OC that is sequestered in these ecosystems and understanding the processes controlling the size of this pool is of major importance in order to understand the global carbon cycle.

5 An important fraction of coastal wetlands is occupied by tidal marshes. These are vegetated intertidal areas located along coastlines and estuaries of ~~sub-Arctic to tropical climates; (although they occur mostly in temperate zones); and extratropical regions and~~ are among the most productive ecosystems on Earth (Rocha and Goulden, 2009; Whigham, 2009). Their elevation increases as a consequence of the deposition of both mineral sediments and allochthonous organic matter (OM) during flooding events on the one hand and ~~the~~ incorporation of in situ produced ~~belowground~~ biomass ~~(both above- and~~
10 ~~belowground)~~ on the other hand (Fagherazzi et al., 2012; Neubauer, 2008). Recently formed young tidal marshes, with a low elevation, receive more mineral sediments than their higher counterparts, with sedimentation rates decreasing through time until the marsh platform elevation is in equilibrium with ~~the~~ local mean high water level (Temmerman et al., 2003).

Despite the fact that the importance of vegetated coastal ecosystems and tidal marshes ~~in particular~~ is now widely recognized, estimates of the total amount of OC stored in tidal marshes are subject to a large uncertainty. Estimates of OC
15 stocks in saltmarshes (i.e. tidal marshes bordering saltwater bodies) range between 0.4 and 6.5 Pg (Bridgham et al., 2006; Chmura et al., 2003; Duarte et al., 2013). To the best of our knowledge, no global estimates are available for brackish and freshwater marshes.

There are multiple reasons for the large uncertainty on estimates of the global OC storage in tidal marsh soils. Firstly, the total area of global saltmarshes currently used to estimate global stocks is poorly constrained, with estimates between 22 000
20 and 400 000 km² (Chmura et al., 2003; Woodwell et al., 1973), while a global inventory for freshwater marshes is lacking (Barendregt and Swarth, 2013).

~~Secondly, the dataset available on soil OC-organic carbon (SOC) stocks is limited, both in terms of the number of samples analysed and the geographical scope: Hhitherto. -M~~most studies were carried out in a limited number of estuaries, mostly located on the south and east coasts of North America. Differences in sampling procedure and depth beneath the soil surface
25 also contribute to uncertainty. Very often only topsoil samples are analysed, with a limited amount of studies considering carbon storage in deeper horizons, although it has been recognized that these also store a significant amount of OC (Elschot et al., 2015; Wang et al., 2011). An additional factor complicating the extrapolation of data to tidal marshes for which no data is available, is the wide range of reported OC contents for tidal marsh soils (Bouillon and Boschker, 2006; Middelburg et al., 1997).

30 A third important reason for the uncertainties mentioned above is that tidal marshes in estuaries are characterized by steep gradients of multiple environmental and ecological factors (Craft, 2007). ~~First, a~~ sharp increase in salinity towards the coast is present, resulting in a longitudinal estuarine gradient from saltmarshes in the most seaward part over brackish marshes to freshwater tidal marshes. As a consequence of this salinity gradient a vegetation gradient develops, with macrophyte biomass generally being higher on freshwater and brackish marshes compared to saltmarshes (Dausse et al.,

2012; Hansen et al., 2016; Weston et al., 2014; Wieski et al., 2010). ~~Third~~In addition, the OC input in tidal marsh soils is a mixture of upland, riverine, estuarine and marine sources and the relative contribution of these sources to the total OC pool varies significantly along the estuary (Middelburg and Nieuwenhuize, 1998).

Currently available data suggest that these environmental gradients along estuaries generally result in decreasing SOC stocks with increasing salinity (Craft, 2007; Hansen et al., 2016; Hatton et al., 1983; Wieski et al., 2010). However, our knowledge on how location along the estuary affects total SOC stocks and which processes control the magnitude of these stocks is, at present, still very limited. ~~Furthermore, M, mainly because m~~ most studies only consider SOC storage down to a depth of 0.3m and generally the reasons for the observed variability are not identified. Nevertheless, understanding the effect of environmental gradients on SOC dynamics in tidal marshes is important. Such understanding may not only help to improve our estimates of current SOC storage but will also be of great help in assessing the effects of sea level rise on these SOC stocks (Morrissey et al., 2014; Poffenbarger et al., 2011; Weston et al., 2011).

Here, we study the variation in SOC inventories in tidal marshes along a salinity gradient in the Scheldt estuary, located in Belgium and the Netherlands. This estuary is characterised by strong gradients in salinity and sediment concentration, making it a suitable location to investigate the impact of these gradients on OC stocks in tidal marsh ~~sediments~~soils. The aims of this study are therefore 1) to determine the SOC stocks of tidal marsh soils along the salinity gradient of a temperate estuary, 2) to determine the main controls on SOC stocks along this gradient and 3) to use this knowledge to assess how future environmental changes may influence SOC stocks in estuarine tidal marsh soils.

2 Materials and methods

2.1 Study sites

The Scheldt river is located in Western Europe and flows into the North Sea in the southern Netherlands (~~Figure 1~~Figure 1). The estuary of the river extends from its mouth up to 160 km upstream where the tide is stopped by sluices near the city of Ghent (Belgium). The estuary is influenced by a semi-diurnal meso- to macrotidal regime, with mean tidal ranges between 3.8 m at the mouth and 5.2 m in the inner estuary (Meire et al., 2005). The estuary has a total length of about 235 km (including tributary tidal rivers) and comprises a salt or polyhaline zone (~~salinity > 18~~practical salinity units, PSU), brackish or mesohaline zone (~~salinity 5 – 18~~PSU) and freshwater/oligohaline zone (~~salinity 0 – 5~~PSU) (~~Figure 1~~Figure 1). The Scheldt estuary is described in detail in Van Damme et al. (2005) and Meire et al. (2005).

Tidal marshes are present along the entire length of the estuary and tributary tidal rivers, resulting in approximately 498 ha of freshwater marshes, 3035 ha of brackish marshes and 652 ha of saltmarshes (Tolman and Pranger, 2012; Van Braeckel et al. 2013). We sampled a salt, brackish and freshwater tidal marsh, and within each marsh we sampled two locations with different but known rates of historical sediment accretion (~~Figure 2~~Figure 2 and Table 1). The first location was at the high marsh with an elevation of 0.1 to 0.3 m above mean high water level (MHWL), which has been accreting during the past decades at a rate that is in equilibrium with the rise of MHWL. At the second location, marsh formation started during the

past decades at heights well below MHWL. Average accretion rates at these locations were therefore significantly higher than sea level rise ([Figure 2](#)). For the period 1955 – 2002, the average accretion rates at low and high marshes were 0.5 and 0.75 cm yr⁻¹ for the saltmarshes 1 – 2 and 0.5 – 1 cm yr⁻¹ for the brackish marshes and 1 – 2 and 1 cm yr⁻¹ for the freshwater marshes (Temmerman et al., 2004). The vegetation history for the different sites is shown in [Figure 3](#).

5 The locations of the sampled tidal marshes are indicated in [Figure 1](#), GPS coordinates of the sampling locations are provided in table S1.

2.2 Sample collection

Depth profiles were collected in November 2014 using a manual gouge auger (0.06 m diameter) down to a maximum depth of 1.4 m. Three replicate soil cores were collected with a maximum distance of 3 m in between the coring locations. The cores were divided into 0.03 m sections [and every soil sample was stored in a resealable bag](#) in the field [before](#) and transported to the lab. Samples for soil bulk density and root density measurements were collected using a Kopecky ring sampler or with the gouge auger if soil wetness prevented the use of Kopecky rings. These samples were collected at the soil surface and at depth increments of 0.1 m up to 0.5 m depth, and [further down at then in](#) 0.2 m increments down to the maximum depth of 1.4 m. Aboveground biomass was collected on a surface area of 0.25 m² (five replicates) at the end of August 2015 at each coring location. [The difference in timing between soil and biomass collections is due to the fact that annual maximum standing biomass production occurs maximum s in August in Western European tidal marshes](#) (Groenendijk, 1984; De Leeuw et al., 1990).

2.3 Soil [and biomass](#) analysis

Before analysis of the soil samples, macroscopic vegetation residues were removed manually using tweezers. The soil samples were oven-dried at 35°C for 48 hours and crushed until they passed through a 2mm sieve. After carbonates were removed with a 10% HCl solution, the samples were analysed for OC, δ¹³C and C:N ratio using an Elemental Analyser (FlashEA 1112 HT, Thermo Scientific). Soil texture was determined using a laser diffraction particle size analyser (LSTM 13 320, Beckman Coulter) and grain size was classified into clay (<2 µm), silt (2 – 63 µm) and sand (>63 µm) fractions. Soil pH was determined after diluting [the 5 g of soil in 25 ml of](#) a 0.01M CaCl₂ solution and electrical conductivity was measured after diluting [5 g of soil in 25 ml of the samples in](#) de-ionized water.

The collected biomass was dried at 60°C for 48 hours after sediments were removed and weighed in order to calculate the total dry weight of the biomass. The total aboveground biomass of one [of the replicates collected on a](#) 0.25 m² surface area was shredded and [split repeatedly divided into equal parts](#) until only a small portion was left. This was further grinded and analysed for OC content, δ¹³C and C:N ratio using [an the](#) Elemental Analyser (FlashEA 1112 HT, Thermo Scientific).

30 Soil bulk density samples were dried at 105°C for 24 hours. After soil bulk densities were calculated, the samples were washed over a 0.5 mm sieve using de-ionized water and all roots were collected. The roots were cleaned using de-ionized water, dried at 60°C and weighed.

2.4 Data analysis

At every location one soil profile was analysed in detail (every other depth sample, i.e. every 0.06 m - 0.03, 0.06 – 0.09m, ...). For ~~the other two~~ all three replicate profiles ~~one sample every 0.09 m~~ every third sample was analysed (i.e. 0 – 0.03, 0.09 – 0.12m, ...) ~~down~~ to a depth of 0.72m. ~~Deeper down the profile, one a sample was analysed every 0.18 m~~ Thereafter ~~Below this depth, samples were analysed every 0.18 m~~ was analysed.

~~Total SOC stocks were calculated for a volume of soil with a surface area of 1 m² and over the total depth of the sampled marsh sediments. Both the average of the three replicate OC percentages and bulk densities were linearly interpolated to construct continuous depth profiles. Continuous depth profiles of OC percentage for layers of 0.01m were obtained by linear interpolation, using the average OC percentage at the depths at which three replicates were analysed (i.e. every 0.09 m). The OC percentages at these depths were linearly interpolated to obtain OC percentages for the intermediate layers/depths. Continuous depth profiles for bulk density were obtained in an identical way. Both~~ These continuous depth profiles were then used to calculate total SOC stocks for a volume of soil with a surface area of 1 m² and over the total depth equal to the total sampling depth of the sampled marsh sediments.

Root biomass was measured at discrete depths as explained above. For every layer the total root biomass for a surface area of 1 m² was calculated by rescaling the average root biomass for the three replicates to the total volume of that soil layer. Linear interpolation between measurements at different depth intervals was used to calculate the total root density per surface area of 1 m².

To test if annual aboveground biomass production ~~is~~ was significantly different between the sites a one-way analysis of variance was used in Matlab[®], after checking for normality ~~using the~~ (-Anderson-Darling test) and homogeneity of variances (Levene's test) with a level of significance of $p < 0.05$. For the other variables only three replicates were available so no reliable significance test could be performed.

3 Results

3.1 Soil characteristics

The studied tidal marsh soils are classified as tidalic Fluvisols with a silt loam ~~texture~~ grain size (detailed grain size data can be found ~~is provided in the Supplementary data)~~. The maximum depth of marsh sediments at the different study sites varies between 0.2 and > 1.4 m (Table 1 ~~Table 1)~~. The average bulk density ranges from 0.40 to 0.99 g cm⁻³ (detailed bulk density data is provided in the Supplementary data), and both the topsoil pH and electrical conductivity increase in the downstream direction, from freshwater- to saltmarshes (Table 2).

3.2 Vegetation biomass production

~~Based on the measured total annual biomass (figure S1, table S2) and reported values of both above- and belowground. The average bulk density ranges from 0.40 to 0.99 g cm⁻³, and both the topsoil pH and electrical conductivity increase from freshwater to saltmarshes (Table 2).~~

5 3.2 Vegetation biomass production

~~Based on the measured maximum annual biomass (figure S1, table S2) and reported values of both above- and belowground annual turnover rates (table S3), annual biomass production for the different sites was calculated, as shown in Figure 4. In this figure, sample locations that do not share a letter have significantly different annual biomass production rates. The average annual aboveground biomass production is the highest for the brackish marshes, followed by the low freshwater marsh and both saltwater marshes. The high freshwater marsh has an aboveground biomass production that deviates from this pattern as a consequence of the fact that only fallen leaves of the willow trees ~~are~~ were taken into account at this site, while ~~the woody parts~~ standing willow vegetation could not be collected, so that we underestimate total biomass production in this case. Upper limits for biomass production on this marsh may be deduced from other studies, which typically result in production rates of 500 - 1000 g dry weight m⁻² y⁻¹ (Kopp et al., 2001). ~~Although~~ No clear pattern in annual production of belowground biomass along the estuary ~~was~~ is observed, large differences between the sites are present. (Figure 4) Figure 4). Belowground biomass production on the low freshwater marsh and the low saltmarsh are two orders of magnitude larger compared to the other tidal marsh sites. At the former locations, most biomass is located belowground, while at the latter locations the majority of the vegetation biomass is located aboveground.~~

3.3 Soil organic carbon depth profiles

20 The depth profiles of SOC show that the depth-averaged concentration ~~decreases~~ eses from freshwater- to saltmarshes, although the highest topsoil OC concentration is observed at the brackish marshes (Figure 5 ~~Figure 5, data on OC and C:N ratios is provided in the supplementary information data~~). In contrast to the freshwater soils, which show a gradual but limited decrease in OC concentration with depth, the brackish- and saltmarshes show a sharp decrease in OC concentration in the ~~top~~ upper 0.25m of the profile.

25 **3.4 Soil organic carbon inventories**

The highest total SOC stocks are found in the freshwater marshes, followed by the brackish- and saltmarshes (Table 3). For every marsh, SOC stocks are greater for the high marshes compared to the low marshes, as a consequence of both deeper marsh soils and higher SOC concentrations. In order to compare the marshes directly to each other the stocks down to the largest common depth have been calculated (Table 3). Using this approach, freshwater- and brackish marshes have comparable SOC stocks, while both locations on the saltmarsh have significantly lower stocks. Depth profiles of cumulative SOC stocks per 0.01 m ~~layer~~, after interpolation as explained in section 2.4, are shown in Figure S2.

3.5 Stable carbon isotopes

The depth profiles of stable OC isotopes ($\delta^{13}\text{C}$) are shown in ~~Figure 6~~ Figure 6, together with the $\delta^{13}\text{C}$ signal of above- and belowground vegetation (data on $\delta^{13}\text{C}$ is provided in the supplementary Information). In general an increase in $\delta^{13}\text{C}$ values with depth is observed, although deviations from this pattern are observed along the profiles. For all sites except the low saltmarsh, which is characterised withby *C4-Spartina anglica* vegetation (C4 type) site at the low saltwater marsh, the $\delta^{13}\text{C}$ signal of SOC in the topsoil layer is similar to the $\delta^{13}\text{C}$ signal of standing vegetation. However, close inspection shows that differences in the $\delta^{13}\text{C}$ signal between vegetation and topsoil can be observed. At the high freshwater marsh the topsoil $\delta^{13}\text{C}$ signal is higher than the signal for both above- and belowground vegetation, while at the low freshwater marsh the topsoil $\delta^{13}\text{C}$ signal is lower than the above- and belowground vegetation signal. At both the low and high brackish marshes, the topsoil $\delta^{13}\text{C}$ is eloselyvery similar -related- to the $\delta^{13}\text{C}$ signal of roots, while it is about 1‰ lower compared to the $\delta^{13}\text{C}$ signal of aboveground vegetation. At the high saltmarsh, the topsoil $\delta^{13}\text{C}$ signal has a value in between the $\delta^{13}\text{C}$ signals of above- and belowground vegetation, while at the low saltmarsh the topsoil $\delta^{13}\text{C}$ signal is significantly lower compared to the signal of both above- and belowground vegetation. -standing vegetation is elosely related to the $\delta^{13}\text{C}$ signal of SOC in the topsoil layer.

4 Discussion

4.1 Soil organic carbon stocks along the estuary Effect of sampling procedure on reported estuarine OC stocks

The results of this study show that both SOC concentrations and stocks of tidal marshes vary significantly along a temperate estuary, with freshwater marshes having the highest stocks, followed by brackish and saltmarshes (Figure 5 and Table 3). This tendency is in agreement with observations in other studies (Table 4). However, the differences reported in previous studies are almost always much smaller than the differences we find. As the estuaries reported in table 4 cover a large geographical range, differences in environmental conditions will have an influence on the reported SOC stocks. For example, the estuaries reported in Table 4 that are located at the south coast of the U.S.A. experience significantly higher average temperatures compared to the Scheldt estuary. In addition, the vegetation species present on the tidal marshes, as well as differences in regional geology and estuarine morphology will play a role. However, as the studies listed in Table 4 report SOC stocks in tidal marsh sediments along a salinity gradient, also similarities in the factors controlling these stocks are present. For example, the freshwater marshes will receive considerably more OC from terrestrial sources, while the influence of OC inputs from marine sources will be the largest in the oligohaline marshes. Moreover, macrophyte production is generally considerably higher at freshwater marshes compared to saltmarshes (refs). In addition to these factors, This may to some extent be related to differences in environmental conditions, but differences in sampling procedures also matter. In most studies, marshes were sampled to a limited depth (Table 4). Generally, the differences in SOC content between different marshes reported in Table 4 are smallest for the top layers. As a consequence, the difference in SOC inventory will

increase if a larger sampling depth is considered. Evidently, considering a larger sampling depth will also lead to higher estimates of SOC stocks. This is one of the factors explaining why our stock estimates are much higher than those reported in the other studies in Table 4, especially for the freshwater marshes.

~~Another issue is whether carbon stocks should be compared by considering stocks down to a certain depth or that the total stock present in the marsh sediments should be taken into account. While it is simpler and more transparent to consider a certain depth, this approach does not account for the differences in dynamics between marshes. As Figure 2 shows, marsh accumulation rates are significantly higher for the freshwater marshes. This automatically implies that, when different marshes are sampled to a common depth, the timeframe that is accounted for will be shorter for those marshes that have the highest accumulation rates (Elschoot et al., 2015).~~

10 4.2.1 Observed patterns in SOC storage

While our data do not allow for a full statistical or mechanistic analysis of the mechanisms controlling the long-term storage of SOC in the studied tidal marshes, some important observations can be made.

A first observation is that low SOC stocks are not systematically related to low biomass production, as no statistical relationship between total annual biomass production (above- and belowground) and SOC stocks is found ($R^2 = 0.01$, figure S3). For example, the annual biomass production at the low saltwater marsh (*Spartina anglica*) is relatively high (Figure 4), while this site is characterised by the lowest SOC stocks. In addition, there is no relationship between annual root carbon production and SOC stocks ($R^2 = 0.004$, data not shown). This is rather surprising, as it has been proposed that roots contribute significantly to the subsoil OC pool in tidal marshes (Craft, 2007; Saintilan et al., 2013).

A second important observation is a the very rapid decrease of SOC with depth at the brackish sites. This decrease is accompanied by a shift in $\delta^{13}\text{C}$ to less negative values with depth in the topsoil of these marshes, suggesting that on the brackish marshes a significant fraction of OC is rapidly decomposed after burial (Figure 6). On the high brackish marsh the decline in SOC and the shift in $\delta^{13}\text{C}$ show the same tendency down to a depth of 0.3 m, while deeper down along the profile, both variables remain approximately constant with depth. This indicates that a significant fraction (approx. 87 %) of deposited OC is decomposed in this top layer. In the low brackish marsh sediments the situation is different. Here the SOC concentration only decreases from the top of the profile down to a depth of 0.15 m, while the $\delta^{13}\text{C}$ signal increases throughout the profile. At this location *Spartina anglica* (a C4 plant) was possibly present during early marsh development, resulting in a more positive $\delta^{13}\text{C}$ signal. This hypothesis is supported by the observation that *Spartina anglica* was indeed present on this marsh before 2000 (Boschker et al., 1999; Middelburg et al., 1997). In contrast, Currently *Elymus athericus*, a C3 plant, is present dominating the marsh vegetation at the marsh surface. This implies that the shift in $\delta^{13}\text{C}$ with depth at the low brackish marsh could partly also be the result of a shift from a C4 to C3 type vegetation, rather than resulting from the decomposition of SOC alone. This is very likely, as in general shifts in $\delta^{13}\text{C}$ as a consequence of kinetic fractionation during decomposition are generally in the order of 1 – 3 ‰ (Choi et al., 2001), while the shift we observe is much larger (ca. 5.7 ‰). However, the decrease in SOC together with the shift in $\delta^{13}\text{C}$ in the top 0.15 m suggests that, also on this marsh

significant decomposition of deposited OC (approx. 68%) took place after burial. ~~It should be noted that the topsoil $\delta^{13}\text{C}$ signal of the most recent sediments found signal of on the intertidal areas can vary throughout the year~~ (Zetsche et al., 2011). ~~However, the observed $\delta^{13}\text{C}$ depth profiles we observe are an integration of these annual cycles, limiting the effect of the timing of sample collection on the observed depth profiles of $\delta^{13}\text{C}$.~~

5 ~~Q~~Also on the high saltmarsh a significant decrease of SOC concentration with depth ~~also~~ occurs. This is again accompanied by a shift in $\delta^{13}\text{C}$ towards more positive values with depth. This location is currently characterised ~~with by~~ a mixture of C3 type vegetation. It is uncertain, however, if the isotopic shift with depth can entirely be attributed to kinetic fractionation caused by OC decay. It is likely that at the beginning of marsh growth ~~also~~ *Spartina anglica* was ~~also~~ present at this location, as it is currently present at the low part of this marsh. This would imply that also at this location the shift in $\delta^{13}\text{C}$ with depth
10 is the result of a combination of decomposition of OC and a shift in vegetation from C4 to C3 type.

Our observations indicate that on both the salt and brackish marshes a significant fraction of OC is lost after burial. Although in the brackish marsh sediments a larger fraction of OC is lost after burial compared to saltmarshes, total SOC stocks in the brackish marsh sediments are significantly higher compared to the saltmarshes.

At the freshwater marshes the situation is different. In both the low and high freshwater marsh sediments the decline in OC
15 concentration with depth is very limited. In addition, the $\delta^{13}\text{C}$ signal does not show a significant shift in the top 0.5 m of the soil profile. Below this depth there is a limited shift in $\delta^{13}\text{C}$ toward more positive values, but the interpretation of this pattern is complicated by the effect of previous land uses on the marsh (~~Figure 3~~Figure 3). These observations indicate that at both locations at the freshwater marsh there is limited decomposition of OC after burial.

4.32 Explanations for the observed patterns in soil organic carbon stocks

20 An explanation for the variation in SOC stocks between salt and brackish marshes on the one hand and freshwater marshes on the other hand needs to account for the differences in depth gradients in both SOC and $\delta^{13}\text{C}$. ~~Several factors may contribute to these differences and their possible role is~~ This may be explained by several factors which are discussed below.

4.32.1 Salinity

Although the Scheldt estuary is characterised by a strong salinity gradient (Van Damme et al., 2005), it is unlikely that
25 salinity as such is a direct factor controlling the difference in decomposition of OC ~~that we observed.~~ If salinity ~~as this would be a direct control on OC decomposition this would~~ imply ~~necessitate that there is~~ a positive relationship between decomposition ~~rate~~ and salinity ~~as decomposition is observed to increase with increasing salinity. However.~~ However, ~~Litterbag~~ litterbag experiments with *Elymus athericus* on ~~a~~ tidal marsh in the Scheldt estuary showed that there was an inverse relationship between soil salinity and decomposition (Hemminga et al. 1991b). In addition, Hemminga et al. (1991b)
30 concluded that there is no significant variation in cellulose decomposition in tidal marsh sediments along the brackish and saltwater portion of the Scheldt estuary.

4.32.2 Vegetation type

The type of vegetation present at the different marshes is another possible controlling factor, as it has been shown that the residues of different macrophytes have a different resistance against decomposition (Buth and de Wolf, 1985; Hemminga and Buth, 1991; Valery et al., 2004). One of the factors that determines the decomposition rate of plant material is the nitrogen content, as whereby plant material with a higher C:N ratio is generally more resistant against decomposition (Hemminga and Buth, 1991; Jones et al., 2016; Webster and Benfield, 1986). The C:N ratio of the vegetation present at the salt marsh (values between 27 and 30) is significantly lower compared to the vegetation present at the brackish- and freshwater marshes (values between 33 and 55) (Table S2). However, our OC and $\delta^{13}\text{C}$ profiles suggest that decomposition rates are highest on the brackish marshes and lowest on the freshwater marshes, while the vegetation present at these locations has comparable C:N ratios. Thus, there does not appear to be a direct relationship between the C:N ratio of the biomass and SOC decomposition.

Although our data do not allow us to isolate the effect of vegetation types on SOC stocks along the estuary, some important observations can be made. Firstly, the low and high freshwater marsh have different vegetation types (*P. australis* and *Salix* resp.). However, both soils show a similar SOC profile. ~~concentration with depth.~~ In addition, the high freshwater marsh, where annual biomass production is significantly lower, has the largest SOC stock. This indicates that the effect of local biomass production on SOC stock is limited in the freshwater marshes. Secondly, both brackish marshes have the same vegetation type (*E. athericus*), while topsoil OC concentrations and total SOC stocks at the high marsh are larger compared to the low marsh. In addition, the high marsh is characterised ~~with~~ by a somewhat lower (although not significant) annual biomass production (although the difference is not statistically significant). Thus, ~~is again indicates that there is only a limited effect of~~ variations in local biomass production also do not explain the differences in ~~vegetation on the~~ SOC stock between young and old ~~at the~~ brackish marshes ~~is limited.~~ Last, ~~On~~ the low saltmarsh ~~The presence of~~ *Spartina anglica* on the low saltmarsh is indeed likely to be responsible for the low SOC stocks. While *Spartina anglica* is characterised by a high net primary productivity, the organic material produced is known to be very labile (Boschker et al., 1999; Bouillon and Boschker, 2006; Middelburg et al., 1997). ~~Taken together, these observations indicate that local biomass production is not likely to be a dominant factor controlling overall variations in SOC stocks along the estuary as variations in OC stocks both along the salinity gradient of the estuary and between old and young marshes at a given salinity level cannot be explained by variations in biomass production. The effect of a specific vegetation type of~~ *Spartina anglica* on the SOC stock of the lower saltmarsh shows, however, that in some cases the presence of a certain vegetation type may be a dominant factor. ~~It may however control local SOC stocks, as is the case on the saltmarshes.~~

~~One of the factors that determine the decomposition rate of plant material is the nitrogen content, as plant material with a higher C:N ratio is generally more resistant against decomposition (Hemminga and Buth, 1991; Jones et al., 2016; Webster and Benfield, 1986). The C:N ratio of the vegetation present at the salt marsh (values between 27 and 30) is significantly lower compared to the vegetation present at the brackish and freshwater marshes (values between 33 and 55) (Table S2).~~

~~However, our OC and $\delta^{13}\text{C}$ profiles suggest that decomposition rates are highest on the brackish marshes and lowest on the freshwater marshes, while the vegetation present at these locations has comparable C:N ratios. Thus, there does not appear to be a direct relationship between the C:N ratio of the biomass and SOC decomposition.~~

4.3.3 Allochthonous organic carbon inputs along the estuary

5 The OC that is present in tidal marsh sediments is not only derived from autochthonous biomass. Estuaries are often characterised by relatively high concentrations of suspended sediment to which a significant amount of particulate organic carbon (POC) is associated (Abril et al., 2002). Due to the long residence time of water in the Scheldt estuary (2-3 months, Soetaert and Herman, 1995), organic matter is intensively processed as it moves through the estuary (Abril et al., 2002; Middelburg and Herman, 2007). In addition, mixing between fluvial and marine particles takes place (Nolting et al., 1999; Regnier and Wollast, 1993). Overall, this leads to significant variations in both the quantity and the quality of the POC that is present in the water and that is deposited on the marshes. Clearly, this variation may not only affect the magnitude of the OC inputs but also the decomposability of the OC that is deposited.

The freshwater marshes are located near the upstream border of the Scheldt estuary close to the maximum turbidity zone (MTZ), with average suspended sediment concentrations of ca. 0.15 g l^{-1} (Van Damme et al., 2001; Temmerman et al., 15 2004). The suspended sediments in this zone contain 7-10% POC (Abril et al., 2002). The higher values are observed in summer, when phytoplankton growth is important, while the lower values are reported in winter. The POC that is present in winter may be assumed to be processed POC from terrestrial origin (Hellings et al., 1999). In addition, during the past decades a large fraction of OC that has entered the freshwater portion of the estuary originated from untreated wastewater from the city of Brussels (Abril et al., 2002; Billen et al., 2005). It has however been shown that this OC is mineralised on a 20 timescale of weeks, possibly even before it enters the estuary (Muylaert et al., 2005; Servais et al., 1987).

Sediment concentrations strongly decline downstream of the MTZ (Abril et al., 2002; Van Damme et al., 2005). At the location of the brackish and saltwater concentrations marshes (ca. 20 km and ca. 50 km from the mouth) sediment concentrations are about 0.05 g l^{-1} (Van Damme et al., 2001; Temmerman et al., 2004). Furthermore, the POC content of these sediments decreases systematically in the downstream direction, except during the spring season when local production of OC due to phytoplankton is important in the marine portion-part of the estuary (Muylaert et al., 2005). As a result, average POC concentrations vary between 4 and 6 % in the brackish water zone and between 2 and 5 % in the saltwater zone (Abril et al., 2002). The overall decline in POC content is not only explained by the progressive downstream mineralization of OC but also by the upstream transport of marine sediments that carry less POC.

The variations in both suspended sediment concentration and POC content have important consequences for the relative 30 importance of allochthonous OC input on the marshes. On the freshwater marshes, both the high suspended sediment concentration and high POC loadings lead to a combination of high sedimentation rates ($10\text{-}20 \text{ mm yr}^{-1}$, with the highest sedimentation rates on the young marshes (Temmerman et al., 2004)) and high inputs of allochthonous POC. On the

saltwater marshes, sedimentation rates are much lower (5-10 mm yr⁻¹ (Temmerman et al., 2004)) and the deposited sediments contain 50 – 70 % less OC than the sediments deposited on the freshwater marsh (Abril et al., 2002).

Evidently, these differences may have important effects on OC storage in tidal marsh sediments (Figure 7). It can be reasonably be assumed that the allochthonous POC that is deposited with the sediments on the freshwater marsh consists for

5 a large fraction of terrestrial, recalcitrant POC. This POC may be expected to have a high burial efficiency (i.e. it will decompose relatively slowly after burial) and will remain in the sediments for a considerable time. The local, autochthonous POC is fresh and, will therefore be less recalcitrant. ~~and may be~~ Consequently it may be expected to decompose much more rapidly with time and contribute much less to long-term OC storage. The latter explains why variations in biomass production and vegetation type on the marshes. Moreover, as both the low and high freshwater marsh are characterised by a very different vegetation (both now and in the past, Figure 3), it is unlikely that local vegetation contributes significantly to long term OC storage, given the fact that the SOC concentration is similar at both sites do not explain variations in SOC storage in different marsh environments. Furthermore, the decomposition rate of both autochthonous and allochthonous POC can be expected to be inversely related to the burial rate as rapid sedimentation will protect OC from decomposition, as high sedimentation rates generally promote the burial efficiency of OC (Hartnett et al., 1998; Wang et al., 15 2014). Thus, OC will be better preserved when sedimentation rates are high.

Figure 7 illustrates how these factors combine. One may indeed expect to find a much less steep decline of the OC content with depth on the freshwater marsh (Figure 7A) due to (1) the dominance of allochthonous, recalcitrant OC and (2) the rapid burial of OC. Therefore, a relatively large fraction of labile autochthonous OC is preserved, as it is advected rapidly to deep sediment layers.

20 On the salt and brackish marshes a low sedimentation rate combines with low OC contents of the deposited sediments (Figure 7B). As a consequence, autochthonous OC is a dominant input, but this OC decomposes rapidly with depth. This results in a significant decline of OC content with depth, combined with a significant increase in $\delta^{13}\text{C}$ due to kinetic isotopic fractionation. In a recent study, Hansen et al. (2016) also attributed decreasing SOC stocks with increasing salinity in the Elbe estuary (Germany) to a decreasing OC content of suspended sediments and decreasing macrophyte biomass with increasing salinity.

25 Thus, both sedimentation rate as well as the rate of allochthonous OC input to the marsh system appear to be important controls on OC preservation in marsh sediments. While other factors such as local biomass production and salinity gradients may also be locally important, they do not appear to be key controls in the Scheldt estuary as most autochthonous POC appears to decompose rapidly, independent of the specific environmental conditions. This finding is similar to the observations of Omengo et al. (2016), who found that the OC preserved at depth in floodplain sediments of the Tana River in 30 Kenya consisted dominantly of processed OC that was deposited by the river, while locally produced OC contributed little to long-term OC preservation.

4.3 Effect of sampling procedure on reported estuarine OC stocks

The results of this study show that both SOC concentrations and stocks of tidal marshes vary significantly along a temperate estuary, with freshwater marshes having the highest stocks, followed by brackish- and saltmarshes (Figure 5 and Table 3). This tendency is in agreement with observations in other studies (Table 4). However, the differences reported in previous studies are almost always much smaller than the differences we find. As the estuaries reported in table 4 cover a large geographical range, differences in environmental conditions will have an influence on the reported SOC stocks. For example, the estuaries reported in Table 4 that are located at the south coast of the U.S.A. experience significantly higher average temperatures compared to the Scheldt estuary. In addition, the vegetation species present on the tidal marshes, as well as differences in regional geology and estuarine morphology, will play a role. However, as the studies listed in Table 4 report SOC stocks in tidal marsh sediments along a salinity gradient, also similarities in the factors controlling these stocks are present. For example, the freshwater marshes will receive considerably more OC from terrestrial sources, while the influence of OC inputs from marine sources will be the largest in the saltmarshes. Moreover, macrophyte production is generally considerably higher at freshwater marshes compared to saltmarshes (e.g. Dausse et al. (2012) and Hansen et al. (2016)).

In addition to these factors, differences in sampling procedures also matter can also explain some discrepancies. In most studies, marshes were sampled to a limited depth (Table 4). Generally, the differences in SOC content between different marshes reported in Table 4 are smallest for the top layers and increase with depth. As a consequence, the difference in SOC inventory will increase if a larger sampling depth is considered. Evidently, considering a larger sampling depth will also lead to higher estimates of SOC stocks. This is one of the factors explaining why our stock estimates are generally much higher than those reported in the other studies in Table 4, especially for the freshwater marshes and why we find larger differences in total SOC stocks between different marshes.

~~Another issue is~~ It is important whether carbon stocks should be compared by considering stocks down to a certain depth or that the total stock present in the marsh sediments should be taken into account. While it is simpler and more transparent to consider a certain depth, this approach does not account for the differences in dynamics between marshes. As Figure 2 shows, marsh accumulation rates are significantly higher for the freshwater marshes. This automatically implies that, when different marshes are sampled to a common depth, the timeframe that is accounted for will be shorter for those marshes that have the highest accumulation rates (Elschot et al., 2015). We suggest that the establishment of a correct time frame, from which sedimentation rates and their variations over time can be deduced, is indispensable for a correct interpretation of differences in SOC stocks (as well as C sequestration rates) between marshes.

4.4 Implications of sea level rise for estuarine soil organic carbon stocks

As global sea level is predicted to continue to rise during the next centuries, progressive intrusion of saltwater further into estuaries may be expected (Robins et al., 2016; Ross et al., 2015). As it is shown that freshwater and brackish tidal marshes store more SOC compared to saltmarshes (Table 3), one may expect that this will lead to a decrease in OC sequestration at locations where brackish marshes are replaced by saltmarshes. Also the MTZ is predicted to shift more inland (Robins et al., 2016). Because the Scheldt estuary is completely embanked and the tidal wave is stopped by sluices at the city of Ghent, the total area of freshwater marshes is likely to decline after sea level rise (Barendregt and Swarth, 2013). As we have shown that SOC sequestration rates are the largest in the freshwater portion of the estuary, the amount of OC sequestration in the freshwater portion estuary is therefore likely to decline with after sea level rise, due to the decline in freshwater marsh area. Moreover, as a consequence of the upstream migration of the MTZ, terrestrial organic matter can travel less for downstream in the estuary. Therefore, tidal marshes which are now located at the downstream end of the MTZ will receive less stable terrestrial OC in the future. ~~However~~ On the other hand, overall sedimentation rates are expected to increase with a rising sealevel, which will automatically lead to an increase in the rate of OC deposition as well as of OC burial rates, resulting in an increase of the OC sequestration rate per unit surface area.

Saltwater intrusion can also influence the decomposition of previously-sequestered OC, with some studies concluding that saltwater intrusion will enhance decomposition of organic matter (Craft, 2007; Morrissey et al., 2014; Weston et al., 2006, 2011), while others find that decomposition rates will decrease (Hemminga et al., 1991a; Weston et al., 2011). From these studies and from the analysis by Chambers *et al.* (2011), it is clear that this effect is highly dependent on local factors, such as the concentration of elements in the sea water that intrudes the estuary. Therefore, no reliable estimation of the impact of direction of OC mineralisation in tidal marsh sediments following saltwater intrusion on OC mineralisation in the Scheldt estuary can be made.

The above illustrates that our current understanding of the future evolution of the Scheldt estuary is still insufficient to make a quantitative assessment of how SOC stocks in the tidal marsh environment may change in the future.

5 Conclusion

As reported data on estuarine gradients of SOC are very scarce and, more importantly, often based on shallow soil sampling, additional research is needed in order to better constrain estimates of global estuarine OC stocks.

This study shows that the quantification of SOC stocks in tidal marsh sediments critically depends on the sampling depth. Gradients in SOC concentrations with depth strongly vary between marsh types so that a full inventory can only be made if sampling is carried out over the entire depth of the marsh sediments. Even if such data are available, interpretation has to be done with care, as sedimentation rates may vary considerably within a single estuary, making it complex to convert inventories to sedimentation or preservation rates.

In the Scheldt estuary, total SOC stocks are largest in a freshwater- and brackish tidal marsh and significantly lower in a saltwater marsh. These variations are to some extent controlled by variations in autochthonous biomass production, but our data strongly suggest that the key control on long-term OC preservation is the relative contribution of [terrestrial](#), allochthonous to total OC input, while OC burial rate may also be important.

- 5 The impact of future sea level rise on OC stocks in tidal marsh sediments will be determined by an interplay of different factors, including the evolution of the spatial extent of marshes in different salinity zones and sediment and OC deposition rates. Our study allowed to identify the factors that are important controls on OC storage and may need further research to resolve this issue.

10 Acknowledgements

We would like to thank Lore Fondu and Jianlin Zhao for their much-appreciated help during field work and lab analysis. We are also grateful to Natuurpunt, Staatsbosbeheer Zeeland and Stichting het Zeeuwse Landschap to provide us the opportunity to sample the tidal marshes. [Furthermore, we greatly thanks one anonymous reviewer and M. Schwarz for their constructive comments on the manuscript.](#)

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Competing interests The authors declare that they have no conflict of interest.

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Table 1: Main properties of the sampled tidal marshes. ^Afrom Meire et al. (2005), ^Bfrom Abril et al. (2002), ^C*Atriplex portulacoides*, *Limonium vulgare*, *Triglochin maritima*, *Elymus athericus*, *Puccinellia maritima*, ^Dbased on depth profiles of texture and OC concentration

Name	Name in this study	Vegetation	Tidal range (m) ^A	Elevation relative to local MHWL (m)	POC% of suspended sediment ^B	Max. Marsh sediment depth (m) ^{CD}
Notelaar marsh	Freshwater low	<i>Phragmites australis</i>	5.14	+0.24	6 - 10	1.2
	Freshwater high	<i>Salix sp</i> + <i>Urtica dioica</i>	5.14	+0.25	6 - 10	> 1.4
Waarde marsh	Brackish water low	<i>Elymus athericus</i>	4.85	+0.01	4 - 5	0.75
	Brackish water high	<i>Elymus athericus</i>	4.85	+0.14	4 - 5	> 1.4
Paulina marsh	Saltwater low	<i>Spartina anglica</i>	4.19	-0.66	3 - 4	0.2
	Saltwater high	Mixed vegetation^C <u>vegetation^D</u>	4.19	+0.11	3 - 4	0.6

5 Notes: ^Afrom Meire et al. (2005), ^Bfrom Abril et al. (2002), ^Cbased on depth profiles of grain size and OC concentration, ^D*Atriplex portulacoides*, *Limonium vulgare*, *Triglochin maritima*, *Elymus athericus*, *Puccinellia maritima*.

Table 2: General characteristics of the soil profiles at the studied sites. Bulk density values are averages for the upper meter of soil, whilst soil pH and electrical conductivity were measured in the topsoil only. ^AAverage for the upper meter, ^BValue for topsoil only, ^CUp to 0.7m depth, ^DUp to 0.2m depth

	Bulk density (g cm ⁻³) ^A	Soil pH ^B	Electrical conductivity (dS cm ⁻¹) ^B
Freshwater low	0.40 ± 0.07	7.47 ± 0.02	0.0271 ± 0.0009
Freshwater high	0.54 ± 0.04	7.35 ± 0.10	0.0262 ± 0.0007
Brackish water low	0.89 ± 0.06 ^C 06 ^A	7.70 ± 0.06	0.0389 ± 0.0048
Brackish water high	0.99 ± 0.06	7.49 ± 0.09	0.0365 ± 0.0023
Saltwater low	0.63 ± 0.07 ^{BD}	7.93 ± 0.02	0.0959 ± 0.0021
Saltwater high	0.96 ± 0.11	7.87 ± 0.03	0.0113 ± 0.0010

5 Notes: ^AAverage for the upper meter, ^BValue for topsoil only, ^CUp to 0.7m depth, ^DUp to 0.2m depth

Table 3: Total organic carbon (OC) stock (kg OC m⁻²) and standard deviations calculated for the full vertical sampling profiles (depths used for calculations are given in brackets), and the upper 0.6m. The depths down to which the stocks are calculated are given between brackets.

	OC stock (kg OC m ⁻²)	
	Low marsh	High marsh
For the entire marsh profile		
Freshwater	32.35 ± 0.65 (1.2m)	46.44 ± 0.80 (1.4m)
Brackish water	20.50 ± 0.72 (0.75m)	32.23 ± 0.31 (1.4m)
Saltwater	2.84 ± 0.10 (0.2m)	9.93 ± 0.34 (0.6m)
Up to 0.6m depth		
Freshwater	16.38 ± 0.54	21.66 ± 0.71
Brackish water	18.63 ± 0.71	19.63 ± 0.27
Saltwater	-	9.93 ± 0.34

Table 4: Reported SOC stocks (kg OC m⁻²) of tidal marsh soils along estuarine salinity gradients.

<u>Estuary</u>	<u>Sampling depth (m)</u>	<u>Freshwater</u>	<u>Oligohaline</u>	<u>Mesohaline</u>	<u>Polyhaline</u>	<u>Reference</u>
<u>Delaware (U.S.A.)</u>	<u>0.16</u>	<u>3.136</u>	<u>2.41</u>	<u>3.528</u>	<u>-</u>	<u>Weston et al. (2014)</u>
<u>Sapelo Dobby, Altamaha</u> <u>(Georgia, U.S.A.)</u>	<u>0.30</u>	<u>8.379</u>	<u>10.692</u>	<u>4.626</u>	<u>5.932</u>	<u>Craft (2007)</u>
<u>Dovey (Wales)</u>	<u>0.10</u>	<u>-</u>	<u>2.8</u>	<u>1.8</u>	<u>2.4 (low),</u> <u>1.4 (high)</u>	<u>Dausse et al. (2012)</u>
<u>Barataria (Louisiana,</u> <u>U.S.A.)</u>	<u>0.38</u>	<u>10.3</u>	<u>24.1</u>	<u>12.9</u>	<u>12.8</u>	<u>Hatton et al. (1983)</u>
<u>Satilla Altamaha Ogeechee</u> <u>(Georgia, U.S.A.)</u>	<u>0.30</u>	<u>8.096 ±</u> <u>1.245</u>	<u>-</u>	<u>6.816 ±</u> <u>0.997</u>	<u>6.069 ±</u> <u>0.482</u>	<u>Wieski et al. (2010)</u>
<u>Barataria basin (Louisiana,</u> <u>U.S.A.)</u>	<u>0.50</u>	<u>5.37</u>	<u>-</u>	<u>4.38</u>	<u>2.90</u>	<u>Williams and</u> <u>Rosenheim (2015)</u>
<u>San Francisco Bay</u> <u>(California, U.S.A.)</u>	<u>0.20</u>	<u>-</u>	<u>-</u>	<u>7.82</u>	<u>5.33</u>	<u>Callaway et al.</u> <u>(2012)^A</u>
<u>Louisiana (USA)</u>	<u>1.5</u>	<u>65.76</u>	<u>-</u>	<u>-</u>	<u>56.65</u>	<u>Wang et al. (2011)</u>
<u>Elbe (Germany)</u>	<u>1.0</u>	<u>-</u>	<u>27.05</u>	<u>16.04</u>	<u>11.31</u>	<u>(Hansen et al.,</u> <u>(2016)^B</u>
<u>Scheldt (Belgium, The</u> <u>Netherland)</u>	<u>0.6</u>	<u>-</u>	<u>21.66 ±</u> <u>0.71</u>	<u>19.63 ±</u> <u>0.27</u>	<u>9.93 ±</u> <u>0.34</u>	<u>This study^A</u>

Notes: ^AData for high marshes only, ^BAverage for all unmanaged sites

Table 4: Reported SOC stocks (kg OC m⁻²) of tidal marsh soils along estuarine salinity gradients. ^AData for high marshes only. ^BAverage for all unmanaged sites

Estuary	Sampling depth (m)	Freshwater	Oligohaline	Mesohaline	Polyhaline	Reference
Delaware (U.S.A.)	0.16	3.136	2.41	3.528	-	(Weston et al., 2014)
Sapelo Doboy, Altamaha (Georgia, U.S.A.)	0.30	8.379	10.692	4.626	5.932	(Craft, 2007)
Dovey (Wales)	0.10	-	2.8	1.8	2.4 (low); 1.4 (high)	(Dausse et al., 2012)
Barataria (Louisiana, U.S.A.)	0.38	10.3	24.1	12.9	12.8	(Hatton et al., 1983)
Satilla Altamaha Ogeechee (Georgia, U.S.A.)	0.30	8.096 ± 1.245	-	6.816 ± 0.997	6.069 ± 0.482	(Wieski et al., 2010)
Barataria basin (Louisiana, U.S.A.)	0.50	5.37	-	4.38	2.90	(Williams and Rosenheim, 2015)
San Francisco Bay (California, U.S.A.)	0.20	-	-	7.82	5.33	(Callaway et al., 2012) ^A
Louisiana	1.5	65.76	-	-	56.65	(Wang et

(USA)						al., 2011)
<u>Elbe</u>	<u>1.0</u>	-	<u>27.05</u>	<u>16.04</u>	<u>11.31</u>	(Hansen et
<u>(Germany)</u>						al., 2016) <u>B</u>
Scheldt	0.6	-	21.66 ±	19.63 ±	9.93 ± 0.34	This study ^A
(Belgium,			0.71	0.27		
The						
Netherland)						

^AData for high-marshes only, ^B Average for all unmanaged sites

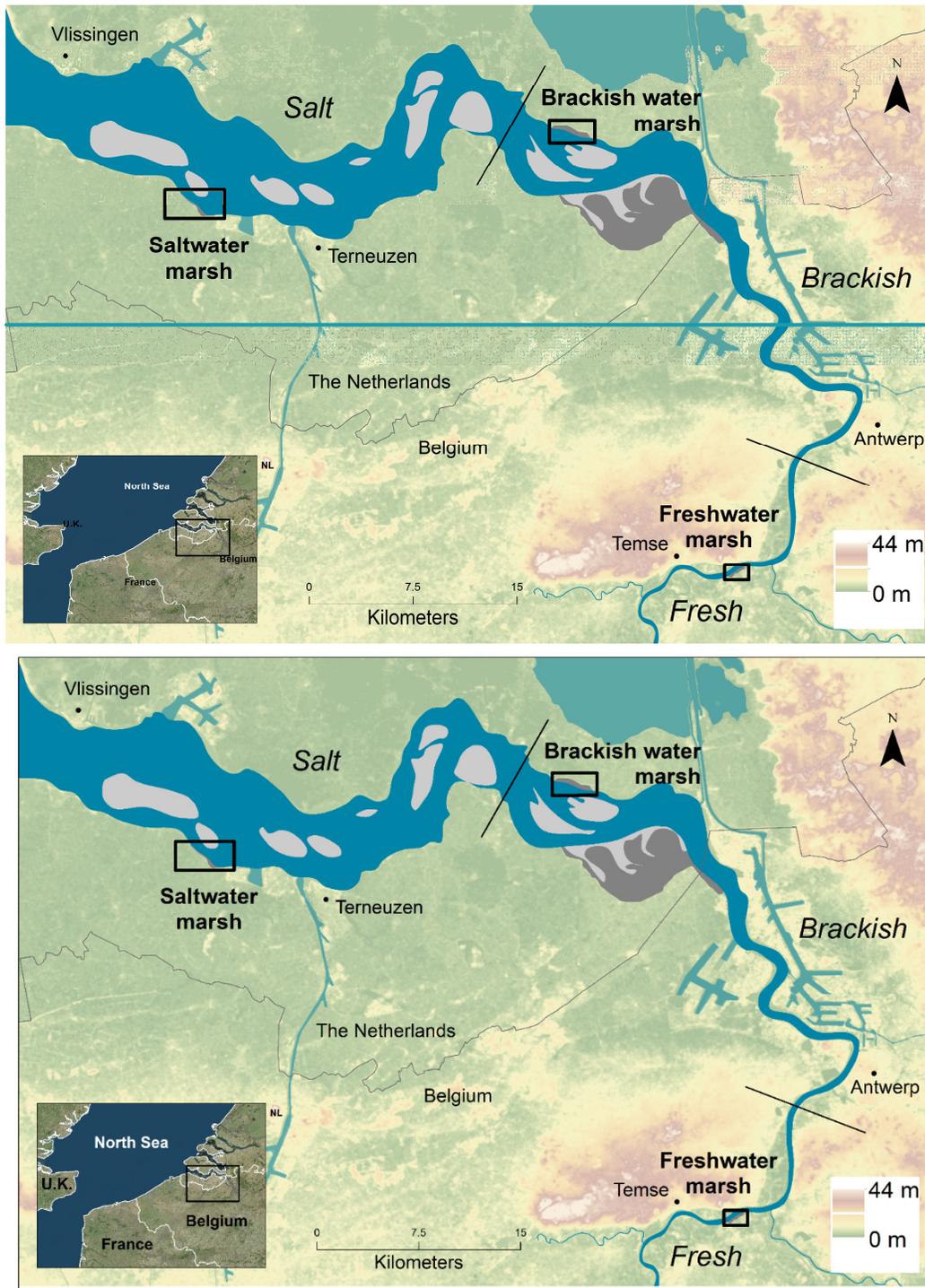


Figure 1: Map of the Scheldt estuary showing the salinity zones and the location of the sampled tidal marshes in a western European context. Intertidal sandflats are depicted in light grey.

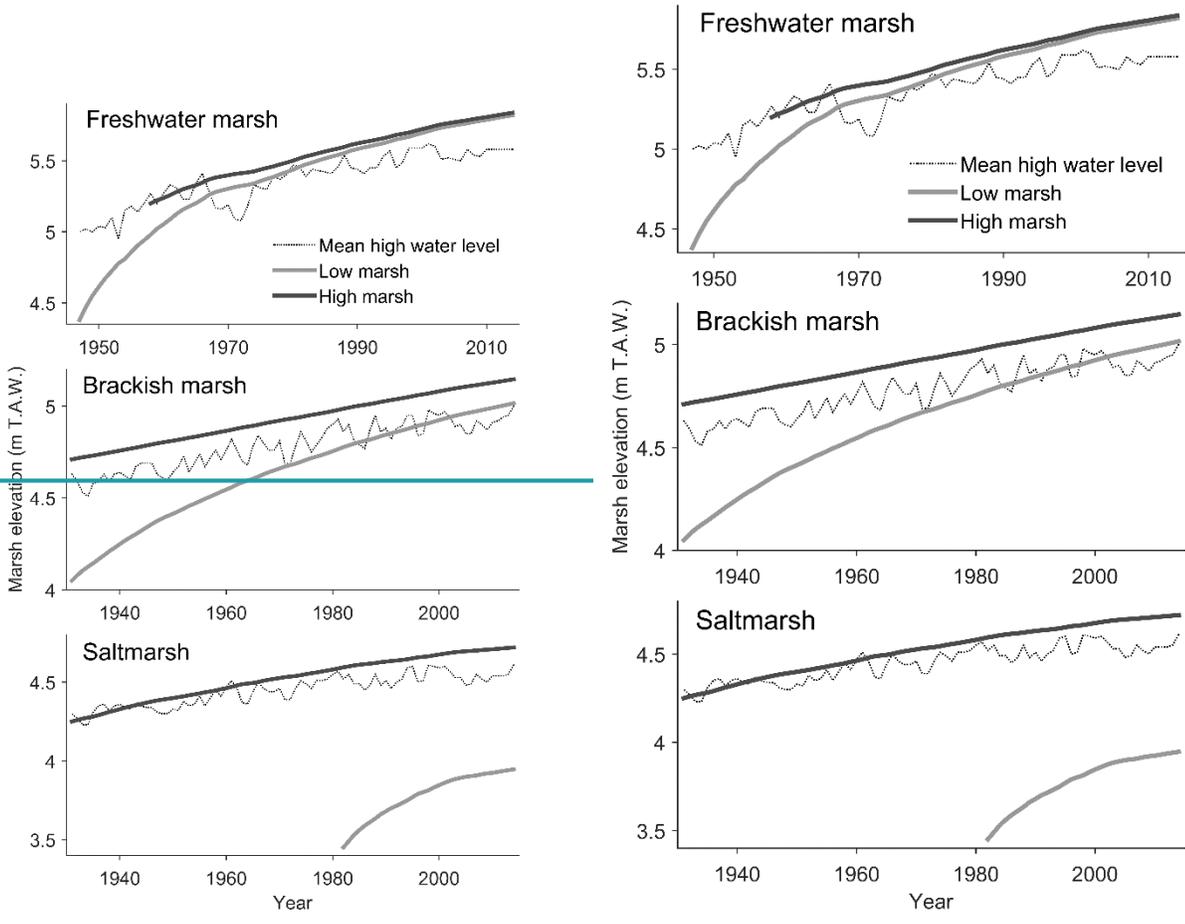


Figure 2: Evolution of marsh surface elevation and mean high water level (relative to Belgian ordnance level, m T.A.W.) at the sampled locations (based on Temmerman et al., 2004).

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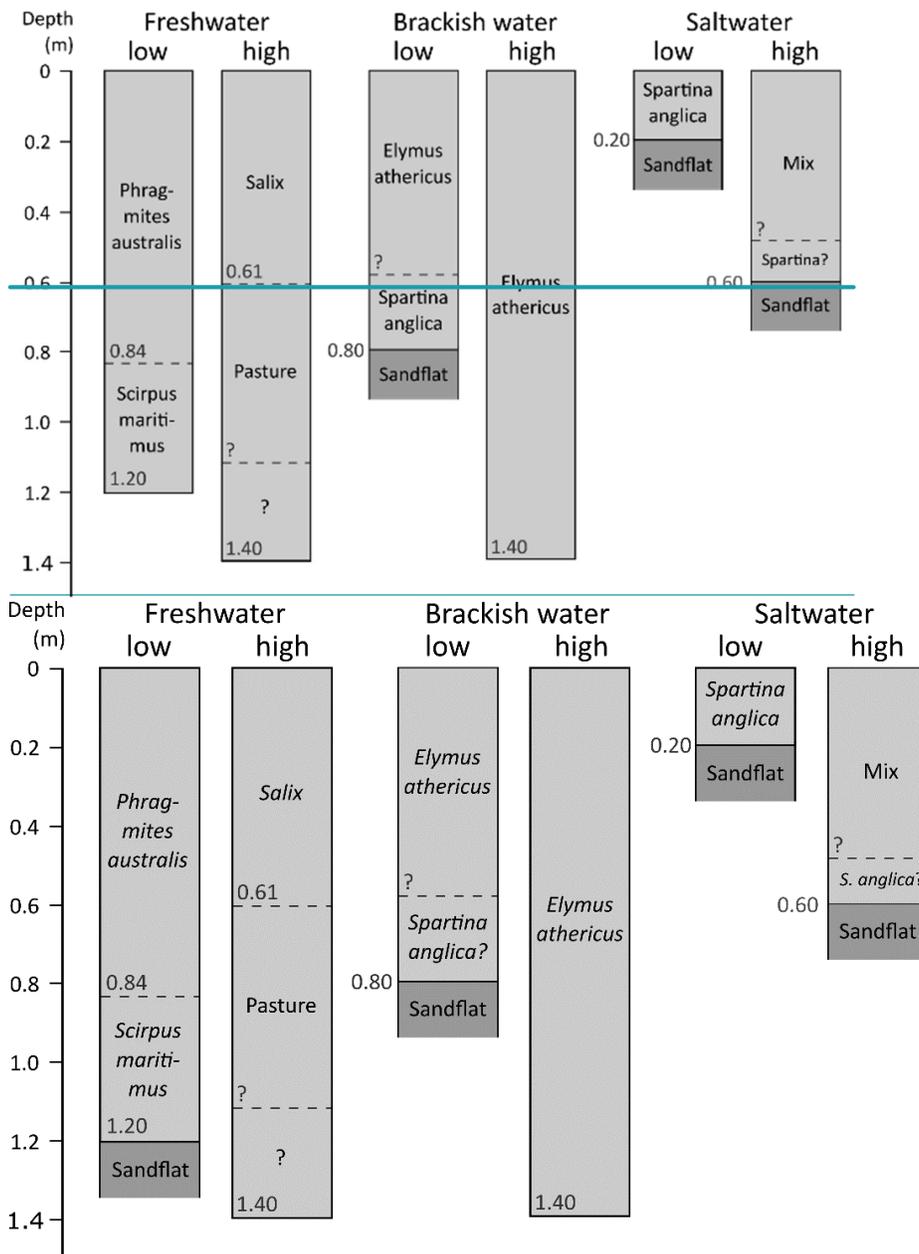
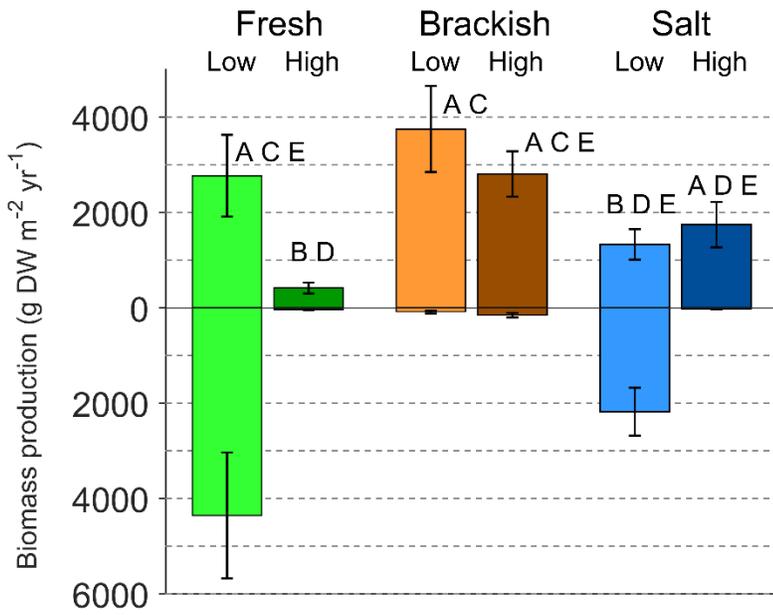
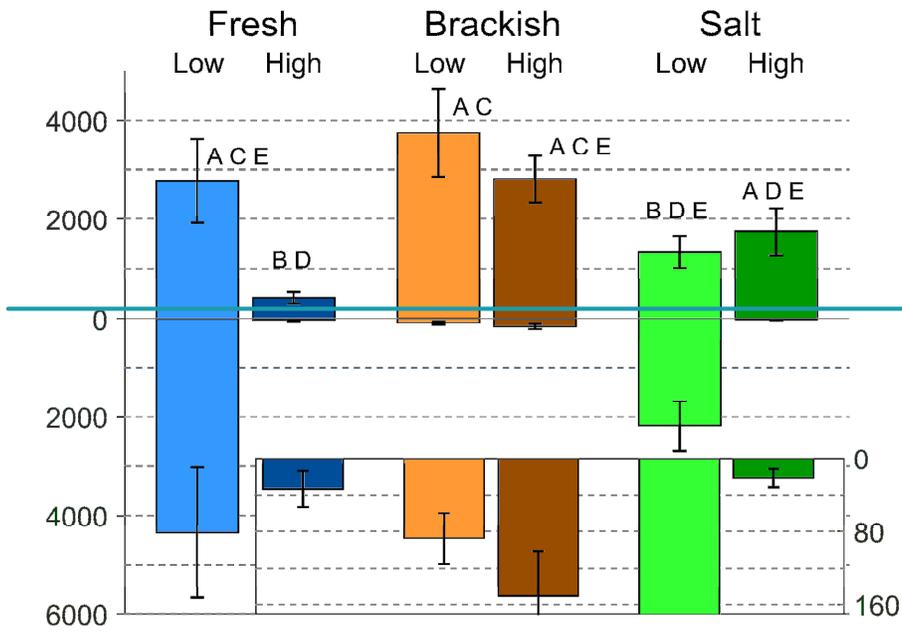


Figure 3: Depth profiles of the sampled tidal marshes showing the vegetation history at each location. At shallow marshes the former tidal sandflat was reached, at other locations the marsh sediments extended below the maximum sampling depth of 1.4 m. At locations where the sandflat was reached this is indicated, at the other locations the marsh sediments extended below 1.4m depth. The vegetation history is based on Temmerman et al. (2003) and information from the $\delta^{13}\text{C}$ profiles from of this study, combined in combination with information from Boschker et al. (1999) and Middelburg et al. (1997). Mix denotes a mixed vegetation which includes *Atriplex portulacoides*, *Limonium vulgare*, *Triglochin maritima*, *Elymus athericus* and *Puccinellia maritima*. A '?' near a dashed line indicates that the exact depth of this line is uncertain, a '?' after species names indicates that the presence of this species was hypothesised.



5 **Figure 4: Annual biomass production (g dry weight m⁻² yr⁻¹), with upward pointing bars representing aboveground biomass production and downward pointing bars representing belowground production (data is provided in table S2) (the inset is a magnification of the root biomass). Standard deviations for aboveground biomass are calculated based on 5 replicates, for root belowground biomass on 3 replicates. Sample locations that do not share a letter have significantly (p < 0.05) different annual aboveground biomass production rates. Significantly different aboveground biomass is denoted with different letters.**

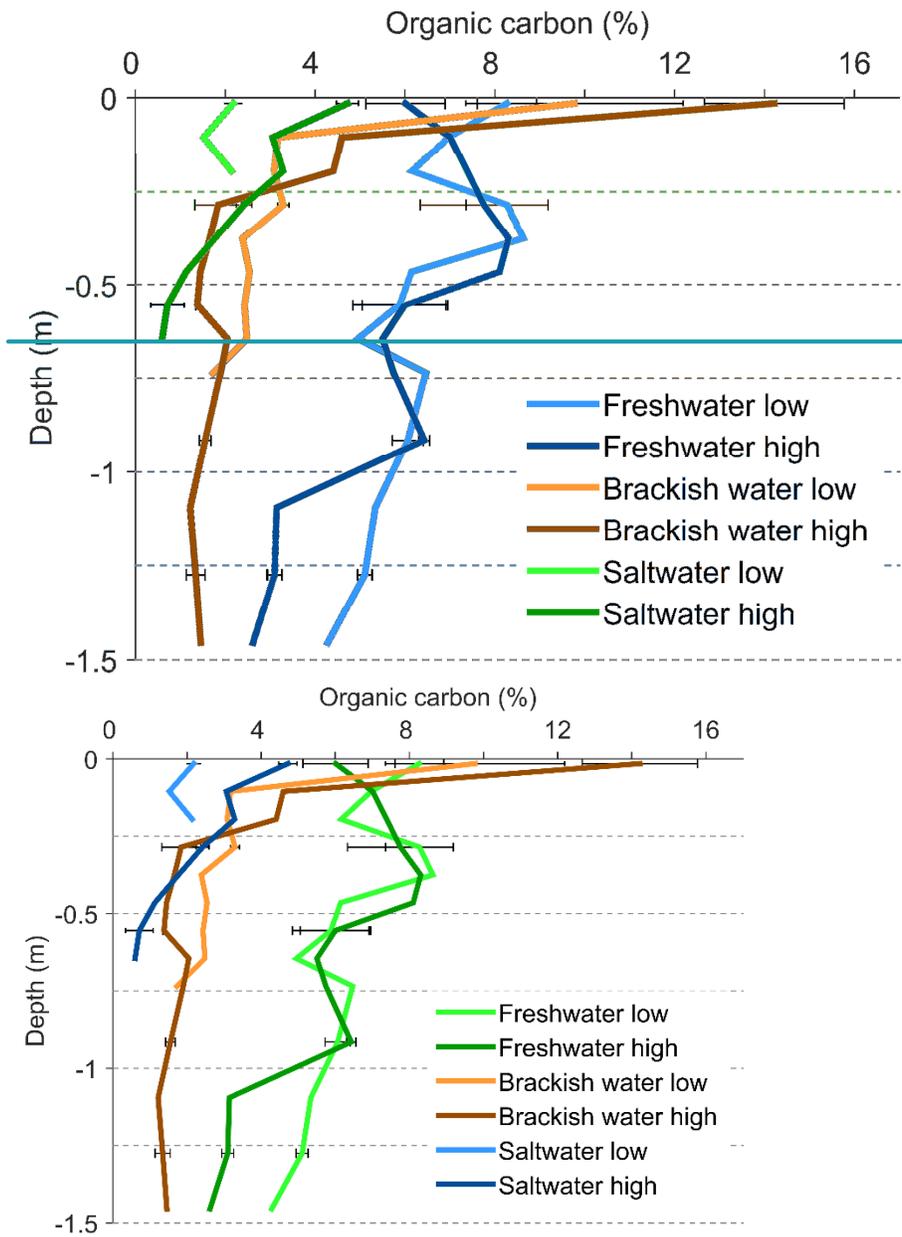


Figure 5: Depth profiles of OC concentration for all study sites. Data points show the average of three replicate soil samples. Error bars for specific depths are shown and represent the standard deviation of three replicate soil profiles.

5

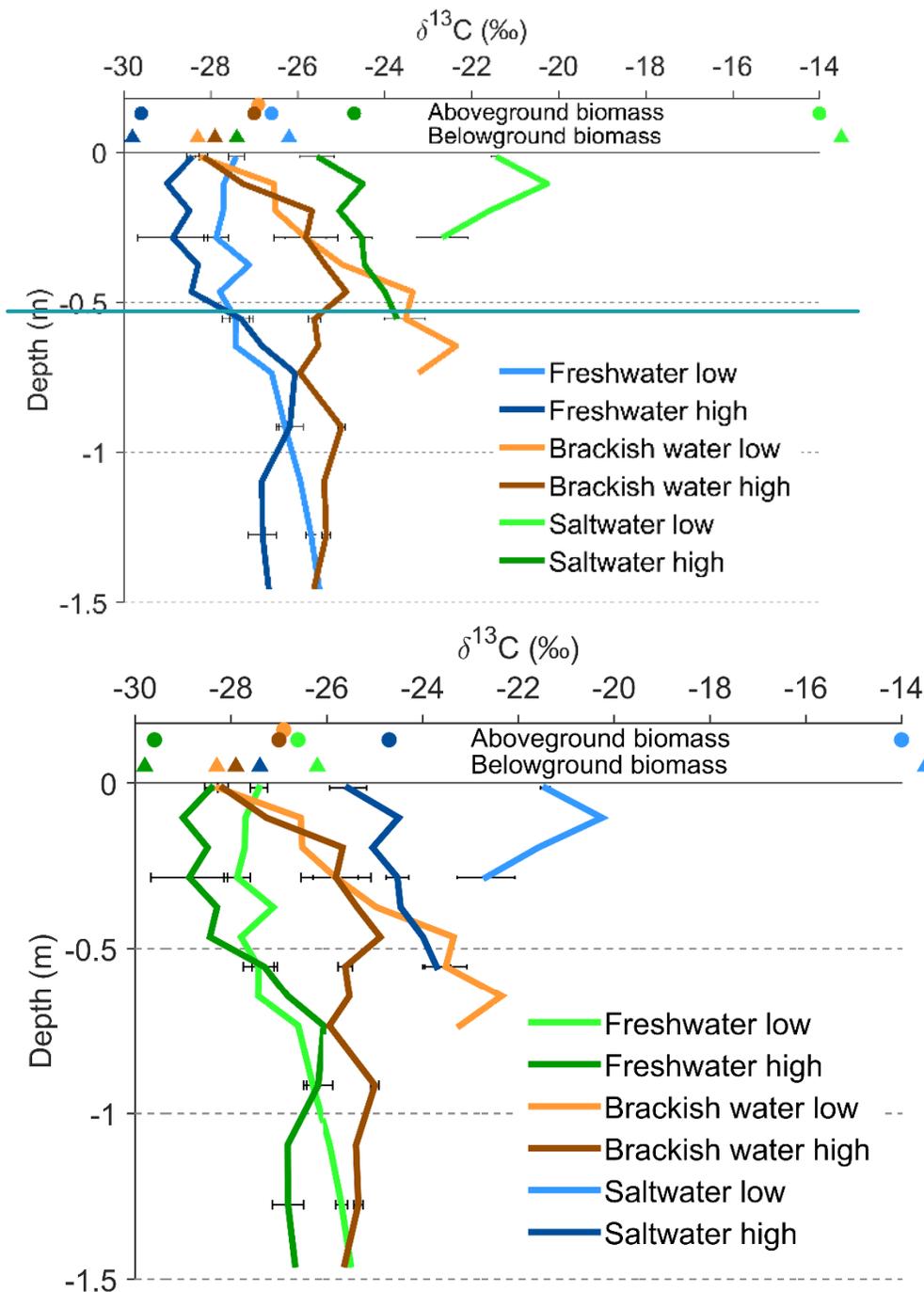


Figure 6: Depth profiles of $\delta^{13}\text{C}$, together with the $\delta^{13}\text{C}$ signal of aboveground (circles) and belowground (triangles) biomass (values are provided in table S1). Error bars represent the standard deviation of three replicate soil profiles.

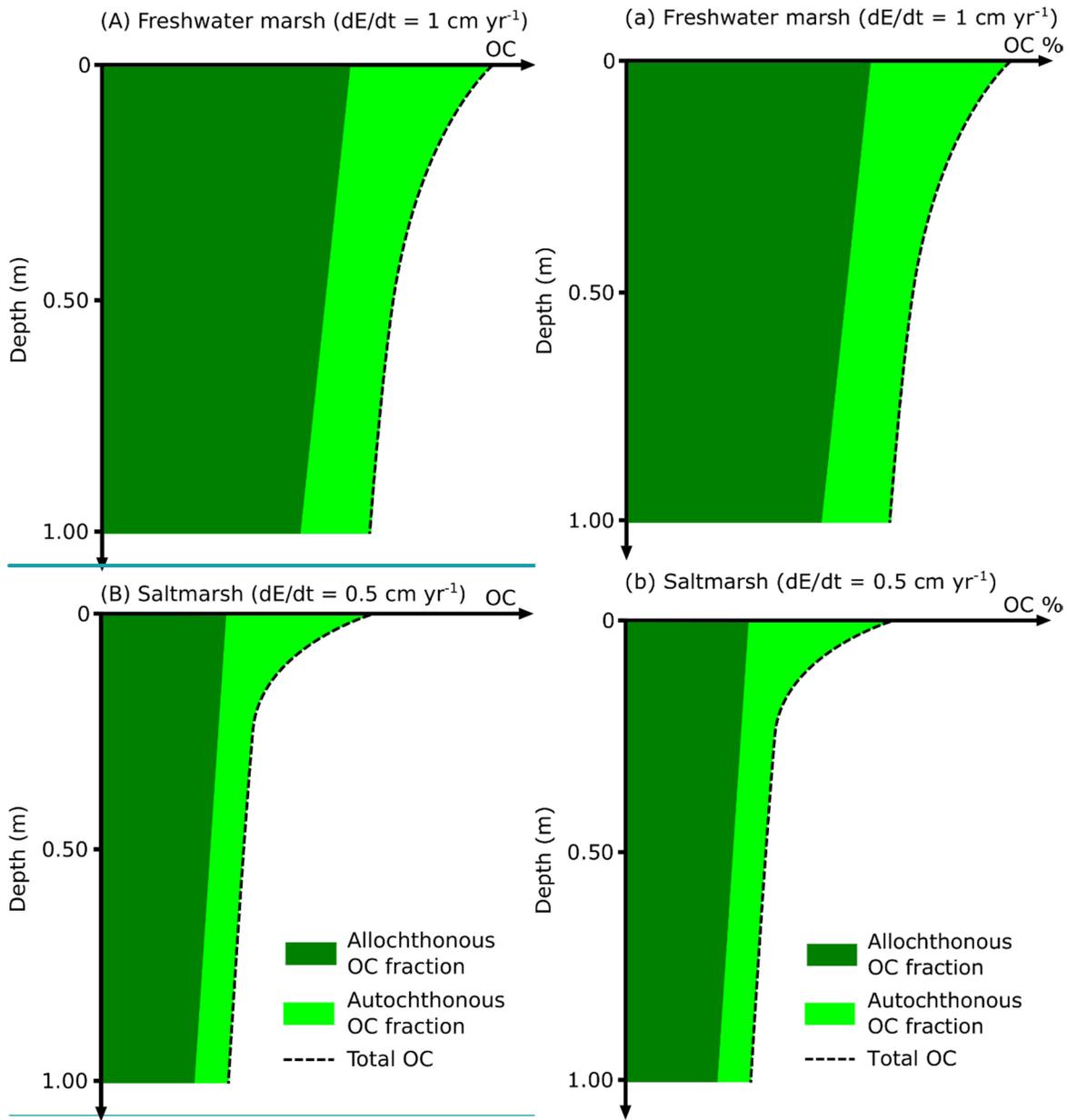


Figure 7: Conceptual diagram of the effect of both sediment deposition rate (dE/dt , E = elevation) and the relative inputs of recalcitrant allochthonous OC-organic carbon and labile autochthonous organic carbon on the fate of buried OC in a tidal freshwater marsh (Aa) and saltmarsh (Bb).

Table S1: GPS coordinates of the sample locations

	Low marsh	High marsh
Freshwater marsh	51° 7' 3.12" N 4° 16' 5.42" E	51° 7' 5.78" N 4° 16' 17.75" E
Brackish-water marsh	51° 24' 10.71" N 4° 6' 22.18" E	51° 24' 17.47" N 4° 6' 22.46" E
Saltwater marsh	51° 21' 0.08" N 3° 43' 14.81" E	51° 20' 59.15" N 3° 43' 10.60" E

Table S2: Average values (\pm standard deviation) for Aboveground, belowground (maximum root depth is given in brackets (m)) and total biomass, and biomass production, maximum rooting depth (m), organic carbon and nitrogen concentration (%), C:N ratio as well as the $\delta^{13}\text{C}$ signal (‰) for vegetation at the study sites. ^AFor below-ground biomass the maximum root depth is given between brackets, ^Bsee Table 1 in main text, ^CTurnover rates are presented in table S3.

	Vegetation type		Biomass (g dry weight DW m ⁻²) ^A	Annual biomass production (g DW m ⁻² yr ⁻¹) ^C	Organic carbon %	Nitrogen %	C:N	$\delta^{13}\text{C}$ (‰)
Freshwater low	<i>P. australis</i>	Above-ground	2775 \pm 858	2775 \pm 858	45.7 \pm 0.5	1.12 \pm 0.03	47.5 \pm 2.0	-26.3 \pm 0.2
		Litter	-	-	45.2 \pm 0.8	1.00 \pm 0.10	53.3 \pm 7.6	-26.6 \pm 0.2
		Below-ground	6400 \pm 1943 (0.8m)	4352 \pm 1321	42.1 \pm 1.0	0.83 \pm 0.11	61.3 \pm 9.6	-26.2 \pm 0.2
		Total	9175 \pm 2124					
Freshwater high	<i>Salix</i> (leaves)	Above-ground	215 \pm 72	215 \pm 72	42.9 \pm 1.6	1.60 \pm 0.02	31.9 \pm 1.2	-30.5 \pm 0.5
		Below-ground	160 \pm 92 (0.35m)	34 \pm 19	42.1 \pm 0.7	1.33 \pm 0.01	36.8 \pm 0.2	-29.8 \pm 0.1
	<i>U. dioica</i>	Above-ground	202 \pm 146	202 \pm 146	43.1 \pm 0.6	1.25 \pm 0.03	40.7 \pm 1.8	-29.6 \pm 0.3
		Below-ground	160 \pm 92 (0.35m)	34 \pm 19	42.1 \pm 0.7	1.33 \pm 0.01	36.8 \pm 0.2	-29.8 \pm 0.1
Brackish water low	<i>E. athericus</i>	Above-ground	2331 \pm 560	3754 \pm 902	45.0 \pm 0.4	0.96 \pm 0.03	54.6 \pm 2.6	-26.9 \pm 0.3
		Below-ground	25 \pm 8 (0.40m)	88 \pm 28	34.4 \pm 4.0	0.55 \pm 0.03	73.1 \pm 7.8	-28.3 \pm 0.4
		Total	2356 \pm 560					
Brackish water high	<i>E. athericus</i>	Above-ground	1746 \pm 295	2811 \pm 475	44.4 \pm 0.7	0.96 \pm 0.06	54.4 \pm 2.1	-27.0 \pm 0.3
		Below-ground	43 \pm 14 (0.20m)	151 \pm 49	35.2 \pm 3.5	0.58 \pm 0.04	68.5 \pm 10.8	-27.9 \pm 0.4
		Total	1789 \pm 295					
Saltwater low	<i>S. anglica</i>	Above-ground	680 \pm 163	1333 \pm 319	39.5 \pm 0.8	1.56 \pm 0.10	29.6 \pm 2.7	-14.0 \pm 0.02
		Below-ground	1728 \pm 399 (0.45m)	2177 \pm 503	40.4 \pm 1.7	1.19 \pm 0.12	40.0 \pm 4.8	-13.5 \pm 0.3
		Total	2408 \pm 431					
Saltwater high	Mixed <u>vegetation^B</u> <u>vegetation^B</u>	Above-ground	1214 \pm 331	1748 \pm 477	40.3 \pm 0.3	1.75 \pm 0.04	26.9 \pm 0.9	-24.7 \pm 0.3
		Below-ground	11 \pm 5 (0.45m)	22 \pm 10	36.8 \pm 1.9	1.67 \pm 0.07	25.7 \pm 0.9	-27.4 \pm 0.2
		Total	1225 \pm 331					

Notes: ^ATurnover rates are presented in table S3, ^B*Atriplex portulacoides*, *Limonium vulgare*, *Triglochin maritima*, *Elymus athericus*, *Puccinellia maritima*

Table S3: Turnover rates for above- and belowground biomass at the study sites. Vegetation type is given in table S2.

Aboveground biomass

Site	Turnover time (yr ⁻¹)	Reference	Remark
Freshwater low	1	Soetaert <i>et al.</i> (2004)	-
Freshwater high	1	-	As only fallen vegetation is sampled the turnover rate is assumed to be 1 /yr
Brackish water low and high	1.61	Groenendijk (1984)	Marsh near Krabbendijke (Oosterschelde Eastern Scheldt), calculated based on the paired-plot data
		Wolff <i>et al.</i> (1979)	Marsh near Stroodorpepolder (Eastern Scheldt Oosterschelde), based on max biomass and biomass production
Saltwater low	1.96	Gray & Benham (1990)	Tidal marsh in the UK, based on primary production
		Groenendijk (1984)	Marsh near Krabbendijke (Eastern Scheldt Oosterschelde), calculated based on his the paired-plot data
Saltwater high	1.44	Groenendijk (1984)	<i>Triglochin maritima</i> , marsh near Krabbendijke (Eastern Scheldt Oosterschelde), calculated based on his the single-plot data
		Wolff <i>et al.</i> (1979)	<i>Elymus athericus</i> , marsh near Stroodorpepolder (Eastern Scheldt Oosterschelde), based on max biomass and biomass production

Belowground biomass

Site	Turnover time (yr ⁻¹)	Reference	Remark
Freshwater low	0.68	Soetaert <i>et al.</i> , 2004)	Average value for roots and rhizomes
Freshwater high	0.21	Gill & Jackson (2000)	<i>Salix bebbiana</i> (Canada); <i>Salix spp.</i> (Alaska)
Brackish water low and high	3.5	Bouma <i>et al.</i> (2002)	Based on root ingrowth cores, marsh near Waarde (Westerschelde Western Scheldt)
Saltwater low	1.26	Bouma <i>et al.</i> (2002)	Based on root ingrowth cores, marsh near Waarde (Western Scheldt Westerschelde)
		Gray & Benham (1990)	Tidal marsh in the UK, based on primary production
Saltwater high	1.99	Bouma <i>et al.</i> (2002)	<i>E. athericus</i> , based on root ingrowth cores, marsh near Waarde (Western Scheldt Westerschelde)
		Groenendijk & Vink-	<i>Triglochin maritima</i> , average for 0-60 cm depth, Oosterschelde Eastern Scheldt , based on biomass

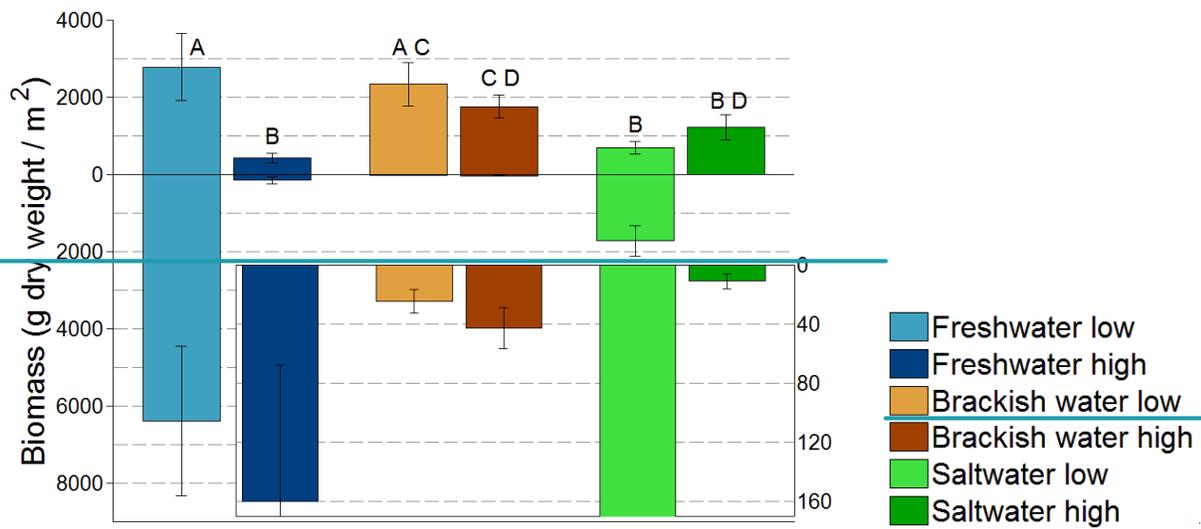
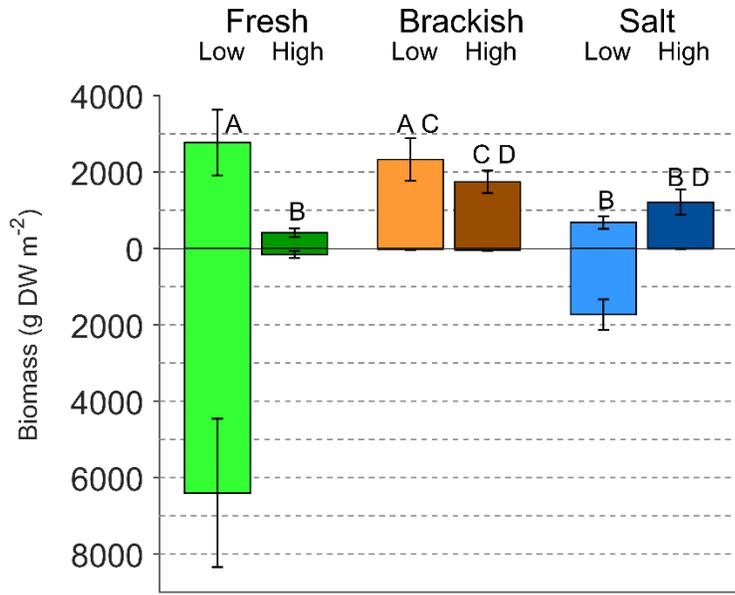
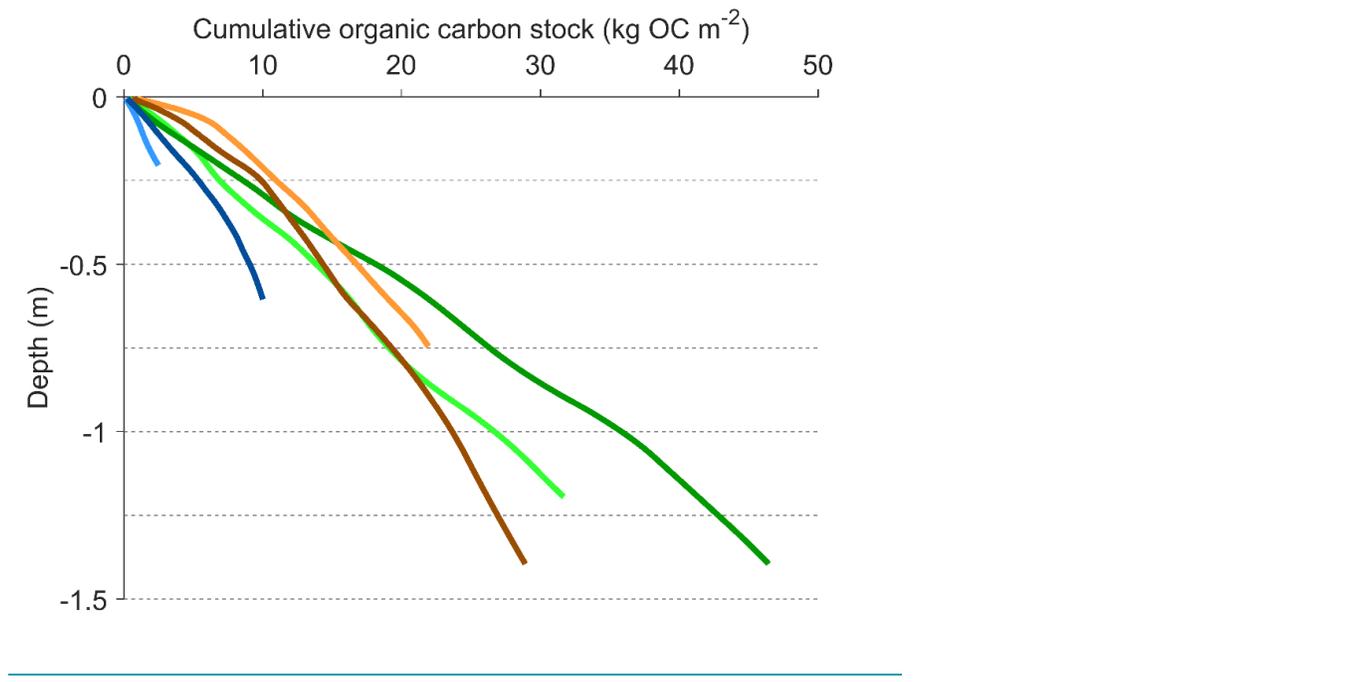
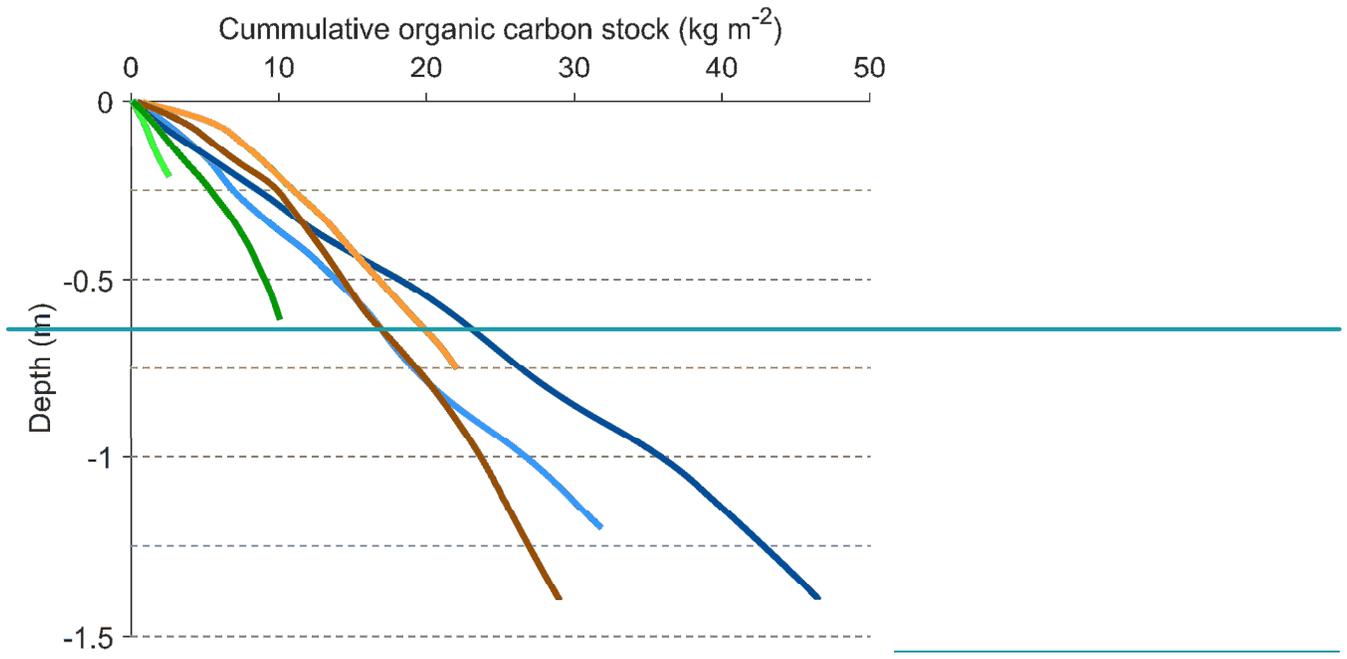


Figure S1: Total above- and belowground biomass for the study sites (g dry weight m⁻²) (the inset is a magnification of the root biomass), with upward pointing bars representing aboveground biomass and downward pointing bars representing belowground biomass (data is provided in table S2). Significantly different aboveground biomass values between sites are denoted by different letters. Upward pointing bars represent aboveground biomass, downward pointing bars represent belowground biomass. Standard deviations for aboveground biomass are calculated based on 5 replicates, for root biomass on 3 replicates. Sample locations that do not share a letter have significantly ($p < 0.05$) different aboveground biomass.

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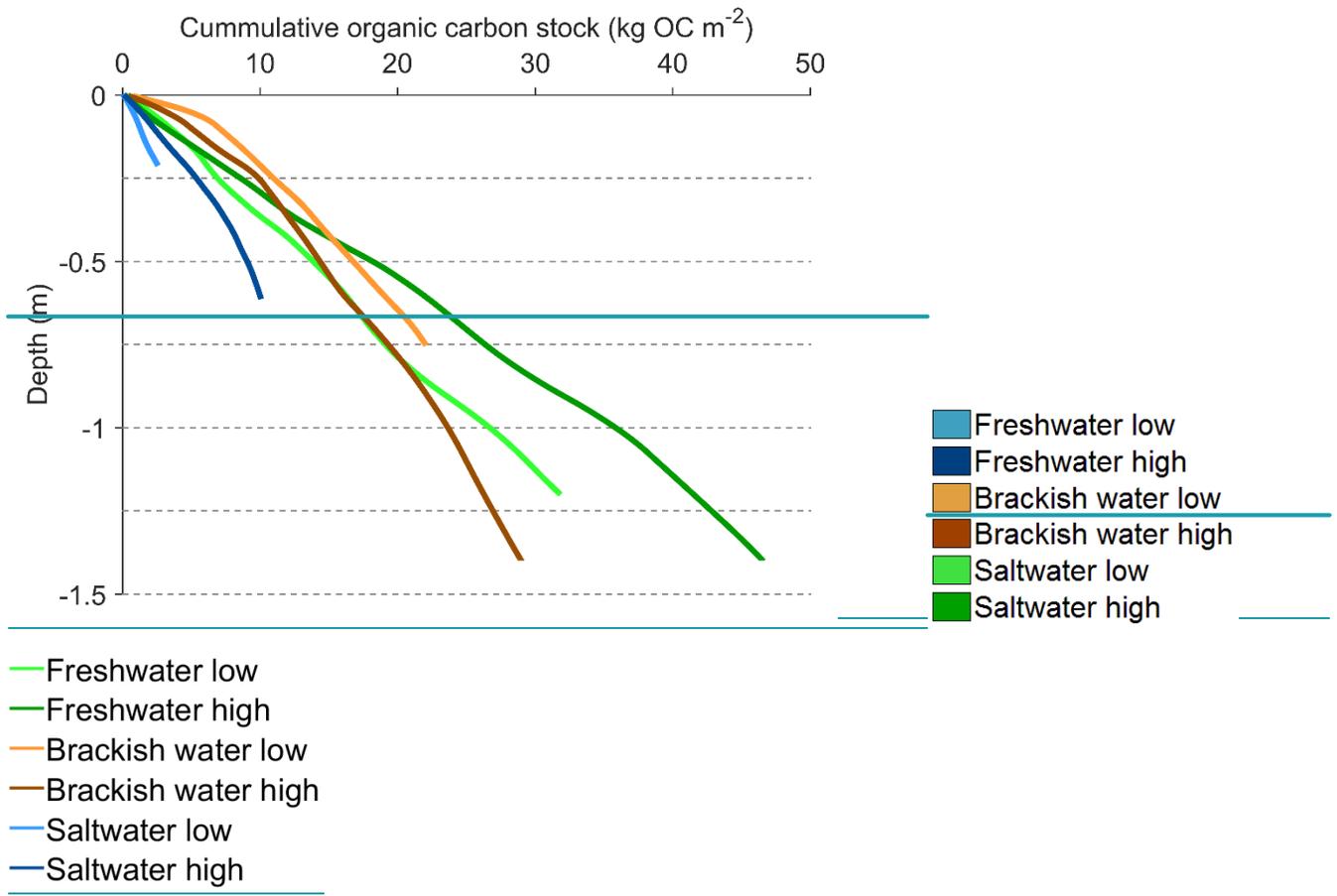


Figure S2: Depth profiles of the cumulative organic carbon stock for depth intervals of 0.01m. No standard deviations are shown to improve readability.

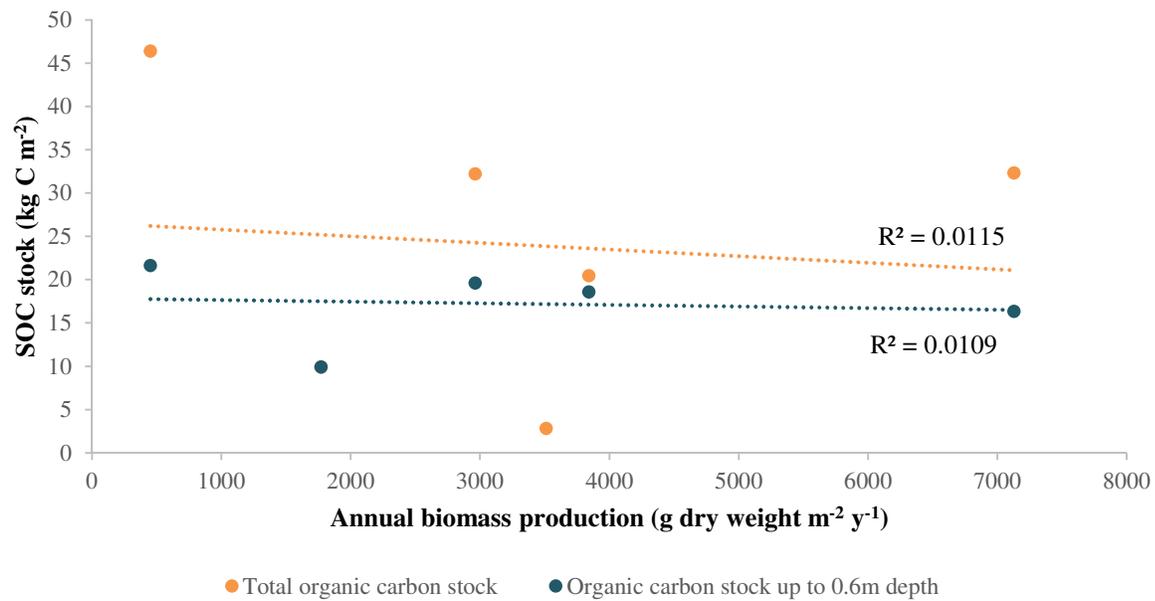


Figure S3: Relationship between the total annual biomass production (above- and belowground) and soil organic carbon stocks, for both total stocks and stocks down to 0.6m depth.

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