

Interactive comment on “Sediment characteristics as an important factor for revealing carbon storage in *Zostera marina* meadows: a comparison of four European areas” by Martin Dahl et al.

Martin Dahl et al.

martin.dahl@su.se

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Dear editor,

We thank the anonymous referees for valuable comments regarding this manuscript. A large part of the manuscript has been changed and we think that it has improved substantially and been given a much broader perspective. A major change that have been made was to include a literature survey on sedimentary carbon and organic matter content in *Z. marina* meadows in Europe and US (see table 5), which put this study into a broader context. We have also reanalyzed the dataset using a general linear

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mixed effect model, as suggested, which in part changed the outcome of this study (for instance carbon content did not decrease with sediment depth). We have also added measurements on core shortening for the Skagerrak area, where the effect of compression is assumed to be highest. This has not been accounted for in the data but has been discussed as a source of error. We argue that this will not change the conclusions made in this manuscript as the variation in carbon content is large between areas.

Below we address the specific comments made by the referees.

Please also see supplement to this comment for an updated version of the manuscript with all the changes (marked in red) including new figures and tables.

Anonymous Referee #1 Received and published: 7 June 2016 General comments: This study provides information on organic carbon at four European sites dominated by *Zostera*. It finds that sites vary among each other in carbon content and that sediment characteristics are an important factor influencing carbon content. However, the four sites chosen vary greatly among each other, comparing one location to another may not be adequate.

Reply: We have chosen these locations with the regard to geographical and physical variation to get areas covering a broad range of environmental conditions that *Z. marina* inhabit within Europe.

Perhaps if more sites were added it would improve the analysis; this is an issue that was not addressed in the discussion.

Reply: We agree that it is always better with more sites but we consider this design and sampling size adequate to answer the questions in this study. We have addressed this in more detail in the discussion and added a table comparing sedimentary carbon content in other areas of Europe and US. The table also show that our data is in line with previous studies.

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At some of the sites *Ruppia* was present and at others *Cymodocea*, how the variation in species presence may be confounding findings was not adequately discussed.

Reply: *Zostera marina* has been the dominating species (approximately more than 80% cover) in all cases. We agree that this could potentially influence the carbon storage and we have addressed this in more detail in the discussion.

The determination of grain size was carried out using three methods, dry sieving with and without removal of organic carbon and through a hydrometer. It is not clear how much the use of these three different methods affects the comparability of the samples, but it certainly can have an unwanted effect and should be avoided.

Reply: We have used the same method for all analyses. This has been clarified in the method section (also see below comment for more details).

The Serrano et al 2016 recent paper on mud content in seagrass meadows that analyses a great number of locations was not discussed, it should have been as well as other literature in greater detail.

Reply: The Serrano et al. (2016) paper was already included in the introduction and discussion in the submitted version, but we have now extended the discussion of this paper in greater detail.

Specific Comments: Methods, Study sites. The sites appear to be chosen bc they are the “edge zones of the *Z. marina* distribution in Europe”, not sure what the justification for this is?

Reply: We realize that edge zone is not a well suited description and we have rephrased this section. The sentence is now written as “The different study areas cover a range of environmental and physical conditions (e.g. salinity and temperature) for *Z. marina* in Europe. “

From the description of the sites, next to labs, it seems this was a sampling of convenience not one designed to address specific questions. One site with presence of

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Ruppia, another with Cymodocea which is not addressed in the discussion. . .

Reply: We selected major *Z. marina* areas in Europe to get a large variation of environmental conditions. The four areas chosen thus represent a broad range of environmental factors in terms of salinity, temperature and sediment characteristics. All of our sites were dominated by *Z. marina* but we have also added a section discussing the potential (minor) effect of other smaller species intermingling with the *Z. marina* plants (L268-272).

P6L119-120.”The sediment samples were cleaned from roots and rhizomes, larger shells and benthic organisms prior of drying and dried in the same way as the biomass.” Correct English, separate into two sentences and provide the details of the drying method used.

Reply: We have corrected the sentence and described the details of drying of sediment.

P6L123-1234. The method of acidification needs to be explained with more detail. Also, were the samples homogenized in any way prior to Corg determination?

Reply: The sediment was homogenized by mixing and grinding using a mixing mill. This has been added to the method section. We have also added more details with regard to the acidification method.

P7L135. “and the sediment of each sieve was weighed to determine the weight of the separate fractions” modify to “Sediment in each fraction was weighed separately”. How much total sediment was dry sieved?

Reply: We have corrected this and added average weight of the sediment sieved.

P7L136-137. Why was organic carbon only removed prior to grain size determination in some samples (referring to those with high Corg, define what “high” is). There is also the potential loss of fine material from the acidification and washing process. Grain size at two regions were measured using a completely different method, instead

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of dry sieving a hydrometer was used. Organic matter in the sample can lead to an overestimation of fine particles how are the treated and untreated samples comparable? How are samples in which grain size was determined using three methodologies comparable? It would appear this is a major flaw in a key point for the study.

Reply: We apologize for not being clear in the section of grain size analysis. We have now rewritten this section and clarified the misunderstandings. Three major issues are highlighted below:

1. Only one method was used, where all samples were dried, sieved and treated with $\text{Na}_4\text{P}_2\text{O}_7$ 2. After sieving the sediment, samples with high amount of particles <0.074 mm were analyzed using the hydrometer method. The data from the hydrometer analysis was only used to more accurately calculate mean grain size. 3. Treating the samples with H_2O_2 is a standard procedure to remove organic carbon that would otherwise interfere with the analysis, and hence there is a risk to underestimate the amount of fine particles due to aggregation of organic matter. The H_2O_2 reacts with organic matter and forms CO_2 and H_2O . This reaction is not affecting other parts of the sediment.

Methods, Stats. Why was a PCA used and not an MDS and Permanova? Include the program that was used.

Reply: PCA was used to explore the variables on a site-specific level and thus explains that on site level several explanatory variables might be of importance, although the overall sediment characteristics were of most importance. We have included the program used (SIMCA 13.0.3)

Results. P9. Sometimes Corg is reported, sometimes g C cm^{-2} , is this one total carbon or should it also be g Corg cm^{-2} ?

Reply: Thanks for noticing this. We have corrected this as it should be g Corg cm^{-2} .

P9 and elsewhere. Instead of using the specific site names it is probably better to

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indicate what they represent, lower or upper thermal limit for the species? east to west sites? etc. . .

Reply: This is an interesting thought but since the sites are not intended to represent any distribution limits we feel that it is better to use the specific site names. However, we have tried to reduce the use of site/area names as much as we could to make it easier for the reader.

P10203-204. Why was mean grain size tested against sediment particles <0.074 mm (%)? they both represent grain size descriptions. . .

Reply: Mean grain size and sediment particles <0.074 mm were not compared. We have added the outcome of the non-significant outcome of %Corg with mean grain size to separate the two analyses and to make this clearer.

Discussion. When comparing with other studies the statistical methods used should be noted and compared as well. Here, nested ANOVAS were used, while other studies use regressions or linear mixed effects models and the statistical findings can vary depending on which models are used. Were the findings using ANOVA replicated using simple regression or linear mixed effects models?

Reply: As suggested (by both referees) we have reanalyzed the data using a mixed effects model (glmm). The result section has been rewritten accordingly (L190-197, 216-221) and table 2 has been changed and is now presenting the outcome of the model.

P13L 278 "In areas without these sediment properties" what does that mean? areas of coarser sediment? larger grain sizes?

Reply: We have clarified this.

Why is this manuscript not comparing the findings to a much larger analysed done recently by Serrano et al 2016? (Serrano, Oscar, et al. "Can mud (silt and clay) concentration be used to predict soil organic carbon content within seagrass ecosystems.")

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Biogeosci Discuss 2016 (2016): 1-24.).

Reply: We agree that the Serrano et al. 2016 paper is important and we have put our results in context with their findings.

Fig. 3. Put both the vegetated and unvegetated into one same graph of each site to be able to compare (different colours or black and grey can be used to differentiate them), they all have different x axis so comparison is not straightforward.

Reply: We have changed figure 3 to include depth profiles of both *Zostera marina* meadows and unvegetated areas within each area in the same figure.

Fig. 4. can be deleted

Reply: We wish to keep figure 4 as we consider it to be a clear visualization of the outcome of the PLS analysis to help the reader to understand and hence easier interpret the conclusions made.

Technical comments: Introduction: P3L49. “Dense meadows have the ability to stabilize the sediment, (and thereby preventing it from eroding)”

Reply: We are sorry but it is not clear what the referee want us to change. The only difference with the sentence in the manuscript is the comma after sediment (which seems strange).

P3L49-51. Separate into three separate sentences.

Reply: We prefer to keep the sentence like it is as the two first statements are the basis for the last dependent clause.

P4L74. “coarser stone-sand bottoms to finer silt and clay sediment” shouldn’t it be coarser?

Reply: This has been changed.

P7L132-133. Should be “each section was analyzed separately”

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Reply: We have rewritten this section.

P7L140. “to be analysed with hydrometer” should be “. . .with a hydrometer (make and model)” P10L186-189. This section needs to be improved as far as clarity and can be separated into 2-3 sentences.

Reply: We have rewritten this section to clarify the types of methods used for grain size analysis.

P12L224. “west coast where 15 times” should be “was”. Also for P13L 277, it occurs several times in the manuscript.

Reply: This has been corrected.

Anonymous Referee #2 Received and published: 8 June 2016 This study provides new insights into the organic carbon storage of *Zostera* meadows in Europe. The dataset compiled may constitute a good overview of the variability in carbon storage within and among sites for this seagrass species. The sites sampled have different habitat characteristics (e.g. geomorphologies, salinity, hydrodynamic energy, water depth, tides, species composition, etc.) and such factors play a role in sediment carbon storage while adding confounding factors and complex interactions between factors that need to be accounted for in the discussion of the manuscript.

Reply: We have considered this carefully and rewritten some parts of the discussion to include a more detailed discussion regarding these factors and also included relevant references.

However, the low number of proxies analysed in the cores precludes such detailed approach, overall leading to relatively vague and well known conclusions: ‘carbon storage in *Zostera* meadows is influenced by sediment grain-size, high porosity and low density of sediments’.

Reply: Regarding the conclusion that there is a correlation between sediment grain size and organic carbon content in seagrass meadows is currently debated and not

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generally accepted; this has been clearly stated in the introduction. We agree that it is well-known that organic content in soil and sediment is positively correlated in general, which is also written in the introduction. However, this has not yet been clearly seen in seagrass ecosystems and is important to highlight as a factor influencing carbon storage.

This manuscript would greatly benefit from the analysis of other proxies in the cores, such as tracers of organic matter origin (e.g. stable C and N isotopes), geomorphological study of the areas to determine whether the Corg stock is derived from the capacity of the plants to trap sediments or simply due to the depositional environment (e.g. fetch, share bottom stress), and dating of the sediments (^{14}C or ^{210}Pb) to determine whether the sediments are mixed (subjected to erosion which may explain lower Corg storage) and to compare 'apples with apples' in terms of period of accumulation rather sediment thickness.

Reply: We agree that this would be excellent proxies to add and if ever possible we will add this to future studies but in the current study we had no possibilities to achieve such technically challenging and costly (some methods very costly) measurements. Regardless, this study is still a contribution to understand carbon storage processes in *Zostera marina* meadows where sediment characteristics should be considered an important factor for carbon storage variability. We do not claim that this is the only factor having an influence on sedimentary carbon content, which we also clearly state in the introduction and discussion.

I consider that this manuscript is not suitable for publication in its present form, and a major overhaul together with additional analyses are required prior to publication. Some of the concerns raised below (e.g. corrections for soil compression) most probably could not be addressed at this stage, which could compromise the reliability of the conclusions derived. I've also provided some tips to improve the manuscript (i.e. to give it a broader perspective), but I am not sure if after all the manuscript could meet the standards of the journal.

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General comments: 1. The methods used for sediment grain-size (e.g. removing coarse plants and other grains that where there and contributed to the accumulation/preservation of Corg, use of H₂O₂ or not, hydrometer vs sieving, etc.) are multiple and probably led to different results with important implications for the conclusions of the paper. It is important to demonstrate that grain-size results are reproducible between methods, since grain-size is the main driver detected in this study. Please discuss the potential implications of using different methods for sediment grain-size analyses, including the hydrometer.

Reply: We apologize for not being clear in the grain size method section. We have now partly rewritten that section to make it clearer that all samples were analyzed using the same methodological strategy. Regarding the concern of sieving and hydrometer methods, all samples were treated with Na₄P₂O₇ prior to analysis and sieved; therefore this should not affect the samples in different ways. The hydrometer analysis was done after the dry sieving in those samples with high fraction of finer grain size particles and was only used to calculate mean grain size. The H₂O₂ is a standard procedure for removing organic matter, which otherwise could lead to an underestimate of fine-sized particles due to aggregation.

2. Compression of loose soils could be as much as 30% and differ between sites, in particular between sites with different sediment grain-size composition. The authors did not account for sediment compression when estimating the Corg stocks for a certain depth, and therefore, this could be a very important but undesired factor explaining part of the conclusions reached by the authors. In other words, meadows in finer sediments most probably experienced higher compression than those growing in coarse sediment. As a result, an overestimation of the Corg content in meadows with finer sediments (more Corg in less space) could led to spurious differences between sites and meadow/bare, in particular when assessing differences in g Corg cm⁻². The fact that the total length of sample recovered during coring differed between sites (i.e. while using 50 cm long pipes in all cases) could evidence such issue.

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Reply: We agree that compression of sediment is relevant and we have measured this in the Skagerrak area, which had the finest grain size and the softest sediment, by measuring the inner and outer part of the core from the top of the corer down to the sediment surface when pressed down in the sediment. The compression in that area is approximately 8% and we have added this measurement to the method section as well as to the discussion section as a source of error. The main reason for the difference in sediment core length was, however, due to the difficulties of manually pressing down the corer without breaking them. This was clearly evident in coarser sediment, for instance at Askö (Baltic Sea), whereas in the Gullmar Fjord on the Swedish west coast the sediment was looser and easy to collect a full-length sediment core. We have explained the sediment sampling more in detail in the method section, including using sharpened corers to reduce the effect of core shortening (Serrano et al. 2012).

3. This study fails to address whether the Corg storage in the meadows is due to the meadow itself (autochthonous inputs) or if it is mainly driven by the depositional environment (allochthonous inputs). In other words, would the Corg stocks be there independently of the presence of the meadow? How much of the Corg stock is due to the grain-size and how much due to the inputs from the plant? Does the plant enhance sediment accumulation and Corg compared to bare sediments? The comparison with bare sediments could be a good approach to address this, but it is not clear whether control bare sediments are realistic controls? (if yes, most probably they should have seagrass meadows in there), or if at least they have similar grain-size (geomorphology/hydrology) so could be used as a reference?

Reply: This is an important factor when determining the trapping of particles in a sea-grass meadow but we did not attempt to address this issue in the present study. The unvegetated reference sites were used to estimate the background carbon content of the areas and areas with higher carbon content in the *Zostera* meadows also had higher carbon content in unvegetated areas in general. We believe that the main reason for lower carbon content and less fine grain size particles in unvegetated areas is

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the absence of seagrass as the seagrass plants affect the sediment characteristics and hydrodynamic properties of the meadow. We have added this to the discussion where we address the complexity between seagrass and sediment.

4. The English needs edition in some parts of the manuscript, in particular the introduction and discussion.

Reply: We have gone through the manuscript and made corrections of the language.

5. Plant cover and biomass refer to present (sampling date), but it seems possible that meadow structure have not been constant over the last years or centuries, so over the ca. 40 cm-thick period of accumulation estimated. The lack of a permanent gradient within each study site (such as water depth driving seagrass density and cover) precludes such approach, and the assumption of constant plant cover etc. over time is hard to believe, in particular considering the dynamics of *Zostera* meadows. Discuss the limitations of using such plant parameters and assumptions involved.

Reply: We agree that plant cover and biomass are highly dynamic and not constant over time. We have added this to the discussion.

6. The discussion of the importance of sediment grain-size in Corg accumulation and preservation is poor (e.g. include the concepts of selective and non-selective preservation in marine sediments). Important papers to look at (and references therein) to enrich the discussion: - Serrano et al 2015 Biogeoscience discussion_Key factors influencing carbon storage in Posidonia. - Lavery et al 2013_PlosOne_Seagrass carbon in Australia

Reply: We agree that these two papers (Lavery et al. 2013; Serrano et al. 2015) are of importance and Lavery et al. 2013 is already included in the discussion. We have now broadened the section where we refer to Lavery et al. 2013 to discuss it more in detail as well as included Serrano et al. 2015 in the discussion section. We have added more in the discussion on grain size and preservation of organic matter.

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[Discussion paper](#)



We have also rewritten the sentence in the introduction where we refer to Serrano et al. 2016 and added the newly submitted manuscript by Röhr et al. 2016 as a reference (L.59-61).

7. What percentage of variability the PCA explained? It is meaningful? This information should be provided. The use of the PCA seems unnecessary.

Reply: We apologize for missing the important detail to include the percentage of variability explained. This has now been added to the results. The aim of using a PCA was to analysis the relationships between predictor variables and sedimentary carbon content on a site level to better understand local variation in factors influencing carbon storage.

8. The manuscript could benefit from some estimates of total Corg storage in Zostera meadows within each site, and maybe within Europe if the authors could argue that the sites sampled are representative of Zostera meadows elsewhere, so they captured the range of variability. Comparisons with Corg storage (and sediment characteristic) in other Zostera (USA) and other plants could also add interesting information to the manuscript.

Reply: Thank you for this suggestion. For comparison, we have done a literature survey compiling sediment carbon and organic matter data for *Z. marina* in Europe and elsewhere in the temperate zone, and putting our data in context with previous studies (making it clear that our data capture the range of variability and are similar to what has previously been seen in the same areas). We have addressed this in the discussion and the comparison of data has been added as a table (table 5).

Particular comments: L74: 'coarser' instead of 'courser'

Reply: This have been corrected.

L124-125: 'Total nitrogen (NT) was measured due to possible alteration of the nitrogen values when treated with HCl (Harris et al., 2001)' This sentence does not make sense,

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[Discussion paper](#)



please clarify.

Reply: We agree that this sentence is not clear and have rephrased it. The new sentence is written as “Total nitrogen (NT) derived from untreated sediment samples was used for estimate of the nitrogen content due to possible alteration of the nitrogen values when treated with HCl (Harris et al., 2001).”

L137-138: Please specify how the samples were washed to remove H₂O₂ and whether this could affect the grain size composition (wet sieving or decanting the supernatant?).

Reply: After the samples were treated with H₂O₂ they were centrifuged in 4500 rpm for a minimum 20 minutes and then the supernatant was carefully removed using a pipette. Distilled water was then added and the samples were centrifuged once more and supernatant was again removed. This has been clarified in the manuscript.

L149-150: provide the equations and explain the potential issues linked to the use of logarithmic regressions to extrapolate corg stocks: could led to over-/under-estimates of Corg stocks compared to cores long enough to estimate the stocks directly? Why not linear regression for example?

Reply: We have added the equations for %Corg and g Corg cm⁻² in the method section for the two sites at Askö which lacked some depth sections down to 25 cm (L181-182). In general, carbon content decreases logarithmically with depth (Fourqurean et al. 2012) due to higher degradation and remineralization in the upper sediment layers (Burdige, 2007; Henrichs, 1992), which we have written in the methods (L182-184). When examining the sediment depth profile taken at Askö (Fig. 3), we noticed that the decrease in carbon content was not linear.

L152-L157: The use of General Linear Mixed Models is a more appropriate and robust analysis, including bare/zostera, etc. as fixed factors in the design, and study site as a random factor. Sediment depth also needs to be included as fixed factors because sediment depth is not independent. Test %Corg, gCorg cm⁻³ and grain-size,

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separately.

Reply: We thank the referee for this suggested analysis. We have done a general linear mixed model as suggested using %Corg, g Corg cm⁻² and grain size as response variables and including sediment depth and habitat as fixed factors, and sites as a random factor. The new result has been added to the result section (L190-197, 216-221) and the statistical section in methods has been rewritten accordingly (L167-170). Table 2 has been changed and is now showing the outcome of this analysis.

L182: replace 'unaffected' by 'constant'

Reply: This sentence has been removed due to the new results from the glmm.

L228-230: Here and in the abstract there is a misleading message: high sediment porosity and low sediment density is the result of high Corg content, not vice-versa, unless you merge the concept of grain-size.

Reply: We apologize for this. We have rewritten the sentence in the abstract as well as on L247-249.

L230-231: Misleading message: as per the comment above, but also consider that fine and Corg-rich bare sediment could also constitute high natural carbon sinks. Therefore, the clue here is to assess seagrass inputs into the sedimentary Corg pool, rather than the Corg sink associated to the natural depositional environment. Data on e.g. stable C and N isotopes of the organic matter that could provide an indication of the origin of the Corg stores within and among sites is missing, which largely constrains the conclusions derived from this study (see e.g. Serrano et al. 2016 Biogeosciences discussions_mud vs seagrass Corg for further details on the topic).

Reply: We have rewritten part of the discussion and added more details on other factors influencing carbon storage as well as more in depth discussed the conclusion made in Serrano et al. 2016.

L249: the age of the sediments and associated sediment accumulation rates could

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also explain the differences found. Are we comparing 25 cm-thick and 500 yr-old with 25 cm-thick and 1000 yr old Corg stocks? Discuss the implications

Reply: We have added this to the discussion.

L259: reference submitted is missing in the list.

Reply: The reference has been added to the reference list.

L254-256: This statement is misleading: from both studies the main message derived is that sediment grain-size has always an influence, but the amount of seagrass-detritus inputs into the sediment makes the difference breaking the linear relationship expected between fine sediments and bulk Corg content. Read the references cited carefully and clarify.

Reply: This statement has been rewritten and corrected. However, in terms of the Samper-Villareal et al. 2015 paper they state that no significant relationship between carbon content and fine sediment fraction was found as they write "...there was only a slight tendency for carbon content to be higher with higher fine sediment fraction (mud) in seagrass sediments ($p = 0.10$, Table 3). Furthermore, when unvegetated sites were removed from the analysis there was no relationship between mud and carbon content ($x^2 = 0.2$, $df = 1$, $p = 0.07$)".

L257-267: This section of the discussion is poor: (i) the relationships between Corg, porosity and density have poor relevance (well know in soil science), in particular if they have not been corrected for compression during coring; (ii) cut this section to provide the main message: Corg content in *Zostera* meadows at Asko is lower due to the coarser sediments in which they inhabit.

Reply: We have cut down this section.

L277: 'was' instead of 'were'

Reply: This has been corrected.

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[Discussion paper](#)



L285: add a comma

Reply: This has been corrected.

- The water depth gradient within and among sites is small, with differing tide regimes, etc. Is there any relationship between meadow structure and water depth that could provide more light into the impact of depth between sites?

Reply: We have examined and tested the relationship between meadow structure (shoot height, shoot density and seagrass cover) and water depth without seeing any clear patterns. There was a tendency for higher shoot length with depth but with a low R^2 -value (0.16).

Table 2: add the results of the ANOVA itself and some descriptives such as df and F.

Reply: This table has been replaced with the outcome of the general linear mixed model (see above comment).

Figure 3: remove negative values from the x-axis (set to zero), and place the graphs of *Zostera* and ungetated in the same plot for each site, reducing figure size and allowing to detect difference visually (the scale of the x-axis differ within and among sites, making interpretation difficult).

Reply: We have changed the figure and included *Zostera* meadows and unvegetated areas within each area in the same figure. We are, however, not using the same scale on the x-axes as the variation is too large for a clear visualization of the differences between *Zostera* meadows and unvegetated sediment within each area.

Table 4: include data on %Corg and Corg density in this table.

Reply: We have included %Corg and g Corg cm⁻² in the table and rewritten the result section to not repeating the information in the table, L195-196.

Figure 4. Define Bg and Ag in the caption

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[Discussion paper](#)



Reply: We have defined belowground (roots and rhizomes) and aboveground (shoots) biomass in the figure text.

Please also note the supplement to this comment:

<http://www.biogeosciences-discuss.net/bg-2016-137/bg-2016-137-AC1-supplement.pdf>

Interactive comment on Biogeosciences Discuss., doi:10.5194/bg-2016-137, 2016.

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