

Interactive comment on “Key biogeochemical factors affecting soil carbon storage in *Posidonia meadows*” by O. Serrano et al.

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Response to Reviewers for “Key biogeochemical factors affecting soil carbon storage in *Posidonia meadows*” by Serrano et al. We would like to thank the Reviewers for their efforts and comments, which have the potential to improve the manuscript. Please find below a detailed response to each of the issues raised. We are looking forward to improve our manuscript based on Editor’s considerations.

Anonymous Referee #1 The manuscript by Serrano et al. describes the trends in organic carbon stocks, burial rates, and origin across a depth gradient in a seagrass (*Posidonia sinuosa*) bed. Overall, it is a well presented and concise study with a clear focus and I agree with the general interpretation of the data. The main message of

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the manuscript is that organic carbon stocks and burial rates vary across the depth gradient in a systematic matter (and hints at underlying mechanisms, such as productivity/density and sediment grain size) – and points out that such variability should ideally be taken into account when upscaling large datasets on OC stocks or burial in seagrass beds to global levels; while the data from this study are insufficient to allow us to do so, it is a valuable point that might stimulate further work in this direction. I have provided a list of minor comments and suggestions below – these mainly relate to some aspects of presenting the data, and I would recommend to include a discussion of how certain analytical aspects might have an influence on some of the data. The novelty of the data compared to earlier work by some of the authors (Serrano et al 2014, Global Biogeochemical Cycles) should also be stressed.

1. A question of semantics – but the manuscript uses a combination of “soils” and “sediments” to refer to the substrate in these seagrass beds or to processes (e.g., sediment accumulation). Considering these are subtidal marine ecosystems, I would be strongly in favour of using the term “sediments” consistently – while it may be a case of preference I feel the use of “sediments” is much more widely accepted in the seagrass/marine community, and in any case there should be consistency throughout the manuscript.

Response comment 1: The definition of the substrate where seagrasses grow is a hot topic among scientists. Despite marine ecologists broadly refer to seagrass sediments, thus it is not necessarily correct. Serrano et al (2012) attempt to classify Posidonia substrates using existing keys of soil taxonomy, and conclude that seagrass substrates meet perfectly the requirements for sediment to be considered a soil. They classify shallow substrates in which Posidonia meadows grow as Limnic Subaquatic Histosols (Calcaric, Eutric). More recently, Kristensen & Rabenhorst (2015) addressed this debate, and concluded that both soil and sediment terms could be used depending on the context. Although further research would be needed for a more robust characterization of seagrass subaqueous soils, we believe that they should be termed soils (an

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extensive discussion on this topic can be found in Serrano et al. (2012)). Therefore, in the manuscript we referred to seagrass substrates as ‘soils’ and bare sediments as ‘sediments’. For the definition of processes and grain-size studied in the cores, we referred to sediment accumulation rates (we would like to change this term to soil accumulation rates to keep consistency throughout the manuscript) and sediment grain-size (we consider that this term was correctly used). One sentence could be added in the introduction to highlight this topic (i.e. definition of seagrass substrates as soils or sediments) to conclude that *Posidonia* substrates could be classified as mineral or organic soils. We consider that developing a section on this topic in the discussion is out of scope in this manuscript, but we are currently working on a manuscript classifying the soils found underneath these and other seagrass habitats. References: Serrano, O., Mateo, M. A., Renom P. and Julià R.: Characterization of soils beneath a *Posidonia oceanica* meadow, *Geoderma*, 185-186, 26–36, 2012. Kristensen, E., Rabenhorst, M.C., 2015. Do marine rooted plants grow in sediment or soil? A critical appraisal on definitions, methodology and communication. *Earth-Science Reviews* 145, 1-8.

2. Reference is made to plant biomass and productivity data at the same site – while I have not checked if the actual depths of the individual sampling sites match, it would be good to make more direct use of these data to support some of the conclusions summarized in Figure 6.

Response comment 2: Collier et al. (2007 and 2008) showed significant variation in plant biomass and productivity, water quality and sediment biogeochemistry parameters across the same depth gradient, matching the depths of coring sites in this study. Adjustments to the discussion will be made to include more detailed comparisons with data reported by Collier et al. (2007 and 2008), after Editor’s considerations. References: Collier, C.J., Lavery, P. S., Masini, R. and Ralph, P.: Morphological, growth and meadow characteristics of the seagrass *Posidonia sinuosa* along a depth-related gradient of light availability, *Mar. Ecol. Prog. Ser.*, 337, 103–115, doi:10.3354/meps337103, 2007. Collier, C.J., Lavery, P. S., Masini, R.J. and Ralph, P.: Physiological characteris-

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tics of the seagrass *Posidonia sinuosa* along a depth-related gradient of light availability. *Mar. Ecol. Prog. Ser.*, 353, 65-79, 2008

3. The acidification procedure deserves some discussion, as the procedure used may result in partial loss of soluble organic C due to the centrifugation and rinsing steps. There is quite a bit of literature discussing/comparing different acidification methods for sediments (fumigation versus in situ acidification in silver cups versus acid treatment + rinsing) and it would be good to at least refer to this and caution that %OC data might be a slight underestimate.

Response comment 3: We agree that the pretreatment procedures used to remove inorganic carbon before organic carbon analysis could lead to an underestimation of organic carbon contents. To reduce the loss of soluble organic carbon we only rinsed the samples once. To our best knowledge, the method used in our study is the best known and commonly used, despite its limitations. Acid-fumigation was not used based on previous experiences, i.e. incomplete digestion of carbonates in samples with 80+% carbonate content. All above plus pertinent references to support statements could be included in the methods section of the final paper, after Editor's considerations.

4. Page 18920, line 9-10: "were they were found": were found

Response comment 4: We will correct this item as suggested.

5. The differences/similarities with a similar study at the same site (Serrano et al. 2014, GBC) should be clarified. They are from the same depth gradient – but are they different sites, different sampling periods? This should be mentioned explicitly. Also, differences in some of the results should be mentioned, e.g. the OC accumulation rates appear to be much higher in the current ms for the 2 and 4 meter depth sites than in the Serrano et al. (2014) paper – these are aspects that need to be elaborated on.

Response comment 5: This manuscript is based on the same cores studied in Serrano et al. (2014), but new variables were analyzed in these cores (i.e. 210Pb dating, sedi-

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ment grain-size, stable carbon isotopes in organic matter) to provide new insights into the factors driving differences in organic carbon storage along a depth gradient. Indeed, we studied all variables explored in a new core sampled in bare sediment within the area of study. Differences in organic carbon stocks and accumulation rates between this and the previous study (Serrano et al. 2014) are related to new age-depth models obtained in the cores (i.e. based on ^{210}Pb dating). Indeed, in this manuscript we argue that in order to assess differences and compare organic carbon storage between meadows it is necessary to normalize organic carbon stocks by a period of accumulation, rather than soil depth as commonly used. Therefore, we present the results and develop the discussion accordingly to the period of accumulation (^{210}Pb -derived, short-term, last 100 years; and ^{14}C -derived, long-term, last 500 years). Serrano et al. (2014) estimated organic carbon storage based on soil thickness, but we consider that this approach could be misleading. In summary, we argue that addressing differences in organic carbon storage among habitats should be based on the period of accumulation rather than substrate thickness, and indeed, it is very important to clearly state the period of accumulation to which the estimates refer (i.e. the larger estimates of organic carbon storage over 100 years compared to 500 years are related to the decomposition of organic carbon with ageing). Although this topic was briefly addressed in the manuscript, but we would like to discuss it further and clarify the differences between this and the previous study (Serrano et al. 2014) in the final manuscript, after Editor's considerations. References: Serrano, O., Lavery, P. S., Rozaimi, M. and Mateo, M. A.: Influence of water depth on the carbon sequestration capacity of seagrasses, *Global Biogeochem. Cycles*, 301–314, doi:10.1002/2014GB004872. Received, 2014.

6. Tables 1 and 3: report $\delta^{13}\text{C}$ data with one decimal only, given the analytical uncertainty on measurements.

Response comment 6: We will include the analytical uncertainty in the methods section and limit $\delta^{13}\text{C}$ data to one decimal as suggested.

Anonymous Referee #2 This study describes variable organic carbon preservation

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rates and stocks across a depth gradient in a *Posidonia sinuosa* meadow in Western Australia. The study is a timely addition to the literature on blue carbon, and adds a much needed element of functional understanding to the issue of carbon preservation in seagrass meadows. The manuscript is well written and the methods employed are robust. More detailed comments on the manuscript are given below.

1. Depth profiles. Am I right in assuming that data reported for sediment properties and Corg are depth integrated values for each site? (maybe this needs better explaining in the methods). If this is so, then it changes interpretations of these contributions across the water depth gradient. I am curious to know whether the contributions of seston and seagrass varied with depth in the sediment profiles. I would suspect that the relative contribution of seston would decrease with depth in the sediment as it is generally more labile than seagrass detritus. The result of more seston detritus across the water depth gradient is generally consistent with our observations, and most likely relates to reductions in bed stress with depth.

Response comment 1: The data reported in Table 1 correspond to average \pm SE values normalized for ca. 500 years old deposits. In Table 3 we reported averaged data for short-term (100 years) and long-term (500 years) periods. Table 4a reports averaged data on d13C signatures of living material analyzed in this study (at each of the four depths studied) plus 'seston' values from the literature. In Figure B (Supporting Information) we presented the trends with age (i.e. depth in the substrate) of the variables studied, including d13C. Individual mixing models to determine the contribution of potential organic carbon sources into seagrass soils were run for each core (i.e. over 500 years of accumulation) to deliver average contributions over the period reconstructed. However, we did not run multiple mixing models at each soil depth within each core. We will clarify all above in the methods as suggested. Figure B (Supplementary information) provides an idea of the changes in d13C signatures with depth over 500 years of accumulation in each core studied, but overall show clear differences in d13C signatures among cores. Although it would be possible to run multiple mixed models

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for each depth (cm) within each core (or for 100 years and 500 years of accumulation) to determine the percentage contribution of autochthonous (plant detritus) and allochthonous (seston, algae+epiphytes) organic carbon sources into the soil organic carbon pool over different time scales, thereby providing some insights on the relative preservation of autochthonous vs. allochthonous sources, we dismissed this option mainly because of the assumptions involved with this approach and its complexity (e.g. lack of fractionation of $\delta^{13}\text{C}$ signatures during diagenesis, impact of European settlement on organic matter inputs, etc.). Despite we agree with the hypothesis raised by Referee #2 in regards to the likelihood of rapid decomposition of allochthonous organic matter compared to the more recalcitrant detritus of seagrass, we consider that addressing this hypothesis is very complex and not possible in this study due to the large assumptions involved. The results obtained (shown in Table 4a) are consistent with previous observations made by referee #2 in the field, i.e. increase in the contribution of seston detritus across the water depth gradient. However, the $\delta^{13}\text{C}$ tends with depth seem to indicate that the contribution of seston increased with depth in the sediment, contrary to what one would expect. We would like to mention the assumptions linked to our conclusions.

2. Comparison with one bare sediment "control". OK, I'm a biogeochemist and am not too picky about ecologist-style statistical designs, but one core taken from one bare sand site 2km away? Can the authors at least provide some justification why this is adequate (e.g. can they confirm that there is absolutely no variation in sediment properties according to depth or location).

Response comment 2: It was difficult or impossible to find a 'pure control' (as per ecological research) for this study. Shallow unconsolidated substrates should be occupied by seagrasses unless there is a biogeochemical reason(s) that precludes their settlement. The reasons could range from anthropogenic disturbance to hydrodynamic energy. In our case, the control site was chosen based on: - Early seagrass mapping showing no presence of seagrass in this area at least since 1960s - Similar water depth

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(4 m) - Small likelihood of seagrass detritus from surrounding meadows being exported and accumulated in the area based on hydrodynamic knowledge in the area. Despite the factors considered above, the site chosen can't still be considered a 'pure control' for comparisons, but a reference core for comparison. For instance, the inclusion of this bare core strengthened our conclusions related to the importance of grain-size (i.e. fine sediments) and seagrass inputs (based on $\delta^{13}\text{C}$ values) on organic carbon storage. Adjustments to the manuscript will be made to highlight the limitations stated above, after Editor's considerations.

3. Biogeochemical factors. The manuscript has one stated aim to "highlight key biogeochemical factors affecting Corg storage in seagrass soils that need to be accounted for when attempting to produce regional or global estimates of Corg storage in seagrass meadows". Unfortunately, there are no real measures of indicators of these factors made, and the discussion around potential factors is sometimes fairly vague (e.g. page 18925 lines 25 – 30).

Response comment 3: The relative importance of the biogeochemical factors identified in this study (i.e. hydrodynamic energy, sediment accumulation rates, fine sediment content, water depth, seagrass net primary production and density) in driving OC storage was not addressed in our study, but rather we discussed the reasons why they can play a role in driving organic carbon storage and highlight potential interactions among them. Understanding the factors controlling Corg storage in seagrasses is at its onset, and a much better understanding (i.e. field and lab detailed studies addressing each factor) are required before being able to disentangle the relative role/importance of each factor identified and synergistic and/or antagonistic interactions among them. We will clarify this question in the discussion.

4. Morphological factors. I feel it is a shame that the authors didn't measure any morphological attributes of the seagrass across the depth gradient, since much is made about the effect of these attributes in both trapping seston and contributing to the Corg pool. I understand that the authors refer to previous work at the site by C. Collier, but

maybe it would be useful to reproduce a more detailed summary of seagrass morphology from this work than what is provided (e.g. page 18925 lines 4 - 5). This would make it much easier to relate the results of this study to other systems and seagrass species around the world.

Response comment 4: Adjustments to the discussion will be made to include more detailed comparisons with data reported by Collier et al. (2007 and 2008), after Editor's considerations. However, it is important to consider the temporal scale of organic carbon accumulation and the dynamics of seagrass meadows, in particular for seagrass species other than *Posidonia*. Seagrass meadow structure (e.g. density, cover, biomass) and even presence/absence can vary over seasonal, annual and decadal time scales. Therefore, assuming that punctual measurements of meadow structure are representative of ca. the characteristics of the study site over ca. 500 years could lead to misleading interpretations. This is particularly true for studies linking meadow structure with organic carbon storage over large areas (e.g. lacking environmental gradients sustained over the period reconstructed) for short-lived and highly dynamic meadows such as those formed by genera *Zostera*, *Halophila* and *Halodule*. However, in our study the presence of a clear and stable environmental gradient (i.e. depth) over the last millennia (Skene et al. 2005), together with the presence of seagrass remains along the cores studied, provide further evidence supporting that the detailed study of meadow structure by Collier et al. (2007 and 2008) linked to irradiance reduction with water depth at our study site is valid for the purposes of this study. References Collier, C.J., Lavery, P. S., Masini, R. and Ralph, P.: Morphological, growth and meadow characteristics of the seagrass *Posidonia sinuosa* along a depth-related gradient of light availability, *Mar. Ecol. Prog. Ser.*, 337, 103–115, doi:10.3354/meps337103, 2007. Collier, C.J., Lavery, P. S., Masini, R.J. and Ralph, P.: Physiological characteristics of the seagrass *Posidonia sinuosa* along a depth-related gradient of light availability. *Mar. Ecol. Prog. Ser.*, 353, 65-79, 2008 Skene, D., Ryan, D., Brooke, B., Smith, J., Radke, L., 2005. The Geomorphology and Sediments of Cockburn Sound. *Geoscience Australia, Record 2005/10*. 88pp

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5. Wind wave energy and bed shear stress. It would be nice to have some description of the environment with regards bed shear stress. I notice that the conceptual model (Fig 6) suggests that hydrodynamic energy increases with water depth. Is this due to tidal currents? Probably best to define what is meant by "hydrodynamic energy", and if wave energy is not important explain why. Generally I would expect much higher bed shear stress at shallow depth due to wind wave action. At least part of the seagrass morphology (e.g. below ground biomass) is likely to be significantly influenced by this bed stress gradient, which presumably has implications for the results of this study. I think this issue needs more comprehensive treatment, given that physical energy is one of the three factors considered.

Response comment 5: We agree with the referee that data on bed shear stress could contribute to this study. However, this type of data is not available (i.e. lack of hydrodynamic models in the region) and indeed, in situ measurements would be required considering the short-distance of the depth gradient studied (ca. 200 m) and the limited resolution and uncertainties associated with models. Therefore, obtaining reliable data on bed shear stress would require the deployment of specific equipment over long time periods, and it is out of scope in this manuscript. In our study, we interpreted sediment grain-size along the core as a proxy of hydrodynamic energy over the period reconstructed, which is a complementary proxy of bed shear stress. Indeed, bed shear stress does not reflect the affect of the canopy on hydrodynamic energy, and therefore the sediment grain size within the meadow could provide a better indication of the hydrodynamic energy within the meadow. The referee misinterpreted the diagram in Figure 6, showing a decrease in organic carbon storage linked to an increase in hydrodynamic energy. Overall, it is difficult or impossible to disentangle the individual role of physical energy and light reduction in driving difference in organic carbon storage along a depth gradient in our study. We will provide more details in the caption of the figure to help interpreting the conceptual model.

6. Decay rates. Could other factors such as bed shear stress and bioturbation impact

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on the estimations of decay rates? My guess is yes, so it would be good to see a little more comprehensive discussion of this.

Response comment 6: We agree with the referee and most probably bed shear stress and bioturbation may also play a role in organic carbon storage in seagrass meadows. In our study, we used sediment grain-size as a proxy of hydrodynamic energy and bed shear stress (see above, response to comment 5), however, we did not account for bioturbation. The ^{210}Pb results provided insights into the degree of mixing of the soils, but it is impossible to decipher biological from physical (i.e. hydrodynamic energy) mixing in our study. We could list other potential variables (i.e. not considered in this study) that could influence organic carbon storage thereby providing new insights for future research, after Editor's considerations.

7. Comparison with other studies. I think it would be good to place the results of this study into context with other studies (e.g. seagrass morphometrics, Corg and grain size properties) so that results have a more global relevance.

Response comment 7: Since this manuscript was accepted for publication in Biogeoscience Discussion there have been a few manuscripts published on the topic. Therefore, we are willing to compare the results of our study with new literature in the following terms (after Editor's considerations): - Comparison with *Posidonia* spp only to keep the focus of our manuscript. - Considering the limitations of comparing punctual measurements of seagrass morphometric characteristics with organic carbon storage over centuries (comment 4, Referee #2), constructive comments will be added when comparing to other studies addressing this topic.

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