Author Response to Referee comments to BG Manuscript egusphere-2023-2784

Spatial patterns of Organic Matter content in the surface soil of the salt marshes of the Venice Lagoon (Italy)

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Reply to Referee #2

Legend:

Referee Comment

Author Response

Modified manuscript text

The paper “Spatial patterns of Organic Matter content in the surface soil of the salt marshes of the Venice Lagoon (Italy)” presents an impressive field campaign, measuring key soil variables at several points along a transect perpendicular to the marsh edge, in ten different marshes of the Venice Lagoon. These results will be useful for the broader salt marsh community.

We thank the Reviewer for his/her positive comments on our manuscript and for his/her insightful suggestions that have contributed to improving the quality and the clarity of our manuscript.

A few important comments to be addressed:

1. There is a description of the outliers in the statistical analysis section missing, as they are only thus far mentioned in the figure legends. How did the authors determine outliers? Were these excluded from all statistical analyses?

   We thank the Reviewer for this useful comment. We included all available data in our analysis without conducting a specific search for outliers. The grey circles in Figures 4, 5 and 6 represent indeed all individual data to provide additional information beyond that provided by the boxplot. To avoid any possible misunderstanding, we specified in the Material and methods section (at the end of section 2.3) that all data are included in the statistical analysis, as follows:

   > “For all the statistical tests, we included all available data in the analysis without attempting to identify and eliminate possible outliers.”

2. In the results presented in Figure 5, it is unclear why the authors binned values in values and b, and how this then influenced their statistical tests. I would think that some sort of linear regression would be more appropriate given that both x and y are continuous data. Thus, I am not convinced by the discussion in L348-352

   The choice of presenting binned values in Figures 5a and 5b was intended to facilitate the visualization of trends and patterns, given the intrinsic variability of the processes at hand. It is important to note that, despite the use of binned values in the visualization, all the data were included in the statistical tests that support the discussion at line 348 (Kendall’s tau test for Shannon Diversity Index H and OC stock in top 20 cm: $\tau = 0.1931$ p-value = 0.0300; with mean SCD in top 20 cm $\tau = 0.1818$ p-value = 0.0412). In consideration of the Reviewer’s comment, we added linear regressions to the plots. Below is Figure 5, revised according to the suggestions of both Reviewers.

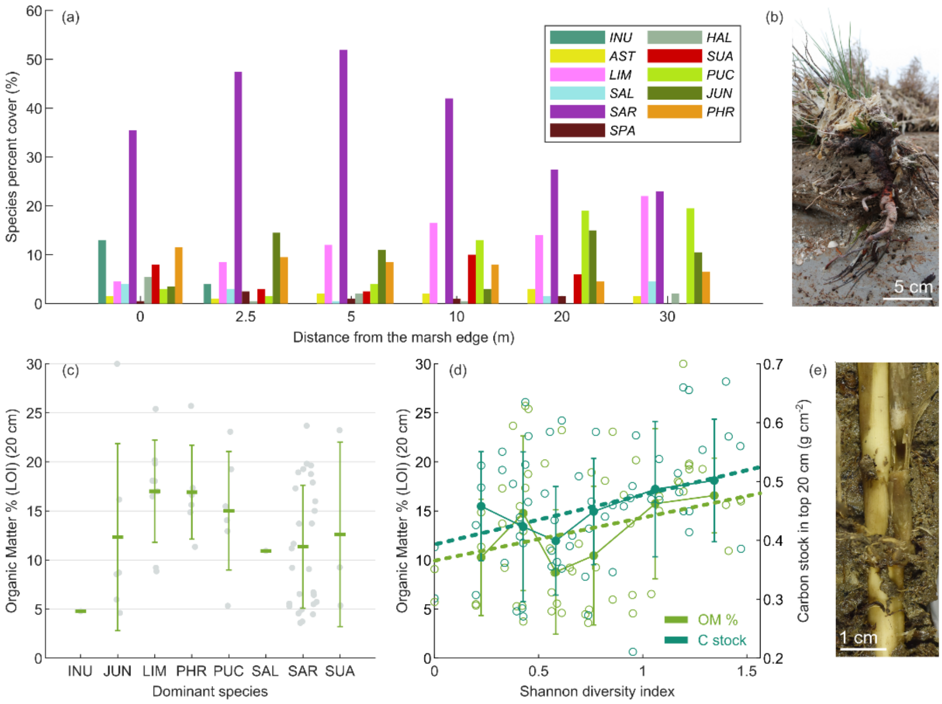

Figure 1. Vegetation distribution and influence on SOM. Species cumulative percent cover at each station along the transect (a) Organic matter content in marsh surface soils (LOI % - mean value in top 20 cm) as a function of dominant species (c) (INU = Inula crithmoides, JUN = Juncus maritimus, LIM = Limonium narbonense, PHR = Phragmites australis, PUC = Puccinellia palustris, SAL = Salicornia veneta, SAR = Sarcocornia fruticosa, SUA = Suaeda maritima). Organic matter content in marsh surface soils (LOI % - mean value in top 20 cm) and OC stock in top 20 cm as a function of Shannon Diversity Index H (d) in 1x1 m plot around core sites. *Limonium narbonense* roots exposed by marsh edge erosion at SE site (b) and *Phragmites australis* roots and stems within PA site soil (e). Panel (c) includes mean values (horizontal-line markers) and standard deviations. Swarm plots show single values (grey closed circles). Panel (d) includes median and standard deviation of binned values. Open circles represent single values and bold lines represent their linear regressions ($OM = 9.91 + H4.43$, $R^2 = 0.0679$, p-value = 0.0444; $C\ stock\ (20\ cm) = 0.39 + H0.08$, $R^2 = 0.0982$, p-value = 0.0147).

The main conclusion from the authors is about the two scales of variation in sedimentary OM content in salt marsh soils. The current version of the discussion lacks the structure for the reader to understand where this conclusion is coming from. One option would be to separate the discussion into two sections based on the marsh scale variation and the basin scale variation, instead of splitting it by variable measured as it is currently. Acknowledging that this would be a lot of work, another option could be to more clearly discuss the two scales of variation in the current discussion to make it evident to the reader the main important results and conclusions of this work.

We thank the Reviewer for his/her suggestion that greatly contributed to improving the discussion of our manuscript. Following the Reviewer’s comment, we rewrote the Discussion to highlight the two scales of OM variation and make this evident to the reader. The revised text reads as follows:

The sources of OM content in salt marsh soils are influenced both by local and non-local processes. Firstly, OM is the result of in-situ production of belowground (root, rhizome and tuber tissue) (Craft et al., 1993; Rybczyk et al., 2002) and aboveground biomass, thus directly depending on the local primary production. OM content is also affected by the accumulation of organic material produced in other sites (Nyman et al., 2006, Mudd et al., 2009, Ewer Lewis 2019, Mueller 2019), which are transported and eventually is deposited on the marsh surface by hydrodynamic processes (i.e. tides and waves) acting at larger spatial scales . Both autochthonous and allochthonous organic materials, once part of marsh soil, are also affected by decomposition resulting from local topographic, sedimentological and environmental conditions (Chen et al., 2016). Due to the intricate interaction of these local and non-local dynamics, two main spatial scales in OM variations can be identified: the marsh scale (meters to tens of meters) and the system scale (ranging from kilometers, encompassing the entire lagoon or estuary). In the following discussion, we will first examine evidence of OM variations in our results at the marsh scale by considering trends along the transects. Subsequently, we will shift our focus to the system scale by comparing results among study sites.

At the marsh scale, our results show that OM content in surface soils displays a significant trend with the distance from the marsh edge, with OM content generally increasing towards the inner marsh (Figure 4a), consistently with previous findings (e.g. Chen et al., 2016; Leonard et al., 2002; Roner et al., 2016). This overall trend in OM with distance from the edge is influenced by various processes acting at the marsh scale, among which the interaction between sediment delivery and local topography plays a preeminent role. Suspended material is primarily delivered onto the marsh platform through inundation by overbank flow along tidal channels (Bayliss-Smith et al., 1979) and by apical flow at creek heads (Torres and Styles, 2007). As soon as the flow reaches the vegetated marsh platform, current velocities and turbulent energy rapidly decrease (D’Alpaos et al., 2007; Mudd et al., 2010), thus promoting the deposition of more abundant sediments during the flooding initial phase in close proximity of the marsh edge (Christiansen et al., 2000; Roner et al., 2016). As a result of the larger deposition close to the edge, inner marsh generally present slightly lower elevation than that of the marsh margin (Figures 2 and 3). Lower elevations promote the persistence of an anaerobic environment, which slows down OM decomposition by reducing microbial respiration (Halupa and Howes, 1995; Kirwan et al., 2013; Puppin et al., 2023a; Roner et al., 2016).

Progressive energy dissipation over the vegetated marsh platform also promotes selective material settling. Coarser, denser inorganic sediment is mainly deposited near the marsh edge, while the inner marsh receives a higher proportion of finer sediments, as supported by the observed grain size distribution along the transect (Figure 4c), and less dense organic material (Leonard et al., 2002; Miller et al., 2022). In addition to the already larger proportion of organic material settling in the inner marsh, the supply of finer inorganic sediment (Figure 4c) may also promote conditions favourable for OM preservation. This can occur due to the reduced oxygen exchange resulting from the lower porosity and drainage capacity of finer sediments, as well as their greater potential for protecting C from decay through organic-mineral interactions and the formation of micro- or macro-aggregates (Kelleway et al., 2016).

Considering all available data along transects allows us to capture global trends and average out variabilities related to local conditions. However, analysing site-specific trends can offer an interesting perspective on driver locally affecting OM dynamics. Overall, seven out of ten analysed transects show an increasing trend of OM with the distance from the marsh edge (CA, PA, SF, SE, CV, CO, VB). At the SA, MI and FO study sites the trend in OM content deviates from the average behaviour and this can be attributed to the effects of local variability. At the SA site, observed OM content is very low (Figure 3n), and its distribution pattern may be masked by the intrinsic variability of the measurements. At the MI site, *Phragmites australis* grows on the marsh edge, providing a contribution of OM that outcompetes that provided by the halophytic vegetation of the inner marsh (Figure S1 in the Supplement). At the FO site, we observed abundant beach-cast seagrass wracks on the marsh edge, which are likely transported from the extensive seagrass meadows located on the tidal flats adjacent to the FO area (Figure 1f). These wracks may serve as an additional source of OM, locally influencing OM trend with the distance from the marsh margin.

Conversely, in some transects where the organic matter trend aligns with the average increasing trend with distance from the marsh margin, we observed particular cases in the behaviour of grain size and topographic variables, which occasionally deviate from the average trend. For example, at the inner end of the SE transect, we can observe an unexpected, slight increase both in sediment grain size (Figure 2l) and in topographic elevation (Figure 2m). This may be related to the presence of a tidal flat at the inner border of the SE marsh (Figure 1j), which can represent an additional source of sediment supply. Two notable exceptions in terms of marsh topography are also represented by the CA and CO marshes, which face a tidal flat (Figure 1b,h) and are exposed to energetic wind waves. As a consequence of the influence of wind waves, the elevation profiles exhibit a subtly convex shape, with the disappearance of the raised margin and a slight inward shift (i.e. between 5 and 10 m from the edge) of the maximum elevation along with the locations of higher median grain size values. However, these variations do not appear to affect the overall increasing trend of OM content towards the inner marsh at SE, CA and CO sites.

We observe variations and patterns of OM also at the system scale. The position within the gradient generated by marine and fluvial influence was previously observed to be a key predictor of OM content (e.g. Van de Broek et al., 2016; Ewers Lewis et al., 2020; Kelleway et al., 2016; Macreadie et al., 2017). At the lagoon scale, our results show lower mean OM content in surface soil in areas which are directly affected by marine influence, being closer to the inlets or along the main channels branching from them (CO, SE, SF, SA) (Figure 1 and Figure 6a). Conversely, higher OM contents were observed at sites closer to the mainland, e.g. CA, MI and PA. In agreement with this result, a significant negative relationship was observed between OM content and water salinity. However, it is unlikely that salinity as such directly controls soil organic content, as previous observations suggest an inverse relationship between soil salinity and decomposition (Hemminga et al., 1991; Wang et al., 2019), ,. The effect of salinity on decomposition may have been overcome by the co-occurring effects of other factors acting at different positions within the lagoon, such as vegetation characteristics, hydrodynamic conditions, sediment supply, freshwater inputs. OM increase at less saline sites is likely minimally related to the supply of already stabilized organic suspended particles from terrestrial sources as suggested for other study areas (e.g. Van de Broek et al., 2016; Gorham et al., 2021; Omengo et al., 2016; Van de Broek et al., 2018), because in the Venice Lagoon, after historical river diversions, fluvial supply of organic and inorganic material dramatically decreased. However, residual freshwater inputs, especially in terms of groundwater, can still locally reduce salinity levels and, consequently, affect vegetation characteristics, with usually increasing macrophyte biomass at lower salinity values (Hansen et al., 2017; Van de Broek et al., 2016).

A relationship between OM and grain size can also be observed at the lagoon scale. Higher values of median grain size were observed at sites closer to the inlet (i.e., SF, SE) or adjacent to first order channel connected to them (SA) (Figure 6d), whereas higher fractions of fine sediments were observed at the lagoon-mainland boundary, near the Dese River mouth (PA) (Figure 2). This pattern is consistent with the general grain-size gradient observed in the Venice Lagoon, reflecting the typical pattern of decreasing hydrodynamic energy conditions from the inlets to the landward shore (Zonta et al., 2018). Considering the landward decreasing grain-size gradient observed within the lagoon, enhanced C preservation capacity of fine sediments (Kelleway et al., 2016) may have a role in the observed organic content pattern. Furthermore, we may hypothesize that at sites where inorganic sediment inputs are greater, where hydrodynamic energy is higher, soil organic fraction is proportionally lower.

Another potentially important factor controlling OM content is vegetation type, as different species exhibit varying biomass production and decomposition resistance (e.g. Van de Broek et al., 2016; Ewers Lewis et al., 2020; Ford et al., 2019; Saintilan et al., 2013; Yuan et al., 2020).

While our data do not allow for a full statistical analysis of the relationship between OM content and vegetation type, we can derive some interesting qualitative observations. We find no discernible trend in vegetation cover along the transects, nor a significant relationship between OM content and vegetation cover.

Considering the relationship between SOM and dominant species, we observed that higher OM percentages in surface soil are not necessarily associated with dominant species having greater aboveground biomass (as reported in the literature, see Table S4 in the Supplement). We speculate that the lack of a relationship between aboveground biomass and OM may be due to the continuous transport and mixing of locally-produced litter by marsh flooding, weakening the effect of local aboveground biomass production. Moreover,

previous studies indicate a major impact from belowground biomass, which inserting into the sediments directly contributes to OM content (Craft et al., 1993; Rybczyk et al., 2002). The highest mean organic contents were observed in the presence of *Limonium narbonense, Phragmites australis* and *Puccinellia palustris* as dominant species (Figure 1c). *Phragmites australis*, characterized by high aboveground biomass and even higher belowground biomass, forms a dense and deep network of leathery stems, roots, and rhizomes (Figure 1e) (Moore et al., 2012; Scarton et al., 2002). *Limonium narbonense,* despite low aboveground biomass (Table S4 in the Supplement), produces massive woody roots (Figure 1b), and *Puccinellia maritima* creates a dense root mat (Brooks et al., 2021). The belowground biomass of these plants may importantly contribute to SOM content quantitatively and qualitatively, as belowground litter decomposition was observed to decline with increasing lignin content (Stagg et al., 2018; Puppin et al., 2023). Interestingly, these species are more abundant in locations associated with higher values of SOM, such as the inner marsh for *Limonium narbonense* and *Puccinellia palustris,* and brackish areas for *Phragmites australis* (Figure 1a and Figure S1 in the Supplement). However, it was not feasible to directly measure or estimate the relationship between aboveground and belowground biomass and their effect on SOM content in this study.
In addition, soil organic content showed a significant positive correlation with vegetation species diversity (Figure 1d), in agreement with Ford et al. (2019). Ford et al. (2016) found that plant species richness was one of the most important explanatory variables of root biomass and Xu et al. (2020) suggested that species richness may increase biomass productivity due to multiple mechanisms including competition reduction, niche complementarity, selection effects, and biotic and abiotic facilitation.

Overall, the interplay of these dynamics at both marsh and system scales also impacts sediment bulk density. We observed higher soil densities along marsh edges and at sites such as CO, SE, SF, and SA, where organic content is generally lower and coarser sediments predominate (Figure 4b and Figure 6b). This observation aligns with previous research indicating that soil density is influenced by both organic matter content and grain size. Indeed, several studies have reported a significant and negative correlation between SOM and bulk density (e.g. Holmquist et al., 2018; Morris et al., 2016), while sand content has been shown to correlate positively with soil bulk density (Tanveera et al., 2016).
As a consequence of the variability in soil organic content and density both at the marsh and at the system scale, SOC density and C stock show significant variations across and within different salt marshes, enhancing the complexity of blue C assessment. Based on our estimates of mean SOC density in top 20 cm, and considering an expected accretion rate of about 0.3 cm yr^{-1} for salt marshes in equilibrium with Relative Sea Level Rise (RSLR) (Day et al., 1998), the average C accumulation rate for the salt marshes in the Venice Lagoon is estimated to be approximately equal to 69 g C m^{-2} yr^{-1}. Our results are consistent with the mean C accumulation rate in the Australian tidal marshes of 54.52 g C m^{-2} yr^{-1}

> calculated by Macreadie et al. (2017) from 323 soil cores to the depth of 30 cm all around Australia and using a mean accretion rate of 0.21 cm yr^{-1}. However, we observed that, under the same accretion rate, estimated SOC from our study may result in C accumulation rates varying up to 50% from one place to another because of the two-scale variability highlighted by our measurements. This underscores the need for careful consideration of local variability when assessing blue carbon sequestration and storage potential in wetland environments."

Comments applicable throughout the paper:

- Choose between the wording “salt marsh” or “salt-marsh” to be used throughout the manuscript (with a preference for the former)

  We agree and we have chosen the wording “salt marsh” to be used throughout the manuscript.

- When reporting very small p-values, it is preferred to use the format $p < 0.0001$ than to give the exact number

  We thank the Reviewer for his/her suggestion. We have substituted very small p-values in the text with "< 0.0001".

Specific edits:

- L44 insert comma after references Done, thank you.
- L58 helps build (remove “to”) Done, thank you.
- L82-83 needs to be rewritten (not sure what is meant here)

  We modified the text as suggested by the Reviewer. The new text reads as follows:

  > “For instance, elevation and hydroperiod importantly affect vegetation characteristics, organic-matter supply and sediment deposition (Chmura et al., 2003) other than microbial community and organic-matter preservation conditions (Kirwan et al., 2013; Marani et al., 2006; Mudd et al., 2009; Yousefi Lalimi et al., 2018).”

- L160 please add the SOM to SOC conversion equation used Done, thank you.
- Figure 4 legend remove text about outliers plotted individually since it seems none are shown in this figure Done, thank you.
- L297-300 split up the sentence (too long) Done, thank you.
- L 300 move parentheses of reference to (2019) Done, thank you.
- L303 remove “to” from near to the Dese River Done, thank you.

- L314 remove “one” before is associated with Done, thank you.
- L319 remove "a” before considerable variability Done, thank you.
- L326 change marsh to marshes Done, thank you.
- L331 consider changing supply to inputs Done, thank you.
- L337-339 I would refrain from concluding on belowground biomass since it wasn’t studied. Or, make it clearer that it’s a variable that likely has an influence but wasn’t measured in this study
  We appreciate the Reviewer's suggestion regarding the limitations of our conclusions based on our biomass estimation approach. Despite our efforts to provide a biomass estimation that is reliable for drawing general conclusions, we recognise that our estimates can be improved and do not account for intraspecific biomass variability. Furthermore, we recognize that the relative conclusions drawn from the biomass estimation are not central to the manuscript's results. As a result, following the suggestions of both Reviewers, we have decided to remove the biomass estimation, along with the corresponding figure and lines in the Material and methods, Results, and Discussion sections. However, we added a Supplement file to the manuscript, where we included a table with literature data on vegetation species biomass. We believe that this information, which has been enhanced and clearly explained, will support some of the general conclusions related to vegetation characteristics and their influence on soil organic matter.
- L349 add parentheses to (2019) Done, thank you.
- L372 change “being this effect overcome” to “as this effect may have been overcome” Done, thank you.
- L376 define RSLR Done, thank you.
- L 375-376 change order to sentence to “Based on our estimates of mean SOC density in the top 20 cm, and considering an expected accretion rate of about 0.3 cm yr−1 for salt marshes in equilibrium with RSLR...” Done, thank you.
- L379-381 What are the implications of your findings? In consideration of the Reviewer’s comment, we added the following statement:

  > “This underscores the need for careful consideration of local variability when assessing blue carbon sequestration and storage potential in wetland environments.”

- Section 4.3 should be combined with the previous section because it is currently too short. Another option is to restructure the discussion. In consideration of the Reviewer’s comment, we have rewritten the discussion as reported in our reply to the second general comment.
- L398-399 This conclusion doesn’t say much. Either further describe or remove We have rewritten this statement and moved it before the preceding paragraph, which further explains it. The new text reads as follows:

> “The observations on morphological, sedimentological and vegetational features emphasize the dynamic feedbacks between hydrological dynamics, sediment supply, surface elevation and vegetation characteristics and allowed us to relate them to SOM spatial patterns.”

- L401 how does the data constrain model representations? It may be more appropriate to write “inform model representations of SOM accumulation, with the possibility to help improve the ability for biogeomorphological models to describe marsh responses to the effects of climate change and anthropogenic perturbations.” Done, thank you.
- L402-403 how do the analyses further elucidate marsh importance within the global C cycle?
- L403-404 how do your findings inform conservation strategies and restoration interventions? Following the Reviewer’s comments, we have rewritten this paragraph as follows:

> “Moreover, our analyses emphasize the potential complications introduced by local variability in assessing the blue carbon sequestration and storage potential in wetland environments. This underscores the importance of carefully considering both the local variability and the factors influencing it. Finally, our findings may inform conservation strategies and restoration interventions, providing information on conditions promoting OM storage and preservation, such as the maintenance or recovery of freshwater inputs, the supply of finer sediments, and the enhancement of vegetation diversity.”

We thank the Reviewer for his/her comments and suggestions that have contributed to improving the quality and the clarity of our manuscript.

Finally, I strongly recommend adding the following to the published dataset:

- Change depth to two separate columns with an upper and lower depth of each sample
- Add a latitude and longitude measurement for each soil core

We thank the Reviewer for his/her suggestions. We modified the database for publication based on his/her feedback.