Response to the letter from the Editor on "Can mud (silt and clay) concentration be used to 1 predict soil organic carbon content within seagrass ecosystems?" by O. Serrano et al. 2 3 4 O. Serrano et al. 5 o.serranogras@ecu.edu.au 6 7 Letter from the Editor: 8 Dear authors 9 10 Having now read your answers to the reviewer's comments and projected changes to the 11 manuscript, I am happy to encourage you to proceed with the full revision of your manuscript. In 12 addition to all the minor adjustments you have mentioned, I recommend you carefully address in 13 your revised MS the following points raised by referee #1: 14 1. Briefly discuss the potential impact of the selected grain size cut off on your conclusions 15 2. Discuss the difference in correlations in surface and deep soils, including the additional figure 16 3. Improve the quality of the figures, if possible avoiding the "ellipses" or "circles", using rather 17 different symbols and objective criteria for samples with "high/low seagrass inputs", etc... 18 19 20 Looking forward to reading this soon. 21 Best regards, Gwenaël Abril 22 23 Response: 24 25 Dear Dr Gwenaël Abril, 26 27 We would like to thank you for reviewing and handling our manuscript. We carefully addressed 28 29 the three points raised by referee #1 in the new version submitted. All other minor comments raised by the two referees have been considered and included in the revised manuscript as 30

suggested, unless stated otherwise. Please find below a detailed response to the comments raised during the review process:

Main adjustments

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- The potential impact of the selected grain size cut-off used in this study has been discussed toward the end of the manuscript, as suggested.
- discussed toward the end of the manuscript, as suggested.

  Text added in the discussion section (doc with changes tracked; L368- L374): 'Previous studies suggested that the relationship between organic matter and the sediment matrix is best seen with clay-sized fractions (<0.004 mm; Bergamaschi et al. 1997; De Falco et al. 2004). However, the grain size cut-off selected in this study (mud, <0.063 mm) is more representative of the bulk soil and their C<sub>org</sub> content (Pedrosa-Pàmies et al. 2013) and therefore a higher correlation is expected when comparing bulk soil C<sub>org</sub> with a larger and
- more representative fraction of the sediment (i.e. including the silt fraction, 0.004-0.063
- mm, also provides binding sites for C<sub>org</sub>; Burdige, 2007).'
- 45 References cited:
- Bergamaschi, B. A., Tsamakis, E., Keil, R. G. and Eglinton, T. I.: The effect of grain size
- and surface area on organic matter, lignin and carbohydrate concentration, and molecular
- compositions in Peru Margin sediments, Geochemica et Cosmochimica Acta 61:1247-
- 49 1260, 1997.
- 50 Burdige, D. J.: Preservation of Organic Matter in Marine Sediments: Controls,
- Mechanisms, and an Imbalance in Sediment Organic Carbon Budgets?, Chem. Rev., 107,
- 52 467–485, doi:10.1021/cr050347q, 2007.
- 53 De Falco, G., Magni, P., Teräsvuori, L. M. H. and Matteucci, G.: Sediment grain size and
- organic carbon distribution in the Cabras lagoon (Sardinia, Western Mediterranean),
- 55 Chem. Ecol., 20, 367-377, doi:10.1080/02757540310001629189, 2004.
- 56 Pedrosa-Pàmies, R., Sanchez-Vidal, A., Calafat, A., Canals, M. and Durán, R.: Impact of
- 57 storm-induced remobilization on grain size distribution and organic carbon content in
- sediments from the Blanes Canyon area, NW Mediterranean Sea. Prog. Oceanograph.,
- 59 118, 122-136, 2013.

 The relevance of soil depth in explaining the relationships between C<sub>org</sub> and mud contents (and the associated figure) have been included as suggested (LX-LX).

 Text added in the results section (L186- L195): 'The relationships between soil  $C_{org}$  and mud contents within different core depths (from 1 to 10 cm-thick deposits, and from 11 to up to 110 cm-thick deposits) for bare sediments and each group of seagrass species were explored in Figure 2. The  $C_{org}$  content increased with increasing mud content in bare sediments for both 1 to 10 cm-thick ( $R^2 = 0.74$ ) and 11 to 110 cm-thick ( $R^2 = 0.81$ ) soils. When combining mud and  $C_{org}$  contents in seagrass meadows of the genera *Halophila*, *Halodule* and *Zostera*, a higher correlation was found for deeper core sections (11 to 110 cm-thick;  $R^2 = 0.74$ ) compared to top core sections (1 to 10 cm-thick;  $R^2 = 0.17$ ). For combined *Amphibolis* and *Posidonia* species, soil  $C_{org}$  and mud contents were only slightly positively correlated in deeper *Amphibolis* spp sections (11 to 110 cm-thick;  $R^2 = 0.23$ ) and not correlated in *Posidonia* spp meadows (Figure 2).'

<u>Text added in the discussion section (L316-L338): 'The soil Corg</u> content tend to decrease with soil depth and ageing in seagrass ecosystems (e.g. Serrano et al. 2012), thereby the persistence of discrete organic detritus within upper soil horizons could lead to organic matter concentrations above those levels explained by the association with clay and silt particles, as previously demonstrated for terrestrial soils (Mayer and Xing, 2001; Gami et al. 2009). The organic matter preserved in most marine sediments is intimately associated with mineral surfaces (i.e. selective preservation by sorption of organic matter into minerals; Keil et al 1994) and therefore the correlation between soil Corg and mud contents in seagrass meadows could vary as a function of soil depth and ageing. The results obtained show that soil depth is not an important factor when attempting to predict soil  $C_{org}$  content based on mud content in bare sediments (i.e.  $R^2 > 0.74$  for all core depths explored; 1 to 110 cm-thick, 1 to 10 cm-thick, and 11 to 110 cm-thick; Figure 2). However, a clearer pattern appeared when exploring the correlation between soil Corg and mud contents in top 10 cm and within 11-110 cm soil depths of combined Halodule, *Halophila* and *Zostera* species ( $R^2 = 0.17$  and  $R^2 = 0.74$ , respectively). These results suggest that the relatively small belowground biomass of these species (i.e. organic detritus) only has an impact on the expected positive correlation between soil Corg and mud content within the top 10 cm, while the correlation for deeper soil depths (11-110 cm) improved ( $R^2 = 0.74$ ) compared to the whole dataset (1 to 110 cm-thick;  $R^2 = 0.56$ ). For combined *Amphibolis* and *Posidonia* species, the results obtained show that soil depth is not an important factor when attempting to predict soil  $C_{org}$  content based on mud content (i.e.  $R^2 < 0.2$  in all cases; 1 to 110 cm-thick, 1 to 10 cm-thick, and 11 to 110 cm thick; Figure 2). These results suggest that the relatively large belowground biomass of these species (i.e. organic detritus) has an impact on the expected positive correlation between soil  $C_{org}$  and mud content within all depths studied.'

101 Reference cited:

Gami, S. K., Lauren, J. G. and Duxbury, J. M.: Influence of soil texture and cultivation on carbon and nitrogen levels in soils of the eastern Indo-Gangetic plains, Geoderm, 153, 304-311, 2009.

Mayer, L. M. and Xing, B.: Organic matter-surface area relationships in acid soils, Soil Sci. Soc. Am. J., 65, 250-258, 2001.

 We improved the quality of the figures by avoiding the "ellipses", and by using different symbols and objective criteria to identify samples with "high/low seagrass inputs" and "mud-Corg saturation", as suggested (Figures 1 to 3).

• We expanded the discussion on how detritus size could influence decay rates of seagrass detritus. We did not compare saltmarsh and seagrass ecosystems in terms of 1) decay rates dependence on detritus size and 2) relationships between plant size and OC content and d13C signatures. Instead, we used existing literature on seagrass to develop these topics in the discussion. We did not address in more detail how plant size (i.e. seagrass species) could influence OC constant and d13C values based because is not the focus of our article. However, Table 2 provides a comprehensive overview of the differences in OC storage and d13C signatures among seagrass species.

<u>Text added (L308-L315):</u> 'In addition, the larger size of detritus within *Amphibolis* and *Posidonia* meadows compared to *Halophila*, *Halodule* and *Zostera* meadows could also contribute to the larger accumulation of  $C_{org}$  in the former, since decay rates of seagrass

detritus increase with decreasing particle size due to larger surfaces available for microbial attack (Harrison, 1989)'

Reference cited: Harrison, P. G.: Detrital processing in seagrass systems: A review of factors affecting decay rates, remineralization and detritivory. Aquat. Bot., 263-288,

### Minor adjustments

1989.

- Line 160. Insert "variance in" between "the" and "trends". Corrected as suggested.
- Line 169. What is a "poor but slightly significant correlation"? Clarified as suggested.

  Text now reads (L197): 'a poor correlation in estuarine ecosystems'.
  - Line 195. A better way of phrasing this idea would be something like "providing more surface area and hence binding sites for  $C_{org}$  per weight of mineral". Also, I don't understand intent of the phrase "increasing the available. . .for  $C_{org}$  aggregates" in line 196. Clarified as suggested.
    - <u>Text now reads (L224-L228):</u> 'The positive relationship found between mud and  $C_{org}$  contents in coastal bare sediments (explaining 78% of the variability) is in agreement with previous studies (e.g. Bergamaschi et al. 1997; De Falco et al. 2004), and is related to their larger surface areas compared to coarse-grained sediments, providing more binding sites for  $C_{org}$  on the surface of minerals'.
  - The sentence in lines 201-204 is confusing the points above this regression are not well-explained by the regression. Clarified as suggested.
     Text now reads (L238-L241): 'The results obtained showed that bare sediment samples with relative high C<sub>org</sub> contents (i.e. >4% C<sub>org</sub>) and relatively low mud contents were also
    - <sup>13</sup>C-depleted (Figure 1), suggesting significant contributions of soil C<sub>org</sub> from allochthonous sources (e.g. terrestrial and sestonic; Kennedy et al. 2010)'.
    - Line 202. Are the authors claiming that the three data points with del-13C of -25 (Figure 1, upper right plot) are the same as the data points with highest OC in the Figure 1, upper left plot? That could be true for only the sample with 6.6% OC, but the other two points must be close to the regression line. Thus these latter two terrestrially influenced samples are close to saturation i.e. predicted by grain size. Clarified as suggested (see comment above).

- Line 210. "obviating" would be a better word than "ending". Corrected as suggested.
- Line 73 & 76: I would prefer the words "significant relationship" instead "positive relationship". Even if it is true that we logically expect a positive relationship between mud content and  $C_{org}$ , rigorously a strong significant negative relationship could be as useful as a positive one. Corrected as suggested.
- Line 132-134: This sentence is not true for P. oceanica. Table 3 shows that for that species, the C<sub>org</sub> content decreases when the mud content increases. Corrected as suggested.
- Text now reads (L169-L172): 'the  $C_{org}$  content increased with increasing mud content in bare sediments ( $R^2 = 0.78$ ) and at species level, except for *Posidonia oceanica* (i.e.  $C_{org}$  content decreased with increasing mud content;  $R^2 = 0.15$ ) and *Amphibolis griffithii* (i.e. no relationship was found,  $R^2 = 0.05$ ; Table 3)'.
- In Table 2: Amphibolis grifficiae or Amphibolis griffithii? Clarified as suggested: the species is Amphibolis griffithii
- Line 148: the "exponential tendency" for combined Amphibolis spp. is speculative, please rephrase or test non linear relationships. Corrected as suggested.
- 170 <u>Text now reads (L201-L202):</u> 'with a tendency of C<sub>org</sub>-rich soils being enriched in <sup>13</sup>C (Figure 1)'.
  - Lines 176 to 182: This is confusing to me. You say before that fine-grained sediment can bind larger amount of  $C_{org}$ . But the capacity for silt and clay to bind  $C_{org}$  is limited, high mud content in sediments provide reducing conditions that can preserve  $C_{org}$  (lower mineralization rates). Then why this could explain relative high  $C_{org}$  contents for some bare sediments with low mud contents? This mud- $C_{org}$  saturation needs to be clarified (specially for non-specialists as me). Clarified for bare sediments, Posidonia and Amphibolis meadows, and Halodule, Halophila and Zostera meadows.
- Text now reads:

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L236-L241: 'However, the maximum capacity of a given soil to preserve  $C_{org}$  by their association with clay and silt particles is limited (i.e. mud- $C_{org}$  saturation; Hassink, 1997). The results obtained showed that bare sediment samples with relative high  $C_{org}$  contents (i.e. >4%  $C_{org}$ ) and relatively low mud contents were also  $^{13}C$ -depleted (Figure 1),

suggesting significant contributions of soil  $C_{org}$  from allochthonous sources (e.g. terrestrial and sestonic; Kennedy et al. 2010)'.

<u>L268-L274</u>: 'The poor relationship between mud and soil  $C_{org}$  contents in *Amphibolis* soils could be explained by samples with relative high  $C_{org}$  contents (i.e. >2.5%  $C_{org}$ ) and relatively low mud contents, as a result of both the contribution of seagrass-derived  $C_{org}$  (i.e. <sup>13</sup>C-enriched) and  $C_{org}$  from allochthonous sources (i.e. <sup>13</sup>C-depleted; Figure 1). In *Posidonia* soils, the poor relationship between mud and soil  $C_{org}$  contents could be explained by samples with relative high  $C_{org}$  contents (i.e. >10%  $C_{org}$ ) and relatively low mud contents, as a result of the contribution of seagrass-derived  $C_{org}$  (i.e. <sup>13</sup>C-enriched; Figure 1)'.

<u>L280-L287</u>: 'The positive relationship between mud and soil  $C_{org}$  contents in *Halodule, Halophila and Zostera* soils could be explained their relatively high mud content and  $^{13}C_{org}$  depleted  $C_{org}$ , indicating that allochthonous  $C_{org}$  inputs and mud content play a major role in soil  $C_{org}$  accumulation in these opportunistic and early-colonizing seagrasses. However, the relative high  $C_{org}$  contents found with relatively low mud contents (i.e. mud- $C_{org}$  saturation) disrupted the correlation found between soil  $C_{org}$  and mud contents in these meadows ( $C_{org} > 1\%$  in samples with 0-20% mud;  $C_{org} > 2\%$  in samples with 20-70% mud and  $C_{org} > 3.5$  in samples with 70-100% mud; Figure 1)'.

- Table 3: please add in caption what na stands for (not available?). Would ns non significant not be better? Corrected as suggested.
- Figure 1 and 2: I don't see any difference between the red and the red-intermittent circles in the manuscript version I received. Please, verify. Clarified as suggested (see above).
- Figure 2, lower-left graph (Mud content vs C<sub>org</sub> for estuarine ecosystems). There are 4 points showing high C<sub>org</sub> contents (around 6%) for very low mud contents. To which type of ecosystem are they related? P. autralis? These four points belong to estuarine P. australis meadows; three out of the four samples contained large amounts of seagrass-derived C<sub>org</sub> (white circles in the top-right of the lower-right graph in Figure 3).

215	MANUSCRIPT WITH CHANGES TRACKED:

# 218 Can mud (silt and clay) concentration be used to predict soil organic carbon

# 219 content within seagrass ecosystems?

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- 221 Paul York<sup>7</sup>, Andy Steven<sup>8</sup>, Peter Macreadie<sup>9,10</sup>

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### ABSTRACT

The emerging field of blue carbon science is seeking cost-effective ways to estimate the organic carbon content of soils that are bound by coastal vegetated ecosystems. Organic carbon (Corg) content in terrestrial soils and marine sediments has been correlated with mud content (i.e. silt and clay), however, empirical tests of this theory are lacking for coastal vegetated ecosystems. Here, we compiled data (n = 1345) on the relationship between  $C_{org}$  and mud (i.e. silt and clay, particle sizes <63 µm) contents in seagrass ecosystems (79 cores) and adjacent bare sediments (21 cores) to address whether mud can be used to predict soil Corg content. We also combined these data with the  $\delta^{13}$ C signatures of the soil  $C_{org}$  to understand the sources of  $C_{org}$  stores. The results showed that mud is positively correlated with soil C<sub>org</sub> content only when the contribution of seagrass-derived Corg to the sedimentary Corg pool is relatively low, such as in small and fastgrowing meadows of the genera Zostera, Halodule and Halophila, and in bare sediments adjacent to seagrass ecosystems. In large and long-living seagrass meadows of the genera Posidonia and Amphibolis there was a lack of, or poor relationship between mud and soil Corg content, related to a higher contribution of seagrass-derived Corg to the sedimentary Corg pool in these meadows. The relative high soil Corg contents with relatively low mud contents (e.g. mud-Corg saturation) in bare sediments and Zostera, Halodule and Halophila meadows was related to significant allochthonous inputs of terrestrial organic matter, while higher contribution of

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seagrass detritus in *Amphibolis* and *Posidonia* meadows disrupted the correlation expected between soil C<sub>org</sub> and mud contents. This study shows that mud (i.e. silt and clay content) is not a universal proxy for blue carbon content in seagrass ecosystems, and therefore should not be applied generally across all seagrass habitats. Mud content can only be used as a proxy to estimate soil C<sub>org</sub> content for scaling up purposes when opportunistic and/or low biomass seagrass species (i.e. *Zostera*, *Halodule* and *Halophila*) are present (explaining 34 to 91% of variability), and in bare sediments (explaining 78% of the variability).

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# 1. INTRODUCTION

The sedimentary organic carbon (C<sub>org</sub>) stores of seagrass meadows – often referred to as 'blue carbon' – can vary among seagrass species and habitats, with reports of up to 18-fold differences (Lavery et al. 2013). Ambiguity remains in the relative importance of the depositional environment and species characteristics contributing to this variability. Seagrasses occur in a variety of coastal habitats, ranging from highly depositional environments to highly exposed and erosional habitats (Carruthers et al. 2007). Since seagrass species differ in their biomass and canopy structure, and occur in a variety of habitat types, this raises the question of whether mud content can be used to predict C<sub>org</sub> content within coastal sediments, or whether the species composition will significantly influence the soil C<sub>org</sub> stores independently of the geomorphological nature of the habitat.

Geomorphological settings (i.e. topography and hydrology), soil characteristics (e.g. mineralogy and texture) and biological features (e.g. primary production and remineralization rates) control soil C<sub>org</sub> storage in terrestrial ecosystems (Amundson, 2001, De Deyn et al. 2008; Jonsson and Wardle, 2009) and in mangrove and tidal salt marshes (Donato et al. 2011; Adame et al. 2013; Ouyang and Lee, 2014). While it is clear that habitat interactions have a large influence on stores of soil C<sub>org</sub>, our understanding of the factors regulating this influence in seagrass meadows is limited (Nellemann et al. 2009; Duarte et al. 2010; Serrano et al. 2014).

The accumulation of  $C_{org}$  in seagrass meadows results from several processes: accretion (autochthonous plant and epiphyte production, and trapping of allochthonous  $C_{org}$ ; Kennedy et al. 2010), erosion (e.g. export; Romero and Pergent, 1992; Hyndes et al. 2014) and decomposition (Mateo et al. 1997). Previous studies demonstrate that both autochthonous (e.g. plant detritus and epiphytes) and allochthonous (e.g. macroalgae, seston and terrestrial matter) sources contribute

to the  $C_{org}$  pool in seagrass soils (Kennedy et al. 2010; Watanabe and Kuwae, 2015). Plant net primary productivity is a key factor controlling the amount of  $C_{org}$  potentially available for sequestration in seagrass ecosystems (Serrano et al. 2014), but the depositional environment is an important factor controlling  $C_{org}$  storage in coastal habitats (De Falco et al. 2004; Lavery et al. 2013).

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Previous studies have shown a large variation in Corg stores among morphologically different seagrass species (Lavery et al. 2013; Rozaimi et al. 2013). Also, that Corg accumulates more in estuaries compared to coastal ocean environments (estimated at 81 Tg  $C_{org} y^{-1}$  and 45 Tg  $C_{org} y^{-1}$ , respectively; Nellemann et al. 2009). This is due largely to estuaries being highly depositional environments, receiving fine-grained particles from terrestrial and coastal ecosystems which enhance Corg accumulation (i.e. silt and clay sediments retain more Corg compared to sands; Keil and Hedges, 1993; Burdige 2007) and preservation (i.e. reducing redox potentials and remineralization rates; Hedges and Keil, 1995; Dauwe et al. 2001; Burdige, 2007; Pedersen et al. 2011). The inputs of seagrass-derived Corg in the sedimentary pool could break the linear relationship among mud (i.e. silt and clay particles) and Corg contents typically found in terrestrial (Nichols, 1984; McGrath and Zhang, 2003) and marine sedimentary environments (Bergamaschi et al. 1997; De Falco et al. 2004). However, the amount of Corg that can be associated with silt and clay particles is limited (Hassink, 1997), which could lead to a poor relationship between mud and soil Corg contents. Also, other factors found to play a key role in controlling soil Corg accumulation in terrestrial and coastal ecosystems, such as chemical stabilization of organic matter (Percival et al. 1999; Burdige, 2007), carbon in microbial biomass (Sparling, 1992; Danovaro et al. 1995), and soil temperature (Pedersen et al. 2011), could also influence Corg storage in seagrass meadows.

A significant relationship between mud (i.e. silt and clay) and  $C_{org}$  contents would allow mud to be used as a proxy for  $C_{org}$  content, thereby enabling robust scaling up exercises at a low cost as part of blue carbon stock assessments. Furthermore, since most countries have conducted geological surveys within the coastal zone to determine sediment grain size, a strong, positive relationship between mud and  $C_{org}$  contents would allow the development of geomorphology models to predict blue carbon content within seagrass meadows, dramatically improving global estimates of blue carbon storage. The purpose of this study was therefore to test for relationships between  $C_{org}$  and mud contents within seagrass ecosystems and adjacent bare sediments.

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# 2. MATERIAL AND METHODS

Data was compiled from a number of published and unpublished studies from Australia and Spain, in seagrass meadows across diverse habitats (Table 1). The study sites encompass monospecific and/or mixed meadows from a variety of temperate and tropical seagrass species of the genera *Posidonia*, *Amphibolis*, *Zostera*, *Halophila* and *Halodule*, and adjacent bare sediments, while including a variety of depositional environments (from estuarine to exposed coastal areas encompassing different water depths, from intertidal to the deep limit of seagrass distribution; Table 1). Data from 100 cores (79 from seagrass meadows and 21 from bare sediments) on sediment grain size, organic carbon ( $C_{org}$ ) content and stable carbon isotope signatures of the  $C_{org}$  ( $\delta^{13}C$ ) was explored in this study (N = 1345).

Sediment cores were sampled by means of percusion and rotation, or vibrocoring (ranging from 10 to 475 cm long). The core barrels consisted of PVC or aluminium pipes (50 to 90 mm inside diamater) with sharpenned ends to cut fibrous material and minimize core shortening

(compression) during coring (Serrano et al. 2012, 2014). All cores were sealed at both ends, transported vertically to the laboratory and stored at 5°C before processing.

The cores were sliced at regular intervals, each slice/sample was weighed before and after oven drying to constant weight at 70°C (DW), and subsequently sub-divided for analysis. The  $C_{org}$  elemental and isotopic composition of the organic matter was measured in milled subsamples from several slices along the cores. The sediment core sub-samples were acidified with 1 M HCl, centrifuged (3500 RPM; 5 minutes) and the supernatant with acid residues was removed using a pipette, then washed in deionized water, centrifuged again and the supernatant removed. The residual samples were re-dried (70°C) before carbon elemental and isotopic analyses. The samples were encapsulated and the organic carbon elemental and isotopic composition was analyzed using an elemental analyzer interfaced with an isotope ratio mass spectrometer. Percentage  $C_{org}$  was calculated for the bulk (pre-acidified) samples. Carbon isotope ratios are expressed as  $\delta$  values in parts per thousand (‰) relative to VPDB (Vienna Pee Dee Belemnite). For sediment grain size analysis, a Coulter LS230 laser-diffraction particle analyzer was used following digestion of the samples with 10% hydrogen peroxide. The mud content in the sediments (silt and clay, <63  $\mu$ m) was determined, and expressed as a percentage of the bulk sample.

Pearson correlation analysis was used to test for significant relationships among  $C_{org}$  and mud contents, and  $C_{org}$  and  $\delta^{13}C$  signatures. Correlations between the variables studied were tested among seagrass species (9 categories) and bare sediments, seagrass genera (4 categories). habitat geomorphology (coastal and estuarine habitats) and soil depth (in 1 to 10 cm-thick and 11 to 110 cm-thick deposits).

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# 3. RESULTS

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370 The soil organic carbon (Corg) and mud contents varied within the seagrass meadows and 371 bare sediments studied in Australia and Spain. The soil Corg and mud contents were higher in seagrass meadows (average  $\pm$  SE,  $1.5 \pm 0.2\%$  and  $18 \pm 2.4\%$ , respectively) compared to bare 372 sediments ( $0.6 \pm 0.1\%$  and  $10.8 \pm 1$  .2%, respectively; Table 2). On average, seagrass meadows 373 of the genera Amphibolis and Posidonia contained higher soil  $C_{org}$  (1.6 ± 0.1%) and lower mud 374  $(7.2 \pm 0.4)$  than meadows of Halophila, Halodule and Zostera  $(1.2 \pm 0.2\%$  and  $34.9 \pm 5.4\%$ , 375 respectively; Table 2). Overall, carbon isotopic ratios from sedimentary organic matter ( $\delta^{13}$ C) 376 were similar between seagrass soils and bare sediments (-17.6  $\pm$  0.3% and -17.3  $\pm$  0.2%, 377 respectively). The Corg in soils from Posidonia and Amphibolis meadows were 13C-enriched (-378  $15.5 \pm 0.3\%$ ) compared with seagrass soils from *Halophila*, *Halodule* and *Zostera* meadows (-379  $20.7 \pm 0.4\%$ ; Table 2). The C<sub>org</sub> content in soils from estuarine and coastal habitats were similar, 380 while mud content in estuarine sediments was higher and δ<sup>13</sup>C values depleted when compared 381 to coastal habitats (Table 2). 382

The relationships between the variables studied (i.e.  ${}^{\circ}$ C<sub>org</sub>,  ${}^{\circ}$ mud, and  ${}^{\circ}$ C signatures of sedimentary C<sub>org</sub>) among different species and habitat geomorphologies, and among different soil depths were explored in Figures 1 to 3 and Table 3. When accounting for the whole dataset (up to 475 cm long cores), the C<sub>org</sub> content increased with increasing mud content in bare sediments ( $R^2 = 0.78$ ) and at species level, except for *Posidonia oceanica* (i.e. C<sub>org</sub> content decreased with increasing mud content;  $R^2 = 0.15$ ) and *Amphibolis griffithii* (i.e. no relationship was found,  $R^2 = 0.05$ ; Table 3). Although most of the correlations at species level were significant, they only explain 2 to 39% of the variance in trends described, except for *Halophila ovalis* (91%; Table 3). In particular, *Posidonia* meadows (P. australis, P. sinuosa and P. oceanica) had the lower

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correlation values ( $R^2$  ranged from 0.02 to 0.15). When combining mud and  $C_{org}$  contents in seagrass meadows of the colonizing and opportunistic genera *Halophila*, *Halodule* and *Zostera* (Kilminster et al. 2015), a relatively high correlation was found ( $R^2 = 0.56$ ; Figure 1), while soil  $C_{org}$  and mud contents in persistent genera were only slightly positively correlated in combined *Amphibolis* spp and not correlated in *Posidonia* spp meadows (Figure 1).

The relationships between soil  $C_{org}$  and mud contents within different core depths (from 1 to 10 cm-thick deposits, and from 11 to up to 110 cm-thick deposits) for bare sediments and each group of seagrass species were explored in Figure 2. The  $C_{org}$  content increased with increasing mud content in bare sediments for both 1 to 10 cm-thick ( $R^2 = 0.74$ ) and 11 to 110 cm-thick ( $R^2 = 0.81$ ) soils. When combining mud and  $C_{org}$  contents in seagrass meadows of the genera *Halophila*, *Halodule* and *Zostera*, a higher correlation was found for deeper core sections (11 to 110 cm-thick;  $R^2 = 0.74$ ) compared to top core sections (1 to 10 cm-thick;  $R^2 = 0.17$ ). For combined *Amphibolis* and *Posidonia* species, soil  $C_{org}$  and mud contents were only slightly positively correlated in deeper *Amphibolis* spp sections (11 to 110 cm-thick;  $R^2 = 0.23$ ) and not correlated in *Posidonia* spp meadows (Figure 2). The classification of habitats based on geomorphology (i.e. coastal and estuarine) showed a lack of correlation between soil  $C_{org}$  and mud contents in coastal ecosystems, and a poor correlation in estuarine ecosystems ( $R^2 = 0.14$ ; Figure 3 and Table 3).

The relationships between soil  ${}^{6}C_{org}$  and  $\delta^{13}C$  signatures were poor for all individual *Amphibolis* and *Posidonia* species studied (R<sup>2</sup> ranging from 0.09 to 0.3; Table 3), and for combined *Amphibolis* spp (Figure 1), with a tendency of  $C_{org}$ -rich soils being enriched in  ${}^{13}C$  (Figure 1). In contrast,  ${}^{6}C_{org}$  and  $\delta^{13}C$  signatures were not correlated in any of the small and fast-growing *Halodule, Zostera, Halophila* meadows studied (Table 3), neither individually nor

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signatures was also found in bare sediments adjacent to seagrass meadows (Figure 3, and Table 3).

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### 4. DISCUSSION

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Overall mud content is a poor predictor of soil Corg in seagrass meadows and care should be taken in its use as a cost-effective proxy or indicator of Corg for scaling-up purposes in the emerging field of blue carbon science. Although we describe some promise for opportunistic and early colonizing *Halophila*, *Halodule* and *Zostera* meadows (i.e. mud content explained 34 to 91% of variability in Corg content) and in bare sediments adjacent to seagrass meadows (explaining 78% of the variability), mud is not a universal proxy for blue carbon content and therefore should not be applied generally across all seagrass habitats. In particular, mud content only explained 5 to 32% of soil Corg content in Amphibolis spp meadows and 2 to 15% of soil Corg content in Posidonia spp meadows, and therefore, mud content is not a good proxy for blue carbon content in these meadows.

A tenet of carbon cycling within the coastal ocean is that fine-grained sediments (i.e. mud) have higher Corg contents. The positive relationship found between mud and Corg contents in coastal bare sediments (explaining 78% of the variability) is in agreement with previous studies (e.g. Bergamaschi et al. 1997; De Falco et al. 2004), and is related to their larger surface areas compared to coarse-grained sediments, providing more, binding sites for Corg on the surface of minerals (Keil and Hedges, 1993; Mayer, 1994a, 1994b; Galy et al. 2007; Burdige 2007). In addition, the predominance of fine sediments reduces oxygen exchange and results in low sediment redox potentials and remineralization rates, contributing to the preservation of

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sedimentary Corg after burial (Hedges and Keil, 1995; Bergamaschi et al. 1997; Dauwe et al. 452 453 2001; Burdige 2007; Pedersen et al. 2011). However, the maximum capacity of a given soil to 454 preserve C<sub>org</sub> by their association with clay and silt particles is limited (i.e. mud-C<sub>org</sub> saturation; 455 Hassink, 1997), The results obtained showed that bare sediment samples with relative high C<sub>org</sub> contents (i.e. >4% C<sub>org</sub>) and relatively low mud contents were also <sup>13</sup>C-depleted (Figure 1), 456 suggesting significant contributions of soil Corg from allochthonous sources (e.g. terrestrial and 457 sestonic; Kennedy et al. 2010). This could have disrupted the correlation found between soil C<sub>org</sub> 458 459 and mud contents in the bare sediments studied. 460

Mud is not a universal proxy for soil  $C_{org}$  content in seagrass meadows, which could be mainly explained by additional inputs of seagrass-derived  $C_{org}$  and/or allocthonous  $C_{org}$  to the sedimentary  $C_{org}$  pool, obviating the linear relationship between mud and  $C_{org}$  contents found in the absence of vegetation. The  $\delta^{13}C$  values indicated that both seagrass- $C_{org}$  and non-seagrass-derived  $C_{org}$  (i.e. epiphytes, algae, seston or terrestrial matter) were buried in the soils of all studied meadows, but are consistent with a model of increasing capture of seagrass-derived  $C_{org}$  at meadows formed by persistent, high-biomass seagrasses (i.e. genera *Posidonia* and *Amphibolis*) relative to opportunistic, low-biomass seagrasses (i.e. genera *Halophila*, *Halodule* and *Zostera*).

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On one hand, the soil  $\delta^{13}$ C signatures measured in these long-living and large seagrass meadows (averaging -15 ± 0.2‰ in both cases) were closer to the  $\delta^{13}$ C signatures of *Posidonia* and *Amphibolis* tissues (ranging from -8 to -14‰; Hyndes and Lavery 2005; Hindell et al. 2004; Cardona et al. 2007; Fourqurean et al. 2007; Collier et al. 2008; Kennedy et al. 2010; Hanson et al. 2010; Serrano et al. 2015) than to  $\delta^{13}$ C values of algae or terrestrial organic matter (ranging from -18 to -32‰; e.g. Smit et al. 2006; Cardona et al. 2007; Kennedy et al. 2010; Hanson et al.

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**Deleted:** which could explain the relative high C<sub>org</sub> contents found in some bare sediment with relatively low mud contents (Figure 1). Also,

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2010; Deudero et al. 2011). The poor relationship between mud and soil Corg contents in 485 486 Amphibolis soils could be explained by samples with relative high C<sub>org</sub> contents (i.e. >2.5% C<sub>org</sub>) 487 and relatively low mud contents, as a result of both the contribution of seagrass-derived C<sub>org</sub> (i.e. <sup>13</sup>C-enriched) and C<sub>org</sub> from allochthonous sources (i.e. <sup>13</sup>C-depleted; Figure 1). In *Posidonia* 488 soils, the poor relationship between mud and soil Corg contents could be explained by samples 489 with relative high C<sub>org</sub> contents (i.e. >10% C<sub>org</sub>) and relatively low mud contents, as a result of 490 the contribution of seagrass-derived Corg (i.e. 13C-enriched; Figure 1). The contribution of 491 492 seagrass-derived Corg (i.e. root, rhizome and sheath detritus) in *Posidonia* soils play a much larger role than the accumulation of fine, organic-rich allochthonous particles. 493 494

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On the other hand, the soil  $\delta^{13}C$  signatures measured in *Halodule*, *Halophila* and *Zostera* meadows (averaging -21 ± 0.4‰) were more similar to  $\delta^{13}C$  values of algae or terrestrial organic matter than to  $\delta^{13}C$  values of their seagrass tissues (ranging from -10 and -14‰; e.g. Hemminga and Mateo, 1996; Kennedy et al. 2010; Hanson et al. 2010). The positive relationship between mud and soil  $C_{org}$  contents in *Halodule*, *Halophila* and *Zostera* soils could be explained their relatively high mud content and  $^{13}C$ -depleted  $C_{org}$ , indicating that allochthonous  $C_{org}$  inputs and mud content play a major role in soil  $C_{org}$  accumulation in these opportunistic and early-colonizing seagrasses. However, the relative high  $C_{org}$  contents found with relatively low mud contents (i.e. mud- $C_{org}$  saturation) disrupted the correlation found between soil  $C_{org}$  and mud contents in these meadows ( $C_{org} > 1\%$  in samples with 0-20% mud;  $C_{org} > 2\%$  in samples with 20-70% mud and  $C_{org} > 3.5$  in samples with 70-100% mud; Figure 1).

The results obtained showed a tendency for high-biomass and persistent meadows (i.e. *Posidonia* and *Amphibolis*) to accumulate higher C<sub>org</sub> stores and seagrass-derived C<sub>org</sub> compared to ephemeral and low-biomass meadows (i.e. *Halophila*, *Halodule* and *Zostera*), suggesting that

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factors (biotic and abiotic) affecting the production, form and preservation of Corg within habitats exert a significant influence on soil Corg content (Lavery et al. 2013; Serrano et al. 2014, 2015). The above- and belowground biomass in meadows of the genus Posidonia (averaging 535 and 910 g DW m<sup>-2</sup>, respectively) is up to 2-fold higher than in Amphibolis meadows (averaging 641 and 457 g DW m<sup>-2</sup>, respectively) and 4 to 18-fold higher than in small and opportunistic seagrasses of the genera Halophila, Halodule and Zostera (125 and 49 g DW m<sup>-2</sup>, on average; respectively; Duarte and Chiscano, 1999; Paling and McComb 2000). Indeed, larger seagrasses tend to have larger and more persistent rhizomes, constituted by more refractory forms of C<sub>org</sub>, more prone to be preserved in soils than simpler, more labile forms of Corg such as seston and algal detritus which are more suitable to experience remineralization during early diagenesis (Henrichs 1992; Burdige, 2007). In addition, the larger size of detritus within Amphibolis and Posidonia meadows compared to Halophila, Halodule and Zostera meadows could also contribute to the larger accumulation of Corg in the former, since decay rates of seagrass detritus increase with decreasing particle size due to larger surfaces available for microbial attack (Harrison, 1989). Differences in above- and belowground biomass and recalcitrance between Posidonia and Amphibolis spp could explain the larger contribution of seagrass-derived Corg (i.e. <sup>13</sup>C-enriched) in the former, thereby obviating the linear relationship between mud and C<sub>org</sub> contents (Figure 1). The soil C<sub>org</sub> content tend to decrease with soil depth and ageing in seagrass ecosystems (e.g. Serrano et al. 2012), thereby the persistence of discrete organic detritus within upper soil horizons could lead to organic matter concentrations above those levels explained by the association with clay and silt particles, as previously demonstrated for terrestrial soils (Mayer and Xing, 2001; Gami et al. 2009). The organic matter preserved in most marine sediments is

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intimately associated with mineral surfaces (i.e. selective preservation by sorption of organic matter into minerals; Keil et al 1994) and therefore the correlation between soil Corg and mud contents in seagrass meadows could vary as a function of soil depth and ageing. The results obtained show that soil depth is not an important factor when attempting to predict soil C<sub>org</sub> content based on mud content in bare sediments (i.e.  $R^2 > 0.74$  for all core depths explored; 1 to 110 cm-thick, 1 to 10 cm-thick, and 11 to 110 cm-thick; Figure 2). However, a clearer pattern appeared when exploring the correlation between soil Corg and mud contents in top 10 cm and within 11-110 cm soil depths of combined *Halodule*, *Halophila* and *Zostera* species ( $R^2 = 0.17$ and  $R^2 = 0.74$ , respectively). These results suggest that the relatively small belowground biomass of these species (i.e. organic detritus) only has an impact on the expected positive correlation between soil Corg and mud content within the top 10 cm, while the correlation for deeper soil depths (11-110 cm) improved ( $R^2 = 0.74$ ) compared to the whole dataset (1 to 110 cm-thick;  $R^2$ = 0.56). For combined *Amphibolis* and *Posidonia* species, the results obtained show that soil depth is not an important factor when attempting to predict soil Corg content based on mud content (i.e. R<sup>2</sup> <0.2 in all cases; 1 to 110 cm-thick, 1 to 10 cm-thick, and 11 to 110 cm thick; Figure 2). These results suggest that the relatively large belowground biomass of these species (i.e. organic detritus) has an impact on the expected positive correlation between soil Corg and mud content within all depths studied.

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Habitat conditions in seagrass meadows not only influence the amount of C<sub>org</sub> accumulation through detrital plant inputs, but the capacity of the plant canopies to retain particles (Gacia et al. 1999). The amount of fine suspended particles available for burial varies among sites, driven by geomorphological features (e.g. run-off, hydrodynamic energy and water depth), while meadow structure (i.e. density, cover and morphology of the canopy) constrains their capacity to

accumulate sediment particles (Hendriks et al. 2010; Peralta et al. 2008). Although the number of cores and species studied in coastal and estuarine ecosystems was unbalanced (i.e. *Amphibolis* and *Posidonia* dominate in coastal habitats and *Halophila*, *Halodule*, *Zostera* dominate in estuarine habitats), the lack of, or poor correlations found within estuarine and coastal ecosystems, precludes the general use of mud as a predictor of blue carbon content based on habitat geomorphology (Figure 3). Seagrass meadows and bare sediments in environments conducive for depositional processes (i.e. estuaries) accumulated up to 4-fold higher amounts of mud compared to other coastal ecosystems, but the saturation of mud with C<sub>org</sub> and the large contribution of seagrass detritus into the sedimentary C<sub>org</sub> pool (<sup>13</sup>C-enriched soils) in some study sites disrupted the positive relationship expected between mud and soil-C<sub>org</sub> contents. In estuarine ecosystems, soil C<sub>org</sub> originated from both mud inputs linked to allochthonous-C<sub>org</sub> via deposition from upstream transport (e.g. Aller, 1998) and seagrass inputs (i.e. in samples with C<sub>org</sub> >5%; Figure 3). The insignificant relationship between mud and soil C<sub>org</sub> contents in coastal habitats could be explained by their relatively low mud content and the accumulation of seagrass-derived C<sub>org</sub>, in particular in samples with C<sub>org</sub> >5% (Figure 3).

In sum, mud is not a universal proxy for blue carbon content in seagrass ecosystems and should not be applied generally across all habitat and vegetation types. Overall, the positive relationship between mud and  $C_{org}$  contents found in bare sediments and in opportunistic and/or low biomass seagrass meadows (i.e. genera *Zostera*, *Halodule* and *Halophila*) allow mud to be used as a proxy for  $C_{org}$  content in these ecosystems, thereby enabling robust scaling up exercises (i.e. benefiting from existing geological surveys and models) at low cost as part of blue carbon stock assessment programs. However, mud content is not a good predictor of  $C_{org}$  content in highly productive meadows such as those constituted by *P. oceanica* in the Mediterranean Sea

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and P. australis, P. sinuosa and Amphibolis spp in Australia. Previous studies suggested that the relationship between organic matter and the sediment matrix is best seen with clay-sized fractions (<0.004 mm; Bergamaschi et al., 1997; De Falco et al. 2004). However, the grain size cut-off selected in this study (mud, <0.063 mm) is more representative of the bulk soil and their Corg content (Pedrosa-Pàmies et al. 2013) and therefore a higher correlation is expected when comparing bulk soil Corg with a larger and more representative fraction of the sediment (i.e. including the silt fraction, 0.004-0.063 mm, also provides binding sites for C<sub>org</sub>; Burdige, 2007). Other biological, chemical and geological factors not explored in detail in this study may also play a key role in Corg storage, and ultimately in the relationship between soil Corg and mud contents. For example, the effects of habitat geomorphology (e.g. hydrodynamic energy, terrestrial mud and Corg inputs, export of seagrass biomass) and species identity (e.g. variation in terms of productivity, oxygen exposure and recalcitrance of Corg stores, and plant influence on sediment retention) within both coastal and estuarine environments, are among the factors identified in this study which might explain significant variation in the C<sub>org</sub> stores of meadows in relatively similar exposure conditions (Serrano et al. 2015). Other factors found to play a key role in controlling soil Corg accumulation in terrestrial ecosystems, such as chemical stabilization of organic matter (Percival et al. 1999; Galy et al. 2008) and microbial biomass carbon (Danovaro et al. 1994), could also influence Corg storage in seagrass ecosystems. Further studies are needed to identify the influences of these other factors on Corg storage in seagrass meadows, and in addition to the mud content, other characteristics should be taken into account when attempting to obtain robust estimates of Corg stores within coastal areas.

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# **Tables and Figures**

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**Table 1.** Data on soil organic carbon and mud contents, and stable carbon isotope from coastal soils were gathered from a variety of seagrass meadows (and also from adjacent bare sediments) and habitat types.

Species	Study site	Geomorphology	Number of cores	Number of samples	Core depth (cm)	Water depth (m)	
Amphibolis (mixed spp)	Rottnest Island, WA, Australia	Coastal	2	68	0-120	2	
impittootis (mixed spp)	Shark Bay, WA, Australia	Coastal	1	38	0-170	2	
Amphibolis antarctica	Shark Bay, WA, Australia Shark Bay, WA, Australia	Coastal	2	63	0-170	2-3	
Amphibolis griffithii	Jurien Bay, WA, Australia	Coastal	2	41	0-200	4	
Posidonia australis	Oyster Harbour, WA, Australia	Estuarine	3	31	0-70	2	
1 Ostaonia austratis	Waychinicup Inlet, WA, Australia	Estuarine	2	79	0-120	2	
	Robbins Island, TAS, Australia	Coastal	6	138	0-130	3	
Posidonia sinuosa	Frenchman's Bay, WA, Australia	Coastal	4	100	0-180	2-8	
r ostaonia sinuosa	Cockburn Sound, WA, Australia	Coastal	3	50	0-30	6	
		Coastal	<i>5</i>	30 147	0-30	2-8	
Posidonia oceanica	Garden Island, WA, Australia		3	192	0-120 475	3	
Postaonia oceanica	Portlligat, Spain	Coastal	1				
77 1 1 1 1	Balearic Islands, Spain	Coastal	6	25	0-270	3	
Halodule uninvervis	Carnarvon, WA, Australia	Estuarine	1	39	0-210	2	
** * * * *	Gladstone, QLD, Australia	Estuarine	6	6	0-10	intertidal	
Halophila decipiens	Gladstone, QLD, Australia	Estuarine	2	2	0-10	intertidal	
Halophila ovalis	Rottnest Island, WA, Australia	Coastal	1	17	0-30	3	
	Swan River, WA, Australia	Estuarine	1	5	0-70	2	
	Leschenault Inlet, WA, Australia	Estuarine	1	8	0-120	1	
	Harvey Inlet, WA, Australia	Estuarine	1	5	0-20	2	
	Gladstone, QLD, Australia	Estuarine	2	2	0-10	intertidal	
Zostera muelleri	Fagans Bay, NSW, Australia	Estuarine	2	20	0-10	intertidal	
	Gladstone, QLD, Australia	Estuarine	23	23	0-10	intertidal	
	Tuggerah Lakes, NSW, Australia	Estuarine	2	64	0-400	3	
Bare	Cockburn Sound, WA, Australia	Coastal	10	131	0-30	2-9	
	Garden Island, WA, Australia	Coastal	1	16	0-30	4	
	Oyster Harbour, WA, Australia	Estuarine	1	26	0-110	3	
	Gladstone, QLD, Australia	Estuarine	9	9	0-10	intertidal	

Species	
Amphibolis (mixed spp)	Rottnest Is
	Shark Bay
Amphibolis antarctica	Shark Bay
Amphibolis grifficiae	Jurien Bay
Posidonia australis	Oyster Ha
	Waychinic
	Robbins Is
Posidonia sinuosa	Frenchmaı
	Cockburn
	Garden Isl
Posidonia oceanica	Portlligat,
	Balearic Is
Halodule uninvervis	Carnarvon
	Gladstone
Halophila decipiens	Gladstone
Halophila ovalis	Rottnest Is
	Swan Rive
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	Gladstone
Zostera muelleri	Fagans Ba
	Gladstone

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Descriptive statistics based on habitat geomorphology (estuarine vs coastal environments). N,

797 number of samples.

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a)									
Habitat	Organ	ic carbo	n (%)	3	5 <sup>13</sup> C (‰)	o) Mud (%)			)
(species)	N	Mean	SE	N	Mean	SE	N	Mean	SE
Posidonia oceanica	217	3.91	0.35	217	-14.92	0.08	217	11.73	0.53
Posidonia australis	248	1.87	0.08	244	-15.79	0.24	248	11.79	0.68
Posidonia sinuosa	297	0.80	0.04	291	-14.08	0.16	297	2.59	0.18
Amphibolis (mixed spp)	106	1.41	0.11	106	-15.20	0.23	106	4.75	0.33
Amphibolis antarctica	63	0.99	0.06	62	-14.62	0.24	63	6.64	0.44
Amphibolis griffithii	41	0.85	0.07	36	-15.83	0.56	41	5.44	0.29
Halodule uninervis	45	0.78	0.12	45	-19.86	0.53	45	17.68	3.04
Zostera muelleri	107	1.10	0.07	43	-20.02	0.30	107	31.68	2.59
Halophila decipiens	2	1.87	0.51	2	-25.60	0.31	2	65.99	9.62
Halophila ovalis	37	0.97	0.23	37	-17.22	0.44	37	24.09	6.23
Bare	182	0.59	0.08	182	-17.25	0.24	182	10.83	1.20
<b>Grand Total</b>	1345	1.56	0.07	1265	-16.18	0.10	1345	10.83	0.43

<u>b</u> )									
Habitat	Organic carbon (%)			δ <sup>13</sup> C (‰)			Mud (%)		
(geomorphology)	N	Mean	SE	N	Mean	SE	N	Mean	SE
Coastal	1026	1.59	0.09	1014	-15.70	0.10	1026	6.85	0.24
Estuarine	319	1.44	0.07	251	-18.10	0.24	319	23.62	1.41

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a)
Habitat
(species)

Posidonia oceanica
Posidonia australis
Posidonia sinuosa
Amphibolis (mixed spp)
Amphibolis antarctica
Amphibolis grifficiae
Halodule uninervis
Zostera muelleri
Halophila decipiens
Halophila ovalis
Bare

o)

**Grand Total** 

Habitat (geomorphology)

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**Table 3.** Pearson correlation analyses to test for significant relationships among soil  $C_{org}$  and mud contents, and soil  $C_{org}$  and  $\delta^{13}C$  signatures in up to 475 cm long cores; based on (a) species identity and (b) habitat geomorphology. *ns*, non significant correlation.

<u>a)</u>								
Habitat	Organic carbon (%) vs mud (%)			Organic carbon (%) vs $\delta^{13}$ C (‰)				
(species)	Formula	$\mathbb{R}^2$	P value	Formula	$\mathbb{R}^2$	P value		
Posidonia oceanica	$C_{org} = -0.26*mud + 6.95$	0.15	***	$C_{\text{org}} = 1.59 * \delta^{13} C + 27.61$	0.13	***		
Posidonia australis	$C_{org} = 0.02*mud + 1.69$	0.02	*	$C_{org} = 0.18*\delta^{13}C + 4.73$	0.30	***		
Posidonia sinuosa	$C_{org} = 0.07*mud + 0.61$	0.09	***	$C_{org} = 0.12*\delta^{13}C + 2.44$	0.23	***		
Amphibolis (mixed spp)	$C_{org} = 0.17*mud + 0.61$	0.26	***	$C_{org} = 0.14*\delta^{13}C + 3.53$	0.09	**		
Amphibolis antarctica	$C_{org} = 0.08*mud + 0.47$	0.32	***	$C_{org} = 0.14*\delta^{13}C + 3.10$	0.29	***		
Amphibolis griffithii	ns	0.05	0.18	$C_{org} = 0.06*\delta^{13}C + 1.79$	0.21	**		
Halodule uninervis	$C_{org} = 0.02*mud + 0.37$	0.34	***	ns	0.00	0.89		
Zostera muelleri	$C_{org} = 0.02*mud + 0.54$	0.39	***	ns	0.08	0.07		
Halophila ovalis	$C_{org} = 0.04*mud + 0.12$	0.91	***	ns	0.00	0.89		
Bare	$C_{org} = 0.06*mud - 0.03$	0.78	***	ns	0.01	0.24		

<u>b)</u>						
Habitat	Organic carbon (%)	vs mud	(%)	Organic carbon (%)	$vs \delta^{13}C$	(%
(geomorphology)	Formula	$\mathbb{R}^2$	P value	Formula	$\mathbb{R}^2$	P
Coastal	ns	0.01	0.85	$C_{\text{org}} = 0.17*\delta^{13}C + 4.14$	0.03	7
Estuarine	$C_{org} = 0.02*mud +1.01$	0.14	*	$C_{org} = 0.17*\delta^{13}C + 4.52$	0.22	
<b>▼</b>						

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Bare

Habitat (species)

Posidonia oceanica

Posidonia australis Posidonia sinuosa

Amphibolis griffithii Halodule uninervis

Zostera muelleri

Halophila ovalis

Amphibolis (mixed spp)  $C_{org}$ Amphibolis antarctica  $C_{org}$ 

For

 $C_{\text{org}} \\$ 

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Figure 1. Relationships among soil  $C_{org}$  and mud contents, and soil  $C_{org}$  and  $\delta^{13}C$  signatures in all, habitats and all soil depths studied: bare sediments, combined *Halodule*, *Halophila* and *Zostera* species, and combined *Amphibolis* and *Posidonia* species. Only correlations with  $R^2$  >0.5 are showed. The grey shaded areas showed the range of  $\delta^{13}C$  signatures of plant detritus (based on literature values; see main text). The white circles indicate the samples obviating the expected correlation between soil  $C_{org}$  and mud contents.

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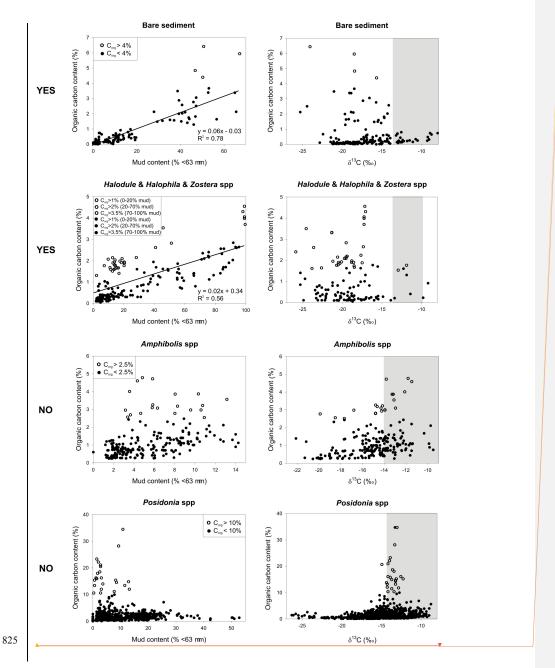
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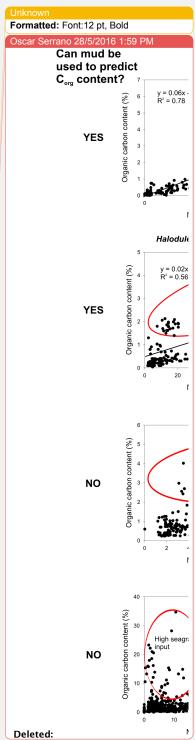
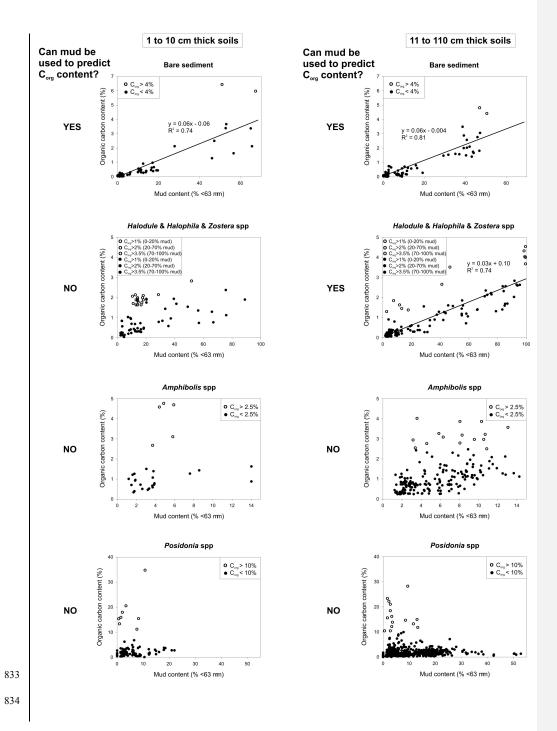


Figure 2. Relationships among soil C<sub>org</sub> and mud contents in 1 to 10 cm and 11 to 110 cm thick soils: bare sediments, combined *Halodule*, *Halophila* and *Zostera* species, and combined *Amphibolis* and *Posidonia* species. Only correlations with R<sup>2</sup> >0.5 are showed. The white circles indicate the samples obviating the expected correlation between soil C<sub>org</sub> and mud contents.

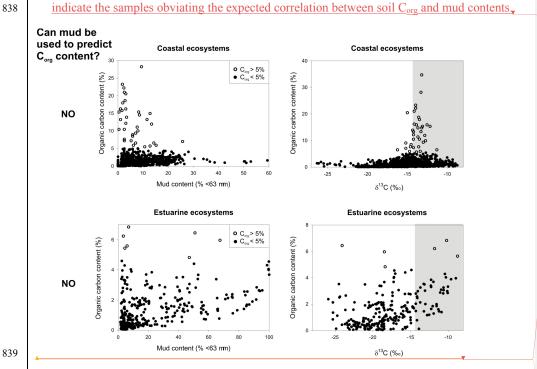


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