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Loss of surficial sedimentary carbon stocks in seagrass meadows subjected to intensive clam harvesting

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ABSTRACT

Seagrass carbon stocks are vulnerable to physical disturbance. We assessed the effect of clam harvesting on the organic carbon (C_{org}) stocks in surface sediments in four intertidal *Zostera noltei* meadows on the Iberian Atlantic coast (Spain and Portugal), by comparing undisturbed and harvested areas. We also monitored the spatial cover of the meadows throughout the growing season. Sedimentary C_{org} content and C_{org} stocks were about four times lower in intensively harvested areas than in control areas, but there were not differences between areas with low harvesting pressure and control areas. Reductions of 53–85% in sedimentary C_{org} stocks of *Z. noltei* meadows were caused by intensive clam harvesting. The effect of intensive clam harvesting on C_{org} stocks increased throughout the growing season, but the area covered by the seagrass increased from 21 to 37%, suggesting rapid recovery of seagrass canopies and potential recovery of sedimentary C_{org} stocks.

1. Introduction

Seagrass ecosystems are valuable for the wide variety of services they provide, including carbon sequestration, fisheries support and water purification, among others (de los Santos et al., 2020). However, seagrass meadows have declined in the past few decades due to anthropogenic impacts such as coastal development, poor water quality and physical disturbance from fishing activities (Orth et al., 2006; Waycott et al., 2009; de los Santos et al., 2019). Among fishing activities, bivalve harvesting takes place in subtidal and intertidal soft-bottom substrates commonly colonized by seagrass meadows (Nordlund et al., 2017). Although bivalve harvesting has historically been a sustainable activity, it can lead to a conflicting interaction with seagrass meadows (Guimarães et al., 2012; Cunha et al., 2013; Bas-Ventín et al., 2015), partly due to the intensive exploitation of shellfish beds carried out in recent decades (Frangoudes et al., 2008). Furthermore, bivalve harvesting involves physical disturbance such as raking, trampling and digging in the seagrass canopies, leading to decreases in seagrass biomass (Peterson et al., 1987; Boese, 2002; McLaughlin et al., 2007; Ferriss et al., 2019) and shoot density (Garmendia et al., 2017; Ferriss et al., 2019).

In the present context of global warming and the urgent need for implementation of mitigation strategies (IPCC, 2019), seagrass meadows, along with salt marshes and mangroves, contribute to climate change mitigation through carbon sequestration. The mitigation is based on the ability of these ecosystems to take up atmospheric CO_2 and store significant amounts of organic carbon (C_{org}) in their sediments on a long-term basis, also known as "blue carbon" (McLeod et al., 2011; Fourqurean et al., 2012). Seagrass meadows slow currents and waves, entrapping particulate matter and enhancing the deposition and accumulation of fine sediments below their canopies. The fine sediments adsorb large amounts of organic carbon and favour development of an anoxic environment that prevents rapid remineralization of the organic carbon (Kennedy et al., 2010; Mazarrasa et al., 2018; Miyajima and Hamaguchi, 2019). Seagrass meadows can accrete vertically, which enables storage of large stocks of carbon below their canopies and makes them among the most efficient biomes for carbon sequestration (Miyajima and Hamaguchi, 2019). Coastal blue carbon ecosystems in the European Union are economically valuable because of their function in preventing carbon emissions to the atmosphere (180 million US$, estimated in 2013) (Luisetti et al., 2013). However, human activities that cause fragmentation and removal of the seagrass canopies increase the

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erosion and oxygenation of the underlying sediment, leading to remineralization and release of the organic carbon stored in the sediments to the atmosphere (Macreadie et al., 2015; Marbà et al., 2015; Serrano et al., 2016a). The CO_2 emissions caused by the loss of seagrasses, estimated to be 0.15 Pg $CO_2 \cdot year^{-1}$, have negative environmental, social and economic consequences (cost of ~6.1 Billion $\$ \cdot year^{-1}$, 2007 US dollars) (Pendleton et al., 2012).

Along the Atlantic and Cantabrian coasts of the Iberian Peninsula, bivalve harvesting on foot takes place in intertidal areas where shellfish beds coexist with seagrass meadows, mainly formed by *Zostera noltei* Hornemann and sometimes interspaced with a few patches of *Zostera marina* Linnaeus. When subjected to clam harvesting, *Z. noltei* canopies suffer fragmentation or removal due to trampling, uprooting and digging (Cabaço et al., 2005; Garmendia et al., 2017, 2021, Bas-Ventín et al., 2015), and they are deliberately covered with sand to create clam farms (Guimarães et al., 2012). These impacts could decrease the sedimentary carbon stocks, as demonstrated in a subtidal *Z. marina* meadow in NW Spain, which suffered a 50% reduction in sedimentary C_{org} stocks due to clam harvesting (Barañano et al., 2018).

Some questions regarding the generalisation and extent to which blue carbon stocks in seagrass meadows are affected by clam harvesting remain to be answered and the effect of different harvesting intensities on the C_{org} stocks is not known. The responses of the sedimentary C_{org} stocks to clam harvesting might be influenced by the spatio-temporal variability of the carbon content in surface sediments of *Zostera* spp. meadows (Couto et al., 2013; Santos et al., 2019; Sousa et al., 2019; Lima et al., 2020; Martins et al., 2021; Potouroglou et al., 2021). This variability of C_{org} stocks is also manifest in the different seagrass species, and although *Z. noltei* is distributed along the eastern Atlantic coast (Borum and Greve, 2004), its blue carbon stocks are underrepresented in the global estimates of carbon storage (Lavery et al., 2013; Santos et al., 2019; Martins et al., 2021; Potouroglou et al., 2021). Also, it is unclear how *Z. noltei* cover would recover after clam harvesting disturbance, since it is a small colonizer seagrass with faster growth rates than larger European seagrasses (Marbà et al., 2004; Roca et al., 2016; Santos et al., 2019).

In this study, we investigated the effects of disturbance caused by clam harvesting on the surficial sedimentary C_{org} stocks in intertidal *Z. noltei* meadows on the Atlantic coast of the Iberian Peninsula, by comparing undisturbed areas and areas subjected to different levels of harvesting pressure in four sites, one in Algarve (S Portugal) and three in Galicia (NW Spain). We specifically assessed the following: 1) the impact of clam harvesting on the sediment properties (grain size, dry bulk density, water content, organic carbon) and sedimentary C_{org} stocks at the end of the *Z. noltei* growing season; 2) the temporal changes in the impact of clam harvesting on C_{org} stocks throughout the *Z. noltei* growing season; and 3) the change in area occupied by the seagrass in harvested clam beds during the *Z. noltei* growing season. We expected to observe a consistent effect of clam harvesting intensity on the sedimentary C_{org} stocks in *Z. noltei* meadows across sites, as well as spatial and temporal differences in the impact of clam harvesting on C_{org} stocks and changes in *Z. noltei* cover throughout the seagrass growing season.

2. Materials and methods

2.1. Description of study sites

Four study sites with intertidal *Z. noltei* meadows subjected to different levels of clam harvesting pressure were selected on the Atlantic coast of the Iberian Peninsula: Noia (Ría of Muros-Noia: 42° 47′ 18″ N, 8° 55′ 12″ W), Cambados (Ría of Arousa: 42° 30′ 3″ N, 8° 49′ 22″ W) and Combarro (Ría of Pontevedra: 42° 26′ 9″ N, 8° 41′ 27″ W), all in NW Spain, and Faro (Ria Formosa: 37° 0′ 7″ N, 7° 59′ 2″ W), in S Portugal (Fig. 1). The locations in NW Spain belong to the Koppen-Geiger climate class Csb (temperate, dry summer, warm summer) and the location in S Portugal to the Csa class (temperate, dry summer, hot summer) (Beck

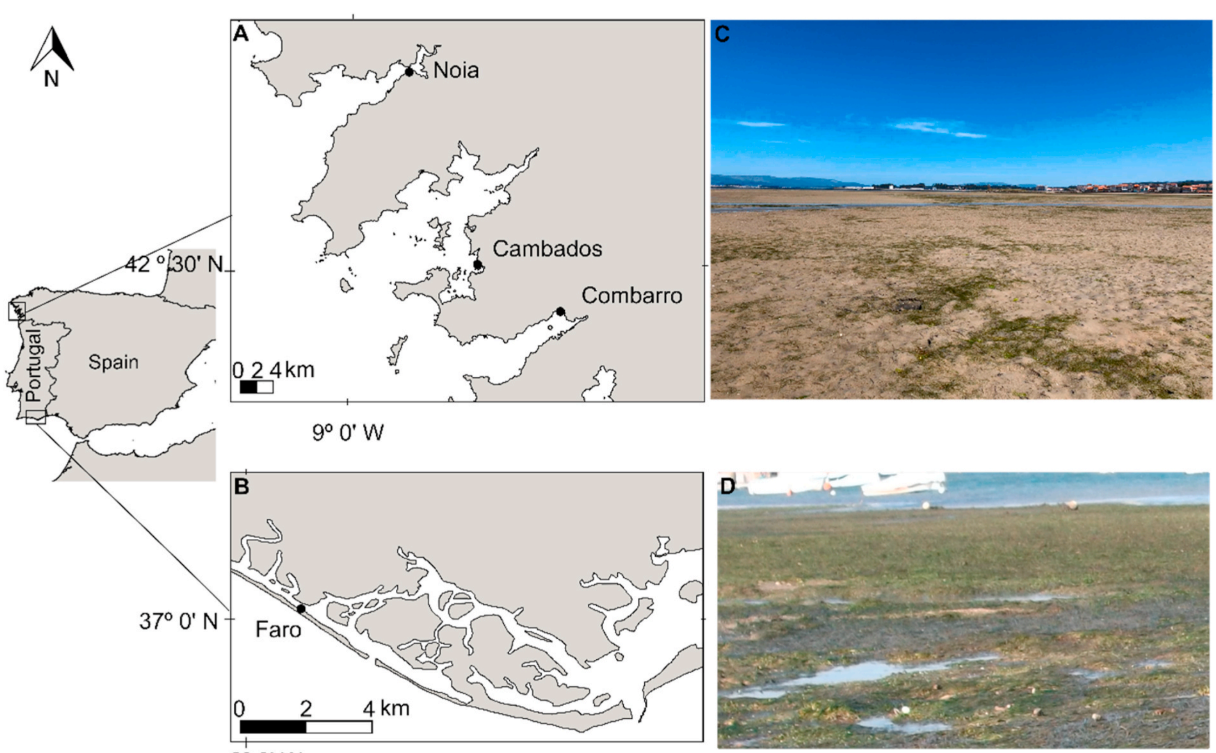

Fig. 1. Location of the *Z. noltei* meadow study sites on the Atlantic coast of the Iberian Peninsula: Noia, Cambados and Combarro in NW Spain (A) and Praia de Faro in S Portugal (B). On the right, pictures of areas affected by intensive clam harvesting in NW Spain (C, Cambados site) and low clam harvesting pressure in Faro site (D).

et al., 2018). Mean annual air temperature and annual precipitation are 12.5–15.0 °C and 1400–2000 mm $year^{-1}$ (for more than 125 days · $year^{-1}$) in NW Spain sites, and 15–20 °C and 400–700 mm $year^{-1}$ (for 50–75 days · $year^{-1}$) in the S Portugal site (AEMET, 2011). All locations are characterised by a semi-diurnal mesotidal regime.

The Noia, Cambados, and Combarro sites are located in the inner area of the Galician rias (Fig. 1) and have extensive *Z. noltei* meadows used as shellfish harvesting areas. The sites are locally managed by fisher organisations (*cofradias*) and are harvested by licenced shellfish gatherers throughout the year in Cambados (211 licences) and Combarro (216 licences), and from September to April in Noia (450 licences) (licences in 2020; www.pescadegalicia.gal; Frangoudes et al., 2008). The bivalves most commonly harvested are the clams *Ruditapes decussatus* (Linnaeus, 1758) and *R. philippinarum* (A. Adams and Reeve, 1850) and the cockle *Cerastoderma edule* (Linnaeus, 1758). In these sites, seagrass meadows situated in the mid and low intertidal zones are uprooted and removed, and the sediment is often stirred up to facilitate settlement of the bivalve larvae and seeding of juveniles of *R. philippinarum* and, to a lesser extent, of *R. decussatus*. Once the bivalves reach commercial size (40 mm for *R. decussatus*, 35 mm for *R. philippinarum*, and 25 mm for *C. edule*), they are harvested manually with hoes and rakes (as regulated by the Galician government Decree 15/2011, from the February 15, 2011, https://www.xunta.gal/dog/), thus destroying the remaining seagrass. This results in a combination of historically strongly impacted areas of bare sediment, impacted or undisturbed areas of fragmented seagrass patches with low shoot densities (annual mean of each meadow from 2246 ± 411 to 4710 ± 682 shoots · m^{-2}, unpublished data) and adjacent undisturbed areas of continuous seagrass canopies with high shoot densities (from 5897 ± 721 to 9881 ± 1299 shoots · m^{-2}, unpublished data).

The Faro site is located in the Ancão Peninsula, in the western zone of the Ria Formosa lagoon (Fig. 1), where *Z. noltei* meadows are commonly subjected to clam harvesting by licensed and unlicensed (occasional) harvesters. The harvesters use a traditional method consisting of manually digging the sediment with a long-bladed shell fishing knife, thus removing the seagrass shoots and rhizomes and leaving shallow holes across the surface of the meadow (Guimarães et al., 2012; Cabaço et al., 2005). The bivalves most commonly harvested in this site are *R. decussatus* and *Polititapes aureus* (Gmelin, 1791) (www.ipma.pt). Based on the differences in the harvesting frequency and techniques, the Noia, Cambados, and Combarro sites were considered to be subjected to intensive clam harvesting pressure, whereas the Faro site was considered to be subjected to low clam harvesting pressure.

2.2. Sampling design

Two zones were selected in each site: a seagrass meadow subjected to clam harvesting (impacted) and an adjacent high-density seagrass meadow where clam harvesting was not carried out (control). Impacted zones in the Noia, Cambados and Combarro sites are known to be harvested monthly, whereas the impacted zone in Faro was selected on the basis of clear marks of the recent clam digging activity (holes in the seagrass meadow of *ca.* 0.5 m^2 and 1 cm depth).

Replicate surface sediment samples ($n = 5$) were collected in the control and impacted zones with small corers (diameter 2.5 cm, depth 5 cm). Seagrass biomass samples ($n = 4$) were taken in the control zones of all sites, using a quadrant (25 × 25 cm, in Noia, Cambados and Combarro) or a core (12 cm of diameter, in Faro). Samples were transported under cool dark conditions to the laboratory (Centro de Ciências do Mar for samples from S Portugal and Universidade de Vigo for samples from NW Spain), where sediment samples were frozen (−20 °C) until further processing, and biomass samples were processed within 24 h. Sediment sampling was carried out at the beginning (March), middle (May) and end (October) of the *Z. noltei* growing season in the Noia, Cambados and Combarro sites (Román et al., 2018) and only at the end of the growing season in Faro (November). Seagrass biomass sampling was conducted in May and October in the Noia, Cambados and Combarro sites and in November in the Faro site.

The change in area covered by the seagrass meadows in the Noia, Cambados and Combarro sites was monitored throughout the growing season (March to October) by a researcher walking along the edges of the seagrass patches with a GIS data collector (Mobile Mapper 50, Spectra Geospatial, sub-metric precision). Seagrass patches were categorized as high- or low-density by visual inspection of their shoot density and fragmentation, two variables that are often interrelated in seagrass meadows (Boström et al., 2006; Barcelona et al., 2021). High-density patches were characterised by continuous, dense cover and high shoot density, whereas low-density patches were fragmented and discontinuous and presented low shoot density, with scars and holes typical of disturbance caused by clam harvesting. The recorded polygons were exported as shapefiles and the areas (hectares) were calculated with QGIS 3.6.

2.3. Analysis of seagrass samples

Samples of *Z. noltei* were processed to determine the shoot density (shoots · m^{-2}) and the above-ground (leaves) and below-ground (roots and rhizomes) biomass (g dw · m^{-2}), the latter after drying the seagrass tissues for 48 h at 60 °C. The carbon stocks in standing *Z. noltei* biomass from NW Spain were calculated by multiplying the above- and below-ground biomass in May and October by the respective average carbon contents (% dw) from *Z. noltei* tissues sampled at each site ($n = 4$) in December 2021. Carbon contents in above and below ground biomass varied slightly between sites (above ground biomass: Noia = 42.1%, Cambados = 37.3%, and Combarro = 42.5%; below ground biomass: Noia = 36.5%, Cambados = 33.1%, Combarro = 36.4%. Standing carbon stocks in Faro were estimated from the average carbon contents (% dw) in *Z. noltei* from the same site, the Ria Formosa (Cabaço and Santos, 2007): above ground biomass = 37.9%; below ground biomass = 36.2%. The organic carbon in below- and above-ground biomass were summed, and the carbon stocks of total live biomass were expressed as g C · m^{-2}.

2.4. Analysis of sediment samples

Sediment samples ($N = 100$) were weighed (fresh weight, fw) on a balance (±0.001 g), dried in an air-oven (48 h, 60 °C) and then reweighed (dry weight, dw). The water content (% of weight) and dry bulk density (DBD, g dw · cm^{-3}) were determined from the weights, the latter by dividing the dry weight by the sediment sample volume (cm^3). The samples were homogenized and ground with a ceramic mortar and pestle, and the organic matter content (OM, % dw) was determined in subsamples of *ca.* 5 g by the loss-on-ignition method in a furnace (450 °C, 4 h).

A subset of 60 samples from the Noia, Cambados and Combarro sites were analysed for carbon content before and after loss-on-ignition method to determine the total carbon and the inorganic carbon, respectively. The analyses were conducted in an elemental microanalyser (Fisons Carlo Erba, mod. EA1108) at the CACTI facilities (University of Vigo). The organic carbon content (C_{org}, %) was calculated as the difference between total and inorganic carbon. Linear regression between the C_{org} content and OM ($C_{org} = 0.399 \cdot OM - 0.136$, $R^2 = 0.86$, $df = 56$) was conducted to estimate the C_{org} content of the remaining 30 samples from the Noia, Cambados and Combarro sites for which only the OM was determined (Supplementary Material, Fig. S1). The mean difference between the C_{org} obtained through the regression and through elemental analyses in those samples where both OM and C_{org} were analysed was −0.067 ± 0.061%, what evidenced the accuracy of the calculations and discarded any overestimation of the C_{org} % (Howard et al., 2019). The C_{org}-OM regression for *Z. noltei* meadows in Galicia was close to those regressions previously reported for sediments in *Z. noltei* (Martins et al., 2021), and in other *Zostera* spp. (Barañano et al., 2018; Potouroglou et al., 2021). The C_{org} contents of the samples from Faro were estimated by applying an existing local regression equation for the Ria Formosa (Martins et al., 2021; C_{org} content = 0.310 · OM - 0.066, R^2 = 0.91, df = 134). The organic carbon stock (g $C_{org} \cdot m^{-2}$) of each surface sediment sample was calculated as the product of the C_{org} content, the DBD and the depth sampled (5 cm), and extrapolated to m^2.

Sediment particle size was measured in a particle size analyser (Beckman Coulter, mod. LS13320) at the CACTI facilities (University of Vigo). The Udden-Wenworth scale: mud (<62 μm), sand (>62 μm, < 2 mm) and gravel (>2 mm) fractions were used to classify the particle size distribution and expressed as % dry weight.

2.5. Data analysis

The loss of C_{org} stocks ($g \cdot m^{-2}$) caused by the clam harvesting activities in May and October–November was estimated as the sum of the loss of C_{org} stocks in the sediment (difference in mean sedimentary C_{org} stocks between control and impacted areas) plus the loss of C standing stocks. Spearman's correlation analysis were used to examine the relationships of the C_{org} content (%) with the DBD and the water content of the sediment. Generalized linear models (GLMs) with a Gaussian error distribution and identity link function were used to analyse the differences in the shoot density, above and below ground biomass, and C

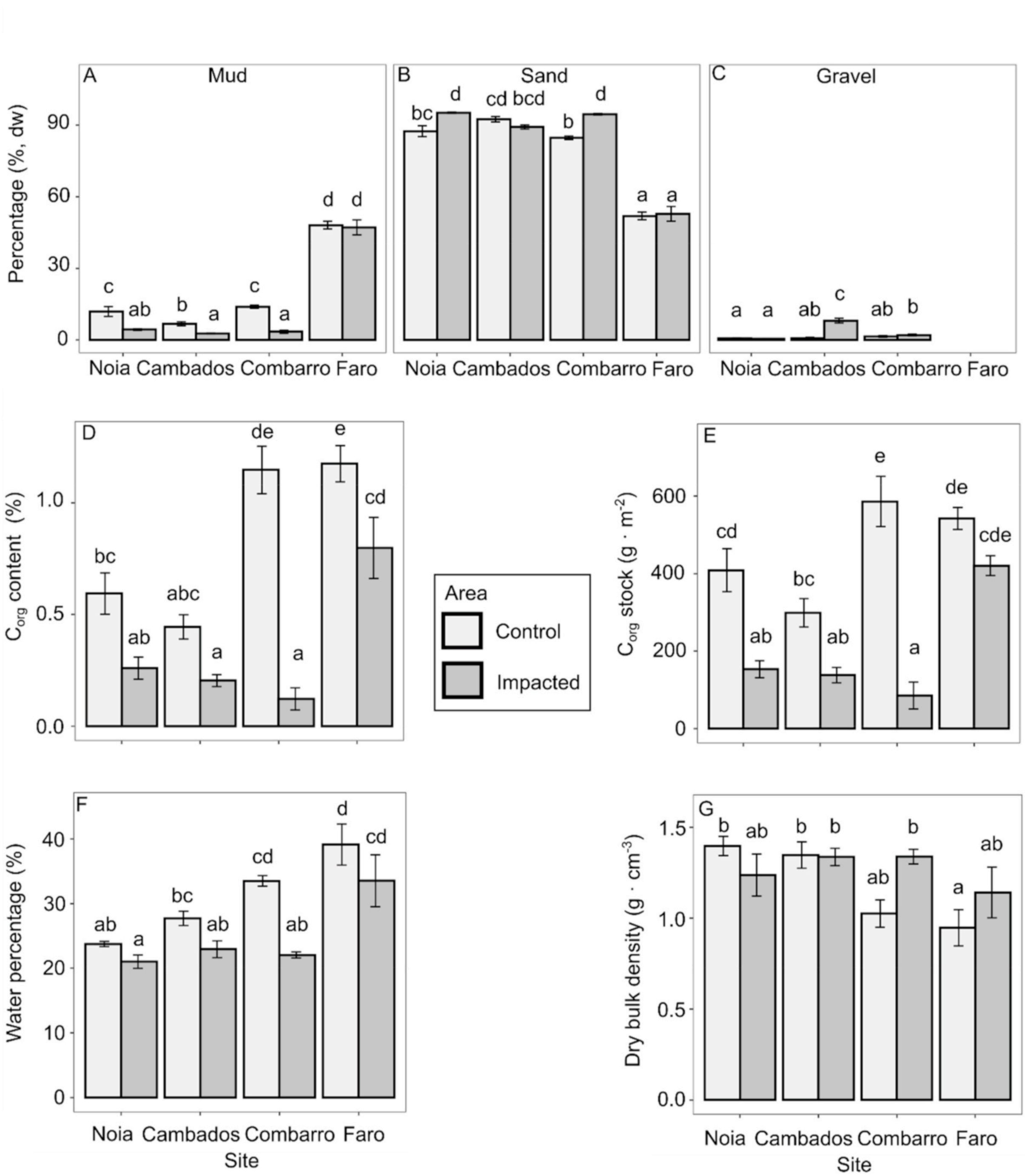

Fig. 2. Mean (±S.E.) values of mud (A), sand (B), and gravel (C) contents, C_{org} content (D), C_{org} stocks (E), water content (F), and dry bulk density (G) in the surface sediment (5 cm) from control (undisturbed) and impacted (clam harvesting) areas in *Z. noltei* meadows at the end of the growing season (October–November). Letters above the bars represent post-hoc pairwise groups indicating differences across study sites and between areas.

standing stock in *Z. noltei* biomass in control areas (undisturbed *Z. noltei* meadows), with Site as a fixed factor (4 levels: Noia, Cambados, Combarro and Faro). Gaussian GLMs were used to analyse the differences in the particle size distribution in surface sediments (mud, sand and gravel), C_{org} content, C_{org} stock, water content and DBD in October–November with Site (4 levels: Noia, Cambados, Combarro and Faro) and Area (Control and Impacted) as fixed orthogonal factors. Gaussian GLMs were used to analyse the differences in the C_{org} stocks throughout the growth season in the Noia, Cambados and Combarro sites, with Site and Area as fixed orthogonal factors. The models were constructed separately for each sampling month (March, May, and October) due to the expected temporal variability in the surface C_{org} stocks during the seasonal growth cycle of *Z. noltei* (Couto et al., 2013; Sousa et al., 2019).

The normality and homoscedasticity of the response variables were previously tested using the Shapiro-Wilk test and Levene's test, respectively. Outliers in the response variables were identified as values that were three times the inter-quartile range above the third quartile and they were removed to increase the confidence of the statistical tests (Crawley, 2013). The percentage of data removed from the variables gravel and C_{org} stock was below 9% and 3%, respectively. The goodness of fit of the models was assessed by checking the normality of the residuals with the Shapiro-Wilk test and graphical analysis. As the residuals of the Gaussian GLMs for mud and water contents in October–November did not meet the normality assumptions, GLMs with a Gamma error distribution and reciprocal link function were applied. When the models were significant, the least-squares means for the combinations of factors were calculated, and pairwise multiple comparisons were carried out. The *p*-values associated with the post-hoc tests were adjusted by Bonferroni correction to minimize the family-wise error rates (Underwood, 1997). The significance was established at $p = 0.05$, and the marginal significance at p between 0.05 and 0.1. All data are reported as means ± S.E. Statistical analyses were performed with RStudio 1.4.1106 software by using the "emmeans" package for the least-squares means calculations and default system packages for the other tests.

3. Results

3.1. Effects of clam harvesting on the sediment properties and C_{org} stocks

In general clam harvesting increased the percentage of coarser sediments and decreased the percentages of mud, C_{org} and water, and the C_{org} stocks. The effects were more pronounced in the intensively harvested meadows than in meadows with lower harvesting pressure (Fig. 2A–G). The Site × Area interaction had a significant effect on the mud, sand, gravel, C_{org} and water contents, and on the C_{org} stocks and DBD in the surface sediment (Table 1). The effect of clam harvesting therefore differed across the study sites.

The mud content was smaller and sand content was greater in the impacted areas than in the control areas in Noia (mud: z.ratio = 5.483, $p < 0.001$; sand: z.ratio = −3.427, $p = 0.017$) and Combarro (mud: z.ratio = 6.700, $p < 0.001$; sand: z.ratio = −4.382, $p < 0.001$), while in Cambados the mud content was smaller in impacted areas (z.ratio = 5.108, $p < 0.001$) but the sand content did not differ between the areas (z.ratio = 1.448, $p = 1.000$), and in Faro there were no differences between areas in either mud or sand content (mud: z.ratio = 0.125, $p = 1.000$; sand: z.ratio = −0.408, $p = 1.000$) (Fig. 2A–B). The gravel content was greater in the impacted areas than in the control areas only in Cambados (z.ratio = −9.914, $p < 0.001$) (Fig. 2C).

Overall, the sedimentary C_{org} content and C_{org} stocks were about 4 times smaller in the highly impacted areas than in the control areas (Fig. 2D–E; Noia, Cambados and Combarro: 0.195 ± 0.028% C_{org} versus 0.728 ± 0.093% C_{org} and 125 ± 16 g m^{-2} C_{org} stocks versus 431 ± 43 g m^{-2}), whereas the sedimentary C_{org} content and the C_{org} stock in the area subjected to low harvesting pressure in Faro were 1.5 times and 1.3 times lower, respectively (Faro: 0.798 ± 0.136% C_{org} versus 1.175 ± 0.081% C_{org} and 420 ± 26 g m^{-2} C_{org} stocks, not significantly different from 542 ± 28 g m^{-2}). Sedimentary C_{org} content in impacted areas was significantly smaller than in control areas in Combarro (z.ratio = 8.857, $p < 0.001$) and Faro (z.ratio = 3.257, $p = 0.032$), but not in Noia (z.ratio = 2.884, $p = 0.111$) or Cambados (z.ratio = 2.074, $p = 1.000$), although the values tended to decrease in all sites (Fig. 2D). The C_{org} stocks in impacted areas were significantly or marginally smaller than in control areas in all sites subjected to intensive clam harvesting (Noia: z.ratio = 4.635, $p < 0.001$; Cambados: z.ratio = 2.919, $p = 0.098$; Combarro: z.ratio = 9.086, $p < 0.001$), whereas in Faro, subjected to low clam harvesting pressure, the C_{org} stocks were not significantly different between areas (z.ratio = 2.215, $p = 0.750$) (Fig. 2E).

The intensity of the harvesting pressure also influenced the C_{org} stocks in impacted areas. At impacted areas the C_{org} stocks were significantly greater at Faro, the site subjected to least intensive harvesting (420 ± 26 g m^{-2}), than in the intensively harvested sites (Noia: z.ratio = −4.850, $p < 0.001$; Cambados: z.ratio = −5.126, $p < 0.001$; Combarro: z.ratio = −6.079, $p < 0.001$), which in turn, were similar to each other (range 85 ± 34 g m^{-2} to 153 ± 22 g m^{-2}) (Fig. 2E).

The water content tended to be lower in impacted areas than in control areas (Fig. 2F), but the difference was only significant in Combarro (z.ratio = 4.698, $p < 0.001$). The DBD tended to be greater in impacted areas than in control areas in Combarro and Faro, whereas the opposite pattern was observed in Noia, and the DBD was similar in impacted and control areas in Cambados (Fig. 2G). The sedimentary C_{org}

Table 1
Summarized results of GLMs used to test the effects of Site (4 levels: Faro, Noia, Cambados, and Combarro), Area (2 levels: control and impacted) and their interaction on sediment particle size categories (n = 4), sedimentary C_{org} content, sedimentary C_{org} stocks, water content, dry bulk density (DBD) in surface sediment (5 cm) (n = 5) in October–November. Results of GLMs testing the effect of Site on the shoot density, above and below ground biomass and C standing stock in control areas (n = 4). Significant effects are shown in bold.

Variable	Factor	*df*	χ^2	*p*
Mud	Site	3	188.916	**< 0.001**
	Area	1	41.275	**< 0.001**
	Site x Area	3	142.115	**< 0.001**
Sand	Site	3	400.290	**< 0.001**
	Area	1	11.740	**< 0.001**
	Site x Area	3	21.750	**< 0.001**
Gravel	Site	2	3.623	0.163
	Area	1	0.089	0.766
	Site x Area	2	69.495	**< 0.001**
Sedimentary C_{org} content	Site	3	63.212	**< 0.001**
	Area	1	8.315	**< 0.01**
	Site x Area	3	28.817	**< 0.001**
Sedimentary C_{org} stock	Site	3	33.812	**< 0.001**
	Area	1	21.486	**< 0.001**
	Site x Area	3	28.592	**< 0.001**
Water content	Site	3	35.509	**< 0.001**
	Area	1	2.006	0.157
	Site x Area	3	8.197	**0.042**
DBD	Site	3	20.345	**< 0.001**
	Area	1	1.714	0.191
	Site x Area	3	8.871	**0.031**
Shoot density	Site	3	51.280	**< 0.001**
Above-ground biomass	Site	3	3.457	0.326
Below-ground biomass	Site	3	5.925	0.115
C standing stock	Site	3	4.046	0.257

content was positively correlated with water content ($\sigma = 0.846$, $p < 0.001$) and negatively correlated with DBD (Spearman correlation coefficient: $\sigma = -0.556$, $p < 0.001$) (Supplementary Fig. S2).

In control areas, the seagrass shoot density differed between sites (Table 1, Fig. 3). Shoot density was significantly higher in the Noia site than in the other sites (Cambados: z.ratio = 5.803, $p < 0.001$; Combarro: z.ratio = 5.790, $p < 0.001$; Faro: z.ratio = 5.943, $p < 0.001$) but there were no significant differences in the biomass or C standing stocks (Table 1, Fig. 3). The estimated loss of C_{org} stocks ($g \cdot m^{-2}$) from *Z. noltei* biomass and sediment caused by the clam harvesting activities in October–November was greater in sites in NW Spain than in the site in S Portugal: 334 $g\ m^{-2}$ in Noia, 216 $g\ m^{-2}$ in Cambados, 561 $g\ m^{-2}$ in Combarro and 165 $g\ m^{-2}$ in Faro.

3.2. Impact of clam harvesting throughout the Z. noltei growing season

The effect of the intense clam harvesting on the C_{org} stocks throughout the seagrass growing season was different across the study sites, as the Site × Area interaction significantly affected the sedimentary C_{org} stocks in *Z. noltei* meadows in NW Spain in March, May and October (Table 2). The effect of intense clam harvesting on C_{org} stocks increased throughout the *Z. noltei* growing season, particularly in Noia, where it became significant in October, whereas in the Combarro site, the differences were always of greater magnitude during the growth season (Table 2, Fig. 4A–C).

In March, the sedimentary C_{org} stocks were significantly smaller in impacted areas than in control areas in Combarro (z.ratio = 10.011, $p < 0.001$), whereas there were no significant differences between the respective areas in Noia and Cambados (Noia: z.ratio = −1.569, $p = 1.000$; Cambados: z.ratio = 1.780, $p = 1.000$) (Fig. 4A). In May, the C_{org} stocks were marginally smaller in impacted areas than in control areas in the Noia (z.ratio = 2.895, $p = 0.057$) and Cambados sites (z.ratio = 2.859, $p = 0.064$) and significantly smaller in impacted areas than in control areas in the Combarro site (z.ratio = 10.223, $p < 0.001$) (Fig. 4B). In October, the C_{org} stocks were significantly smaller in impacted areas than in control areas in Noia (z.ratio = 4.278, $p < 0.001$) and Combarro (z.ratio = 8.386, $p < 0.001$) (Fig. 4C).

Different patterns in seasonal variability were observed, as throughout the growing season the sedimentary C_{org} stocks in control areas were stable in Cambados, decreased slightly in Combarro and increased by 2.5-fold in Noia, with stocks reaching higher levels than in Cambados in May and October (Fig. 4A–C). The water and C_{org} contents tended to be smaller and DBD tended to be greater in impacted areas than in control areas (Supplementary Fig. S3).

In control areas, the shoot densities and the above ground biomass differed significantly between sites in October and in May, respectively (Fig. 5A–D, Table 2). The C standing stocks increased between May and October in Noia and Cambados, whereas the values decreased in Combarro (Fig. 5E–F). The C standing stocks in Combarro were significantly greater than in Cambados in May (z.ratio = −3.432, $p < 0.01$), but there were no significant differences in the C standing stocks between sites in October (Table 2, Fig. 5E–F).

The estimated loss ($g \cdot m^{-2}$) of C_{org} stocks from *Z. noltei* biomass and sediment caused by the clam harvesting activities increased throughout

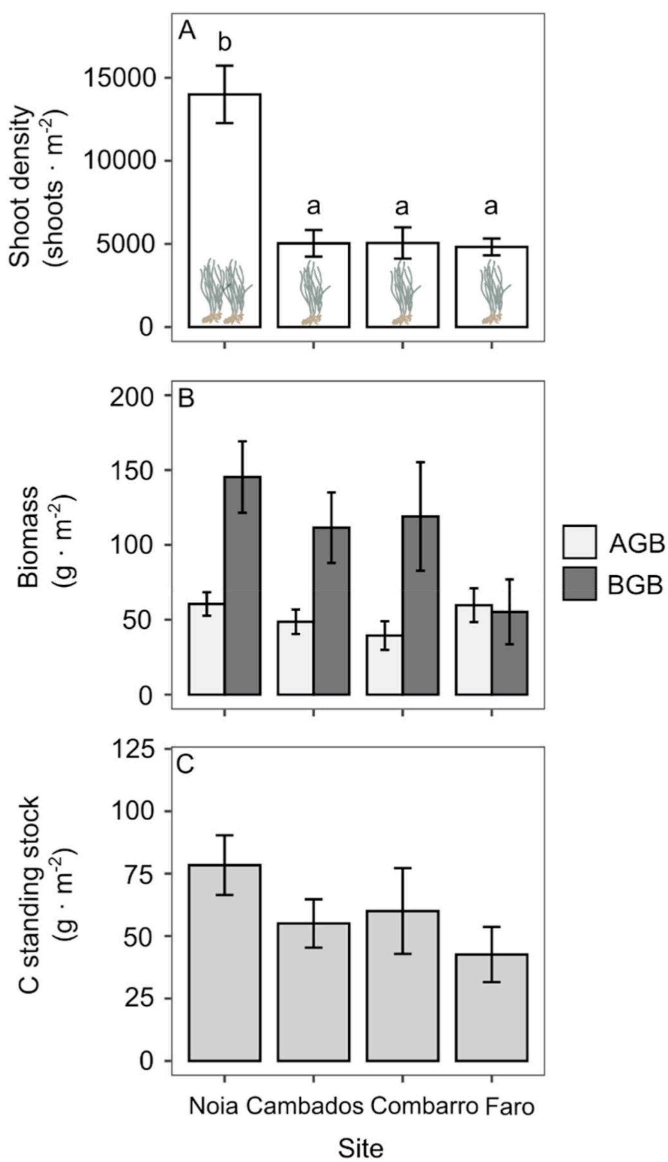

Fig. 3. Mean (±S.E.) values of shoot density (A), above (AGB) and below ground (BGB) biomass (B), and C standing stock (C) in biomass in control areas of *Z. noltei* meadows in October–November (n = 4). Letters above the bars are post-hoc pairwise groups indicating differences in the shoot densities and biomass between study sites.

Table 2
Summarized results of GLMs used to test the effects of Site (3 levels: Noia, Cambados and Combarro), Area (2 levels: control and impacted), and their interaction, on sedimentary C_{org} stocks in surface sediment (5 cm) (n = 5) in March, May and October, and on the shoot densities, above and below ground biomass and the C standing stocks in *Z. noltei* biomass (n = 4) in May and October in NW Spain. Significant effects are shown in bold.

Variable	Month	Factor	*df*	χ^2	*p*
Sedimentary C_{org} stock	March	Site	2	70.399	**< 0.001**
		Area	1	2.462	0.117
		Site x Area	2	83.320	**< 0.001**
	May	Site	2	49.049	**< 0.001**
		Area	1	8.381	**< 0.01**
		Site x Area	2	39.051	**< 0.001**
	October	Site	2	23.589	**< 0.001**
		Area	1	18.302	**< 0.001**
		Site x Area	2	17.263	**< 0.001**
Shoot density	May	Site	2	1.227	0.541
	October	Site	2	35.513	**< 0.001**
Above-ground biomass	May	Site	2	27.098	**< 0.001**
	October	Site	2	3.032	0.219
Below-ground biomass	May	Site	2	2.488	0.228
	October	Site	2	0.776	0.678
C standing stock	May	Site	2	11.830	**< 0.01**
	October	Site	2	1.706	0.426

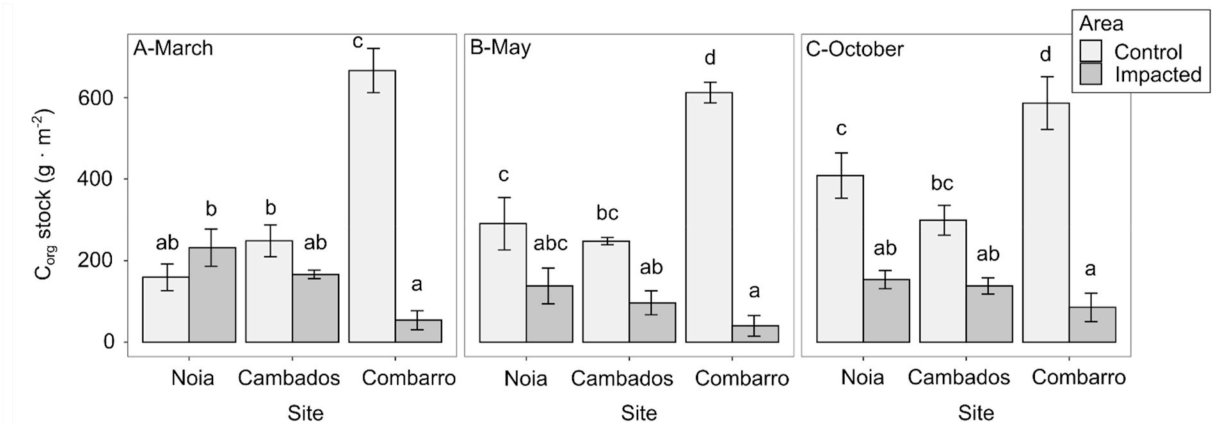

Fig. 4. Mean (±S.E.) organic carbon stocks (g C_{org} · m^{-2}) in surface sediment (5 cm) in control (undisturbed) and impacted (clam harvesting) areas of *Z. noltei* meadows in March (A), May (B) and October (C) (n = 5). Letters above bars are post-hoc pairwise groups indicating differences between levels of study sites and clam harvesting impact.

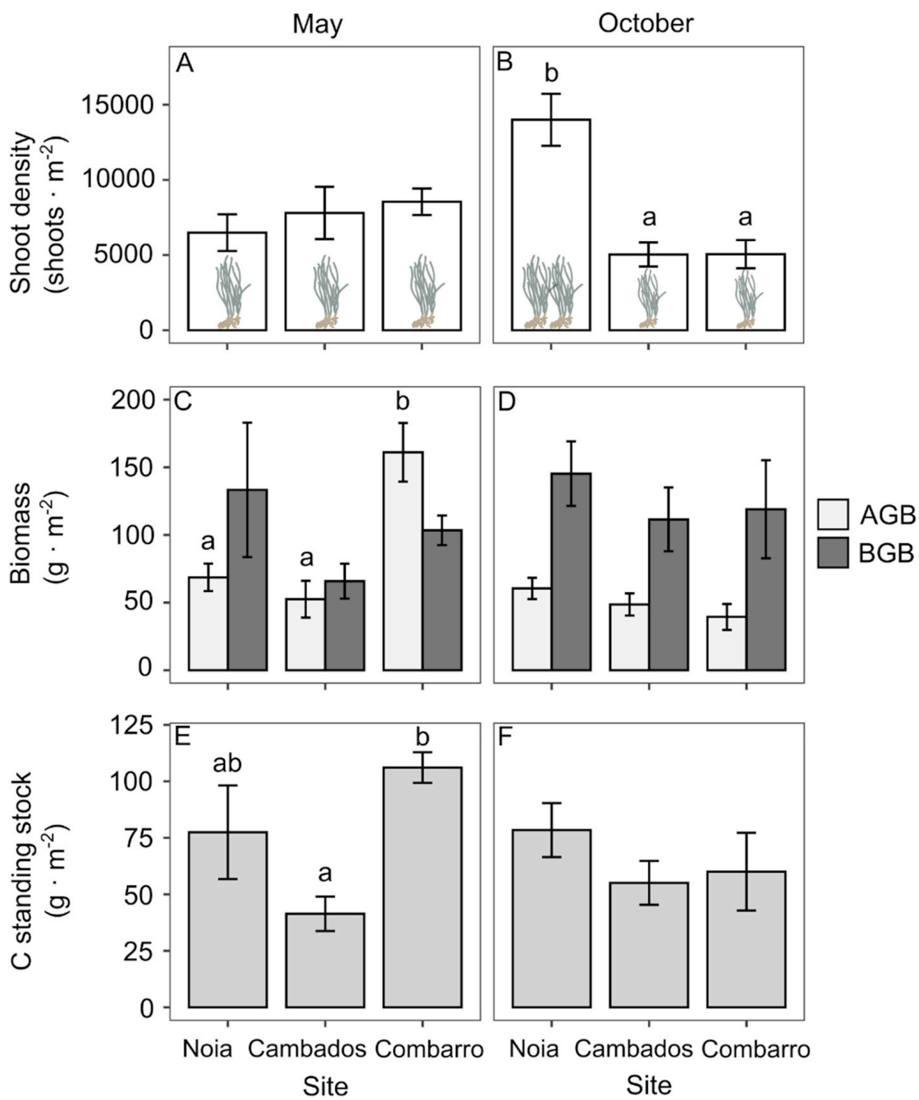

Fig. 5. Mean (±S.E.) shoot density (A–B), above (AGB) and below ground (BGB) biomass (C–D) and C standing stock (E–F) in *Z. noltei* biomass in May and October (n = 4) in control areas in seagrass meadows in NW Spain. Letters above the bars represent post-hoc pairwise groups indicating differences in shoot densities, biomass or C standing stocks between study sites.

the growing season in Noia (May: 230 g m^{-2}, October: 334 g m^{-2}) and Cambados (May: 192 g m^{-2}, October: 216 g m^{-2}), but not in Combarro (May: 678 g m^{-2}, October: 561 g m^{-2}).

3.3. Change in seagrass area in harvested clam beds during the *Z. noltei* growing season

The total area covered by *Z. noltei* during the growing season (i.e. March to October) within the shellfish beds increased by 37% in Noia, 21% in Cambados and 32% in Combarro. In all three sites, the area covered by high density patches increased, whereas some low density patches in March became high density patches in October, resulting in a net decrease in the area occupied by low-density patches (Table 3, Fig. 6).

4. Discussion

We showed that intense clam harvesting in *Z. noltei* meadows on the Atlantic coast of the Iberian Peninsula decreased the surficial sedimentary C_{org} stocks by between 53 and 85%. The decrease in the sedimentary C_{org} stock was consistent with general decreases in the mud and water contents. We also observed that the effects of clam harvesting may be site-specific and vary throughout the growing season. Despite the intensive harvesting activity, the area covered by seagrass *Z. noltei* recovered up to 37% during the growing season, with potential recovery of loss of stored carbon both in the sediment and in the living biomass, in the absence of further disturbance.

The mean carbon content in control areas in NW Spain was close to the minimum values observed in surficial sediments from nearby *Z. noltei* meadows in the Ria de Vigo (from 0.549 ± 0.091% to 2.439 ± 0.027%; M. Román unpublished data), whereas the mean percentages of carbon in impacted areas were well below this range. In the Faro site, the surficial sedimentary C_{org} content in control areas was close to the 1.24 ± 0.84% reported by Santos et al. (2019) in *Z. noltei* meadows for sites elsewhere in Ria Formosa, whereas the carbon content in impacted areas was much smaller. The results from the Faro site are consistent with a previous hypothesis suggesting that clam harvesting disturbs the surface sediments and potentially decreases carbon storage in the Ria Formosa (Martins et al., 2021).

Our findings are consistent with those of previous studies in which the effect of physical disturbance on the sedimentary C_{org} stocks of seagrass meadows were found to vary depending on the intensity of the impact. For example, in Rottnest Island (W Australia), the mechanical destruction by mooring activities in meadows composed of *Posidonia sinuosa* Cambridge & J.Kuo and *Amphibolis griffithii* (J.M.Black) Hartog caused 4- to 5-fold decreases in the C_{org} stocks in the upper 50 cm of sediment (Serrano et al., 2016b). Similarly, in the Lower Laguna Madre (USA), C_{org} stocks were significantly smaller in the upper 20 cm of sediment in areas of *Thalassia testudinum* K. D. Koenig meadows scarred by boat propellers than in undisturbed areas (Arney et al., 2020). By contrast, in other cases, physical disturbance did not affect C_{org} stocks in the sediment, such as in Port Phillip Bay (SE Australia), where the C_{org} in the upper 5 cm did not show a detectable decrease 2 years after simulated boat anchor damage to meadows of *Zostera nigricaulis* (J. Kuo) S. W. L. Jacobs & Les (Macreadie et al., 2014). In *Z. noltei* beds in the Mira estuary (W Portugal), the organic matter content, which is a proxy for C_{org} content, did not differ between undisturbed plots and plots dug in a manner simulating traditional harvesting (Branco et al., 2018). This result is similar to our findings in the Faro site, characterised by low harvesting pressure.

Table 3
Area (ha) occupied by high and low density *Z. noltei* patches in meadows subjected to clam harvesting (on foot) in the three sites in the NW Iberian Peninsula in March and October 2020.

Site	March			October		
	High density	Low density	Total	High density	Low density	Total
Noia	1.552	1.915	3.514	2.950	1.878	4.828
Cambados	0	8.787	8.787	7.403	3.261	10.664
Combarro	6.785	3.849	10.634	13.459	0.629	14.089

The effect of clam harvesting on the surficial sedimentary C_{org} stocks generally increased throughout the growing season, because stocks increased in control areas but not in harvested areas. At the beginning of the growing season, in March, significant decreases in the C_{org} stocks in impacted areas were only observed in the Combarro site. However, in May and October, as the growing season advanced, C_{org} stocks were marginally or significantly lower in impacted areas in all sites (Noia, Cambados and Combarro). Furthermore, the C_{org} stocks and the shoot density in control areas of the seagrass meadows tended to increase in Noia throughout the growing season, whereas at impacted areas these trends were not observed. This finding suggests that the increase in surficial sedimentary C_{org} stocks in control areas may be related to greater inputs of allochthonous (not derived from seagrass) and autochthonous (seagrass-derived) organic matter towards the end of the growing season. Although the contribution of autochthonous carbon to the sediment is smaller in small and fast-growing seagrasses than in larger seagrass species (Serrano et al., 2016b; Mazarrasa et al., 2018, 2021), the meadows become denser and more productive as the growing season advances, which can lead to greater inputs of autochthonous matter to the sediment.

The effect of clam harvesting on the particle size distribution varied depending on the intensity of the activity and was consistent with the decrease in the surficial sedimentary C_{org} stocks. In areas subjected to intensive clam harvesting, the uprooting and removal of the *Z. noltei* canopies and the stirring of the sediment may have hindered stabilization of the sediment, favouring loss of the mud fraction and enhancing the penetration of oxygen into the sediment. All of these processes favour remineralization of the stored C_{org} and prevent the further sequestration of C_{org} (Miyajima and M. Hamaguchi, 2019). By contrast, in the Faro site (subjected to less intensive clam harvesting), the mud content and sedimentary C_{org} stock in impacted areas did not decrease significantly. The correlation between the fine sediments and the organic carbon accumulation appears to be a general characteristic in small and fast-growing seagrasses (Dahl et al., 2016; Serrano et al., 2016a; Mazarrasa et al., 2018; Lima et al., 2020). In our study, the observed variability in the surficial sedimentary C_{org} stocks in the undisturbed areas across the four sites may be mainly driven by the variability in sediment traits, such as the mud content, and local environmental factors (Mazarrasa et al., 2021).

The spatial cover of *Z. noltei* recovered after the disturbance caused by the intense clam harvesting during the seagrass growing season, increasing up to 37%. Previous studies have shown a rapid recovery of the shoot density and biomass of *Zostera* spp. after clam harvesting, as in *Zostera japonica* Ascherson & Graebner in Korea (Park et al., 2011) and in *Z. noltei* in Portugal (Alexandre et al., 2005; Cabaço et al., 2005), where the recovery after disturbance was attributed to increased vegetative growth and reproductive effort. These processes finally lead to recovery of the spatial cover, as observed in the Ria de Vigo (NW Spain), where the cover of a *Z. marina* meadow increased by up to 20% after closure of the bivalve extraction, from April to October (Barañano et al., 2017). Quantification of the *Z. noltei* cover in our study comprised from spring to early autumn, when the recovery was favoured by the greater irradiance and higher water and air temperatures, which provide optimum conditions for growth (Moore and Short, 2006; Park et al., 2011; Román et al., 2018) and reproduction (Alexandre et al., 2005; Moore and Short, 2006). In winter, the harsher environmental conditions and the slowdown of the seagrass growth may hinder recovery after physical disturbance, probably leading to a decrease in *Z. noltei* cover. It must also be taken into account that clam harvesting pressure was considerably reduced in 2020 due to the COVID-19 pandemic. Future surveys

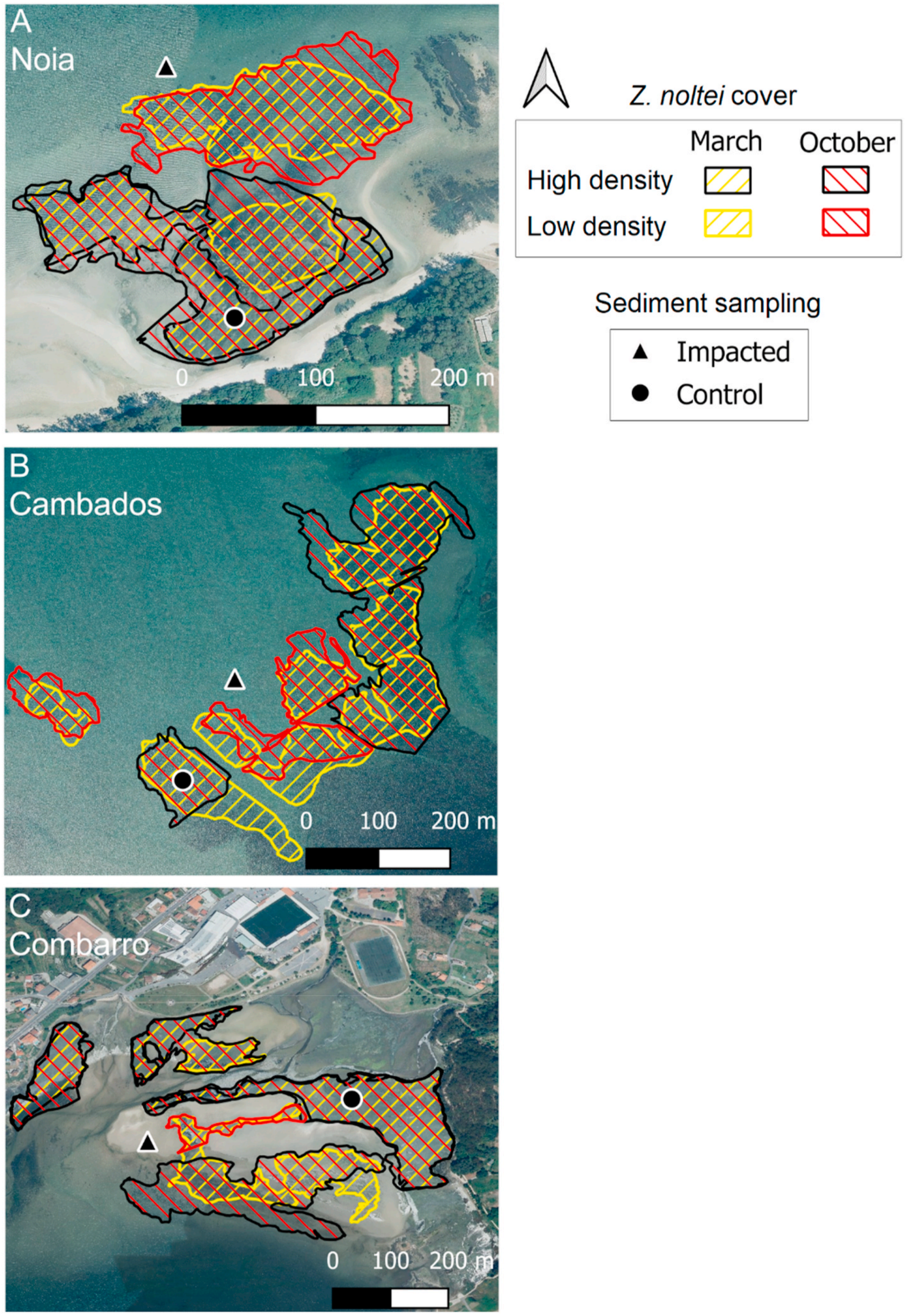

Fig. 6. Spatial cover of the *Z. noltei* meadows located within the shellfish beds in the NW Iberian Peninsula (Noia, A; Cambados, B; Combarro, C) in March and October 2020, showing the sampling points on the surface sediment (5 cm) in control (high-density seagrass meadow) and impacted zones (harvested meadows).

comprising months to years would be necessary to determine the net effect of clam harvesting on the *Z. noltei* cover.

The rapid recovery of the spatial cover in seagrass meadows implies some recovery potential for the surficial sedimentary C_{org} stocks, if no further clam harvesting takes place in the meadows. However, the C_{org} stocks in deeper layers could be also disturbed, since accumulation, stabilization and storage of organic carbon in blue carbon ecosystems are slow processes that require recalcitrance of the organic matter, biotic suppression, anoxia and physical stabilization (Belshe et al., 2017; Miyajima and Hamaguchi, 2019). In NW Spain, intensive clam harvesting has been carried out since the 1960s (Frangoudes et al., 2008) and, together with other anthropogenic factors, has probably contributed to fragmentation of the meadows (Alexandre et al., 2005; Boström et al., 2006). The fragmentation reduces the C_{org} storage in surface sediments within the meadow (Ricart et al., 2017), increases the number of edges, where C_{org} stocks are smaller than in the innermost parts (Ricart et al., 2015), and destabilizes the sediment (Suykerbuyk et al., 2016), which can inhibit the recovery of C_{org}. Analysis of C_{org} stocks in long sediment cores from impacted and recently recovered meadows would be useful to confirm the negative effect of intensive clam harvesting on the long-term storage of C_{org} in deep layers, which is particularly important for climate change mitigation (Belshe et al., 2017).

Less intensive harvesting of clams, involving digging only, rather than previously stirring the sediment and uprooting the seagrass, could reduce the loss of C_{org} stocks, as shown in the Faro site. Our findings help clarify the dynamics of surficial organic carbon stocks in seagrass meadows affected by clam harvesting. This information is necessary for designing management plans aimed at use of seagrass meadows that optimizes the continuity of the carbon sequestration service.

Author statement

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.marenvres.2022.105570.

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