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Mestrado em Biologia Marinha

Supervisors:

Prof. Rui Santos - Centro de Ciências do Mar (CCMAR) (rosantos@ualg.pt)

Co-supervisor

Dr. Carmen Barrena de los Santos – Centro de Ciências do Mar (CCMAR) (cbsantos@ualg.pt)

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Declaration of Authorship

Declaro ser a autora deste trabalho, que é original e inédito. Autores e trabalhos consultados estão devidamente citados no texto e constam da listagem de referências incluída.

I declare to be the author of this work, which is original and unprecedented. Authors and works that were consulted are properly cited in text and are part of the reference list included.

27/09/2021

(Ángela Herrero Fernández)

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Abstract

Seagrass meadows are coastal ecosystems situated almost throughout all of the world's coastlines. They provide important ecosystem services such as coastal protection, nutrient cycling, pathogen reduction and provision of nursery grounds, among others. They share the ability of mangroves and tidal salt marshes to sequester carbon from the atmosphere and water column and to store it into their biomass and into the soil. Moreover, seagrasses enhance nitrogen burial within the sediment, creating a natural filter that improves water quality. Despite the benefits that seagrass meadows provide to human well-being, they are experiencing a global decline due to coastal development and increased water pollution, among other threats. Restoration of seagrass meadows can be a global feasible strategy to mitigate climate change by restoring their role as carbon and nitrogen sinks, compensating anthropogenic CO_2 emissions and excessive nitrogen loads, while preserving these important ecosystems. However, information about the effectiveness of these projects on C and N sequestration service recovery is still needed. This study analyzed sediment biogeochemistry and sedimentary organic carbon (OC) and total nitrogen (TN) stocks within natural and open coast restored *Zostera marina* meadows in the Portuguese coast. The comparison between sites revealed that the amount of OC and TN deposited in the sediment, slightly increased through time although differences between natural and restored sedimentary stocks were not significant. Higher mud percentage was found within the superficial sediment layers of natural meadows pointing out that the simpler structure and higher hydrodynamics conditions of restored meadows, influence deposition of fine sediment. Further research will analyze the causes of variability and establish accurate sediment accretion rates, leading to a more complete evaluation of the restored seagrass meadows in recovering their ecological function as carbon and nitrogen sinks.

Keywords: Blue carbon, nitrogen, seagrass, restoration, carbon sequestration.

Resumo

As ervas marinhas são angiospermas marinhos distribuídos ao longo de águas rasas, de latitudes subárticas a tropicais em todo o mundo, formando extensas pradarias quando as condições são adequadas. Assim como outros ecossistemas costeiros com vegetação, como os mangais e os sapais, as ervas marinhas são um dos ecossistemas mais diversos e produtivos do planeta. Esses ecossistemas suportam uma grande diversidade e são fontes essenciais de alimento para espécies ameaçadas de extinção, como dugongos e tartarugas verdes. Eles fornecem importantes serviços ecossistémicos, como proteção costeira, ciclos de nutrientes, redução de organismos patógenios e habitat para rerodução e desenvolvimento de estados larvares e juvenis, entre outros. Assim como outros habitats costeiros com vegetação (mangais e sapais), as ervas marinhas são conhecidas como ecossistemas de Carbono Azul (BC) devido à sua capacidade de capturar carbono orgânico e armazená-lo no solo, na biomassa viva acima do solo, na biomassa viva abaixo do solo e dentro da biomassa não viva. As condições anóxicas e a diminuição da exposição à erosão nos sedimentos das ervas marinhas permitem um sequestro de carbono mais longo em comparação com os habitats terrestres. Além disso, os baixos níveis de oxigénio aumentam o soterramento de azoto no sedimento, criando um filtro natural que melhora a qualidade da água, removendo as quantidades excessivas de azoto que podem aumentar o risco de proliferação de algas e eutrofização. A estrutura dos prados aumenta a atenuação da corrente e o aprisionamento de partículas, levando à deposição de matéria orgânica produzida dentro e fora do habitat das ervas marinhas. A presença de ervas marinhas promove a sedimentação de sedimentos finos, capazes de reter maiores quantidades de material orgânico e, portanto, de carbono orgânico e azoto.

Apesar da tendência de recuperação observada em alguns locais, as ervas marinhas ainda estão diminuindo em muitos lugares devido a eventos climáticos extremos, doenças naturais, danos mecânicos, desenvolvimento costeiro e diminuição da qualidade da água. A deterioração desses importantes habitats pode levar à perda da função de filtro de azoto costeiro, à libertação de carbono anteriormente acumulado no sedimento e reduzir sua capacidade de sequestro. O número de iniciativas que tentam proteger esses ecossistemas tem crescido em todo o mundo e, recentemente, o restauro de prados de ervas marinhas tem sido considerado uma estratégia viável para mitigar as mudanças climáticas, restaurando seu papel como sumidouros de carbono e azoto, compensando as emissões antropogênicas de CO_2 e cargas excessivas de azoto. Uma combinação de parâmetros de ervas marinhas externos (número e intensidade de

estressores, ambiente físico) e internos (taxa de crescimento, ciclo de reprodução, comprimento da folha) afetam a eficácia da restauração e existe incerteza quanto ao potencial de recuperação da capacidade de sequestro de carbono e nitrogênio. Portanto, evidências sobre os fatores que afetam o restauro de ervas marinhas e sua influência na recuperação dos serviços ecossistêmicos ainda são necessárias.

A decorrer desde 2007, o programa Biomares teve como objetivo a recuperação e gestão da biodiversidade do Parque Marinho Professor Luiz Saldanha, uma AMP (Área Marinha Protegida) pertencente ao Parque Natural da Arrábida. Aqui, a Zostera marina estava em declínio desde os anos oitenta e o último prado foi avistado em 2006. O restauro começou em 2007 com transplantes, no entanto, as fortes tempestades durante o inverno de 2009/2010 removeram as plantas do restauro e apenas a maior das manchas plantadas anteriormente, sobreviveu até hoje. Em 2017, foi plantada outra pradaria com origem na Ria Formosa, que é uma lagoa costeira mesotidal localizada 250 km a sul da Arrábida. Situada a 5km do local de restauração está a Ponta do Adoxe, um dos poucos locais em Portugal onde ainda podem ser encontrados os prados da Z. marina, e que também serviu de dadora mas não persistiu, ao contrário da Ria Formosa.

O projecto de recuperação de ervas marinhas no Parque Marinho da Arrábida oferece uma excelente oportunidade para estudar como se recuperam os serviços ecossistémicos de *Z. marina* ao longo do tempo. Este estudo investigou a capacidade dos prados restaurados na Arrábida de recuperarem a sua capacidade de sequestro de carbono azul e azoto. As análises geoquímicas foram realizadas em amostras de sedimento coletadas em prados naturais (doadores e nas proximidades) e aquelas revegetadas há 3 e 10 anos. Todos os parâmetros estudados mostraram valores mais elevados em prados naturais, embora os resultados indiquem que as diferenças na biogeoquímica dos sedimentos entre prados naturais e restaurados diminuem com o tempo. O carbono orgânico sedimentar e os stocks de azoto total estão dentro da gama descrita em estudos anteriores para outros prados de Z. marina, e não mostraram diferenças significativas entre os locais. As taxas de acumulação de sedimentos foram estimadas de maneira qualitativa e não muito rigorosa devido à falta de dados. Pesquisas futuras irão analisar as causas da variabilidade e estabelecer taxas de acúmulação de sedimentos precisas, levando a uma avaliação mais completa dos prados de ervas marinhas restaurados na recuperação de sua função ecológica como sumidouros de carbono e azoto.

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1. Introduction

1.1 Seagrasses

Seagrasses are marine angiosperms composed approximately by 76 species (Alongi, 2018) distributed along shallow waters, from subarctic to tropical latitudes around the world, forming extensive meadows (Green et al., 2003). Although they occupy less than 0.2% of the world's ocean area (Duarte, 2002), seagrasses are among one of the most productive ecosystems on earth (Duarte, 2017).

1.1.1 Ecosystem services

Seagrasses support high biodiversity and provide important ecological services such as coastal protection, nutrient cycling, pathogen reduction and provision of nursery grounds (de los Santos et al., 2020). As well as other coastal vegetated habitats (mangroves and saltmarshes), seagrasses have become known as Blue Carbon (BC) ecosystems due to their ability to capture organic carbon (OC) and store it within the soil, the living biomass above ground (leaves, branches, stems), the living biomass below ground (rhizomes and roots), and within the non-living biomass (litter and dead wood) (Howard et al., 2014). Differing from terrestrial ecosystems, which mainly accumulates organic carbon in their biomass, seagrasses accumulate higher amounts inside the sediment fraction (Fourqurean et al., 2012). One of the main causes of this difference is the anaerobic environment within the seagrass sediment that prevent aerobic microbial carbon oxidation and allows for a long-term carbon sequestration (Duarte et al., 2005).

The structural complexity of seagrass meadows promotes particle trapping and wave attenuation, enhancing its deposition (Hendriks et al., 2008). As a consequence, they not only sequester OC produced in the meadow (autochthonous) by photosynthetic processes, but also the one originated elsewhere (allochthonous). It is estimated that between 50 and 72 % of seagrass meadows sedimentary OC is allochthonous (Kennedy et al., 2010; Samper-Villarreal et al., 2016). Besides by the structure of the canopy, deposition and preservation of sedimentary OC are influenced by other biotic and abiotic parameters (Burdige, 2007; Serrano et al., 2016). Sediment particle size influence the aggregation of organic particles, being mud particles (silt and clay) the ones able to retain higher amounts of organic matter (OM) (Keil & Hedges, 1993). Increased deposition rates of OM are not only related with higher sedimentary OC, but also with enhanced microbial activity that promotes nitrogen (N) recycling, removal and denitrification processes (Jordan et al., 2011). N uptake into the seagrass sediment reduces N availability to ephemeral algae species, limiting its growth thus decreasing the risk of algal blooms (Gurbisz et al., 2017). Through this process, seagrass help to remove excess anthropogenic nitrogen loads in coastal areas (Jordan et al., 2011), that come mainly from the use of fertilizers for agriculture and the discharge of human sewage (Smith and Schindler, 2009).

Globally, the average stored OC in seagrasses has been estimated to be between 4.2 and 8.4 Pg (Fourqurean et al., 2012) and proportional estimates regarding N stocks are still missing despite the importance of N sequestration for water quality improvement. There is large variation between species and locations (Lavery et al., 2013). For example, OC stock for the seagrass species present in the Red Sea is 7.2 ± 0.4 Mg C ha^{-1} (Garcias-Bonet et al., 2019) while for the south-eastern Australian species is 24.3 ± 1.82 Mg C ha^{-1} (Ewers Lewis et al., 2018). Even the same species can show large variability within locations as show the results of Dahl et al (2016) for *Zostera marina;* 35 ± 4.1Mg C ha^{-1} in the Sweden (Gullmar Fjord) vs 5 ± 0.9 Mg C ha^{-1} in the Black Sea (Sozopol, Bulgaria).The same occurs regarding N stocks, Martins et al. (2021) estimated total nitrogen (TN) within the *Zostera noltei* meadows of Ria Formosa (south Portugal) to be between 7-11 Mg N ha^{-1}, whereas Kindeberg et al. (2018) estimated values of 0.2 to 4 Mg N ha^{-1} for *Z. marina* along the Danish coast.

1.1.2 Threats and global trends

Despite their important role as carbon and nitrogen sinks, and the fact that a recovering trend has been observed in some locations (de los Santos et al., 2019), seagrasses are still declining in many places (Dunic et al., 2021). Concretely in Europe, it has been estimated that 1/3 of the seagrass meadows have been lost since 1869, mainly due to extreme climatic events, natural diseases, mechanical damage, coastal development and decreased water quality (de los Santos et al., 2019). Deterioration of these important habitats can lead to the loss of the coastal N filter function (Aoki et al., 2020), to the release of carbon accumulated in the sediment over centennial or millennial time scales at estimated rates that could be up to 299 Tg C yr^{-1} (Fourqurean et al., 2012), as well as reduce future capacity for carbon capture and storage (Pendleton et al., 2012; Thorhaug et al., 2017). Consequently, greenhouse gases concentrations could rise, and acidification processes will be enhanced by increased CO_2 concentrations in the water column (Spivak et al., 2019).

1.2 Seagrass restoration

Concern about the decline of seagrass populations globally has risen in the last decades and many conservation attempts are being done (Prentice et al., 2020) aiming to reestablish the ecosystem services lost. Either by seagrass transplantation in unvegetated areas with previously known suitable habitat or by revegetation of existing but degraded meadows (Greiner et al., 2013; Lundquist et al., 2018; Reynolds et al., 2016), these initiatives seek to induce a change in the ecological state of the meadows by increasing its structural complexity (Paulo et al., 2019) thus enhancing its posterior self-recovery.

Seagrass restoration is generally considered successful when it is possibly to quantify an increase in terms of biological features like canopy density or regarding the recovery of ecosystem services such as biodiversity or sequestration capacity (Duarte et al., 2020; Suding, 2011). Even though the number of positive outcomes has been increasing in the last decade (Orth et al., 2020; Rezek et al., 2019; Tan et al., 2020), restoration attempts have had a modest success rate (37–38%) as yet (Bayraktarov et al., 2016; Duarte et al., 2020). The fact that seagrass growth, and hence, the effectiveness of seagrass restoration is affected by a combination of factors including species-specific (e.g., growth rate and reproduction cycle) and donor population characteristics (e.g., genetic diversity) (Bekkby et al., 2020), recovery potential (e.g., number and intensity of stressors) (Roca et al., 2016), location and physical environment (e.g., current velocity and sediment type) (van Katwijk & Wijgergangs, 2004), has derived in uncertainty regarding the possible outcomes of restoration projects which has constrained conservation efforts and prevent the deployment of BC initiatives. Therefore, both positive and negative outcomes, evidence on the factors affecting seagrass restoration and its influence on their ecosystem services recovery are still needed (Griffiths et al., 2020; Macreadie et al., 2019).

1.2.1 Effects of restoration on Blue Carbon and Nitrogen sequestration capacity

Coastal vegetated ecosystems have received increased attention for their sequestration capacity (Lavery et al., 2019; Macreadie et al., 2017). Data regarding restored seagrass meadows is still scarce, however, recent publications have proven the feasibility of recovering C and N sequestration within manageable periods of time. In a study of a restored *Z. marina* seagrass meadow in Virginia bays (USA), OC burial accelerated 5 years after the seagrass planting, reaching rates of 36.7 g C m^{-2} yr^{-1} after 10 years (Greiner et al., 2013). The authors anticipated that 12 years after planting, burial rates in restored seagrass would equal those of natural meadows. The N sequestration capacity of seagrass meadows after restoration was studied by Aoki et al. (2020) also in Virginia bays where they found that the N burial rate of *Z. marina* 10 years after seeding (3.52 g N m^{-2} yr^{-1}) was comparable to rates in natural meadows and more than 20-fold the rate in adjacent bare sediments (0.17 g N m^{-2} yr^{-1}). In Oyster Harbour (Western Australia), Marbá et al. (2015) demonstrated that the carbon stocks of *Posidonia australis* meadows eroded after their decline, and that OC burial rates 18 years after planting new seagrass were similar to those found in nearby vegetated meadows (26.4 ± 0.8 g C m^{-2} yr^{-1}).

1.2.2 Seagrass restoration in Portugal

In Europe four native fully-marine seagrass species are found, three of them being present in the Portuguese coasts (Cunha et al., 2013). *Cymodocea nodosa* and *Zostera marina* develop in the subtidal zone, and *Z. noltei* in the intertidal one, and most of them are found in sheltered coasts, coastal lagoons, and estuaries (Cunha et al., 2013). This region of the Atlantic represents the northern and western limit for *C. nodosa* and the southern distributional limit for *Z. marina* (Alberto et al., 2008; Cabaço and Santos, 2010). Because of its location, these meadows are exposed to genetical mixture with other seagrasses species that extend from the south, allowing for a unique genetic population (Alberto 2008; Diekmann et al., 2005; Olsen et al., 2004). The three species have been listed as vulnerable in the OSPAR Convention (OSPAR Convention, 2008).

Zostera marina, with a total coverage of 0.075 km^2 (Cunha and Serrão, 2011), appears to be the most endangered one within Portugal. In 2010 it had disappeared from six of the eight sites where the species was once abundant being left only the populations in Lagoa de Óbidos and in the Ria Formosa lagoon (Cunha et al., 2013). However, in 2013, new meadows were observed in Ponta do Adoxe and Costa da Galé during the explorations carried out by the Biomares program, in 2015 also in Ria Formosa lagoon (Rui Santos, personal observation), and recently in Aveiro where new and bigger patches than those spotted a decade ago have been observed (Guerrero-Meseguer et al., 2021). This fact indicates that the species may recover if disturbances decrease, as observed in many other parts in Europe (de los Santos et al., 2019). Nevertheless, its location within its southern distributional limit, make *Z. marina* meadows highly vulnerable against climate change due to the negative influence of increased temperatures on its sexual reproduction (Cabaço and Santos, 2010). Therefore, it is important to assess its current condition and the potential recovery of the ecosystems services they provide, as well as promote its self-recovery and restoration (Cunha et al., 2013).

Up to date, only two seagrass restoration projects have been carried out in Portugal (Cunha and Serrão, 2011). The first one was conducted in the Mondego Estuary where *Z. noltii* meadows had decreased considerably (Martins et al., 2005). Also, in 2007, LIFE Biomares project aimed to restore and manage the biodiversity of the Marine Park Professor Luiz Saldanha, an MPA belonging to the Arrábida Natural Park with high biodiversity richness (Cunha et al., 2014; Henriques et al., 2009). *Zostera marina,* that was once covering large extensions of the area, has been declining since the eighties mainly due to dredging for bivalves and anchoring of fishing and recreational boats, was last spotted growing naturally in 2006 (Cunha et al., 2013). The restoration started in 2007 using transplants of three species (*Z. marina, Z. noltei* and *C. nodosa*) from two different donor populations (Sado Estuary and Ria Formosa). However, the strong storms during the winter of 2009, interfered with the restoration plans and only patches of *Z. marina* planted in 2010 survived. In 2017, another attempt was made using only this species from the donor population in Ria Formosa, which is a mesotidal coastal lagoon located 250 km south from the Arrábida. Ponta do Adoxe is one of the other few places in Portugal where *Z. marina* meadows can still be found. It is situated only 5 km away from the Arrábida, on the outer coast of Tróia peninsula (Cunha et al., 2013). There, the meadows disappeared after the winter of 2009 but, after seedling germination, they recovered and were spotted again in 2013 (Cunha et al., 2013; Paulo et al., 2019).

The seagrass restoration project in the Arrábida Marine Park offers an excellent opportunity to study how *Zostera marina* ecosystem services recovery through time, allowing the comparison between parameters of natural meadows to 3-year and 10-year-old transplanted ones.

1.3 Aim and objectives

The aim of this thesis is to investigate the capacity of *Zostera marina* meadows in Portugal to recover their blue carbon and nitrogen sequestration ability after a restoration project carried out through transplantation. To do so, geochemical analyses were performed in sediment samples collected over depth of natural meadows (donor and nearby) and those revegetated 3 and 10 years ago. Specifically, I aim to:

a) Investigate and reconstruct the trajectory of the (expected) effect of the revegetation on the sediment geochemistry of restored meadows over time, by assessing the carbon and nitrogen contents and grain size along the sediment depth profile.

b) Estimate and compare the sedimentary carbon and nitrogen stocks in restored and natural (nearby and donor) meadows.

c) Estimate sediment accumulation rates based on the trajectory reconstruction of restored meadows.

The findings are expected to shed light on the feasibility of restoring open coast seagrass meadows of *Z. marina* to restore their carbon and nitrogen sequestration potential within a reasonable period of time.

2. Methodology

2.1 Study Sites

Sediment cores in *Zostera marina* seagrass meadows were collected in four sites (*Figure 2.1*). Ponta do Adoxe (PA) natural meadows (38.492º, -8.909º), Arrábida Natural Park (AR) meadows, where seagrasses were transplanted 3 (AR3; 38.481º, -8.968º) and 10 (AR10; 38.481º, -8.969º) years ago, and in the donor populations from those, located in the inner side of Culatra island (37.003º, -7.823º), within the Ria Formosa coastal lagoon (RF).

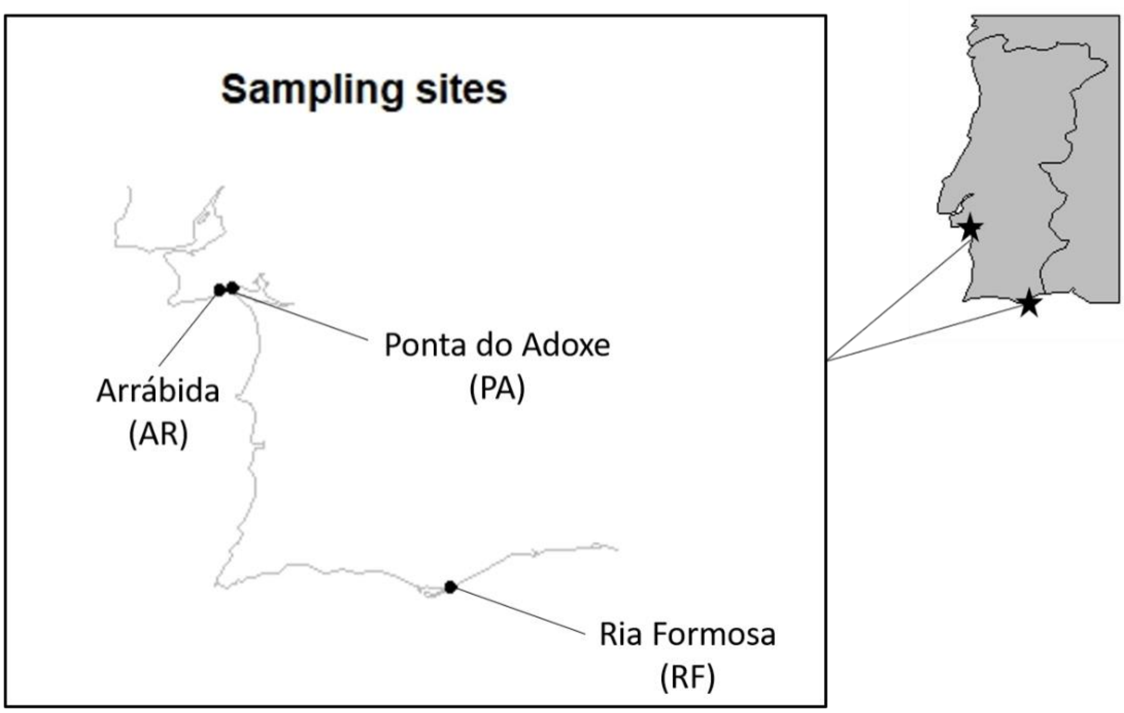

Figure 2.1. Sampling sites of the study showing the location of the donor meadow in Ria Formosa (RF), the natural meadow in Ponta do Adoxe (PA) and of the transplanted meadows in Arrábida (AR).

2.2 Core extraction and sub-sampling

A total of twelve sediment cores were collected (PA=3; AR3=3; AR10=3; RF=3) by inserting into the sediment PVC tubes (4.6 cm diameter, 150 cm length) with sharpened ends to cut fibrous material and minimize core compression (Howards et al., 2014). Cores were extracted by SCUBA divers from Arrábida and from Ponta do Adoxe meadows. The cores from Ria Formosa donor meadows were extracted during low tide. All cores were sealed at both ends, transported to the laboratory, and stored at -20 ºC before processing. The cores were opened in two halves using a wire saw, one half was sliced at regular depth intervals of 2 cm and the other was sliced at intervals of 1cm only in the upper 10 cm. The remaining part of the core was archived at -20 ºC. In order to correct the compression applied to the sediment during the sampling (compaction range 12-46%), a compaction corrector factor was determined by dividing the length of sample recovered by the length of core penetration (Howards et al., 2014). Then the compaction factor was applied to correct the volume and depth of each slice.

2.3 Geochemical analysis

Subsamples of 1 cm corresponding to the superficial 10 cm of the core and a selection of the 2 cm subsamples following series of 4 cm spacing between them along the depth of the core were used for the analysis. Grain size analysis was only conducted on the 2-cm subsamples.

2.3.1 Dry bulk density and water content

Wet sediment subsamples were weighted (g fw) in their pre-weighted labelled zip bags and oven dried at 60 ºC until constant weight, then weighted again to obtain the dry weight (g dw). Water content was obtained from the dw and fw and expressed in percentage (%). Dry Bulk Density (DBD; g dw cm^{-3}) was determined from the dry weight (g dw) and the original volume of each slice (cm^3), which was estimated by geometrical approximation.

2.3.2 Organic matter, organic carbon, and total nitrogen contents

Organic matter content in the sediment samples was estimated by using the loss-on-ignition method. First, each subsample was homogenized by grinding them to fine powder using a ceramic mortar and pestle. The mortar was cleaned between processing samples to avoid cross-contamination. The subsamples (5-30 g dw) were transferred into pre-weighted aluminum containers, then weighted and burnt at 450 ºC for 4 hours in a muffle furnace. Organic matter content (OM, % dw) was determined as percentage of dry weight (Heiri et al., 2001).

A local linear relationship from the Ria Formosa (Martins et al. 2021, *Figure 2.2*) was used to estimate the organic carbon (OC, % dw) and the total nitrogen (TN, % dw) contents based on the organic matter content (Howards et al., 2014). CHN analysis was performed in a selection of samples (n = 10) with OM (%) values in the whole OM range obtained from the sediment in Ponta do Adoxe and Arrábida to verify the appropriateness of the use of the linear relationship from the Ria Formosa.

Considering the known effect of depth on the amount of sedimentary organic matter (Mazarrasa et al., 2017; Samper-Villarreal et al., 2016) and given sediment cores' depths (from 43.48 to 114.54 cm), the investigation was focus on the upper 50 cm, to make possible the comparison between locations. Although profiles along depth were represented for maximum depth reached (~120cm)

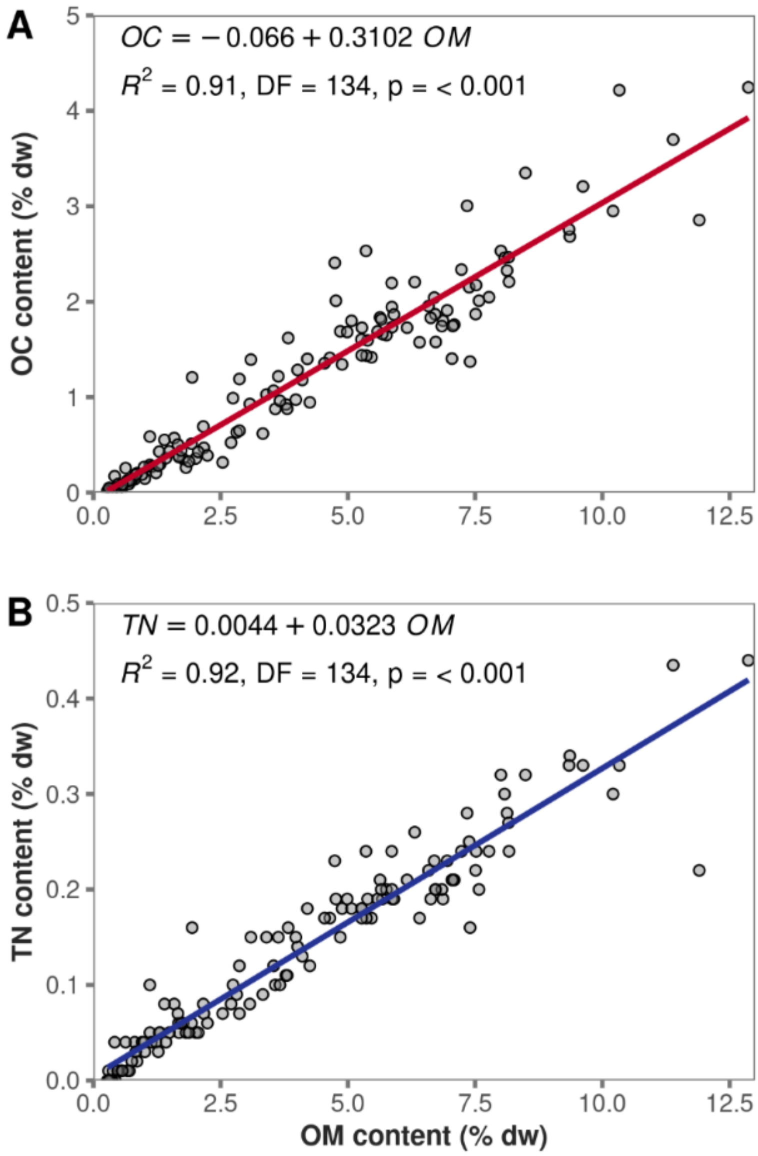

Figure 2.2. Linear relationship between organic matter (OM) content with organic carbon, OC (A) and total nitrogen, TN (B) contents in sediment samples of coastal vegetated habitats in the Ria Formosa. Source: Martins et al., 2021.

2.3.3 Organic carbon and total nitrogen stocks

Sedimentary stocks of OC and TN in the sampled meadows were estimated by integrating, along the sediment depth, the product of OC or TN contents (% dw) and DBD (g cm^{-3}), using the trapezoidal rule (Howards et al., 2014). Stocks were expressed in Mg ha^{-1}.

2.3.4 Granulometry analysis

Sediment particle-size analyses were performed on the remaining sediment samples of the 2-cm slices (approximately 3-24 g dw). Samples were weighted and exposed to organic matter digestion with hydrogen peroxide in increasing concentrations, up to 35 % (v/v). The samples were washed repeatedly with deionized water and dried at 70 ºC.

Dry sieving was carried out using a sieve shaker for 15 minutes to separate particles of 8, 4, 2, 1, 0.5, 0.125, and 0.063 mm. Sediment samples with particle size smaller than 63 µm (mud), were wet sieved and subjected to the pipette sampling method based on Stokes' law. This method relies on the relationship that exists between settling velocity and particle diameter (Gee and Bauder, 1986). The mud particles were allocated in a graduated cylinder, mixed with deionized water up to 800 mL, agitated, mixed with hexametaphosphate (70 mL, 3.04 g L^{-1}) up to 870 mL, agitated again and filled up to 1000 mL with deionized water. After 15 - 48 h the samples were agitated for 2 min and subjected to six timed withdrawals of pipette samples (20 mL) at a certain height and at a constant temperature. Sediment concentrations were measured at combinations of time and depth corresponding to particle diameters of 62, 32, 16, 8, 4 and 2 mm in order to get the silt and total clay ratios (Blott and Pye, 2012). The separated fractions were oven dried (100 ºC) during approximately 12 h. The fraction of each particle size category (*Table 2.1*) was weighted and expressed as percentage of dry weight (% dw).

Table 2.1.. Particle size scale proposed by Blott and Pye (2012), both in mm and phi units.

Particle size			
mm	Limits in Phi units (ϕ)		(Blott & Pye, 2012)
8	-4	-3	Medium gravel
4	-3	-2	Fine gravel
2	-2	-1	Very fine gravel
1	-1	0	Very coarse sand
0.5	0	1	Coarse sand
0.25	1	2	Medium sand
0.125	2	3	Fine sand
0.063	3	4	Very fine sand
<62	<4		Very coarse silt
<32	<5		Coarse silt
<16	<6		Medium silt
<8	<7		Fine silt
<4	<8		Very fine silt
<2	<9		Total clay

2.4 Estimation of accumulation rates

Based on visual inspection of the sediment colour and sediment layers, the OM deposition profile along depth, and the historical data collected from the bibliography, it was estimated the depth at which sediment and OM after the transplantation started to accumulate. Taking into consideration that superficial sediment is characterized by higher content in fine (< 63μm) and darker sediment, peaks of coarser sediment along depth were considered intrusions caused by disturbances on the meadows. This method is far from being precise, so the precaution and conservative principles were used when interpreting the results.

2.5 Data analysis

Data obtained through geochemical and grain size analysis was checked for normality by inspection of residual plots and for homoscedasticity using Levene's test. When normal distribution was not met, non-parametric tests were used. Differences in DBD for the top 50 cm among the seagrass sites were tested by a one-way ANOVA and Tukey´s HSD test. To analyze the differences between OM, OC, and TN contents among the sites, Kruskal-Wallis and Wilcoxon tests were used on 0-50 cm samples. Differences in the OC and TN stocks among sites were tested with Kruskal-Wallis test. R programming language (R Core Team 2019) was used for the statistical analysis.

3. Results

3.1 Depth profiles in restored and natural meadows

The sediment DBD along 0-50 cm depth in the sampling meadows presented a mean value of 0.91±0.27 g cm^{-3} (± SD). Sediment density presented lowest values within the surface of natural meadows and increased with depth for all sites, although disturbances in the pattern are shown below 15cm in AR10 and below 30cm depth in RF (*Figure 3.1*). Minimum and maximum values were found within RF meadows (0.35-1.64 g cm^{-3}). One-way ANOVA showed significant variability between meadows (F= 16.37, p<0.001). Tukey's HSD test revealed great similarities in DBD between the donor meadow (RF) and the meadow transplanted 3 years ago (AR3), as well as between the transplanted meadow 10 years ago (AR10) and the nearby natural meadow (PA).

In general, OM (%dw) content was higher in the natural meadows studied (PA, RF), reached highest values within the superficial sediment layers (0-20 cm), and decreased relatively steadily until a point where it stabilizes (*Figure 3.2*). This pattern was more evident for PA and RF, while for AR10 and AR3 the variability of OM with depth was higher (*Figure 3.2*).

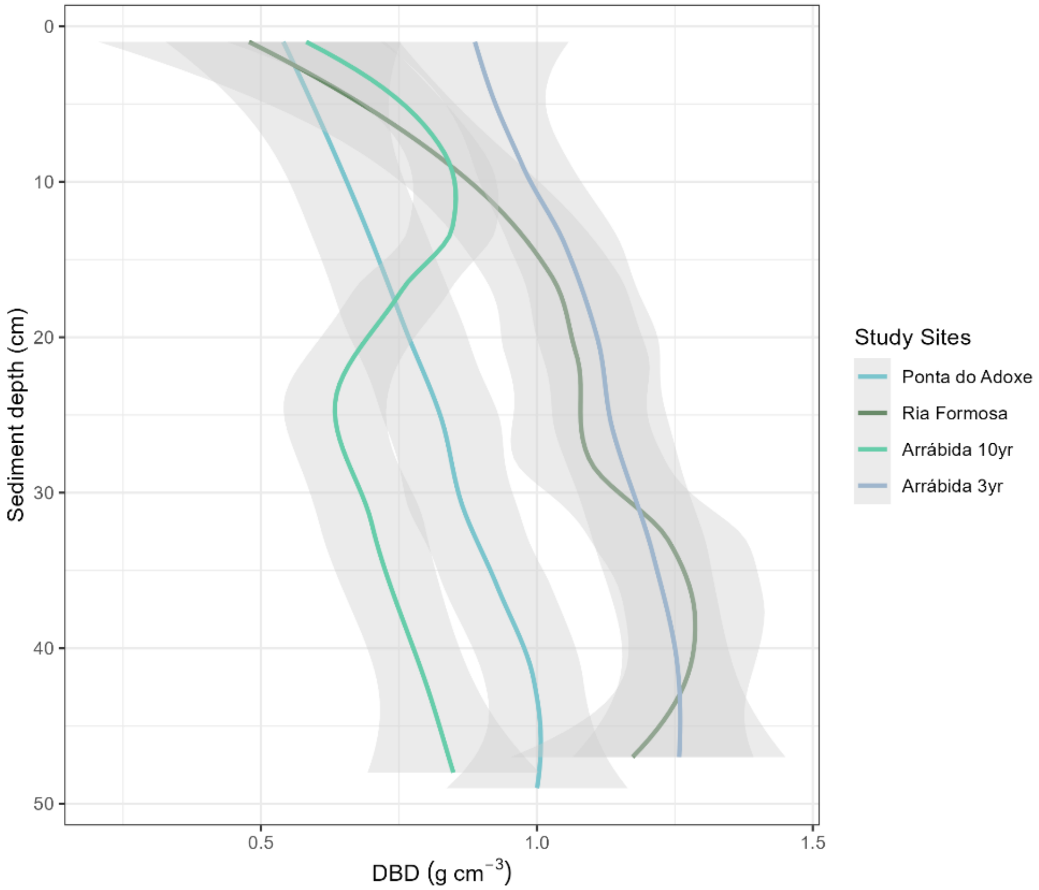

Figure 3.1. Sediment dry bulk density (g dw cm^{-3}) of the different study sites along sediment depth (cm).

Following the same pattern found in the OM profiles, TN and OC in the sediment presented higher values in PA and RF than in AR10 and AR3 (*Figure 3.2*). The OC content (0.03-2.46 %dw) was in general one order of magnitude higher than the TN content (0.01-0.27%dw). Highest OC values were found within natural meadows (PA: 0.03 – 2.46 %dw; RF: 0.06 –1.87 %dw) as well as for TN (PA: 0.02 – 0.27 %dw; RF: 0.02 – 0.21 %dw). Transplanted meadows in general, presented lower OC (AR10: 0.17-0.97 %dw; AR3: 0.10-0.77%dw) and TN (AR10: 0.03-0.11 %dw; AR3: 0.02-0.09 %dw). Mean values of 0.41 (OC %dw) and 0.05 (TN %dw) correspond to the 10 years old meadows while values for the 3 years old are 0.31 (OC %dw) and 0.04 (TN %dw), showing a slightly increase through time.

Results from the non-parametric Kruskal Wallis and Wilcoxon post-hoc tests on the top 0-50cm depth sediment layers, showed significant differences in the amount of OM, OC and TN deposited within the natural meadows, PA and RF ($p \leq 0.0001$) (*Figure 3.3*). Differences between the restored meadows and the nearby PA were significant only for the comparison with the 3 years old meadow ($p \leq 0.01$). Significant differences were also observed between meadows restored 10 and 3 years ago ($p \leq 0.01$) *(Figure3.3).*

Granulometry analyses (*Figure 3.4*) show predominant values of sand for all study sites (91 % dw average). Gravel content varied the most between locations, showing lower values within natural meadows. Mud values followed a gradient of depth, presenting highest amounts within the surface of natural meadows. Within the 10-yr restored meadow, a slight increase in the content of muddy sediments was noticeable around 30 cm depth, followed by a peak in the % of gravel around 20 cm occurring simultaneously for all replicates. This gravel intrusion was related with 2009 winter storm and established as base point for the 1st approximation strategy when developing the trajectory reconstruction.

Transplant operation consisted in the revegetation of bare sediment with sods (20x20x5cm) coming from Ria Formosa (Paulo et al., 2019). This technique could lead to intrusions of smaller and richer in OM sediment particles and is thought to be reason of slight increases in %OM (*Figure 3.2*) and fine sediment (*Figure 3.4*) around 15cm depth for Arrábida 10yr and around 5 cm for Arrábida 3yr. This conclusion has been used to establish the 2nd approximation strategy. Finally, the increase in %OM and fine sediment above 5cm depth has been considered as deposition in situ and the base for the 3rd approximation strategy (*Figure 3.4*)

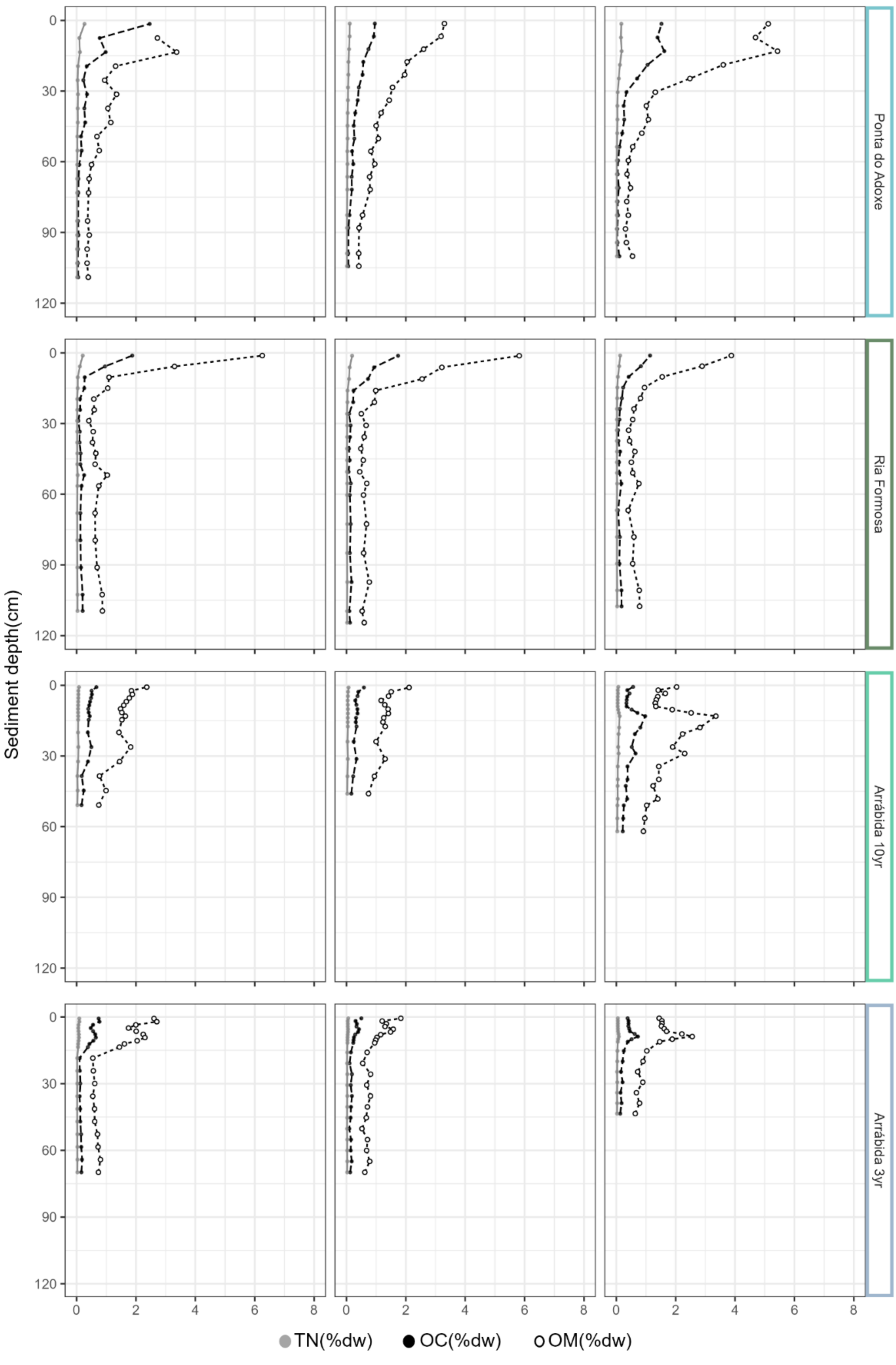

Figure 3.2. Total nitrogen (TN), organic carbon (OC) and organic matter (OM) contents expressed as % of dw are represented along sediment depth, showing the 3 replicates per study site.

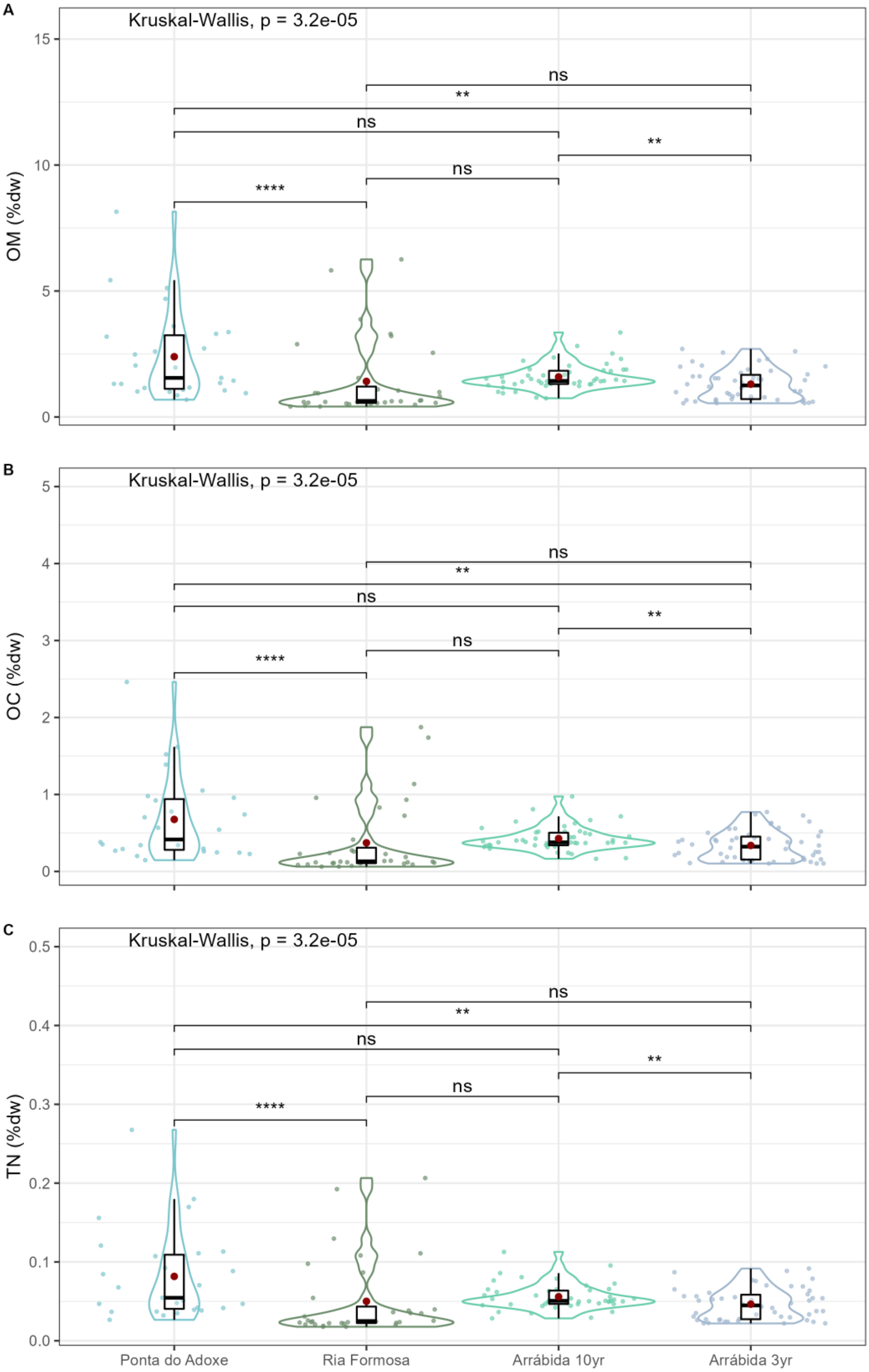

*Figure 3.3. A- Organic matter (OM), B- organic carbon (OC) and C- total nitrogen (TN)) contents expressed as % of dw in the superficial 50-cm sediment layer for the study sites. Red dots correspond to mean values and the black lines inside the boxplots correspond to the medians within each group. ****: $p \leq 0.0001$; ***: $p \leq 0.001$; **: $p \leq 0.01$; *: $p \leq 0.05$; ns: $p > 0.05$.*

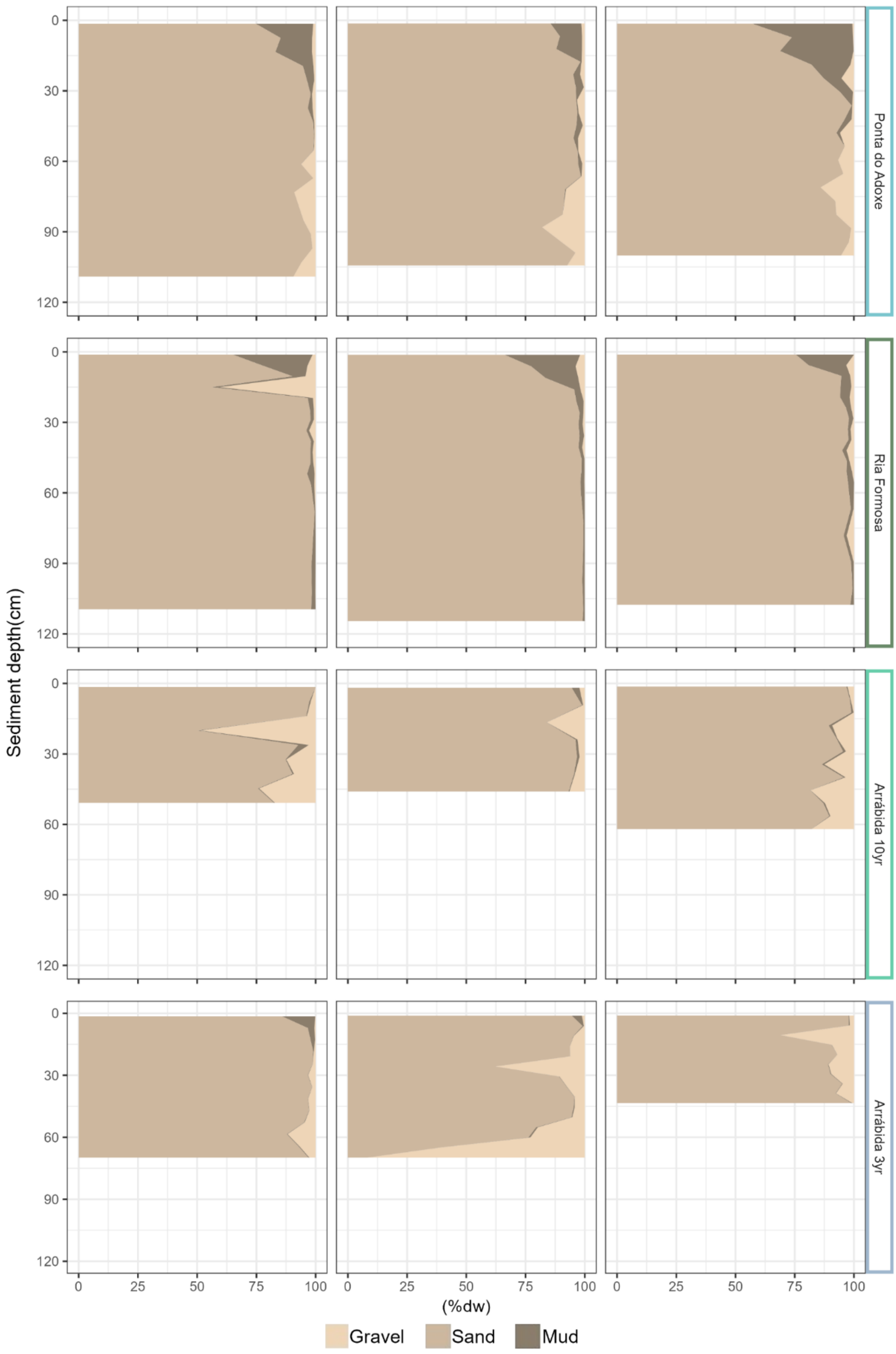

Figure 3.4. Gravel, sand and mud (%dw) are represented following sediment depth gradient for the different core replicates per study site.

3.2 Sedimentary stocks of organic matter, organic carbon, and total nitrogen in restored and natural meadows

Stocks (Mg ha^{-1}) integrated for the top 0-50cm of depth present the highest average values within PA natural meadows (OM stock=75.95 Mg ha^{-1}, OC stock=22.28 Mg ha^{-1}, TN stock= 2.77 Mg ha^{-1}) (*Figure 3.5*). Average values within the other study sites are lower (RF: OM stock= 50.01 Mg ha-1, OC stock=13.22 Mg ha-1, TN stock= 1.97 Mg ha-1; AR10: OM stock=51.09 Mg ha-1, OC stock=14.18 Mg ha-1, TN stock= 1.90 Mg ha-1; AR3: OM stock=48.76 Mg ha-1, OC stock=12.06 Mg ha-1, TN stock= 1.89 Mg ha-1), although differences between sites were not significant (Kruskal Wallis, p>0.05) (*Figure 3.5*). Certain variability existed between replicates per site (*Table 3.1*). Extrapolation applied in cores where maximum depth did not reach 50cm might resulted in overestimates for replicates AR010 and AR06. Other causes of variability need to be addressed in further research.

Table 3.1. Characteristics of the sediment cores collected from the Zostera marina meadows in the study sites: collection date, compaction factor, maximum depth reached during the extraction, OM, OC and TN stocks expressed in Mg ha¯1.

Core	Study Site	Date	Compaction factor	Max Depth (cm)	OM stock (Mg ha¯1)	OC stock (Mg ha¯1)	TN stock (Mg ha¯1)
AR02	Ponta do Adoxe	30/6/2020	0.67	109.03	70.31	20.45	2.59
AR03	Ponta do Adoxe	30/6/2020	0.74	104.33	75.43	21.78	2.77
AR04	Ponta do Adoxe	30/6/2020	0.69	100.10	82.11	24.61	2.97
SS07	Ria Formosa	22/9/2020	0.87	109.54	42.43	11.14	1.70
SS08	Ria Formosa	22/9/2020	0.81	114.54	55.23	15.22	2.13
SS09	Ria Formosa	22/9/2020	0.88	107.60	52.37	13.32	2.07
AR05	Arrábida 10yr	1/7/2020	0.65	50.84	44.89	12.39	1.67
AR06	Arrábida 10yr	1/7/2020	0.54	45.98	41.57	10.94	1.57
AR07	Arrábida 10yr	1/7/2020	0.73	62.00	66.82	19.19	2.44
AR08	Arrábida 3yr	1/7/2020	0.70	69.87	38.36	9.45	1.50
AR09	Arrábida 3yr	1/7/2020	0.82	69.87	47.60	11.41	1.87
AR010	Arrábida 3yr	1/7/2020	0.85	43.48	60.32	15.31	2.30

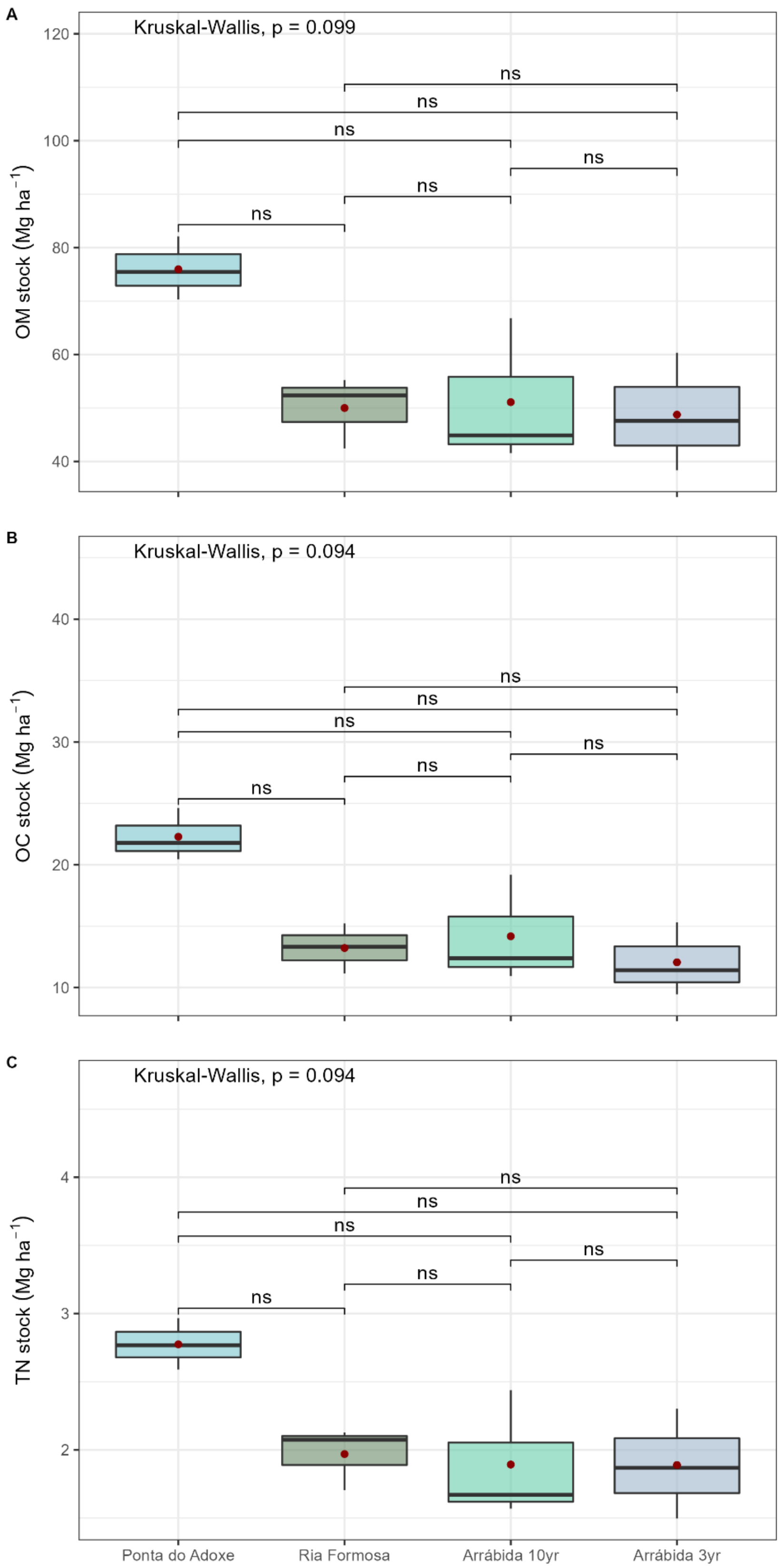

*Figure 3.5. A- Organic matter (Mg ha¯1), B- organic carbon (Mg ha¯1^{2}) and C-total nitrogen stocks (Mg ha¯1) in the study. Red dots correspond to mean values and the black lines inside the boxplots correspond to the medians within each group. ****: $p \leq 0.0001$; ***: $p \leq 0.001$; **: $p \leq 0.01$; *: $p \leq 0.05$; ns: $p > 0.05$.*

3.3 Estimated deposition rate in the restored meadows

The 1st approximation, based on the effect of the strong storm at Arrabida during the 2009 winter on sediment granulometry, i.e. the intrusion of gravel at ~20cm depth (Fig. 3.4), revealed an average value of 20.71 mm yr^{-1} on the 10 yr old meadows (*Table 3.2*). This signal is not present on the 3 yr old because the meadow was transplanted in 2017 and thus the deposition rates cannot be estimated in this case using this approximation. The 2nd approximation, based on the prompt increase in the amount of OM caused by the transplant (~ 15cm depth for Arrábida 10yr Fig. 3.2 and ± 5cm for Arrábida 3yr, Fig. 3.2), led to average values of 11.37 mm yr^{-1}. Following the 3rd approximation which only considers the superficial constant increase, treated as accumulation *in situ*, the SAR average for the 10-yr meadows is 2.38 mm yr^{-1}. For the 3rd approximation on the 3-yr meadow only the shallower sample (~ 0.5 cm) was used, giving an average SAR of 2.12 mm yr^{-1}.

Table 3.2. Estimated sediment accumulation rates (SAR) expressed as mm yr^{-1} for the 10- and 3-yr old restored meadows following 3 different approximation strategies.

Age (yr)	Core	1st Approximation	2nd Approximation	3rd Approximation
10	AR05	20.03	10.01	2.31
10	AR06	23.91	10.11	2.76
10	AR07	17.91	7.57	2.07
3	AR08	---	16.63	2.38
3	AR09	---	10.21	2.04
3	AR10	---	13.7	1.95

4. Discussion

4.1 Effect of restoration on sediment biogeochemistry

In order to understand the effect of restoration on the sediment biogeochemistry of *Z. marina* meadows, DBD, %OM, %OC, %TN contents and particle size among natural and transplanted meadows were analyzed and compared. DBD was higher within restored meadows, agreeing with previous studies (Greiner at al., 2013) in which it was shown that DBD decreased with the age of the meadow. Obtained DBD mean (±SD) values (0.95 ± 0.27 g cm^{-3}) were similar to those (0.99 ± 0.03 g cm^{-3}) reported by Lima et al. (2020) for temperate meadows in the UK where *Z. marina* appears mixed with other seagrass species and lower than values reported

(1.57 ± 0.08 g cm^{-3}) by Ricart et al. (2015) for *Zostera muelleri* meadows in Queensland (Australia).

OM (1.32 ± 1.08 % dw), OC (0.34 ± 0.34 % dw) and TN (0.05 ± 0.03 % dw) mean values for all sites and depths were within the range of previously reported values for other *Z. marina* meadows (Dahl et al., 2016; Prentice et al., 2020; Röhr et al., 2016), although great variability is shown between studies and most of the studies were based on superficial sediment samples (~ 0-20 cm). The comparison among sites based on the sediment depth profiles together with the OM, OC and TN content in the top 50 cm showed that PA presented higher amounts for all parameters, RF reach highest values in the top 30 cm and then decreases sharply. However, restored meadows, especially AR10, showed peaks of OM along depth leading to higher mean values than those in RF and that AR3 present lower mean values than AR10 and less disturbances along depth.

Several factors can influence sediment deposition in seagrass meadows (Burdige, 2007; Mazarrasa et al., 2018; Mazarrasa et al., 2021). RF and PA are located in a more closed coastal environment where current velocities are slower and sediment deposition is enhanced (Lavery et al., 2013; Martins et al., 2021; Santos et al., 2019). Contrarily, restoration site in the coast of the Arrábida is an open ocean setting where prevalence of southern winds and strong storms during winter are relatively normal thus affecting the stability of the sediment. This can explain why the seagrass sediment in the Arrábida meadows contained less mud than those found in the sheltered locations of Ria Formosa and Ponta de Adoxe.

Hydrodynamics are closely related with sediment grain size (Cabaço and Santos, 2010; Mazarrasa et al., 2017). Previous studies found that canopy and shoots densities are positively correlated with the trapping effect (Greiner at al., 2013) and inversely correlated with sediment erosion (Marbá et a., 2015), which ultimately will affect deposition and storage capacities of the meadows. Seagrass meadows with higher shoot density, will be more effective decreasing current velocity thus enhancing deposition of muddy sediment (Gacia et al., 2002; Hendriks et al., 2008; Dahl et al., 2016; Serrano et al., 2016). Mud particles have smaller interstitial spaces, which reduces permeability and oxygen penetration (Hedges, 1995). Therefore, high mud content within seagrass soils will enhance an anoxic environment where organic particles such us carbon and nitrogen will be less exposed to microbial action (Mayer, 1994; Burdige, 2007; Serrano et al., 2016; Miyajima et al., 2017). Consequently, RF and PA natural meadows, where shoot density is higher (personal observation), showed higher mud content than restored meadows where the canopy structure might need to increase in order to show greater amounts of fine, rich sediment (Lundquist et al., 2018; Orth et al., 2020).The study carried out by Greiner et al. (2013) on *Z. marina* meadows showed that densities achieved 4 years after planting, not only were insufficient to reduce resuspension and shallow mixing of sediment but accelerated flow around individual shoots and created turbulence.

Statistically significant differences on the amount of OM, OC and TN for the top 50 cm between Ria Formosa and Ponta do Adoxe indicate that even if both ecosystems are less exposed to the hydrodynamics effect than the Arrábida, other factors less perceptible, such as dissolved nutrients or turbidity, might be implicated and further research is needed. The fact that no significant differences were found between the donor and the transplanted meadows could be explained by the transplantation technique, leading to increased amounts of rich sediment in restored meadows that might belong to the donor meadow in the Ria Formosa. Further analysis will be needed to explore this possibility, for example comparing the sources of OM along the cores. The significant differences found between the transplanted meadow 3 years ago and the nearby natural meadow of Ponta do Adoxe, together with the unsignificant differences between the 10-year-old meadow and Ponta do Adoxe, indicates that differences between natural and restored meadows decreased with time. As it has been proved in previous studies regarding restored *Zostera marina* (Greiner et al., 2013; Orth et al., 2020; Aoki et al., 2020), the complexity of the meadow needs time to develop and become more functional and reach the sequestration rates observed in natural meadows. The time required to equal the donor or nearby seagrass meadow will depend on biotic and abiotic factors. Significant differences between restored meadows show that restoration can help increase deposition even in an open coast setting.

4.2 Effect of restoration on stocks of organic matter, organic carbon and total nitrogen

OM stock in the natural meadow of PA (75.95 ± 4.83 Mg OM ha^{-1}) was higher than those for the other study sites (RF: 50.01 ± 5.49 Mg OM ha^{-1}; AR10: 51.09 ± 11.09 Mg OM ha^{-1}; AR3: 48.76 ± 9.00 Mg OM ha^{-1}). Same pattern was observed for OC and TN stocks although the statistical tests revealed that the differences were not significant for any of the parameters studied. Average OC stock for all study sites (15.43 ± 4.73 Mg C ha^{-1}) falls within the range of previous ones calculated for other *Z. marina* meadows in Europe (Dahl et al. 2016). However, it is slightly lower than OC stocks estimated for different temperate seagrass species (33.80 ±

18.70 Mg C ha^{-1}; Lima et al., 2020) but higher than those estimated for the seagrass species present in the Red Sea (7.2 ± 0.4 Mg C ha^{-1}; Garcias Bonet et al., 201). Average TN stock calculated within this study (2.13 ± 0.47 Mg N ha^{-1}) is comprised within the range calculated by Kinderberg et al. (2018) for *Z. marina* meadows in the Danish coasts (0.2 to 4 Mg N ha^{-1}) and lower than the range calculated for *Z. nolti* meadows in Ria Formosa (7-11 Mg N ha^{-1}; Martins et al., 2021).

Similarities between RF donor meadow OC average stock (13.22 ± 1.67 Mg C ha^{-1}) and those estimated for restored meadows (AR10: 14.18 ± 3.59 Mg C ha^{-1}; AR3: 12.06 ± 2.44 Mg C ha^{-1}), might be cause by an intrusion of OC rich sediment coming from RF into the AR sediment during transplant operations. The fact that the standard deviation was 2 times higher in restored than in natural meadows for all parameters, indicates the instability of the sediment within the restoration site. It can be due to several factors, including the compaction pressure applied while collecting the cores, and the fact that this location has suffered from many disturbances along the years, including weather and human impacts (Cunha et al.,2014), to which this site was still exposed after the restoration (Paulo et a., 2019). Last *Z. marina* meadow in the Arrábida was spotted in 2006 (Cunha et al., 2013) and since then, the place has been subjected to greater erosion forces due to the absence of vegetation than places where seagrass meadows were present (Marbá et al., 2015).

Data collected within this study is not enough to clarify the effect of restoration on sedimentary stocks, although contributed to emphasize the need of further research regarding restored seagrass ecosystems, especially those located in open coast environments. Based on the information available in the literature, seagrass meadows with higher sedimentary stocks are those with higher deposition and burial capacity. These processes are enhanced by an environment with low O_2 and hydrodynamics, as well as high mud content and low DBD (Dahl et al., 2016; Mazarrasa et al., 2018). Therefore, restored seagrass meadows situated in enclosed environments, with a structure well developed and able to trap fine sediment particles, will have a better sequestration capacity that those meadows restored in places with high hydrodynamics and poor/ damaged structure.

4.3 Sediment accumulation rates in restored meadows

Given the lack of more precise results from dating methods, SAR (mm yr^{-1}) was roughly estimated based on a re-construction method with different approaches. The three approximation strategies revealed values that go from 23.91 mm yr^{-1}to 1.95 mm yr^{-1}. Even if only an estimate, the mean value obtained with the 3rd approximation (2.25 mm yr^{-1}) is similar to global values reported for seagrass meadows (2.0 mm yr^{-1}, Duarte et al. 2013). It is within the range of reported range values (0.8-9.5 mm yr^{-1}) for Z. *marina* meadows by Prentice et al. (2020), but lower than those reported for *Z. marina* in 10-yr-old meadows (6.6 mm yr^{-1}, Greiner et al., 2013) and those calculated by Martins et al. (2021) for *Z. noltei* meadows (3.69 - 15.76 mm yr-1). Estimates of SAR in the PA and RF meadows were not obtained (yet samples are being analysed in an external laboratory), but it is expected to be higher in these sites as a result of lower wave exposure (Nelleman et al., 2009; Mazarrasa et al., 2018) and a higher and denser canopy structure able to attenuate the current effect and facilitate sediment deposition (Rozaimi et al. 2013; Serrano et al., 2016).

Sediment accumulation rates are required to establish sequestration rates, one of the necessary steps when trying to develop carbon budgets following carbon financial guidelines (e.g., Verified Carbon Standard) that could lead to increase the benefits provided by the mitigation capacities of these seagrass meadows.

5. Conclusions

To sum up, findings within this study show that differences in sediment biogeochemistry between natural and restored meadow decreased with time, pointing out that overall, restoration is enhancing the recovery of the seagrass habitat in the Arrábida. Sedimentary stocks were similar between study sites, falling within the range of previous estimates for temperate *Zostera marina* meadows. However, the effect of restoration on the recovery of carbon and nitrogen sequestration remains unclear. Conservation of these valuable habitats will benefit from further research on potential sources of variability among the study sites such as hydrodynamics, canopy structure, human pressures, turbidity and sources of organic matter by eDNA analysis. Sediment accretion rates within this study were roughly estimated in order have a first advance and the accuracy will be improved in future research with dating techniques.

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