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Contribution of Seagrass Blue Carbon Toward Carbon Neutral Policies in a Touristic and Environmentally-Friendly Island

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Estimates of organic carbon (C_{org}) storage by seagrass meadows which consider inter-habitat variability are essential to understand their potential to sequester carbon dioxide (CO_2) and derive robust global and regional estimates of blue carbon storage. In this study, we provide baseline estimates of seagrass extent, and soil C_{org} stocks and accumulation rates from different seagrass habitats at Rottneest Island (in *Amphibolis* spp., *Posidonia* spp., *Halophila ovalis*, and mixed *Posidonia/Amphibolis* spp. meadows). The C_{org} stocks in 0.5 m thick seagrass soil deposits, derived from 24 cores, were 5.1 ± 0.7 kg C_{org} m^{-2} (mean \pm SE, ranging from 0.05 to 12.9 kg C_{org} m^{-2}), accumulating at 23.2 ± 3.2 g C_{org} m^{-2} $year^{-1}$ (ranging from 0.22 to 58.9 g C_{org} m^{-2} $year^{-1}$) over the last decades. There were significant differences in C_{org} content (%) and stocks (mg C_{org} cm^{-3}), stable carbon isotope composition of the soil organic matter ($\delta^{13}C$), and soil grain size among the seagrass meadows studied, highlighting that biotic and abiotic factors influence seagrass soil C_{org} storage. Mixed meadows of *Posidonia/Amphibolis* spp. and monospecific meadows of *Posidonia* spp. and *Amphibolis* spp. had the highest C_{org} stocks (ranging from 6.2 to 6.4 kg C_{org} m^{-2}), while *Halophila* spp. meadows had the lowest C_{org} stocks (1.2 ± 0.6 kg C_{org} m^{-2}). We estimated a total soil C_{org} stock of 48.1 ± 8.5 Gg C_{org} beneath the 755 ha of Rottneest Island's seagrasses, and a C_{org} sequestration capacity of 0.81 ± 0.06 Gg C_{org} $year^{-1}$, which is equivalent to the sequestration of $\sim 22\%$ of the island's current annual CO_2 emissions. Our results contribute to the existing global dataset on seagrass soil C_{org} storage and show a significant potential of seagrass to sequester CO_2 , which are particularly relevant in the context of achieving carbon neutrality through conservation actions in environmentally-marketed, tourist destinations such as Rottneest Island.

Keywords: organic carbon, coastal vegetated ecosystems, *Posidonia*, *Amphibolis*, *Halophila*, Rottneest Island, Western Australia

INTRODUCTION

The carbon storage capacity of seagrass has been recognized since the early 1980s (e.g., Smith, 1981) but interest has recently intensified with the recognition of blue carbon ecosystems and their potential to contribute to climate change mitigation (Duarte et al., 2005, 2013; Nellemann and Corcoran, 2009; Mcleod et al., 2011; Fourqurean et al., 2012a). While occupying only 0.1% of the ocean surface, seagrass ecosystems have been estimated to bury 27–44 Tg organic carbon (C_{org}) year⁻¹ globally, accounting for 10–18% of the total carbon burial in the oceans, and have soil C_{org} stocks comparable to those of temperate and tropical forests, mangroves, and tidal marshes (Duarte et al., 2005; Fourqurean et al., 2012a). However, there has been a trend to estimate seagrass C_{org} storage potential from a limited dataset, based largely on the C_{org} content of superficial soils and a limited range of seagrass habitats (Fourqurean et al., 2012a; Lavery et al., 2013).

Seagrasses comprise a wide variety of species across a range of depositional environments (Carruthers et al., 2007), and the variability in the sedimentary C_{org} stocks among seagrass habitats had been found to be high (up to 18-fold; Lavery et al., 2013). The seagrass itself may exert a primary control on C_{org} storage through its biomass, productivity, and nutrient content (Mateo et al., 1997; Lavery et al., 2013; Miyajima et al., 2015). In addition, both autochthonous (e.g., plant detritus and epiphytes) and allochthonous (e.g., seston and terrestrial matter) sources contribute to the C_{org} pool in seagrass soils (Hendriks et al., 2008; Kennedy et al., 2010). Moreover, once C_{org} is buried in the soil, biotic and abiotic factors are likely to control the degree of C_{org} accumulation and preservation (Mateo et al., 2006). These factors include the rates of sediment accumulation, sediment grain-size, and biochemical composition of the organic matter (Keil and Hedges, 1993; Torbatinejad et al., 2007; Serrano et al., 2016a), and also vary among seagrass meadows (De Falco et al., 2000; Kennedy et al., 2010). As such, considerable variation exists in the estimates of C_{org} storage in seagrass soils worldwide (ranging from 4.2 to 8.4 Pg C_{org} ; Fourqurean et al., 2012a) and for any given location (Lavery et al., 2013; Campbell et al., 2015; Röhr et al., 2016). In order to improve existing estimates of C_{org} storage in seagrass ecosystems, further studies that expand the current knowledge on geomorphological and biological factors driving C_{org} storage are needed (Serrano et al., 2016a; Gullström et al., 2018; Mazarrasa et al., 2018).

The recent focus on carbon trading provides the opportunity to avoid or mitigate CO₂ emissions through the conservation and restoration of seagrass meadows, which rank among the most endangered habitats in terms of global loss rates. Despite recent studies showed that seagrass extent remained stable or increased since 2000s (Carmen et al., 2019), seagrass losses have been estimated at 29% of their global extent since 1880 (Waycott et al., 2009), largely resulting from coastal eutrophication and mechanical disturbance (Short and Wyllie-Echeverria, 1996; Orth et al., 2006). Australia has one of the largest areas of seagrass worldwide (Carruthers et al., 2007) but over the last decades has experienced severe seagrass loss (e.g., Cambridge and McComb, 1984; Arias-Ortiz et al., 2018), even in relatively pristine environments such as in Rottnest Island, Western

Australia, a tourism destination but where the deployment of permanent moorings led to fragmented meadows (Walker et al., 1989; Hastings et al., 1995) and CO₂ emissions from seagrass soil C_{org} stocks (Serrano et al., 2016d).

While there is considerable interest in bringing seagrasses and other blue carbon ecosystems into national accounting and mitigation frameworks, there is also significant interest at a more local scale, with local or regional governments, or even private companies, often exploring the potential to become carbon neutral or offset their carbon emissions (e.g., Gössling, 2009). Therefore, Rottnest Island represents an important target area due to its large seagrass meadows and management strategies to mitigate climate change (RIA, 2018). Rottnest Island Authority (RIA) aims to implement actions to reduce greenhouse gas emissions and to investigate offset plans through revegetation programs (RIA, 2018); hence, the results from this study would be useful to guide potential carbon sequestration strategies in Rottnest Island. As with larger scale assessments, the known variability in seagrass carbon stocks requires that the area and the stocks of the different habitats are accounted for when making assessments.

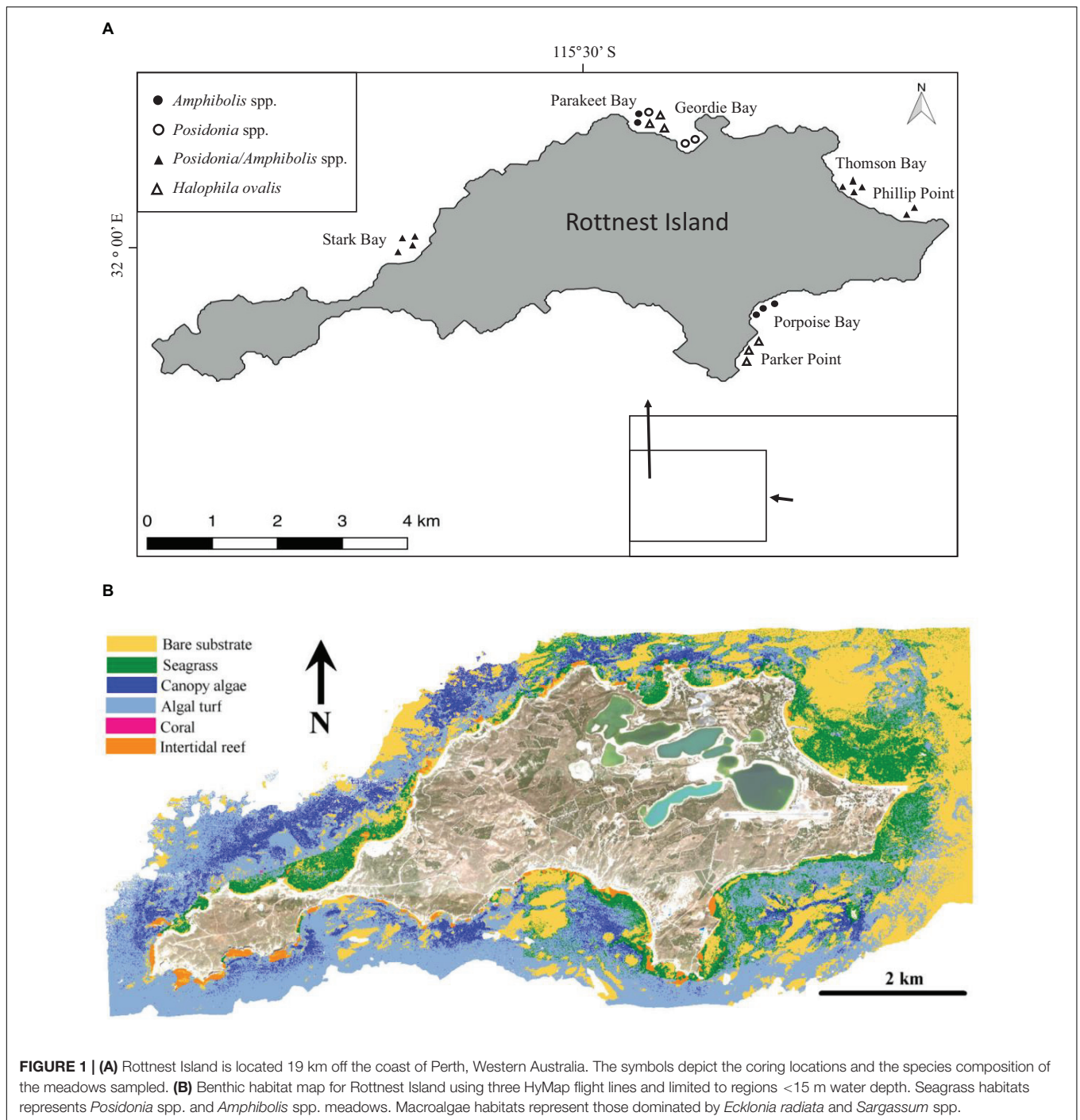
Here we present the results of an extensive survey of seagrass meadows along Rottnest Island (Perth, Western Australia), which included habitat mapping, to produce regional estimates of soil C_{org} stocks and sequestration rates, and also to provide insights into the contribution of seagrass conservation to achieve a carbon neutral policy in the Island. While this study focuses on one region, the results obtained contribute to understand the differences in C_{org} storage between seagrass species, highlighting the importance of accounting for habitat variability when scaling up estimates. Finally, to place our results within a broader context, we compare these data to estimates from global datasets, emphasizing recognized variation across seagrass habitats.

MATERIALS AND METHODS

Study Site, Sampling, and Laboratory Procedures

Rottnest Island is a marine reserve located 19 km off the coast of Perth (Western Australia, 32°00'07" S, 115°31'01" E), surrounded by extensive sub-tidal seagrass ecosystems generally found in sheltered bays and areas protected by reefs (Wells et al., 1993; RIA, 2015; **Figure 1**). Nine species of seagrass have been recorded at Rottnest Island, the dominant species belonging to the genera *Posidonia* and *Amphibolis* (Wells et al., 1993). *Posidonia sinuosa*, *Posidonia australis*, *Amphibolis antarctica* and *Amphibolis griffithii* all form mono-specific meadows and are considered to be “climax” species (Lavery and Vanderklift, 2002), but can also be found in mixed meadows (Kendrick et al., 2000; Carruthers et al., 2007).

To assess seagrass soil C_{org} stocks and accumulation rates, in 2014 we sampled soil cores ($N = 24$) from extensive seagrass meadows comprising mono-specific meadows of *Halophila ovalis* (6 cores), mixed meadows of *P. australis* and *P. sinuosa* (3 cores), mixed meadows of *A. antarctica* and *A. griffithii* (5 cores), and mixed *Posidonia* spp. and *Amphibolis* spp. meadows (10 cores)



along the north and southeast shores of the island (i.e., Thomson Bay, Stark Bay, Phillip Point, Porpoise Bay, Parakeet Bay, Parker Point, and Geordie Bay; **Figure 1** and **Supplementary Table S1**). The sites were chosen to be representative of seagrass meadows from a variety of habitats, including differences in biotic (e.g., species composition) and abiotic (e.g., hydrodynamic energy) settings. The cores were sampled within extensive seagrass meadows, and meadow edges were located > 50 m away in all cases.

Undisturbed soil cores were randomly sampled within continuous meadows using PVC pipes (70 mm in diameter, 75 cm long) that were gently hammered into the seafloor at 2–4 m water depth while being rotated to assist with penetration. The corers were sealed before retrieval (i.e., using lids and PVC tape) to avoid losing the unconsolidated sediments contained within the corers. Following retrieval, the core samples were cut into 1 cm thick slices. Each slice was weighed before and after oven drying at 60°C until constant weight [dry weight (DW)] to estimate the

dry bulk density (DBD). Then, every second slice was divided into two subsamples by quartering. One subsample was milled and analyzed for C_{org} and stable carbon isotope composition ($\delta^{13}C$), while the other subsample was used for sediment grain-size analyses.

For C_{org} and $\delta^{13}C$ analyses, 1 g of ground sample was acidified with HCl 4% until bubbling stopped to remove inorganic carbon, centrifuged (5 min at 3,400 r/min), and the supernatant removed carefully by pipette. Then, the sample was washed with Milli-Q water, centrifuged, and the supernatant removed. The residual samples were re-dried and then encapsulated for C_{org} analysis using a Micro Cube elemental analyzer (Elementar Analysensysteme GmbH, Hanau, Germany) interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, United Kingdom) at the University of California Davis. Carbon isotope ratios are expressed as δ values in parts per thousand (‰) relative to the Vienna PeeDee Belemnite. Replicate assays and standards indicated measurement errors of 0.01% for C_{org} content and 0.06‰ for $\delta^{13}C$. Content of C_{org} was calculated for bulk (pre-acidified) samples.

For sediment grain-size analysis, a Mastersizer 2000 laser-diffraction particle analyzer was used following digestion of the samples with 10% hydrogen peroxide to remove organic matter. Sediments were classified as coarse sand (<1 and >0.5 mm) medium sand (<0.5 and >0.25 mm), fine sand (<0.25 and > 0.125 mm), and very fine sand plus mud (<0.125 mm), according to a scale adapted from Brown and McLachlan (2010).

Data Analyses

The length of core barrel inserted into the soil and the length of retrieved soil were recorded in order to correct the core lengths for compression effects. The corrected core lengths ranged from 22 to 68 cm, and all variables studied here are referred to the corrected, uncompressed depths. C_{org} density ($g C_{org} cm^{-3}$) was calculated for each soil depth in each core by multiplying the DBD ($g cm^{-3}$) by the C_{org} concentration (%). For soil depths where C_{org} content (%) was not analyzed, we extrapolated the % C_{org} (i.e., by averaging the % C_{org} between above and below depths) and multiplied the % C_{org} by the DBD to obtain C_{org} density ($g C_{org} cm^{-3}$). To allow direct comparisons among sites, the standing stocks per unit area (cumulative stocks; $kg C_{org} m^{-2}$) were standardized to 50 cm thick deposits, which involved extrapolating linearly integrated values of cumulative C_{org} stocks with depth in 12 out of the 24 cores sampled (i.e., from 30 to 50 cm; $r^2 = 0.98$, $P < 0.001$; **Supplementary Figure S1**). Accumulation rates of C_{org} were estimated by dividing the inventories in the 50 cm thick soils by the mean soil accretion rate estimated for seagrass meadows at Rottneest Island (i.e., based on ^{210}Pb dating) by Serrano et al. (2016d).

The proportion of autochthonous and allochthonous C_{org} in the seagrass soil C_{org} pool was estimated using Stable Isotope Mixing Models in R (“simmr” and “rjags” packages; Parnell et al., 2010). The average $\delta^{13}C$ values within the top 50 cm of each core were analyzed for the probability of relative organic matter contribution to soil stocks using a one-isotope three-source mixing model (Zencich et al., 2002; Phillips and Gregg, 2003). The $\delta^{13}C$ signatures of potential autochthonous

and allochthonous C_{org} sources (mean \pm SD; $-10.8 \pm 1.6\%$ for seagrass, $-17.2 \pm 1.1\%$ for epiphytes plus macroalgae and microphytobenthos, and $-24.2 \pm 0.6\%$ for seston) were obtained from Waite et al. (2007), Ricart et al. (2015), and Serrano et al. (2016c).

All statistical analyses were performed using univariate general linear mixed model (GLMM) procedures in SPSS v. 14.0. GLMMs were used to accommodate the potential non-independence of samples taken at different depths within the same core, since depth is a proxy for time in the cores, and the unbalanced sampling design. The GLMMs were performed to test for significant effects of species composition (*Amphibolis* spp., *Posidonia* spp., *H. ovalis*, and *Posidonia/Amphibolis* spp.) on DBD ($g cm^{-3}$), C_{org} concentration (%), C_{org} content ($mg cm^{-3}$), $\delta^{13}C$ signatures, and sediments < 0.125 mm. Species composition and soil depth (cm) were treated as fixed factors, and study site (Thomson Bay, Stark Bay, Phillip Point, Porpoise Bay, Parakeet Bay, Parker Point, and Geordie Bay) was treated as a random factor (probably distribution: normal; link function: identity). A separate GLMM was run to test the effect of species composition (fixed factor) on cumulative C_{org} stocks ($kg C_{org} m^{-2}$). All response variables (DBD, C_{org} concentration, content and stock, $\delta^{13}C$ signatures, and sediment grain size fractions) were square-root transformed prior to analyses and had homogenous variances. Normality and homoscedasticity of model residuals were determined by visual estimation. Pearson correlation analysis was used to test for significant relationships among the variables studied.

Estimates of Seagrass Area and C_{org} Storage at Rottneest Island

Three flight lines of HyMap hyperspectral data were flown over Rottneest Island by HyVista Corporation in April 2004 with a ground resolution of 3.5 m. The data were corrected to remove sun-glitter and the influence of the atmosphere and water column, using the Modular Inversion and Processing System (MIPS; Heege and Fischer, 2004; Pinnel, 2007). MIPS uses a physically based process to extract information from the data on the water constituents, bathymetry, bottom cover type, and bottom reflectance, with no external inputs. The hyperspectral data were corrected using three generic bottom cover types: bare sediment, and light and dark submerged aquatic vegetation represented by spectral signatures extracted directly from the image.

The output of MIPS provides the spatial distribution of bare sediment and vegetated regions < 15 m depth within the Rottneest Island Marine Park boundaries. The vegetated regions were further classified to identify seagrass meadows and macroalgae using a hierarchical spectral separation classification algorithm in conjunction with a library of spectral reflectance signatures, measured *in situ*, describing the dominant benthic habitat components for Rottneest Island (Harvey, 2009). The classification algorithm calculated the probability of each benthic habitat component (e.g., *Posidonia* spp.) being the dominant component for each image pixel and assigning the pixel to the component with the highest probability. The results of this classification

required an additional step using the abiotic variables of mean sea level and wave exposure to systematically correct areas misclassified as seagrass as either intertidal reef (areas above mean sea level) or macroalgae (areas highly exposed to wave action). The seagrass meadows were further classified to those dominated by *Posidonia* spp. or *Amphibolis* spp., and macroalgae habitats were classified into those dominated by *Ecklonia radiata* or *Sargassum* spp. It should be noted it was not possible to identify *Halophila* spp. meadows using the data obtained due to its sparse growth and size, which means its spectral reflectance at a pixel level is dominated by the bare substrate. The extent of each habitat type was calculated using the number of pixels multiplied by the area of each pixel. Regional seagrass soil C_{org} stocks and accumulation rates at Rottnest Island were estimated considering inter-species variability, by multiplying the average C_{org} stocks and accumulation rates of *Posidonia* spp., *Amphibolis* spp., and mixed *Posidonia/Amphibolis* spp. by their respective extents around the Island.

RESULTS

The total area of seagrass within the Rottnest Island Marine Park was estimated to be 755 ha, with *Posidonia* spp. occupying 113 ha, *Amphibolis* spp. 257 ha, and mixed *Posidonia/Amphibolis* spp. meadows 385 ha. The sediments under seagrass meadows at Rottnest Island were mainly composed of medium and fine sands (70–80% of DW). The DBD ranged from 0.5 to 2.1 g cm^{-3} , while the C_{org} content (%) of the soils ranged from 0.05 to 3.8% and the $\delta^{13}C$ signatures of soil organic matter ranged from -8.6 to -24.5‰ . The soil C_{org} stocks and accumulation rates (in 50 cm thick deposits) across the 24 meadows studied ranged 200-fold from 0.05 to 12.9 kg $C_{org} m^{-2}$ and 0.22 to 58.9 kg $C_{org} m^{-2} year^{-1}$ (mean \pm SE, 5.1 ± 0.7 kg $C_{org} m^{-2}$ and 23.2 ± 3.2 g $C_{org} m^{-2} year^{-1}$, respectively; **Table 1**).

The DBD, proportion of sediment particles < 0.125 mm, and $\delta^{13}C$ signatures increased with soil depth within the top 10 cm of *Amphibolis* spp. and *H. ovalis* cores, and were relatively constant or declined in the *Posidonia* spp. and mixed *Posidonia/Amphibolis* cores (**Supplementary Figure S2**). Below 10 cm soil depth, DBD, sediment particles < 0.125 mm and $\delta^{13}C$ signatures remained constant or slightly declined down-core in all meadows, except for the increase in sediment particles < 0.125 mm and $\delta^{13}C$ signatures after 30 cm depth in *H. ovalis* cores. The soil C_{org} content (%) in *Posidonia* spp. and *Amphibolis* spp. showed no clear trends with soil depth, but C_{org} content declined in *Posidonia/Amphibolis* spp. and *H. ovalis* cores below 20 cm depth. Overall, there were no significant relationships ($P > 0.05$) between % C_{org} and the proportion of sediment particles < 0.125 mm ($R^2 = 0.02$), nor between % C_{org} and $\delta^{13}C$ signatures ($R^2 = 0.07$), except for *Halophila* cores that showed a weak but statistically significant positive relationship between % C_{org} and $\delta^{13}C$ signatures ($R^2 = 0.37$; **Supplementary Figure S3**).

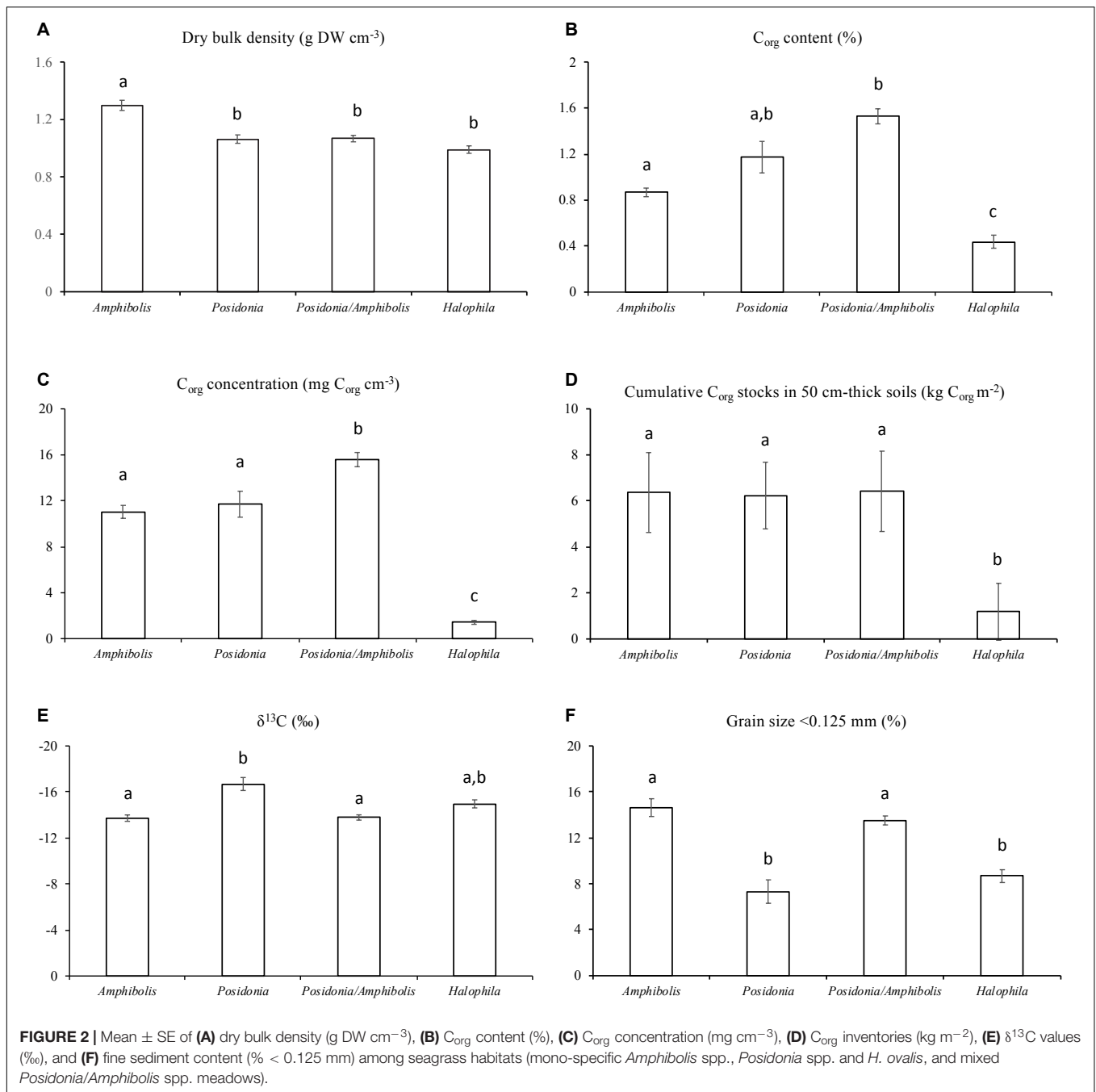
Amphibolis spp. meadows had significantly higher DBD (1.3 ± 0.03 g cm^{-3}) than *Posidonia* spp., *Posidonia/Amphibolis* spp., and *H. ovalis* meadows (ranging from 1.0 ± 0.03 to

TABLE 1 | Cumulative organic carbon (C_{org}) stocks [kg $C_{org} m^{-2}$; mean \pm standard error (SE)] and accumulation rates (g $C_{org} m^{-2} year^{-1}$) in 50 cm thick soil deposit in seagrass meadows at Rottnest Island, Western Australia.

Core ID	Species	kg $C_{org} m^{-2}$		
		Mean	Mean	SE
RT1	<i>Amphibolis</i> spp.	4.94	22.62	4.45
RT2	<i>Amphibolis</i> spp.	4.28	19.61	3.85
RT3	<i>Amphibolis</i> spp.	3.72	17.05	3.35
RT4	<i>Amphibolis</i> spp.	10.87	49.81	9.79
RT5	<i>Amphibolis</i> spp.	8	36.62	7.2
RT6	<i>Posidonia</i> spp.	5.79	26.5	5.21
RT7	<i>Posidonia</i> spp.	8.52	39.01	7.67
RT8	<i>Posidonia</i> spp.	4.39	20.1	3.95
RT9	<i>Posidonia/Amphibolis</i> spp.	3.5	16.05	3.15
RT10	<i>Posidonia/Amphibolis</i> spp.	2.75	12.6	2.48
RT11	<i>Posidonia/Amphibolis</i> spp.	3.5	16.02	3.15
RT12	<i>Posidonia/Amphibolis</i> spp.	9.51	43.55	8.56
RT13	<i>Posidonia/Amphibolis</i> spp.	6.73	30.82	6.06
RT14	<i>Posidonia/Amphibolis</i> spp.	5.7	26.11	5.13
RT15	<i>Posidonia/Amphibolis</i> spp.	5.6	25.65	5.04
RT16	<i>Posidonia/Amphibolis</i> spp.	7.45	34.13	6.71
RT17	<i>Posidonia/Amphibolis</i> spp.	6.53	29.91	5.88
RT18	<i>Posidonia/Amphibolis</i> spp.	12.85	58.86	11.57
RT19	<i>H. ovalis</i>	1.24	5.68	1.12
RT20	<i>H. ovalis</i>	4.17	19.08	3.75
RT21	<i>H. ovalis</i>	0.97	4.47	0.88
RT22	<i>H. ovalis</i>	0.28	1.28	0.25
RT23	<i>H. ovalis</i>	0.37	1.69	0.33
RT24	<i>H. ovalis</i>	0.05	0.22	0.04
Average \pm SE		5.07 \pm 0.69	23.23 \pm 3.15	

The C_{org} accumulation rates were estimated based on the seagrass soil accumulation rates from Rottnest Island provided in Serrano et al. (2016b).

1.1 ± 0.02 g cm^{-3} ; $P < 0.001$; **Figure 2A** and **Table 2**). The C_{org} content was significantly lower in *H. ovalis* meadows ($0.4 \pm 0.1\%$ C_{org} and 1.4 ± 0.2 mg $C_{org} cm^{-3}$) compared to the other meadows (ranging from 0.9 ± 0.004 to $1.5 \pm 0.1\%$ C_{org} and 11.0 ± 0.5 and 15.6 ± 0.6 mg $C_{org} cm^{-3}$; $P < 0.001$), while mixed *Posidonia/Amphibolis* spp. meadows contained higher C_{org} content ($1.5 \pm 0.1\%$ C_{org} and 15.6 ± 0.6 mg $C_{org} cm^{-3}$) than mono-specific *Amphibolis* spp. meadows ($0.9 \pm 0.03\%$ C_{org} and 11.0 ± 0.6 mg $C_{org} cm^{-3}$; $P < 0.001$; **Figures 2B,C**). The $\delta^{13}C$ values of organic matter in *Posidonia* spp. soils were significantly lower ($-16.7 \pm 0.6\text{‰}$) than in *Posidonia/Amphibolis* spp., *Amphibolis* spp., and *H. ovalis* meadows (-13.8 ± 0.2 , -13.7 ± 0.3 , and $-15.0 \pm 0.3\text{‰}$, respectively; $P < 0.001$; **Figure 2E** and **Table 2**). Therefore, the contribution of seagrass-derived organic matter to the soil C_{org} pool was relatively lower in *Posidonia* spp. ($33 \pm 2\%$) compared to the other meadows (ranging from 53 to 66%; **Figure 3** and **Supplementary Table S2**). The proportion of sediment particles < 0.125 mm was significantly higher in *Amphibolis* spp. and *Posidonia/Amphibolis* spp. (14.6 and 13.5%, respectively) than in *Posidonia* spp. and *H. ovalis* meadows (7.3 and 8.7%, respectively; $P < 0.001$; **Figure 2F** and **Table 2**).



The C_{org} stocks and accumulation rates in *H. ovalis* meadows (1.2 ± 0.6 kg C_{org} m⁻² and 5.4 ± 0.4 g C_{org} m⁻² year⁻¹) were up to fivefold lower than in *Posidonia/Amphibolis* spp. (6.4 ± 1.0 kg C_{org} m⁻² and 29.4 ± 1.8 g C_{org} m⁻² year⁻¹), *Amphibolis* (6.4 ± 1.3 kg C_{org} m⁻² and 29.1 ± 2.6 g C_{org} m⁻² year⁻¹), and *Posidonia* (6.2 ± 1.2 kg C_{org} m⁻² and 28.5 ± 3.2 g C_{org} m⁻² year⁻¹) meadows ($P < 0.001$), which were similar among them (Figure 2D and Table 1). The total seagrass C_{org} stocks in 0.5 m thick soils in Rottneest Island, accounting for inter-species variability, were estimated to be 48.1 ± 8.5 Gg C_{org}. Of this total stock, 34% was contained within

monospecific *Amphibolis* spp. meadows, 14% in monospecific *Posidonia* spp. meadows, and the remaining 51% in mixed meadows of *Posidonia/Amphibolis* spp. These contributions are directly proportional to the area of each seagrass habitat. The seagrass meadows in Rottneest Island were estimated to have accumulated 220 ± 17 Mg C_{org} year⁻¹ over the last decades. As with C_{org} stocks, the contribution of each habitat to the total annual sequestration was in direct proportion to their area. The seagrass C_{org} stocks in Rottneest Island are equivalent to 177 ± 31 Gg CO₂ captured at a rate of 0.81 ± 0.06 Gg CO₂ year⁻¹ (Table 3).

TABLE 2 | (A) Results of statistical testing (GLMMs) for significant effects of seagrass species and soil depth (cm) on the dry bulk density (g DW cm⁻³), organic carbon content (%C_{org} and g C_{org} cm⁻³), cumulative soil C_{org} stocks at 50 cm depth (kg m⁻²), stable carbon isotope values (δ¹³C), and the content of soil particles < 0.125 mm. (B) *Post hoc* tests showing differences in the variables studied among seagrass species.

(A)		df	SS	F	P
Density (g DW cm ⁻³)	Seagrass species	3	0.241	13.705	<0.001
	Soil depth	41	0.013	0.755	0.86
C _{org} content (%)	Seagrass species	3	6.324	68.664	<0.001
	Soil depth	41	0.080	0.873	0.69
C _{org} content (mg cm ⁻³)	Seagrass species	3	127.94	183.853	<0.001
	Soil depth	41	0.751	1.080	0.35
Cumulative mass C _{org} (kg m ⁻²)	Seagrass species	3	3.620	10.627	<0.001
δ ¹³ C (‰)	Seagrass species	3	1.782	11.801	<0.001
	Soil depth	41	0.173	1.147	0.26
<0.125 mm (%)	Seagrass species	3	5.291	10.726	<0.001
	Soil depth	38	0.227	0.46	0.98

(B)		C _{org} content (%)			
		Amphibolis	Posidonia	Posidonia/Amphibolis	Halophila
Density (g DW cm ⁻³)	Amphibolis		NS	<0.001	<0.001
	Posidonia	<0.001		<0.05	<0.001
	Posidonia/Amphibolis	<0.001	NS		<0.001
	Halophila	<0.001	NS	NS	
		δ ¹³ C (‰)			
		Amphibolis	Posidonia	Posidonia/Amphibolis	Halophila
C _{org} concentration (mg cm ⁻³)	Amphibolis		<0.001	NS	<0.01
	Posidonia	NS		<0.001	<0.001
	Posidonia/Amphibolis	<0.001	<0.01		NS
	Halophila	<0.001	<0.001	<0.001	
		Cumulative mass C _{org} (kg m ⁻²)			
		Amphibolis	Posidonia	Posidonia/Amphibolis	Halophila
< 0.125 mm (%)	Amphibolis		NS	NS	<0.001
	Posidonia	<0.001		NS	<0.001
	Posidonia/Amphibolis	NS	<0.001		<0.001
	Halophila	<0.001	NS	<0.001	

DISCUSSION

Variability in Seagrass C_{org} Storage Among Habitats

The soil %C_{org} content in *Posidonia* spp. (0.9–1.4%), *Amphibolis* spp. (0.6–0.9%), *Halophila* spp. (0.05–1.4%), and mixed *Posidonia/Amphibolis* spp. (0.7–2.3%) meadows measured in this study (Figure 2B) are comparable to global values from meadows dominated by the seagrass *P. sinuosa* and *P. australis* (0.1–2.1%), *A. antarctica* and *A. griffithii* (0.4–3%), and *Halophila* spp. (0.6–1.2%; Fourqurean et al., 2012b; Lavery et al., 2013; Serrano et al., 2014; Campbell et al., 2015). The relatively low soil C_{org} content in Rottne Island seagrasses (1.15% C_{org}) compared to the global average (2% C_{org}; Fourqurean et al., 2012a) resulted mainly from the low C_{org} content in *Halophila* spp. meadows

and to the fact that global estimates are likely biased by extremely high C_{org} storage values from some temperate seagrass meadows, especially *Posidonia oceanica*.

The seagrass soil C_{org} stocks at Rottne Island (0.05–12.9 kg C_{org} m⁻² in 0.5 m thick soils) are in the low range compared to global estimates (ranging from 12 to 83 kg C_{org} m⁻² in 1 m thick soils; Fourqurean et al., 2012a). However, the global estimates were derived from a limited data set biased by the extremely high C_{org} content of soils from Mediterranean *P. oceanica* meadows (Fourqurean et al., 2012a). Recent estimates of soil C_{org} stocks in seagrass meadows encompassed by low biomass species (e.g., *Halodule uninervis*, *H. ovalis*, *Halophila stipulacea*, *Zostera* spp., and *Thalassia hemprichii*) ranged from 0.1 to 5.4 kg C_{org} m⁻² (in 0.5 m thick deposits; estimated from Campbell et al., 2015; Macreadie et al., 2015; Serrano et al., 2018), which are within

TABLE 3 | Differences in area (ha), organic carbon (C_{org}) stocks (in 0.5 m thick soils; $kg C_{org} m^{-2}$) and accumulation rates ($g C_{org} m^{-2} year^{-1}$) per unit area, C_{org} stocks (in 0.5 m thick soils; $Gg C_{org}$), and C_{org} accumulation rates ($Mg C_{org} year^{-1}$) and CO_{2-eq} ($Gg CO_2$, $Gg CO_2 year^{-1}$) among seagrass habitats at Rottneast Island.

	<i>Amphibolis</i> spp.	<i>Posidonia</i> spp.	<i>Posidonia/Amphibolis</i> spp.	<i>Halophila ovalis</i>
Area (ha)	257 (34%)	113 (15%)	385 (51%)	–
C_{org} stock ($kg C_{org} m^{-2}$)	6.4 ± 1.3	6.2 ± 1.2	6.4 ± 1.0	1.2 ± 0.6
$g C_{org} m^{-2} year^{-1}$	29.1 ± 2.6	28.5 ± 3.2	29.4 ± 1.8	5.4 ± 0.4
$Gg C_{org}$ (Rottneast Is.)	16.4 ± 3.3	7.0 ± 1.4	24.6 ± 3.8	–
$Mg C_{org} year^{-1}$ (Rottneast Is.)	74.8 ± 7	32.2 ± 3.6	113.2 ± 6.9	–
$Gg CO_2$ (Rottneast Is.)	60.4 ± 12.3	25.7 ± 5.0	90.4 ± 14.1	–
$Gg CO_2 year^{-1}$ (Rottneast Is.)	0.27 ± 0.02	0.12 ± 0.01	0.42 ± 0.03	–

The values in parentheses indicate the percentage of the total.

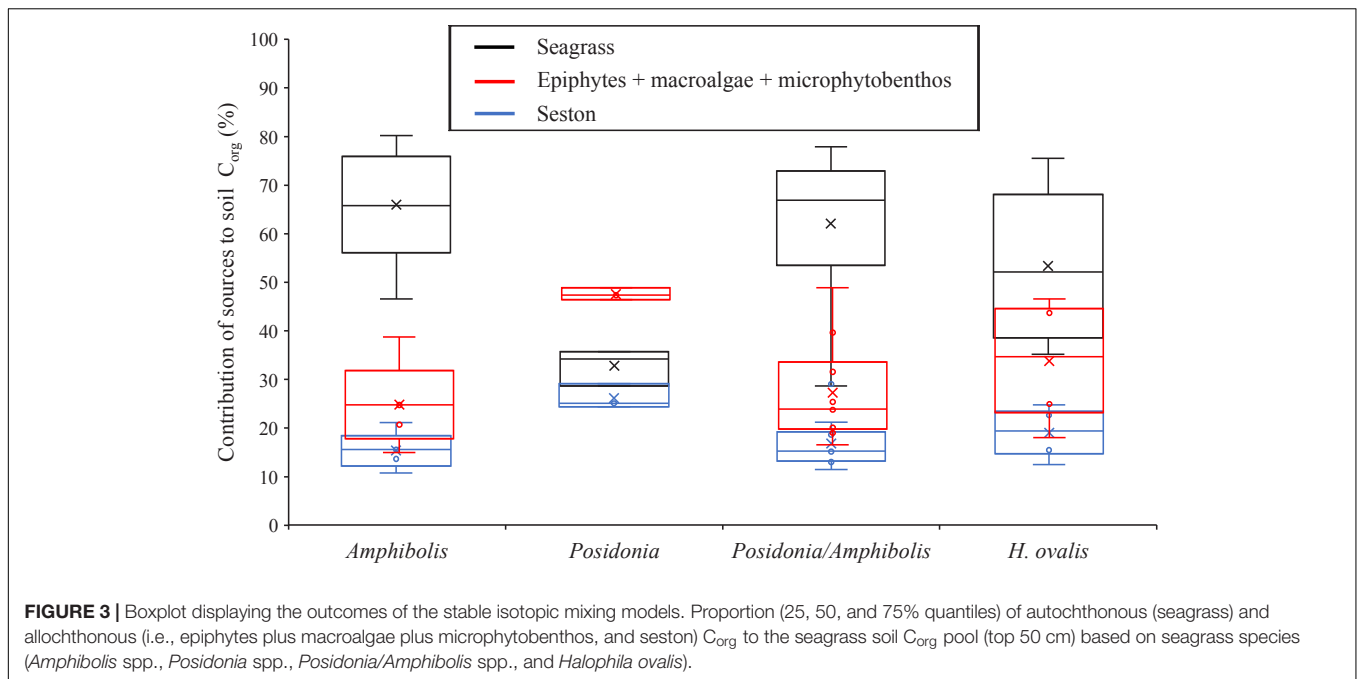
the range of C_{org} stocks estimated for low biomass *H. ovalis* meadows at Rottneast Island (ranging from 0.05 to 4.2 $kg C_{org} m^{-2}$). In addition, the C_{org} stocks in meadows with high biomass species (*P. australis* and *P. sinuosa*) at Rottneast Island (ranging from 2.7 to 12.9 $kg C_{org} m^{-2}$) is in the higher range of other meadows with similar species composition (e.g., ranging from 0.9 to 3.6 $kg C_{org} m^{-2}$; estimated for 0.5 m thick soils from Rozaimi et al., 2016 and Serrano et al., 2016b), but fourfold higher than previous studies on *Amphibolis* spp. meadows (1.6 $kg C_{org} m^{-2}$ in 0.5 m thick soils; estimated from Lavery et al., 2013). Hence, despite C_{org} stocks in Rottneast Island seagrass soils tending to be in the lower range of global estimates, the comparisons above highlight the need to update the global estimate of C_{org} content in seagrass soils using a more balanced geographical distribution of seagrass meadows, but also accounting for habitat variability (i.e., diversity of morphological traits across species and geomorphology; Mazarrasa et al., 2018).

Seagrass biomass and net primary production have been found to play a key role in C_{org} storage, with dense meadows formed by large species storing larger amounts of C_{org} in their soils (Fourqurean et al., 2012a; Lavery et al., 2013; Serrano et al., 2016c). The results obtained in this study support this generalization, with up to fivefold higher C_{org} stocks in *Posidonia* spp. and *Amphibolis* spp. meadows compared to *H. ovalis* meadows. Seagrass meadows of the genera *Posidonia* (*P. australis* and *P. sinuosa*) and *Amphibolis* (*A. antarctica* and *A. griffithiae*) are composed of large and long-living species with high above- and below-ground biomass (averaging 500 and 1,000 $g DW m^{-2}$, respectively), whereas *H. ovalis* is a small and fast-growing species which forms relatively low biomass meadows (76 $g DW m^{-2}$; Duarte and Chiscano, 1999). These differences in biomass likely contributed to the higher C_{org} stocks in *Posidonia/Amphibolis* spp. meadows compared to *H. ovalis* meadows. Another factor that may contribute to higher C_{org} stocks in monospecific and mixed *Posidonia/Amphibolis* spp. meadows is their capacity to form bulky roots and rhizomes that can penetrate deep into the soil, helping to more rapidly bury dead roots and rhizomes in deeper, anoxic soils, which favors their preservation (Mateo et al., 2006), further enhanced by the higher recalcitrance of these tissues (Trevathan-Tackett et al., 2017). The higher C_{org} content (% and $mg C_{org} cm^{-3}$) in mixed *Posidonia/Amphibolis* spp. meadows compared to monospecific meadows could be related to the higher functional performance (e.g., productivity) of meadows formed by multiple species (Duarte, 2000), as observed in mangroves

where monotypic forests have lower C_{org} stocks than those comprising multiple species (Atwood et al., 2017).

Mud content is a good predictor of soil C_{org} content in *Halophila* spp. meadows but not in mixed *Posidonia/Amphibolis* spp. meadows (Serrano et al., 2016a), where the larger contributions of seagrass matter to soil C_{org} pools dominates the contributing factors. Consequently, the higher $\delta^{13}C$ values (typical of seagrasses compared to other source of carbon) in meadows of larger seagrass species have been associated with higher C_{org} storage (Serrano et al., 2016a). The higher contribution of seagrass-derived C_{org} to the soil C_{org} pool in *Amphibolis* spp. and *Posidonia/Amphibolis* spp. meadows (ranging from 62 to 66%) support this hypothesis (Figure 3). However, the relatively higher soil C_{org} stocks in *Posidonia* spp. meadows were not linked to relatively high seagrass- C_{org} contributions (33% on average), while the relatively high contribution of seagrass-derived C_{org} in *H. ovalis* meadows (averaging 53%) did not entail relatively higher soil C_{org} stocks. The significant positive relationship between % C_{org} and $\delta^{13}C$ values in *Halophila* spp. meadows is consistent with the previous observation that soil C_{org} stocks increase with increasing contribution of seagrass-derived C_{org} in the soil pool (Serrano et al., 2016a). However, the lack of significant relationships between % C_{org} and particles < 0.125 mm, and between % C_{org} and $\delta^{13}C$ within monospecific and mixed *Posidonia* spp. and *Amphibolis* spp. meadows precludes any generalizable conclusion about the influence of sediment grain-size and seagrass-detritus contribution on soil C_{org} storage. Other habitat characteristics known to play a role in seagrass soil C_{org} storage (e.g., differences in hydrodynamic energy, plant cover, density, and biomass; Mazarrasa et al., 2018) among study sites, which were not considered in this study, may help to explain the observations.

The C_{org} accumulation rates in Rottneast Island seagrass ($23.1 \pm 3.2 g C_{org} m^{-2} year^{-1}$ on average) are much lower than global estimates ($138 \pm 38 g C_{org} m^{-2} year^{-1}$; Duarte et al., 2013). However, the C_{org} accumulation rates in *Posidonia* spp. meadows at Rottneast Island ($28 \pm 3 g C_{org} m^{-2} year^{-1}$) are similar to previous estimates for *Posidonia* spp. meadows in Australia (12 ± 7 to $26 \pm 0.8 g C_{org} m^{-2} year^{-1}$; Marbà et al., 2015; Serrano et al., 2016b), but lower than previous estimates for *P. oceanica* meadows in the Mediterranean Sea ($84 \pm 20 g C_{org} m^{-2} year^{-1}$), which are the highest in the world (Serrano et al., 2016b). The low accumulation rates in *Halophila* meadows ($5.4 \pm 0.4 g C_{org} m^{-2} year^{-1}$), are similar to previous



estimates for low biomass and fast-growing seagrass species (i.e., *Zostera* spp., *T. hemprichii*, *Enhalus acoroides*, and *Cymodocea serrulata*) from Japan, ranging from 2.1 to 10.1 g C_{org} m⁻² year⁻¹ (Miyajima et al., 2015).

Contribution of Seagrasses Toward Carbon Neutrality at Rottneast Island

Seagrasses are widely distributed along the coast at Rottneast Island, occupying 755 ha within the Marine Park (excluding meadows formed by small seagrasses such as *Halophila* spp.), and store considerable amounts of C_{org} in their soils. In this study, it was not possible to incorporate inter-species variability in C_{org} storage (i.e., higher C_{org} stocks in *Posidonia* and *Amphibolis* spp. compared to *H. ovalis* meadows) because of mapping constraints for *Halophila* spp. meadows. Thus, there is a need to improve seagrass mapping (i.e., including extent of small and large seagrasses) to robustly estimate seagrass blue carbon ecosystem service at Rottneast Island and globally.

Accounting for C_{org} storage, we estimated that seagrass meadows at Rottneast Island store $\sim 48 \pm 8$ Gg C_{org} within the top 0.5 m soils, which is equivalent to roughly 48 years of local CO_2 emissions at 2015 rates (3,650 Mg CO_2 year⁻¹; RIA, 2015). Soil C_{org} stocks at Rottneast Island have been accumulating at a rate of 0.81 ± 0.06 Gg C_{org} year⁻¹ over the last decades, thereby sequestering C_{org} at a rate equivalent to $\sim 22\%$ of current annual CO_2 emissions from the Island (ranging from 20 to 24%). This highlights the significant role of seagrass meadows in sequestering CO_2 .

The estimates of total C_{org} storage and CO_2 sequestration in Rottneast Island provided here are overall conservative because: (1) we did not incorporate C_{org} storage within small meadows such as those formed by *H. ovalis* due to the lack

of knowledge on their extent, (2) we restricted our estimates within the 755 ha of mapped seagrass area within the Rottneast Marine Park (up to 15 m depth), and (3) our estimates only considered the C_{org} stocks within the top 50 cm of soil, while some habitats may contain deeper seagrass C_{org} stocks. In addition, the large extent of macroalgae at Rottneast Island (190 ha for canopy-forming macroalgae and 1,192 ha for turf algae; Harvey, 2009) could also contribute to C_{org} sequestration in marine sediments and the deep ocean (Krause-Jensen and Duarte, 2016). In particular, our results showed that macroalgae (plus epiphytes) contributed 12–45% (28% on average) to the seagrass soil C_{org} pool, suggesting that the export of macroalgae biomass largely contributes to seagrass blue carbon (Kennedy et al., 2010).

Seagrass ecosystems around Rottneast Island were under threat and declining due to anchoring damage (Hastings et al., 1995), which lead to the loss of seagrass, and their associated carbon sink capacity, while eroding the carbon stocks (Serrano et al., 2016d). The deployment of 893 moorings along the coast of Rottneast Island since 1950s has resulted in the loss of 4.8 ha of seagrass meadows (RIA, 2015), which led to the erosion of existing sedimentary C_{org} stocks and the lack of further accumulation of C_{org} (Serrano et al., 2016d). Assuming an average loss of 48 Mg C_{org} ha⁻¹ following mooring deployment (Serrano et al., 2016d), we estimate cumulative emissions of 845 Mg CO_2 -eq from soil C_{org} stocks (within 4.8 ha of seagrass loss), and a lack of sequestration of 3.9 Mg CO_2 -eq ha⁻¹ year⁻¹ (cumulative lack of sequestration over 60 years estimated at 235 Mg CO_2 -eq year⁻¹). Seagrass conservation and restoration measures resulting in enhanced C_{org} sequestration and/or avoided emissions at Rottneast Island can help offset CO_2 emissions by human activities in the island. For example, the revegetation of the 4.8 ha mooring scar extent could result in

enhanced sequestration of 19 Mg CO₂-eq year⁻¹, which would offset 0.5% of annual CO₂ emissions at Rottneast Island (3,650 Mg CO₂ year⁻¹; RIA, 2018).

CONCLUSION

Rottneast Island is a popular tourism destination, with approximately half a million visitors per year, with 150,000 arriving by private vessel (Smallwood and Beckley, 2008). The RIA is committed to increasing the environmental, social, and economic sustainability of the Island through increasing the placement of renewable energy, increasing energy efficiency and actions to promote carbon sequestration through potential revegetation programs. This is reflected in the lower CO₂ emissions at Rottneast Island (0.007 Mg CO₂ per capita¹) compared to the average emissions in Australia (16 Mg CO₂ per capita). Therefore, through the incorporation of seagrass C_{org} sequestration in national greenhouse gas inventories (IPCC, 2003), Rottneast Island is well positioned to improve its carbon neutral policy. The CO₂ sequestration capacity of seagrass ecosystems provides one means of valuing these ecosystems and would provide economic and development opportunities for coastal communities in Rottneast Island and around the world, thereby potentially generating economic income through carbon trading schemes based on the conservation and restoration of seagrass meadows resulting in enhanced C_{org} sequestration and/or avoided CO₂ emissions (Kelleway et al., 2017). For instance, the replacement of existing moorings at Rottneast Island for environmental-friendly moorings could contribute to minimize seagrass fragmentation and enhance recolonization of bare areas (Demers et al., 2013), thereby enhancing C_{org} sequestration and reducing CO₂ emissions.

Despite the low C_{org} stocks of *Halophila* meadows it is important not to neglect their contribution to ecosystem dynamics. As they are small pioneer species, in temperate regions they are extremely important in colonizing the gaps and scars created by human and natural disturbances, facilitating the establishment of long-living and large biomass seagrasses such as *Posidonia* spp. and *Amphibolis* spp. after disturbance (Duarte et al., 1997; Duarte, 2000). Our results showed that there is a need to incorporate inter-habitat variability in regional estimates of seagrass blue carbon resource, in particular accounting for differences between large and long living seagrass such as *Posidonia* spp. and *Amphibolis* spp. vs. small and short living species such as *H. ovalis*.

Our results contribute to the existing global dataset on seagrass soil C_{org} storage. Furthermore, the results show a significant potential of seagrass to sequester CO₂, which are particularly relevant in the frame of achieving carbon neutral in environmentally-friendly tourist destinations. Indeed, conservation actions aimed to enhance C_{org} sequestration and/or avoid CO₂ emissions from disturbed seagrass ecosystems could contribute to offset CO₂ emissions at Rottneast Island, and it will also help to protect one of the most valuable ecosystems

in the world considering the ecological services they provide (Costanza et al., 1997).

DATA AVAILABILITY STATEMENT

All datasets generated for this study are included in the article/Supplementary Material.

ETHICS STATEMENT

The Ethics Committee of Edith Cowan University has approved this study.

AUTHOR CONTRIBUTIONS

OS designed the study. CB, OS, MH, and CD carried out the field and laboratory measurements. MH mapped habitat types. CB and OS analyzed the data and drafted the first version of the manuscript. All authors contributed to the writing and editing of the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fmars.2020.00001/full#supplementary-material>

FIGURE S1 | Relationship between extrapolated and measured organic carbon stocks (a) from 30 cm to 50 cm in seagrass soil cores \geq 50 cm depth.

FIGURE S2 | Changes along soil depth (mean \pm SE) in the variables studied based on seagrass species at Rottneast Island.

FIGURE S3 | Biplots showing the relationships among the variables studied in the seagrass cores based on seagrass species.

TABLE S1 | Details of the seagrass cores sampled, including the % compression during coring operations. Core length recovered (cm compressed) and core length corrected for compression (cm decompressed).

TABLE S2 | Mean (\pm SE) isotopic carbon values (‰) in seagrass soil organic matter. Percentage (%; Mean \pm SE) contribution of potential organic sources (i.e., seagrass, epiphytes & macroalgae & microphytoenthos, and seston) into seagrass soils.

TABLE S3 | Dataset used to produce this manuscript.

¹<http://data.worldbank.org/indicator/EN.ATM.CO2E.PC>

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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