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Mixed provenance of organic carbon in Northeast Atlantic temperate intertidal seagrass sediments

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Blue carbon accreditation for climate mitigation services provided by coastal ecosystems, such as seagrass beds, typically account only for autochthonous organic carbon, potentially underestimating the total carbon sequestration capacity of seagrass ecosystems. Here, a multi-proxy approach is used to determine the provenance of organic carbon in two intertidal temperate seagrass ecosystems in the Northeast Atlantic. The organic carbon to nitrogen ratio (C_{org}/N) and carbon isotope composition ($\delta^{13}C$) of seagrass tissues and sediments from an open coastal sandy site (Ryde, UK) and a muddy tidal inlet site (Farlington Marshes, UK) were measured. Sedimentary C_{org}/N was higher at the muddy site than the sandy site, suggesting a greater contribution of marine algal organic matter in the latter. Isotopic mixing model analysis showed that seagrass biomass contributes between 12 and 25% to accumulated sedimentary C_{org}. These findings demonstrate that temperate Northeast Atlantic seagrass sediments are dominated by allochthonous C_{org} (75–88%) and that current blue carbon accreditation frameworks undervalue these ecosystems. Supporting the estimation and uptake of high integrity field-derived allochthonous deduction evidence would assist uptake of these frameworks to support implementation of nature-based solutions.

Keywords Carbon accreditation, Blue carbon, GHG mitigation, Sustainable development goal 13

The concept of blue carbon accreditation, namely payment for climate mitigation services, is attractive to many because it appears to be a plausible mechanism to increase financial investment into blue carbon habitat protection and restoration. The financial investment associated with carbon accreditation typically excludes research and development, with the rationale that these may not result in verifiable emission reductions[1]. Therefore, carbon-accredited finance specifically refers to financial investment into projects demonstrating a quantifiable reduction in carbon emissions; within the context discussed here, this refers to seagrass habitat protection or restoration. Methodological frameworks for carbon accounting exist for seagrass restoration, including the Verified Carbon Standard's (VCS) 'Methodology for Tidal Wetland and Seagrass Restoration VM0033'[2]. Unlike other habitats, VCS standards stipulate that seagrass projects demonstrate empirical evidence of carbon provenance[3]. This is due to seagrasses' capacity to capture carbon originating outside the seagrass ecosystem, termed allochthonous carbon. In its simplest iteration, this means distinguishing seagrass-derived carbon from allochthonous carbon. Ultimately, seagrass restoration projects require more detailed data on carbon provenance than other coastal habitats to qualify for carbon-accredited financing[3].

The VCS VM0033 carbon accreditation methodology allows the use of peer-reviewed published data as evidence to generate a value of the percentage of allochthonous sediment organic carbon to be deducted for carbon accounting[2]. Whilst the use of peer-reviewed literature values is allowed, it has been highlighted that caution should be taken as there is the potential for carbon offsets to be sold at an overestimated capacity versus its actual sequestration capacity[4]. Preliminary assessments of carbon provenance in existing seagrass meadows near the project area would indicate whether carbon-accredited finance represents a cost-effective financial

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strategy for restoration in that locality. Ultimately the implementation of local carbon provenance assessments increases the technical barriers which must be overcome to evaluate the potential of seagrass accreditation[3].

To date, isotope analysis (e.g., carbon and nitrogen isotopes, $\delta^{13}C$ and $\delta^{15}N$) of bulk tissue is the most well-tested and cost-effective technique for determining carbon provenance, in comparison to alternative techniques such as environmental DNA and compound-specific isotope analysis[5]. For these reasons, bulk $\delta^{13}C$ and $\delta^{15}N$ analysis remains the most widely utilised technique for the determination of sediment carbon provenance. However, inferences about the contribution of sources to a mixture from bulk isotope analyses can be strengthened through the inclusion of subsidiary data from other sources of evidence. This is commonplace in other stable isotope applications[6–10]. Furthermore, elemental analysis of organic carbon and nitrogen and the subsequent ratio of the elements (C_{org}/N) can provide additional information on the sources of organic matter and are an ideal supplementary tool given elemental analysis is usually completed alongside bulk isotope analysis[11]. Bacteria and marine algae have lower C_{org}/N ratios (< 10) which are distinct from terrestrial vegetation (> 12)[11]; higher C_{org}/N ratios form in terrestrial plants due to an increased proportion of C-rich structural compounds (e.g., cellulose) found within vegetation[12]. It is important to note that lignin can be found in seagrass, which appears to protect the lacunal system from water pressure[13] and that seagrass lignin can contribute to the organic carbon found within seagrass sediments[14].

Photosynthetic growth is determined by concentrations of dissolved nutrients (including carbon and nitrogen). During the assimilation of Dissolved Inorganic Carbon (DIC) and Dissolved Inorganic Nitrogen (DIN) for photosynthetic growth, the high availability of these nutrients relative to biological demand can lead to discrimination between the lighter and heavier isotopes causing isotopic fractionation. When isotope fractionation occurs, plants become depleted in heavier stable isotopes (e.g., ^{13}C and ^{15}N). If the isotope values of organic sources are distinct from each other, their relative contribution to unknown mixtures can generally be resolved with stable isotope mixing models (SIMMs). However, increasing the number of potential sources of organic matter, which may have high variability and/or overlap in their isotope values, can increase uncertainty and lower the diagnostic power of SIMMs[15]. As such, the successful application of SIMMS for resolving carbon provenance in sediments depends on the system in question and is most appropriate in isotopically well-constrained systems. In some cases, bulk isotope analysis cannot distinguish the contributions of different primary producers[5] and does not allow the discernment of sources to finer taxonomic ranks (e.g., species-level) as noted for eDNA[16,17]. However, applications of allochthonous deductions within the VCS blue carbon accreditation methodology are used primarily to distinguish seagrass-derived carbon from allochthonous carbon. Therefore, source contribution to the species level is not necessarily needed. If the seagrass and other organic sources isotope values are well-constrained, SIMMs remain a valuable tool for resolving the relative contribution of seagrass-derived versus allochthonous organic carbon within seagrass sediment. The use of a multi-proxy approach (e.g., elemental C_{org}/N) can support inferences made about bulk isotope analysis and strengthen the decision process behind the SIMMs applied.

The aims of this study were (1) to determine $\delta^{13}C$ of the temperate intertidal *Zostera marina* and *Zostera noltii* above and below-ground tissues, (2) to compare these seagrass $\delta^{13}C$ values with the seagrasses' sediment organic carbon $\delta^{13}C$ values, (3) to assess the provenance of the organic carbon within these seagrass sediments with a multi proxy approach utilising C_{org}/N ratio and isotope analysis, and (4) apply SIMMs to estimate the relative contribution of each organic carbon source to the temperate seagrass sediments. Finally, the implications of our results are discussed in the context of carbon-focused accreditation and the potential financial investment available for the restoration of similar temperate intertidal seagrass beds.

Results

Seagrass tissue $\delta^{13}C$ values and C_{org}/N ratios

The seagrass below ground tissue $\delta^{13}C$ values range from − 11.3‰ for *Z. marina* at Farlington Marshes to -8.1‰ for *Z. noltii* at Ryde. Shapiro Wilk tests confirmed that when grouped by site, seagrass species, and seagrass tissue type, the data followed a normal distribution ($P > 0.05$), except for Ryde's *Z. marina* and *Z. noltii* below ground tissue $\delta^{13}C$ data (RY Zm BG: $W = 0.750$, $P < 0.001$; RY Zn BG: $W = 0.750$, $P < 0.001$) (Fig. 1). Therefore, when the data was grouped by all three factors (tissue type, seagrass species and site) the median was the best measure of central tendency (Fig. 1). When grouped by seagrass species, and seagrass tissue type, the Farlington Marshes $\delta^{13}C$ data followed a normal distribution ($P > 0.05$) and met the assumptions for homogeneity of variances ($P > 0.05$). The *Z. noltii* below ground tissues had observationally higher $\delta^{13}C$ values when compared to *Z. marina* below ground tissues at Farlington marshes (FM BG: $\Delta\delta^{13}C_{Zm\text{-}Zn} = +0.6‰$). ANOVAs confirmed there was no significant interaction between the species and tissue type (above ground and below ground) and ($F_{1,8} = 2.006$, $P = 0.194$) or significant main effect of species ($F_{1,10} = 1.103$, $P = 0.318$) or tissue type ($F_{1,10} = 0.679$, $P = 0.429$). When grouped by seagrass species, and seagrass tissue type, the Ryde $\delta^{13}C$ data did not meet the assumptions of normality ($P < 0.05$). The above and below ground tissue $\delta^{13}C$ values were not significantly different when comparing the two-seagrass species (*Z. marina* and *Z. noltii)* at Ryde (AB $W = 4.5$, $P = 1$; BG $W = 2.5$, $P = 0.4936$).

When seagrass species is removed as a factor so that the $\delta^{13}C$ data is only grouped by site and type of seagrass tissue, Shapiro Wilk test confirmed that the data followed a normal distribution ($P > 0.05$) and bartletts test confirmed that the data met the assumptions for homogeneity of variances ($P > 0.05$). Overall, the seagrass below ground tissues at Ryde have higher $\delta^{13}C$ values (BG: $\delta^{13}C$ = -8.70‰ ± 0.64) than the below ground tissues at Farlington Marshes (BG: $\delta^{13}C$ = -10.92‰ ± 0.60). Seagrass above ground tissues also have higher $\delta^{13}C$ at Ryde (AB: $\delta^{13}C$ = -9.27‰ ± 0.47) than at Farlington Marshes (AB: $\delta^{13}C$ = -10.65‰ ± 0.51). However, ANOVAs confirmed there was no significant interaction between the tissue type (above and below ground) and site ($F_{1,20} = 3.298$, $P = 0.08$) or significant main effect of tissue type ($F_{1,22} = 0.111$, $P = 0.742$). There was a significant difference in the seagrass $\delta^{13}C$ values between sites ($F_{1,22} = 57.08$, $P < 0.001$, $d = 3.08$ large effect) (Fig. 1, FM $\delta^{13}C$ = -10.78; RY $\delta^{13}C$ = -8.98).

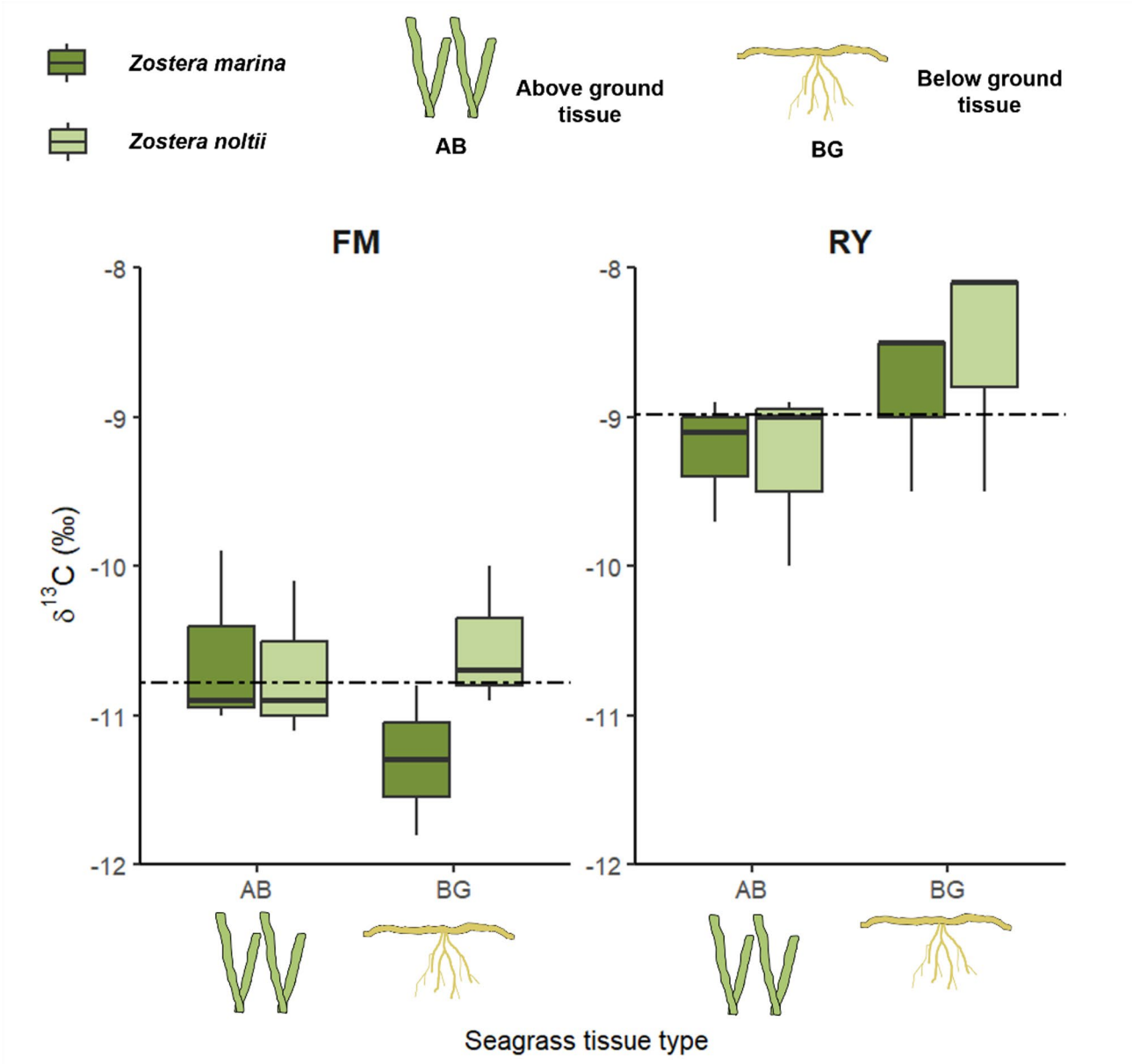

Fig. 1. $\delta^{13}C$ (‰) values for seagrass above ground (AB) and below ground (BG) tissues for each seagrass species (*Zostera marina* and *Zostera noltii*) at Farlington Marshes and Ryde. Dashed line = mean $\delta^{13}C$ value of seagrass at each site.

Shapiro Wilk tests confirmed that when grouped by site, species and seagrass tissue type, the seagrass C_{org}/N data followed a normal distribution ($P > 0.05$), except for Ryde's *Z. noltii* above ground tissue C_{org}/N data (RY Zn AB: $W = 0.750$, $P < 0.001$). Therefore, site was removed as a factor and each site's C_{org}/N data was analysed separately. When grouped by seagrass species, and seagrass tissue type, the Farlington Marshes C_{org}/N data followed a normal distribution ($P > 0.05$), but did not meet the assumptions for homogeneity of variances ($P < 0.05$). Welch's t- test found no significant difference in C_{org}/N at Farlington Marshes between species (t_{10} = -0.63, $P = 0.54$), but a significant difference between C_{org}/N values depending on the tissue type (t_5 = -6.60, $P = 0.001$), with higher C_{org}/N in the below ground tissues. When grouped by seagrass species, and tissue type, the Ryde C_{org}/N data did not meet the assumptions of normality ($P < 0.05$). Wilcoxon test determined that at Ryde there was no significant difference in C_{org}/N values between species ($W = 18$, $P = 1$), but a significant difference between C_{org}/N values depending on the tissue type ($W = 0$, $P = 0.005$), with higher C_{org}/N in the below ground tissues. All seagrass leaf C_{org}/N ratios were < 20 which indicates reduced light availability in the British Isles[18] (Table 1).

Site	Tissue type	Spp.	$\%C_{org}$	%N	C_{org}/N	n
FM	AB	*Zm*	35.5 ± 2.65	2.5 ± 0.35	13.8	3
		Zno	39.4 ± 1.15	2.5 ± 0.17	15.9	3
		Subtotal	37.4 ± 2.8	2.5 ± 0.2	15.0 ± 1.3	6
	BG	*Zm*	30.3 ± 1.25	0.9 ± 0.32	38.2	3
		Zno	33.8 ± 0.35	0.76 ± 0.12	44.9	3
		Subtotal	32.1 ± 2.1	0.9 ± 0.2	39.8 ± 9.1	6
RY	AB	*Zm*	34.7 ± 0.75	1.8 ± 0.25	19.3	3
		Zno	36.5 ± 0.91	2.3 ± 0.26	16.8	3
		Subtotal	35.6 ± 1.3	2.1 ± 0.3	17.6 ± 2.4	6
	BG	*Zm*	31.4 ± 0.66	1.3 ± 0.10	22.8	3
		Zno	31.6 ± 1.90	0.8 ± 0.10	39.0	3
		Subtotal	31.5 ± 1.3	1.1 ± 0.3	32.0 ± 9.5	6

Table 1. Average seagrass % C_{org}, % N and C_{org}/N ratio at each site (FM = Farlington marshes, RY = Ryde) for each species (*Zm* = *Zostera marina*, *Zno* = *Zostera noltii*) and tissue type (AB = above ground, bg = below ground) ($n = 3$).

Sediment organic carbon content and δ ^{13}C downcore profiles

The seagrass sediment $\delta^{13}C$ values at Ryde were highest in the top 1 cm of sediment (median = -18.9‰) and higher than those in the top 1 cm of sediment at Farlington Marshes (median = -19.8‰). The seagrass sediment $\delta^{13}C$ values at Ryde tended to decrease with depth consistently, as such $\delta^{13}C$ was lowest at Ryde within the 4–5 cm depth interval (median = -20.6‰) (Fig. 2A). At Farlington Marshes sediment core two and three had $\delta^{13}C$ values which decreased with depth (0–1 cm depth $\bar{x}$ = -19.9‰; 4–5 cm depth $\bar{x}$ = -21.4‰), but core one showed a slight decrease in $\delta^{13}C$ at 1–2 cm depth (-19.8 to -20.2‰) before increasing with depth (4–5 cm depth − 18.4‰) (Fig. 2A). The $\%C_{org}$ depth profile within Farlington Marshes' core 1 followed the pattern seen in its $\delta^{13}C$ depth profile, a slight decrease in $\%C_{org}$ (at 1–2 cm depth = 1. 5 %) before increasing with depth (2.2 %) (Fig. 2B). Often the samples with the highest $\%C_{org}$ were those associated with the highest $\delta^{13}C$ values (Fig. 2C). The average sediment $\delta^{13}C$ value was lower at Farlington Marshes compared to Ryde (Table 2). When grouped by site, Shapiro Wilk tests confirmed that the sediment $\delta^{13}C$ data followed a normal distribution ($P > 0.05$) and bartletts test confirmed that the data met the assumptions for homogeneity of variances ($P > 0.05$). However, the difference in sediment $\delta^{13}C$ between sites was not significant (t_{23} = -1.9656, $P = 0.06$). With the Rayleigh model, we obtained an estimated fractionation factor ε = −0.17‰ (SE = 0.12‰) and an intercept $\delta^{13}C0 = -20.34$‰ (SE = 0.25‰). However, the relationship was weak (adjusted $R^2 = 0.036$) and not statistically significant ($P > 0.05$). On the other hand, the depth regression gave a positive slope of 0.233‰ per cm (SE = 0.105, $P < 0.05$), with an adjusted $R^2 = 0.119$, indicating that depth alone explains a modest but significant fraction of the isotopic variability.

Presence and provenance of allochthonous organic carbon

On average seagrass $\delta^{13}C$ values were higher (FM: $\bar{x}$ = -10.78‰ SD ± 0.55; RY: $\bar{x}$ = -8.98‰ SD ± 0.61) (Fig. 1) than those of seagrass sediment (FM: $\bar{x}$ = -20.38‰ SD ± 1.01; RY: $\bar{x}$ = -19.78‰ SD ± 0.62;) (Fig. 2A). The difference between $\delta^{13}C$ values of seagrass and seagrass sediment produced a positive average at both Farlington Marshes ($\Delta\delta^{13}C_{\text{seagrass-sediment}}$ $\bar{x}$ = +9.60‰ SD ± 1.01) and Ryde ($\Delta\delta^{13}C_{\text{seagrass-sediment}}$ $\bar{x}$ = +10.80‰ SD ± 0.55) confirming the presence of allochthonous organic carbon within the seagrass sediment at both sites.

Whilst the quantity of $\%C_{org}$ and %N is lower at Ryde versus Farlington Marshes (Table 2), the quantity of carbon present relative to nitrogen (C_{org}/N ratio) also differs at the two sites. The C_{org}/N ratio is lower in Ryde's sediment versus Farlington Marshes (Table 2), although the low prevalence in %N at Ryde (Supp., Fig. 1), does increase the C_{org}/N ratio variation at this site (Supp., Fig. 2). When grouped by site Shapiro Wilk tests confirmed that the sediment C_{org}/N ratio data followed a normal distribution ($P > 0.05$), whilst bartletts test showed the data did not meet the assumptions of homogeneity ($P < 0.05$). Despite increased variation in C_{org}/N ratio at Ryde, Welch's test confirmed the difference in sediment C_{org}/N between sites was significant ($t_{17} = 5.6858$, $P < 0.0001$) (Table 2; Fig. 3). The lower sediment C_{org}/N ratio at Ryde identifies its sediment as more closely associated with marine organic inputs such as that expected from an open coastal environment (Fig. 3)[11]. The higher C_{org}/N at Farlington Marshes indicates that different or additional organic sources with higher C_{org}/N are mixed within the sediment (e.g., higher plants mixed with marine algae/POC), more typical of an estuarine environment.

Potential contribution of organic carbon sources to seagrass sediments

The range of $\delta^{13}C$ of seagrass sediment samples from Ryde (-20.7 to -18.4‰) lie within the iso-space created by the potential organic carbon source's $\delta^{13}C$ values and C_{org}/N ratios when you consider the sources error of margin (two SD) (Fig. 4A) (Table 3). The iso-space ranges from the more negative terrestrial grasses $\delta^{13}C$ values ($\bar{x}$ = -27.8‰ SD ± 0.331[19]) to Ryde's highest $\delta^{13}C$ values represented by the site's seagrass below ground tissue $\delta^{13}C$ values ($\bar{x}$ = -8.70‰ SD ± 0.61) (Table 3). The maximum $\widehat{R}$ value was 1.01, suggesting satisfactory convergence (Supp., Figs. 3 and 4). The Bayesian mixing model identified suspended particulate matter (SPM) and epiphytic material as the highest potential contributors of organic carbon to Ryde's seagrass sediments

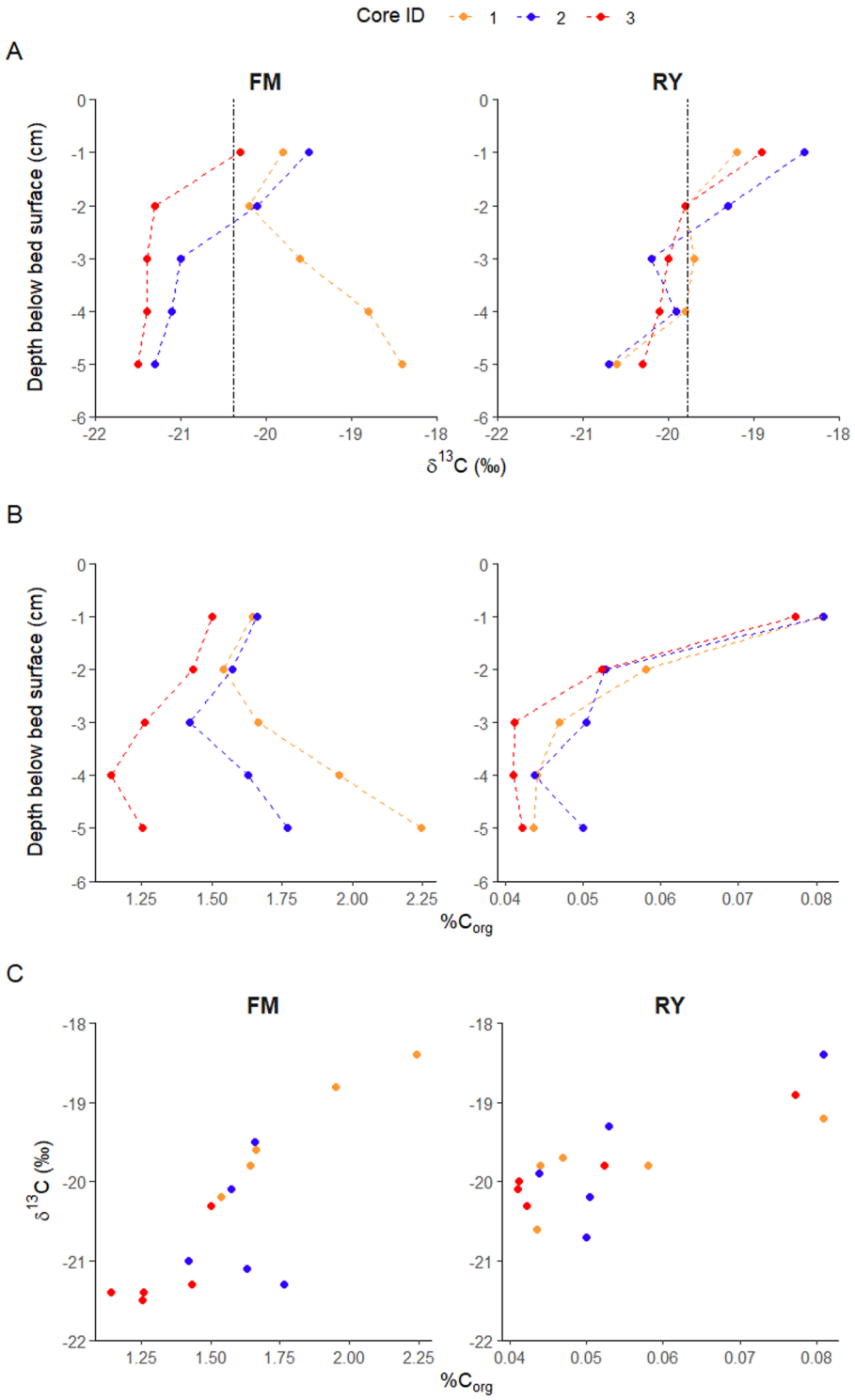

Fig. 2. Sediment down core profiles at Farlington Marshes (FM) and Ryde (RY) **A**$\delta^{13}C$ values (Black dashed line = average $\delta^{13}C$ at each site) **B** Sample percentage concentration of carbon ($\%C_{org}$) **C**$\delta^{13}C$ values against sample $\%C_{org}$ concentration.

(SPM proportion $\bar{x}$ = 0.42% SD ± 0.11; epiphytic material proportion $\bar{x}$ = 0.39% SD ± 0.19), although the model struggles to resolve the contribution from epiphytic material. Above ground seagrass tissue is identified most confidently as the next most important source of organic carbon to Ryde's seagrass sediments (proportion $\bar{x}$ = 0.09% SD ± 0.08) and could contribute as much as 16% (75th percentile) of the organic carbon present (Fig. 4B). On average, the Bayesian mixing model predicted that 88% of the organic

Site	$\%C_{org}$	%N	C_{org}/N	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)
Farlington Marshes	1.58 ± 0.28	0.15 ± 0.02	10.39 ± 0.56	-20.38 ± 1.00	+ 6.58 ± 0.14
Ryde	0.05 ± 0.01	< 0.01	7.61 ± 1.81	-19.78 ± 0.62	-

Table 2. Average sediment $\%C_{org}$, % N, C_{org}/N ratio, $\delta^{13}C$, $\delta^{15}N$ at each site ($n = 15$). There was insufficient material to analyse $\delta^{15}N$ accurately in Ryde's sandy sediment.

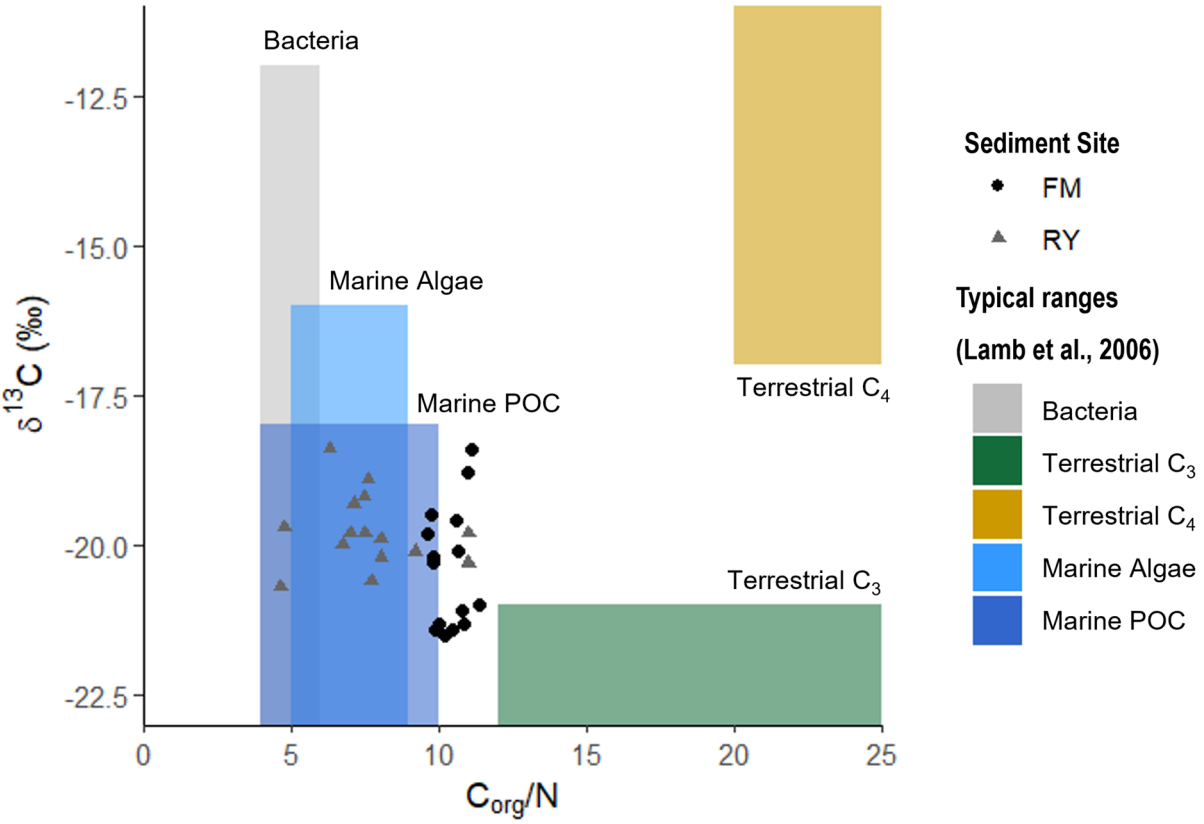

Fig. 3. Sediment C_{org}/N ratio and $\delta^{13}C$ values from the coastal site Ryde (RY) and tidal inlet site Farlington Marshes (FM). A selection of typical C_{org}/N ratios and $\delta^{13}C$ ranges for marine (POC and algae), terrestrial (C_3 and C_4 plants) and bacterial organic inputs to coastal environments, (modified from Lamb et al., 2006)[11].

carbon present in Ryde's seagrass sediment is allochthonous (red algae, epiphytic material, brown algae, terrestrial, SPM).

Mean $\delta^{13}C$ and $\delta^{15}N$ values of Farlington Marshes seagrass sediments laid within the iso-space defined by $\delta^{13}C$, $\delta^{15}N$ and C_{org}/N mean values of potential organic sources (Fig. 5A, B, C). The SIMMs $\widehat{R}$ values were all 1.00, indicating convergence (Supp., Figs. 5 and 6). At Farlington Marshes, the major potential contributors to seagrass sediments were SPM (proportion $\bar{x}$ = 0.60% SD ± 0.03) and above ground seagrass tissue (proportion $\bar{x}$ = 0.22% SD ± 0.04). In fact, the model predicted above ground seagrass tissue could contribute as

much as 26% (75th percentile) of the organic carbon present (Fig. 5D). The Bayesian mixing model predicted that on average higher plants (seagrass, saltmarsh and terrestrial sources) contribute 33% of the organic carbon present. Whilst on average 75% of the organic carbon present within Farlington Marshes' seagrass sediment is allochthonous (algal mat, brown algae, saltmarsh, terrestrial, SPM).

Discussion

The findings demonstrate that both autochthonous and allochthonous organic carbon were present in the temperate intertidal *Z. marina* and *Z. noltii* seagrass sediments at our study sites. Seagrass-derived organic carbon is not the dominant source of organic carbon in either of these UK temperate intertidal seagrass meadows (12–25% seagrass-derived C_{org}) versus non-seagrass derived allochthonous organic carbon (75–88% C_{org}). This agrees with previous research which demonstrated that within temperate *Z. marina* sediments often < 50% of

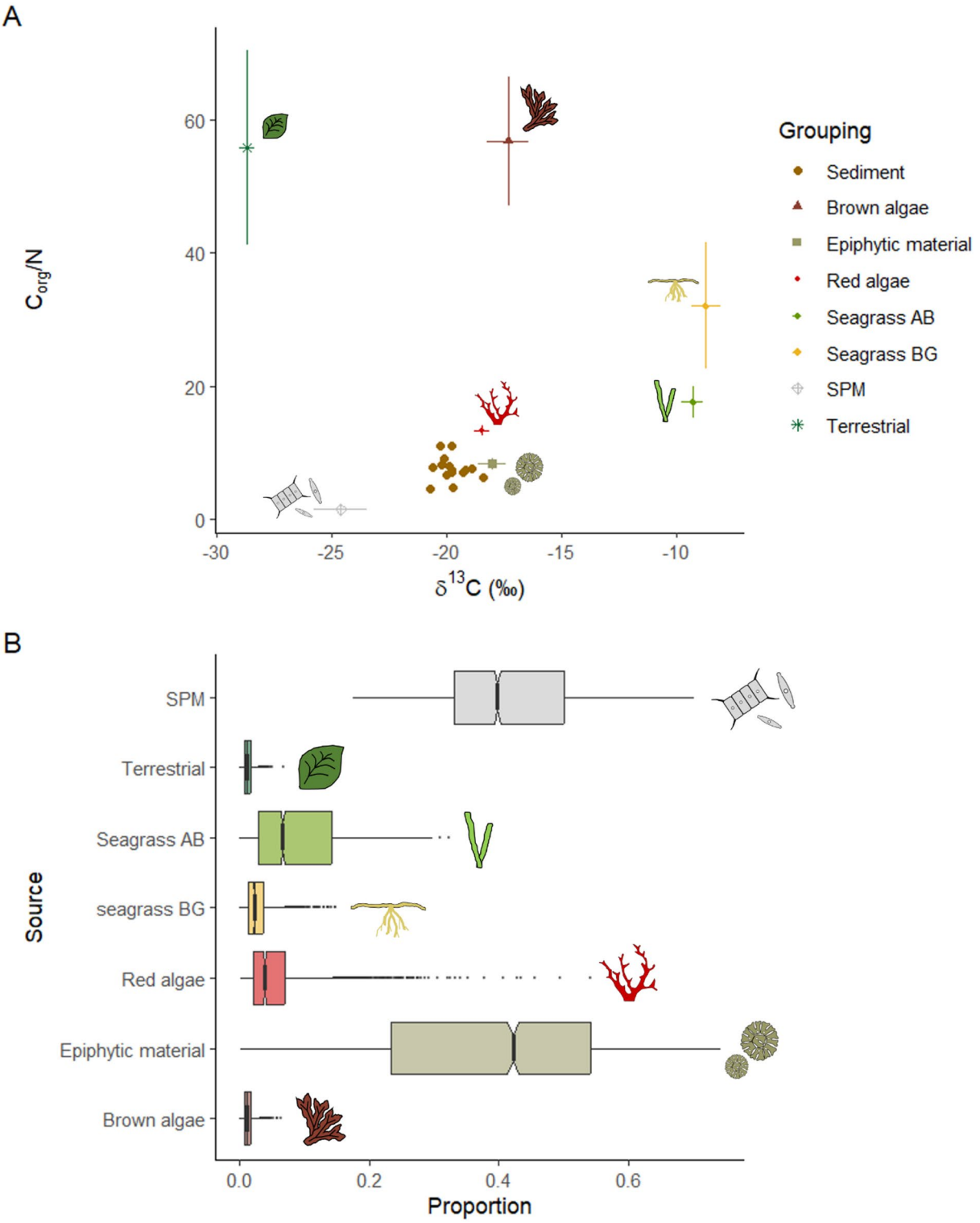

Fig. 4. Northeast Atlantic Open Coastal Seagrass Meadow: **A** Mean $\delta^{13}C$ and C_{org}/N values of organic carbon sources (Suspended Particulate Matter (SPM), Terrestrial grasses, Seagrass above (AB) and belowground (BG) material, Red algae, Epiphytic material and Brown algae) utilised in SIMMR, error bars indicate standard deviation used in the input of Bayesian mixing models. Alongside $\delta^{13}C$ and C_{org}/N values the values in Ryde's seagrass sediment. SPM and Terrestrial data[19]. **B** Contribution of primary producers to the organic carbon in Ryde's seagrass sediments calculated using Bayesian mixing models. Boxes show the 25th, 50th and 75th percentiles.

Grouping	Location	Sample type	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	C_{org}/N	n	Source
Green algae	Farlington Marshes	Algal mat	**-18.23 ± 1.16**	**+9.58 ± 0.23**	**10.53 ± 0.61**	3	This study.
Red algae	Ryde	Red filamentous algae	**-18.23 ± 0.12**	-	**13.33 ± 0.76**	3	This study.
Brown algae	Farlington Marshes	*Ascophylum nodosum*	-18.04 ± 0.88	+8.18 ± 0.21	54.90 ± 8.91	3	This study.
		Fucus vesiculous	-17.67 ± 1.20	+8.72 ± 0.17	54.67 ± 5.25	3	
		Average	**-17.95 ± 0.83**	**+8.45 ± 0.34**	**54.78 ± 6.54**	6	
	Ryde	*Fucus serratus*	-16.53 ± 0.31	-	62.17 ± 10.43	3	
		Pelvetia canaliculata	-18.10 ± 0.40	-	51.00 ± 5.70	3	
		Average	**-17.32 ± 0.92**	-	**56.58 ± 9.69**	6	
Epiphytic material	Ryde	Epiphytic material	**-18.00 ± 0.40**	-	**8.23 ± 0.91**	3	This study.
Seagrass	Farlington Marshes	Above ground tissue	**-10.65 ± 0.51**	**+4.77 ± 2.00**	**15.02 ± 1.34**	6	This study.
		Below ground tissue	**-10.92 ± 0.60**	**+5.18 ± 4.77**	**39.80 ± 9.10**	6	
	Ryde	Above ground tissue	**-9.27 ± 0.47**	-	**17.58 ± 2.40**	6	This study.
		Below ground tissue	**-8.70 ± 0.64**	-	**32.02 ± 1.81**	6	
Saltmarsh	Farlington Marshes	*Spartina* spp.	**-13.87 ± 0.11**	**+6.81 ± 0.22**	**33.6 ± 2.61**	3	This study.
SPM	Southampton Water	SPM inc., Phytoplankton, zooplankton, and detritus.	**-24.62 ± 1.17**	**+8.41 ± 1.05**	**11.352 ± 4.37**	45	[19]
Terrestrial	Southampton Water	Leaf litter	**-27.8 ± 0.31**	**+4.31 ± 0.70**	**55.80 ± 14.62**	-	[19]

Table 3. Mean (± SD) $\delta^{13}C$, $\delta^{15}N$ and C_{org}/N of potential organic carbon sources for seagrass sediments. Some samples were merged into collective groups (e.g., brown algae) and where possible these values were site specific. The values in bold were input into bayesian mixing models (SIMMR). SPM = Suspended particulate matter. All locations are within the solent, UK. Insufficient material to analyse $\delta^{15}N$ accurately, in ryde's sediment meant vegetative samples from this site were only analysed for $\delta^{13}C$ by isotope ratio mass spectrometry.

sedimentary organic carbon is derived from seagrass and seagrass-derived contributions can be as low as 3% at some seagrass sites[20].

Seagrass growing within the estuarine (Farlington Marshes) versus the open coastal environment (Ryde) have distinct seagrass tissue $\delta^{13}C$ values. This follows trends seen in fjordic versus open ocean *Zostera noltii* $\delta^{13}C$ leaf values, which showed lower $\delta^{13}C$ (by 5‰) in the innermost part of the fjord versus the open ocean, following a gradient of change to lower (~ 3–4‰) DIC $\delta^{13}C$ values of seawater from those systems[21]. Estuarine environments receive inputs of inorganic carbon from the coastal waters and/or terrestrial or freshwater inorganic carbon upstream. Where there are high inputs of terrestrial DIC, seagrass tissues can have lower $\delta^{13}C$ values as they assimilate the inorganic carbon from terrestrial sources. For example, seagrass leaves grown near mangroves have lower $\delta^{13}C$ compared to those further from mangrove areas[22,23]. The relative influence of these terrestrial and freshwater inputs will determine the extent to which the estuarine DIC $\delta^{13}C$ value changes relative to a fully marine DIC $\delta^{13}C$ value. However, changes in DIC $\delta^{13}C$ between the estuary and open ocean environment have been shown to only partially explain the variation in the seagrass leaf $\delta^{13}C$ values[21]. Other environmental factors (e.g., light availability, heat stress and nutrient availability) may cause differences in seagrass $\delta^{13}C$ between Ryde and Farlington Marshes and should be considered when interpreting seagrass $\delta^{13}C$ values[24]. The site specificity within our study emphasises the appropriate use of site-specific seagrass $\delta^{13}C$ values within our SIMMs and that ^{13}C values of marine vegetation should ideally be taken from the location of interest or consider the geomorphology of the coast and its influence on DIC $\delta^{13}C$ values[4]. It is important to acknowledge that future studies may also benefit from acquiring direct SPM samples, however it is appropriate practice to use suitable literature values where samples are not directly acquired and, in this scenario, using regional data can be suitable[6,25]. The SPM and terrestrial isotopic values used in this study's models were representative of samples from the Solent.

Considerable seasonal changes in $\delta^{13}C$ values have been recorded in *Zostera* spp. seagrass tissues related to seasonal changes in productivity[21,26]. Seasonal increases in productivity and carbon demand, as witnessed in these seagrass sites[27] can lead to increased $\delta^{13}C$ discrimination and potentially lower $\delta^{13}C$ values in summer after growth has been at its highest. Given higher seagrass leaf $\delta^{13}C$ is generally witnessed in spring and lower leaf $\delta^{13}C$ in the summer[21]collecting seagrass tissue outside these two extremes (e.g., October, as in this study) may be more representative of an annual $\delta^{13}C$ seagrass value. Often, seasonal isotope seagrass leaf studies specifically measure new seagrass leaves as these reflect the production of organic carbon within the previous month. In contrast, the $\delta^{13}C$ values of old leaves reflect the accumulation of organic carbon over a longer period[26]. Therefore, utilising $\delta^{13}C$ values from older leaves, or at least not preferentially selecting young leaves, to determine the $\delta^{13}C$ values would ensure the seagrass $\delta^{13}C$ values utilised in the SIMMs for sediment carbon provenance reflect a longer time period and avoid any seasonal $\delta^{13}C$ variation found in young seagrass leaves.

For the seagrass species *Thalasia testudinum*, seagrass leaves have been recorded as having lower $\delta^{13}C$ (-10.7 ± 0.2) than seagrass rhizomes (-8.6 ± 0.1) by ~ 2‰, even though they came from the same plants[28] therefore, the $\delta^{13}C$ of all seagrass tissues should not be assumed to be equal. Temperate seagrass roots have higher lignin-associated organic carbon and consequently higher refractory organic matter than other temperate seagrass tissues[29]. Therefore, seagrass roots may also form a larger part of the organic carbon stored in sediments, as it is

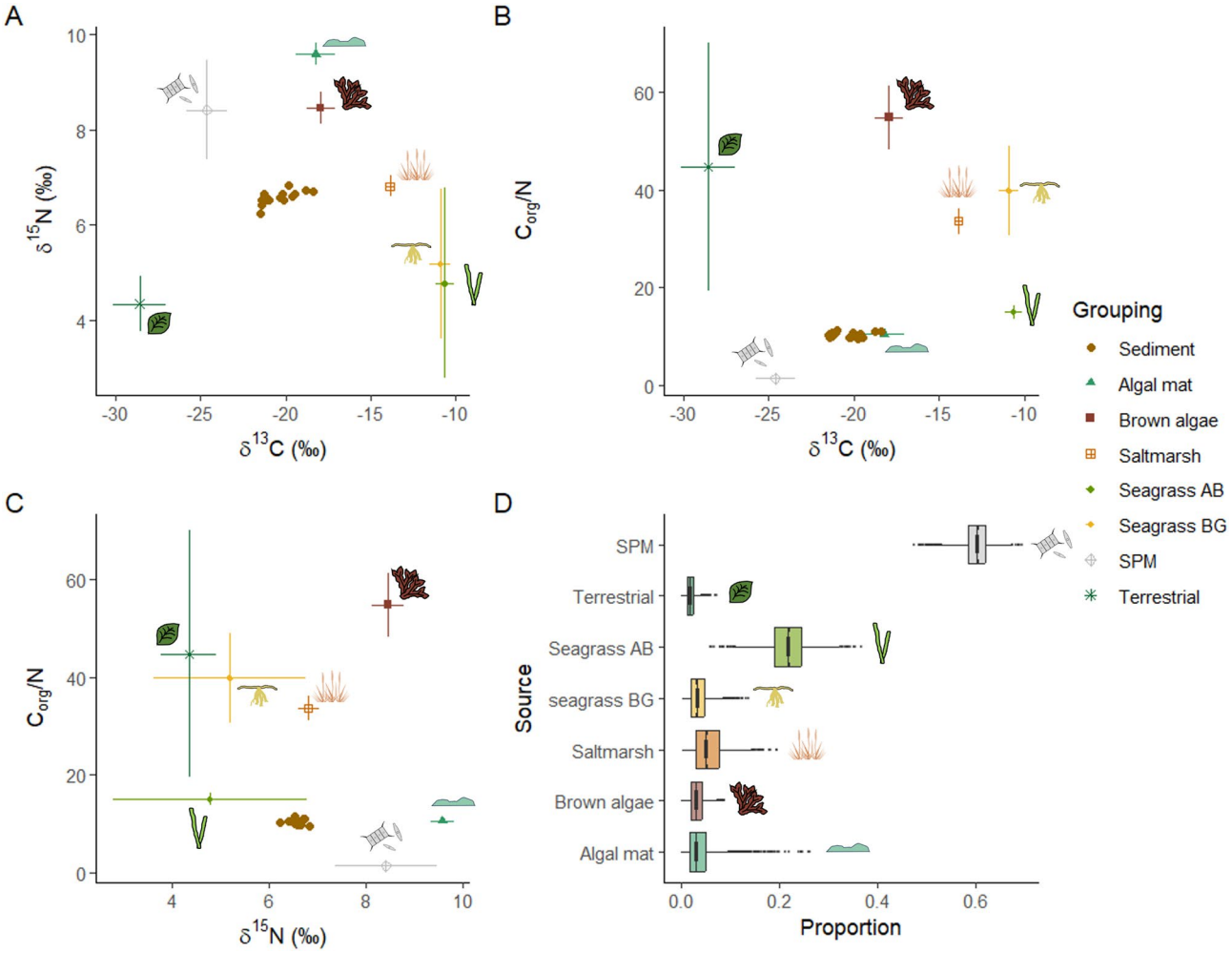

Fig. 5. Northeast Atlantic Tidal Inlet Seagrass Meadow: **A** Iso-space plot showing mean $\delta^{13}C$, $\delta^{15}N$ and C_{org}/N values of of organic carbon sources (Suspended Particulate Matter (SPM), Terrestrial grasses, Seagrass above (AB)and belowground (BG) material, Red algae, Epiphytic material and Brown algae) utilised in SIMMR, error bars indicate standard deviation used in the input of Bayesian mixing models. Error bars indicate standard deviation used in the input of Bayesian mixing models. Alongside $\delta^{13}C$, $\delta^{15}N$ and C_{org}/N values from Farlington Marshes' seagrass sediment. SPM and Terrestrial data[19]. **B** Contribution of primary producers to the organic carbon in Farlington Marshes' seagrass sediments calculated using Bayesian mixing models. Boxes show the 25th, 50th and 75th percentiles.

more recalcitrant and less likely to be remineralised. This reinforces that below-ground tissue $\delta^{13}C$ should not be assumed to be equal to above-ground $\delta^{13}C$ values, as this would influence SIMMs outputs. However, our results match studies of other *Zostera* spp. which display no significant difference in $\delta^{13}C$ values between the leaves and the below-ground tissue (e.g., root, rhizome)[30–32]. In our case, it is appropriate to aggregate the $\delta^{13}C$ values of both above and below-ground tissues to provide holistic seagrass $\delta^{13}C$ values to utilise in the SIMMs.

A major consideration when using mixing models to determine the organic carbon provenance within sediments is that 'modern' or live vegetation is sampled to determine the $\delta^{13}C$ values of the organic sources, even though the deposited organic carbon will not be in its original live state as at least some decomposition of the vegetative material would be expected. *Zostera* spp. seagrass tissues have been reported in some cases to show little difference in $\delta^{13}C$ values between live and decomposed material[31,33]whilst in other instances, decomposed *Zostera* spp. tissues have shown up to 4‰ lower $\delta^{13}C$[34]. One of the reasons for such variability in seagrass $\delta^{13}C$ response to decomposition may be linked to the 'short-term periods' (< 120 days) at which decomposition studies are conducted, given the decay rates in seagrass below-ground tissues stabilise after 12–18 months[35]. In blue carbon studies, it is this long-term seagrass detritus in the refractory or stable phase of decomposition which is most likely to contribute to the long-term organic carbon accumulating in seagrass sediments. It is important to consider that the rate of organic carbon remineralisation will differ between our sites given the difference in their sediment types (e.g., muddy vs. sandy) as sediment type has been shown to influence the retention of labile carbon[36]. In coarse sandy seagrass sediments, the organic carbon pool is predominantly refractory (80%), whilst in muddy seagrass sediments, refractory organic carbon is a much smaller proportion (30%) of the organic carbon pool[37]. Sediment pore space size influences fluxes of O_2, such that permeable sediments are

subject to advective flow, whereas fine-grained muds are typically diffusive[38,39]. This means that in silty sediment, with a small sediment particle size, when the detritus is buried, the aerobic decomposition is reduced, leading to retention of the labile elements of the detritus alongside the refractory carbon, whereas in coarse sandy sediments, enhanced aerobic decomposition results primarily in the retention of the more stable refractory detritus. Further to this, fine-grained sediments support higher physical protection from decomposition and adsorption to mineral surfaces[40,41]. This ultimately means sandy sediment retains less organic carbon than fine-grained sediments[42].

In some contexts, it might be appropriate to apply post-hoc fractionation correction factors to account for the decomposition of the organic sources within the sediment, but these correction factors may be situation dependent. Carbon provenance studies would benefit from the application of decomposition correction factors, where appropriate, but this is not possible without strong data availability for a given organic source and/or setting, and knowledge of the nature and strength of decomposition relationships[34]. Although the Rayleigh-fractionation model shows a slight ^{13}C enrichment with decomposition ($\varepsilon < 0$), it accounts for very little of the observed $\delta^{13}C$ variation. Sediment depth proves a better predictor, suggesting that processes correlated with burial (for example, mixing of source materials or selective preservation) may dominate over simple microbial fractionation in controlling the isotopic profile. Since appropriate fractionation correction values are not available for our organic sources for each contextual setting, and it is not certain whether a strong decomposition $\delta^{13}C$ relationship exists for our organic sources, it would not be appropriate to apply or extrapolate correction factors into our SIMMs. However, filling this data knowledge gap would remove one of the major caveats to the application of SIMMs for organic carbon provenance in sediments.

C_{org}/N ratio analysis has been used in both paleo sediment reconstruction and on modern sediments to discern between terrestrial and marine inputs[43–45]. In this study, we utilise C_{org}/N ratios alongside isotope data to make inferences on the contribution of organic carbon sources to seagrass sediment. There is a clear distinction between the sediment C_{org}/N ratio at our open coastal seagrass site versus the site found within a tidal inlet. Our C_{org}/N data reflects trends seen in other coastal areas whereby the C_{org}/N ratio increases with exposure to freshwater and terrestrial inputs[42]. Farlington Marshes' raw sediment C_{org}/N (10.39 ± 0.56) suggests that the sediment is a mix of marine and terrestrial inputs (Fig. 3) and indicate that the inputs most closely relate to typical C_{org}/N ratios for angiosperms specifically C_3 vegetation, but also partially C_4 vegetation[11]. This matches the vegetation found within and adjacent to Farlington Marshes; namely the presence of saltmarsh[46,47]. Ryde's sediment C_{org}/N (7.61 ± 1.81) suggests that the inputs are dominated by organic carbon from marine sources, although some individual C_{org}/N sediment points at Ryde (Fig. 3) are low enough (< 5) to suggest bacterial influences are reworking the sediment. Bacterial reworking of the organic carbon is plausible given the sandy sediment would experience increased flushing of oxic water and, as such, aerobic respiration, relative to the cohesive muddy sediment at Farlington Marshes, which is quickly anoxic. Demonstrating that interpreting the raw C_{org}/N sediment values can provide insights made in this context about the contribution and provenance of organic carbon sources to seagrass sediment.

The low %N in Ryde's sandy sediment (Supp., Fig. 1), meant there was insufficient material to analyse $\delta^{15}N$ accurately, and its low prevalence meant the slightest heterogeneity in the sample could lead to misinterpretation of the values. Therefore, $\delta^{13}C$ and C_{org}/N ratio were utilised within a two-tracer mixing model, although as this still incorporates sedimentary nitrogen data (Supp., Fig. 2), the outputs of the SIMM should be interpreted with caution. Basic mass-balance mixing models were restricted to systems involving a single unknown (or the mean of multiple unknowns), and where the number of sources was less than or equal to the number of isotopes + 1, the contributions of the different sources could be solved exactly. In this context, we would only be able to distinguish between three sources at Ryde using a two-tracer mixing model. However, SIMMs can now estimate source proportions regardless of how underdetermined the mixing system is (e.g., many more sources than tracers)[49]. Although we can increase the number of sources and still produce a contribution estimate, we should still aim to minimise the number of sources applied as this can diffuse probability distributions for many of the proportional contribution estimates, limiting the interpretability of the results[50]. As such, at Ryde, our seven-source two-tracer model is the most conservative for answering our targeted research question.

One of the major caveats to mixing models is the selection of appropriate sources, as missing a source can lead to biases in the model, while including inappropriate sources will misguide the mixing model predictions. Therefore, SIMMs rely on the ability of the ecologist to observe the system adequately[48]. In our case, collecting macro vegetation samples on-site, ensures appropriate vegetation types are included within our SIMM. At Farlington Marshes this included seagrass above and below ground tissue, green algal mat, saltmarsh and brown algae. Whilst at Ryde the *in situ* vegetation included seagrass above and below ground tissue, red algae, epiphytic material and brown algae. We also utilise SPM; a broad grouping of phytoplankton, zooplankton, and detritus concentrations[20]. SIMMs lose power with increasing numbers of sources; therefore, it is often necessary to aggregate or combine multiple sources[6]. SPM is a broad group and good representative value for the organic carbon suspended in the water column in the Solent. Although a rare source could be missed, being scarce its contribution to the sediment is most likely negligible and its omission from the mixing model should make negligible difference[48].

Providing the scientific evidence base for complex incentive schemes such as VCS is challenging due to many knowledge gaps surrounding seagrass carbon sequestration, such as carbon provenance, which need to be addressed to create efficient and operational payment programs[51]. Carbon provenance studies such as this need to be undertaken to estimate the proportions of autochthonous and allochthonous organic carbon stored within seagrass sediment. In fact, most of the organic carbon stored within these temperate intertidal seagrass beds, irrespective of whether the seagrass sites were open coastal seagrass sites or estuarine, predominantly held non-seagrass derived allochthonous organic carbon (on average 75–88% C_{org}). This highlights that local estimates of carbon provenance diverge far from the global average of ~50% autochthonous C_{org} in seagrass

sediments[52]. It has been argued that utilising the global value of 50% provides a conservative approach that can underestimate blue carbon accumulation[53], but it this context it would risk vastly overestimating its capacity. The VCS VM0033 requires a deduction value to be applied in estimates of CO^2 emissions from the SOC pool to account for allochthonous carbon[2]. However, the framework allows for this to be estimated using published values, field-collected data and modelling. Whilst there is guidance within the framework (e.g., same or similar region, similar geomorphic, hydrologic and biological properties)[2], introducing proxies, whilst often necessary economically, can introduce uncertainty. Direct estimates relative to a locality or site should be considered those to produce carbon credits with the most integrity. Under the VCS frameworks, our field-derived estimates of autochthonous organic carbon suggest only a small proportion of the organic carbon stored within these seagrass sediments would be applicable to trade-in the carbon accreditation scheme.

The instability of the voluntary carbon market in which schemes such as VCS operate impacts carbon prices, which makes a purely carbon-based approach questionable; fluctuating carbon prices mean projects cannot guarantee financial returns on investment or adequate payments to meet participants' needs[51]. Only accounting for seagrass-derived autochthonous organic carbon also negates accounting for any organic carbon derived from other primary producers found in situ within the seagrass (e.g., epiphytic material). Epiphytic material represents a potential *in situ* contributor of organic carbon, whose presence is enabled by the seagrass acting as a substrate. Therein many of these carbon credit frameworks would benefit from introducing flexibility to include other organic carbon (e.g., epiphytic material) fixed *in situ* within the seagrass habitat as autochthonous, at least where there is data to justify its inclusion. However high epiphytic loads can be associated with seagrass deterioration[54] and therefore reduced carbon sequestration capacity, which misaligns with the ethos of seagrass restoration for climate mitigation benefits, so this is a context dependent example. Considering only seagrass-derived carbon works as a conservative approach but applying the methodology in VM0033 to estimate allochthonous deductions underestimates the actual mass of carbon sequestered *in situ*[55]. To distinguish other *in situ* primary producer contributions to the sediment would require applying isotopic analysis spatially or utilising other techniques, such as compound-specific isotope analysis or eDNA, which are not utilised in this study. Thus, supporting the estimation and uptake of high integrity allochthonous deduction evidence would assist uptake of these frameworks to support nature-based solutions.

Based on our current understanding of carbon provenance in this locality, it would suggest that a solely carbon-accredited funding scheme for seagrass restoration would not be suitable or provide the funds necessary for restoration. Given the array of ecosystem services seagrass ecosystems provide, the most viable restoration funding scheme would complement carbon payments with payments for additional ecosystem services[52] adopting a holistic strategy which considers the level of each ecosystem service provided by that specific meadow rather than relying on carbon sequestration alone. Policy targets could also shift away from habitat specific accounting; the benefits of wider seascape accounting have been suggested to remove some of the complications arising from prescribing certain materials as allochthonous or autochthonous[56]. Ultimately disparity in terms of what to consider allochthonous and accounting for allochthonous deductions likely limits uptake of carbon accreditation but also undervalues these ecosystems.

There are caveats to utilising stable isotope analysis (e.g., the influence of diagenesis on fractionation) and situationally dependent mixing models (e.g., selecting the appropriate contributing sources) to determine carbon provenance in coastal sediments. Further research could enhance our confidence in these estimates (e.g., diagenesis correction factors), our multi-proxy approach considers both C_{org}/N and isotope analyses to increase our confidence in the inferences made about the contribution and provenance of organic carbon sources to seagrass sediment. As such, this study highlights some discernible differences between our estuarine and open coastal seagrass sites, including (1) seagrass tissue $\delta^{13}C$ values likely partially related to DIC $\delta^{13}C$ values in these environments; (2) different sediment C_{org}/N ratios related to the influence of different allochthonous organic carbon sources at each site; (3) different sediment $\delta^{13}C$ depth profiles which highlight a potential knowledge gap related to the remineralisation of vegetative material in different sediment types (e.g., sandy versus muddy) and how this may influence $\delta^{13}C$ values. This highlights why it is appropriate to take a site-specific point of view when formulating and interpreting our SIMMs. This approach has delivered conservative estimates of carbon provenance in intertidal seagrass sediments based on our current knowledge and data availability in this context and locality. The interpretation of isotope values remains a valuable tool for determining carbon provenance in sediments, but the isotope values themselves represent an integrated value of several processes which cannot always be fully disentangled and as such, care should be taken when interpreting $\delta^{13}C$ for this purpose. Ultimately this study provides locally important field-derived estimates of autochthonous organic carbon which suggest only a small proportion of the organic carbon stored within these seagrass sediments would be applicable to trade-in existing carbon accreditation schemes. These field estimates of allochthonous carbon demonstrate that current blue carbon accreditation frameworks undervalue these ecosystems. Carbon accreditation frameworks would benefit from a broader interpretation of what is considered autochthonous carbon where data is clearly available to demonstrate such and should be designed so that complementary payments for additional ecosystem services can be more easily integrated. Finally, supporting the estimation and uptake of high integrity field-derived allochthonous deduction evidence would encourage uptake of these frameworks to support implementation of nature-based solutions.

Methods

This study was conducted at two intertidal seagrass meadows within the Solent, South of England: Ryde, an open coastal sandy seagrass site on the Isle of Wight and Farlington Marshes, a muddy seagrass site located within Langstone Harbour a tidal inlet.

Sample collection

All sediment sampling occurred in summer 2022 (July - August) within the two seagrass meadows at Farlington Marshes and Ryde. Sediment cores were collected 70 m from the meadow strandline perpendicular to the shoreline. At Farlington Marshes, the three sediment cores were collected across the seagrass bed (100 m apart), whilst at Ryde permission was only granted to collect cores within the land owned by Wightlink ferries (cores ~ 10 m apart). The cores were collected on foot by driving a metal split corer (Van Walt) into the sediment to a depth of 30 cm (internal core diameter = 6.5 cm). The collection of cores was repeated until 30 cm cores showing no compaction were obtained. The split corer was opened onsite for subsampling to prevent sediment mixing within the core, which can occur during transport off-site. From the three 30 cm seagrass sediment cores collected at each site, only the sediment from 0 to 5 cm below the sediment surface was utilised in this study. Each of the 0–5 cm core sections had been split into subsamples following 1 cm intervals. After slicing, subsamples were placed into sterile sample bags and returned to the Institute of Marine Science, where they were frozen at -20 °C.

Higher seagrass leaf $\delta^{13}C$ is witnessed in spring and lower leaf $\delta^{13}C$ in the summer[21] therefore collecting seagrass tissue outside these two extremes provides samples which have $\delta^{13}C$ values more representative of the average annual $\delta^{13}C$ seagrass value. Furthermore, the seasonal senescence of seagrass leaves documented at these sites[27] suggests the main deposition period for seagrass detritus occurs in Autumn. Therefore, vegetative samples of both seagrass species were collected alongside other primary producers found adjacent to the seagrass meadows at Ryde and Farlington Marshes on the 11th and 14th October 2022. All samples were stored at the Institute of Marine Sciences, frozen at -20 °C.

Elemental and isotope analysis

The sediment samples were left to defrost overnight and then dried in an oven at 40 °C for at least 96 h. Macroscopic items such as roots, rhizomes and large shell fragments were removed from sediment samples to isolate the sedimentary carbon[57]. The samples were homogenised, initially by pestle and mortar, then milled using a Fritsch Pulverisette 7-ball mill (Fritsch International), spun at 700 rpm for up to seven minutes. Approximately 10 ml of 5% HCl was added to 1 g of each sediment sample, agitated then incubated overnight. The addition of acid causes bicarbonate and carbonate ions to be converted to carbon dioxide (inorganic carbon), leaving the organic carbon present within the sediment sample. Subsequently, samples were centrifuged, supernatant removed, and water added. This washing step was repeated until the sample reached a neutralised pH. The sediment samples were then dried in an oven at 40 °C for at least 24 h and ground by pestle and mortar.

The seagrass shoots were separated into the above-ground tissue (leaves) and below-ground tissue (rhizome and roots). Seagrass leaves were gently scraped to remove any epiphytic material. At Farlington Marshes the seagrass epiphytic material was negligible, but at Ryde, the *Z. marina* leaves had sufficient epiphytic material that was removed and retained to be considered as another organic carbon source. All vegetative samples (seagrass and other primary producers) were acid-washed in 5% HCl to remove carbonates and rinsed in deionised H_2O prior to lyophilisation and homogenisation using a mortar, pestle and liquid nitrogen to produce a fine powder.

Each homogenised sample was weighed directly into tin capsules using a microbalance with their weight recorded to the 0.001 mg level. The quantity of sample weighed out was relative to each material type (e.g., sandy sediment, muddy sediment, vegetative) to ensure each sample contained approximately 500 µg of organic carbon and 100 µg of nitrogen. Across the runs, 10% of the samples were analysed in duplicate. Vegetative samples were all acid washed and these were utilised for $\delta^{13}C$ and $\delta^{15}N$ determination. Whilst acidified sediments were used for $\delta^{13}C$ determination, and non-acidified sediments were used for $\delta^{15}N$ determination. Analysis was completed on an Elementar vario ISOTOPE cube elemental analyser coupled to an isoprime precisION isotope ratio mass spectrometer with an onboard centrION continuous flow interface system at the Isotope Geosciences Laboratory, British Geological Survey, UK. Carbon isotope ratios were corrected for[17]O interference & linearity effects, then normalised to Vienna Pee Dee Belemnite (VPDB) using USGS61 (-35.05‰), USGS62 (-14.79‰), and USGS63 (-1.17‰). Normalisation and linearity were checked using well-characterised internal laboratory standard BROC3 (-27.6‰). Nitrogen isotope ratios were normalised to atmospheric nitrogen (AIR) using USGS61 (-2.87‰), USGS62 (+20.17‰), and USGS63 (+37.83‰). Carbon isotope data are reported in delta (δ) notation in per mille (‰) relative to the VPDB international reference scale, and for N measurements, the standard is AIR.

The stable isotopic composition is reported as δ values:

$$\delta_{\mathrm{sample}} = 1000[(\mathrm{R}_{\mathrm{sample}}/\mathrm{R}_{\mathrm{standard}}) - 1]$$

where $R = {}^{13}C/{}^{12}C$ for $\delta^{13}C$, and $R = {}^{15}N/{}^{14}N$ for $\delta^{15}N$ values. Internal laboratory standard BROC3 (41.25%C and 4.85%N) was used to calculate the elemental content of samples and C_{org}/N is reported as the mass ratio. Given the low %N in the sandy sediment samples (Supp., Fig. 1), there was insufficient material to analyse $\delta^{15}N$ accurately, as the low prevalence means even the slightest heterogeneity in the sample could lead to misinterpretation of the values. Therefore, both the sandy sediment (Ryde) and vegetative samples from this site were only analysed for $\delta^{13}C$ by isotope ratio mass spectrometry.

Data analysis

Seagrass $\delta^{13}C$ values and C_{org}/N ratios according to species and tissue type

Initially, the seagrass $\delta^{13}C$ and C_{org}/N data were grouped by site (FM or RY), seagrass species (*Z. marina* or *Z. noltii*) and seagrass tissue type (Above ground and below ground). Shapiro-Wilk test was used to test the normality of the data. Initially site was removed as a factor and the sites were assessed separately to determine if there was a significant difference between the seagrass species and tissue type. Where a site's data conformed

to normality and met the bartlett's test for homogeneity of variance ANOVAs were applied. Where a site's data conformed to normality but did not meet the assumptions of homogeneity both the factors (seagrass species and tissue type) were assessed individually by Welch's t-test. Where a site's data did not conform to normality a Wilcoxon test was applied.

Thereafter, the seagrass $\delta^{13}C$ data was grouped only by site (FM or RY) and seagrass tissue type (Above ground and below ground). Shapiro-Wilk tests confirmed that the data conforms to the normal distribution and meet the assumptions of homogeneity when grouped by site and tissue type. Therefore, ANOVAs were used to determine whether there was a significant difference in $\delta^{13}C$ based on the two factors, site and tissue type, as well as on the interaction between these two factors. Cohen's *d* is reported alongside the ANOVA output as a measure of effect size.

Sediment organic carbon content $\delta^{13}C$ downcore profiles

The sediment $\delta^{13}C$ value was averaged across the five sediment depth subsamples collected from three cores of each site ($n = 15$) to produce the average sediment $\delta^{13}C$ value at each site. Shapiro-Wilk test confirmed that the data conforms to the normal distribution when grouped by site. Therefore, a t-test was used to assess if there was a significant difference between sites.

We computed the remaining fraction of organic carbon, f, for each sample as the ratio of its organic-C density to the maximum density observed in the dataset. The natural logarithm of this fraction, ln(f), was then used as the predictor in a linear Rayleigh-distillation model:

$$\delta^{13}\mathrm{Ci} = \delta^{13}\mathrm{C0} + \varepsilon\ \mathrm{In(fi)},$$

fitted by ordinary least squares to our measured $\delta^{13}C$ values. For comparison, we also fitted a simple linear regression of $\delta^{13}C$ against sediment depth (cm) to assess whether depth itself could better explain isotopic variation.

Presence and provenance of allochthonous organic carbon

The $\delta^{13}C$ values from both seagrass species (*Z. marina*, *Z. noltii*) and tissue types (above ground, below ground) were collated to provide an average seagrass $\delta^{13}C$ value at each site. The differences between $\delta^{13}C$ seagrass sediment values and the site-specific average seagrass $\delta^{13}C$ were calculated ($\Delta\delta^{13}C_{\text{seagrass-sediment}}$).

The sediment $C_{org/}N$ was averaged across the five sediment depth subsamples collected from three cores of each site ($n = 15$) to produce the average sediment $C_{org/}N$ value at each site. Shapiro-Wilk test confirmed that the data conforms to the normal distribution when grouped by site, but the bartlett's test showed the data did not meet the assumptions of homogeneity. Therefore, Welch's t-test was used to assess if there was a significant difference between sites. The individual sediment $C_{org/}N$ and $\delta^{13}C$ data points were overlaid onto typical C_{org}/N ratios and $\delta^{13}C$ ranges for marine (Particulate Organic Carbon (POC) and algae), terrestrial (C_3 and C_4 plants) and bacterial organic inputs to coastal environments according to Lamb[11].

Potential contribution of primary producers to seagrass sediments

The in situ primary producers from Ryde were merged, where appropriate, into five groups with similar $\delta^{13}C$ and C_{org}/N values: seagrass above ground (AB) and below ground (BG) tissue, red algae, epiphytic material and brown algae and selected as potential organic carbon sources for this site's mixing model. For Farlington Marshes the in situ primary producer $\delta^{13}C$, $\delta^{15}N$ and C_{org}/N values were merged into five groups seagrass above ground (AB) and below ground (BG) tissue, green algal mat, saltmarsh and brown algae and selected as potential organic carbon sources for this site's mixing model. At low lipid concentrations, lipid extraction has very little impact on the $\delta^{13}C$ of plant samples[58]. For plants, $\%C_{org}$ can be used as a predictor of the % lipid in samples when $\%C_{org} > 40\%$, but little relationship between $\%C_{org}$ and % lipid exists among samples with $\%C_{org} < 40\%$[58]. Whilst this seminal paper on lipid extraction suggests it should generally be performed on plant samples with $\%C_{org}$ used to normalize lipid content[58] as all our vegetative samples collected *in situ* had $\%C_{org}$ values < 40% (only one value above this threshold at 40.7 $\%C_{org}$) it seems inappropriate to apply lipid correction values where there is little relationship between the response and the predictor. Also included as allochthonous sources were reported stable isotopic compositions of suspended particulate matter (SPM) and terrestrial leaf litter[19]. The relative contribution of different primary producers as potential sources of organic matter to UK seagrass sediments was estimated from Bayesian mixing models[46,59] run with the stable isotope mixing model (simmr) package[60] fit by Markov chain Monte Carlo. The mixing models were run separately for each site, due to differences in the number of isotopic tracers and the number of potential organic sources. To determine whether multiple chains of a SIMM were converging to the same distribution $\widehat{R}$ values were calculated:

$$\widehat{R} = \sqrt{\frac{Variance\ between\ chains}{Variance\ within\ chains}}$$

The final SIMM; used was a two-tracer ($\delta^{13}C$ and C_{org}/N) seven-source SIMM for Ryde and a three-tracer ($\delta^{13}C$, $\delta^{15}N$ and C_{org}/N) seven-source SIMM for Farlington Marshes.

Data availability

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

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Author contributions

Research conceptualisation was undertaken by EAW, SER, MJL, JHL, MC, FR and JP. Research methodology was determined by EAW, SER, MJL, JHL. Investigation (fieldwork and laboratory processing) was undertaken by EAW, JHL and BP. Writing the original draft, formal analysis and visualisation were undertaken by EAW, with the supervision of JP, SER, MC and FR. All authors were included in the review and editing.

Declarations

Competing interests

The authors declare no competing interests.

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