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Soil Carbon Stocks Vary Across Geomorphic Settings in Australian Temperate Tidal Marsh Ecosystems

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ABSTRACT

Tidal marshes rank among the ecosystems with the highest capacity to sequester and store organic carbon (C_{org}) on earth. To inform conservation of coastal vegetated ecosystems for climate change mitigation, this study investigated the factors driving variability in carbon storage. We estimated soil C_{org} stocks in tidal marshes across temperate Western Australia and assessed differences among geomorphic settings (marine and fluvial deltas, and mid-estuary) and vegetation type (*Sarcocornia quinqueflora* and *Juncus kraussii*) linked to soil biogeochemistry. Soil C_{org} stocks within fluvial and mid-estuary settings were significantly higher (209 ± 14 and 211 ± 20 Mg C_{org} ha⁻¹, respectively; 1-m-thick soils) than in marine counterparts (156 ± 12 Mg C_{org} ha⁻¹), which can be partially explained by higher preservation of soil C_{org} in fluvial and mid-estuary settings rich in fine-grained (< 0.063 mm) sediments ($49 \pm 3\%$ and $47 \pm 4\%$, respectively) compared to marine settings

($23 \pm 4\%$). Soil C_{org} stocks were not significantly different between *S. quinqueflora* and *J. kraussii* marshes (185 ± 13 and 202 ± 13 Mg C_{org} ha⁻¹, respectively). The higher contribution of tidal marsh plus supratidal vegetation in fluvial (80%) and intermediate (76%) compared to marine (57%) settings further explains differences in soil C_{org} stocks. The estimated soil C_{org} stocks in temperate Western Australia's tidal marshes (57 Tg C_{org} within ~ 3000 km² extent) correspond to about 2% of worldwide tidal marsh soil C_{org} stocks. The results obtained identify global drivers of soil C_{org} storage in tidal marshes and can be used to target hot spots for climate change mitigation based on tidal marsh conservation.

Key words: Saltmarsh; Carbon storage; Temperate ecosystems; Habitat characteristics; Blue carbon; Coastal ecosystems; Biogeochemical cycles; Western Australia.

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Author's Contribution CG and OS conceived the ideas and designed the study; CG, OS, CS, PL and JJK collected the data; CG analysed the samples and performed the statistical analyses; OS, PL and JJK contributed to interpreting results; CG wrote the first draft of the manuscript, and all authors reviewed and edited the manuscript.

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HIGHLIGHTS

- Soil organic carbon stocks were 1.3-fold higher in upstream tidal marsh
- Mud content and halophyte contribution enhanced the soil organic carbon stocks
- Temperate Western Australia holds about 2% of worldwide tidal marsh soil carbon stocks

INTRODUCTION

Recent emphasis on climate change mitigation has focused attention on the valuation of ecosystems that naturally act as carbon sinks (Ullman and others 2013). Blue carbon ecosystems, such as tidal marshes, mangroves and seagrasses, have been identified as some of the most efficient carbon sinks on earth (McLeod and others 2011). Although terrestrial ecosystems typically reach maximum soil organic carbon (C_{org}) sequestration capacity within decades or centuries, blue carbon ecosystems are capable of accumulating C_{org} in their soils over millennia (Nellemann and others 2009; McLeod and others 2011). On a per unit area basis, tidal marshes are one of the most efficient C_{org} sinks globally (McLeod and others 2011). However, global estimates of the total soil C_{org} inventories in tidal marsh ecosystems remain poorly understood. This can be attributed to a lack of below-ground C_{org} data from globally relevant regions and limitations in tidal marsh areal coverage and distribution data (Chmura and others 2003; Duarte and others 2013; Mcowen and others 2017).

Tidal marsh ecosystems occur on all continents except Antarctica and though they persist across all climatic regions, they are most prevalent in temperate zones (Mcowen and others 2017). Tidal marshes inhabit a wide variety of geomorphic and depositional environments and can exhibit significant variability in soil C_{org} stocks across multiple spatial and temporal scales. At the global scale, variability of tidal marsh soil C_{org} stocks is primarily driven by historic rises in sea-level, which controlled vertical accretion of mineral and organic materials (Rogers and others 2019). In addition, estimates of tidal marsh soil C_{org} storage capacity at continental or regional scales can be highly variable (up to 68-fold; Macreadie and others 2017), which points to the importance of local environmental drivers influencing soil C_{org} storage.

Variability in soil C_{org} stocks at the regional scale has been associated with differences in climatic and physical variables (for example, temperature,

salinity, precipitation, sediment type, elevation, periodicity and intensity of inundation; Christiansen and others 2000; Chmura and others 2003; Butzeck and others 2014; Baustian and others 2017). For example, cooler climates and greater rainfall, characteristic of temperate regions, minimise salinity-induced stress and enhance growth rates and survivorship of tidal marsh plants, contributing to autochthonous C_{org} inputs into the soil (Adam 1990; Saintilan 2009). Although plant community structure, topography and resulting inundation regimes likely influence C_{org} availability in tidal marsh habitats, variability at the within-estuary spatial scale is largely attributed to variation in geomorphic settings and their influence on physical and physico-chemical characteristics of tidal marsh habitats (Kelleway and others 2016; Baustian and others 2017; Alongi 2018; van Ardenne and others 2018). Differences in fluvial and marine influence on tidal marsh can alter the supply of nutrients, salinity and sediment availability (Roy and others 2001; Saintilan and others 2009; Kelleway and others 2016). These factors can impact rates of tidal marsh primary production and decomposition of plant biomass and other organic matter within systems (Saintilan and others 2013), and thereby soil C_{org} storage. In estuaries, the depositional characteristics of tidal marsh habitats are often associated with their geomorphic setting, including marine flood tidal deltas, mid-estuarine tidal flats, fluvial deltas and riverine habitats (Roy and others 2001; Saintilan and others 2009; Kelleway and others 2016). Previous studies have shown that tidal marsh habitats situated near fluvial deltas can store up to twofold larger soil C_{org} stocks than marine-dominated counterparts (Kelleway and others 2016; Macreadie and others 2017).

Furthermore, differences in geomorphic setting (topography and hydrology) influence soil characteristics (for example, sediment grain size and mineralogy), suspended sediment supply, allochthonous carbon inputs and the production and/or deposition of biogenic carbonates in tidal marsh habitats, which contribute to spatial variability in soil C_{org} at the within-estuary scale (Kelleway and others 2016; Macreadie and others 2017). For example, an abundance of fine-grained sediments, characteristic of fluvial-influenced tidal marsh, are likely to limit oxygen exchange and redox potential within soils, thereby limiting organic matter remineralisation rates and enhancing soil C_{org} preservation (Hedges and Keil 1995).

Other potential drivers of within-site variability of C_{org} stocks are spatial patterns in vegetation,

historic shifts of habitat type (for example, the presence of mangrove soils below contemporary tidal marsh), and the elevation of collected samples from within a site (low, mid and upper marshland; Kelleway and others 2017b; van Ardenne and others 2018). However, factors influencing soil C_{org} in tidal marshes remain understudied when compared to those of mangrove and seagrass habitats (Donato and others 2011; Fourqurean and others 2012a; Alongi and others 2016; Serrano and others 2016; Mazarrasa and others 2018; Rovai and others 2018). To allow spatial modelling of regional or global soil C_{org} stocks from limited data sets, there is a need for improved habitat distribution data and to better understand the biophysical conditions which control soil C_{org} variability in tidal marshes at each spatial scale (Kelleway and others 2016).

Australia contains 5–11% of the global blue carbon stocks, spread over a wide range of climatic and geomorphic regimes, including up to 35% of the global tidal marsh soil C_{org} stock (Serrano and others 2019). The Western Australia (WA) coast-line spans approximately 21,000 km (about one-third of the Australian coastline; Bucher and Saenger 1991; Geoscience Australia. 2004), encompassing about 22% of mapped tidal marsh extent in Australia and about 5% of total world-wide tidal marsh extent (Bucher and Saenger 1991; Mcowen and others 2017; Serrano and others 2019). Tidal marshes within southern WA differ from the more intensely studied temperate tidal marshes of eastern Australia in that they occur along a microtidal coast almost completely devoid of mangroves and subject to strongly seasonal (Mediterranean) climatic conditions (Brearley 2006). Further, owing to the persistent and high wave energy regime of the temperate south-west coastline, the estuaries within this region are collectively wave-dominated barrier estuaries with tidal marsh distributed across multiple geomorphic settings (for example, marine flood tidal deltas and fluvial deltas; Hodgkin and Hesp 1998), presenting the opportunity for a robust comparison of within-estuary variability in soil C_{org} stocks.

Previous studies found that soil C_{org} stocks in WA tidal marsh ecosystems were up to twofold lower compared to other temperate regions within Australia and worldwide (Chmura and others 2003; Zhang and others 2008; Holmquist and others 2018; Macreadie and others 2017; Serrano and others 2019), including those with similar relative sea-level histories over the Holocene (Rogers and others 2019). However, the WA estimates were based on a limited data set comprising only 10 cores from four tidal marsh ecosystems. The paucity of

soil C_{org} stock estimates for tidal marsh in the western third of Australia has been recognised as a key limitation in the most comprehensive assessment of blue carbon resources in the country (Serrano and others 2019).

The primary goal of this study was to provide robust estimates of blue carbon stocks for temperate Australian tidal marshes which occur in a highly seasonal, Mediterranean climate zone, and have variable levels of connectivity to the ocean. To this aim, we quantified soil C_{org} stocks across temperate WA tidal marshes and assessed potential factors (that is, climate and habitat characteristics) driving carbon storage in tidal marshes. We hypothesise that soil C_{org} stocks in temperate WA tidal marshes would be below national and global estimates (as per pre-existing regional estimates). Furthermore, we expected that soil C_{org} stocks would be significantly higher in upper estuary locations compared to those situated in seaward locations.

MATERIALS AND METHODS

Study Setting and Experimental Design

The mean annual rainfall and temperature of WA's temperate region are 824 mm (dominated by winter rain and dry summers) and 15 °C, respectively (Department of the Environment 2015). The variability of localised rainfall across the temperate coastline is high, with the south coast of WA varying from 450 mm to 1400 mm of precipitation as you move from east to west (Hodgkin and Hesp 1998).

Ten estuaries were sampled along the temperate coast of WA, from Perth to Esperance (Figure 1). The sampled estuaries were all wave-dominated barrier estuaries fronted by sandy barrier islands and/or spits. Each estuary maintained either permanently open or intermittently open entrances to the ocean, with some remaining closed for decades at a time (Table 1). Estuarine evolution in WA was greatly influenced by the enhanced sedimentation yields during the Holocene marine transgression (8000 to 4000 years before present), with mid-Holocene shell deposits previously identified in six of the estuaries sampled in this study (Hodgkin and Hesp 1998). Each of the sampled estuaries exhibits relatively low tidal energy, with a microtidal (0.4 m) range (Hodgkin and Hesp 1998; Ryan and others 2003). Instead, wave energy near the barrier entrance and river inflow in upstream areas are the main contributors of energy, though these inputs are largely dissipated towards the broad central

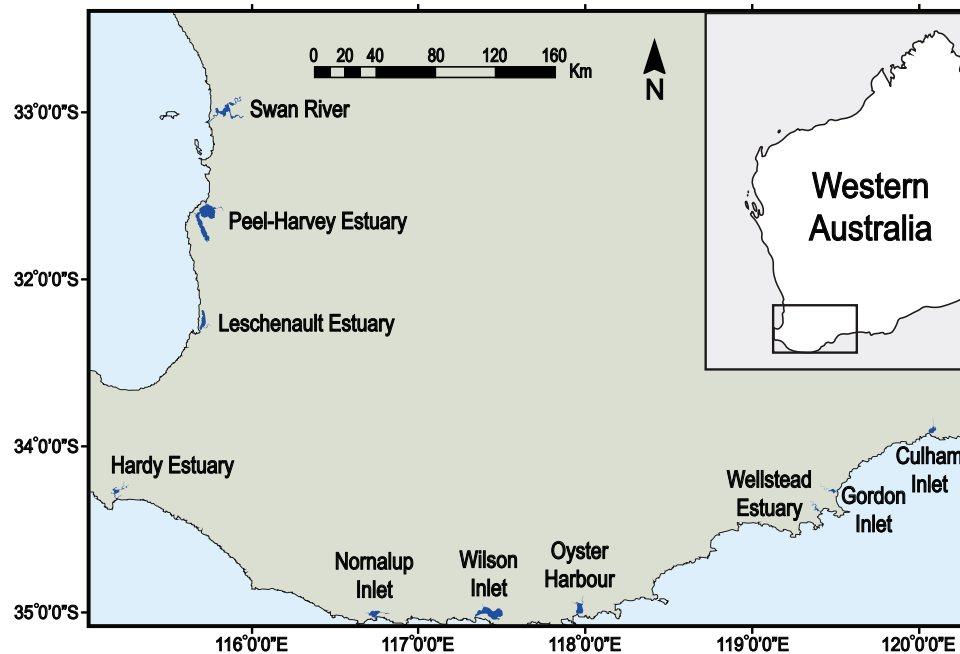


Figure 1. Location of the ten estuaries sampled along the temperate southwest coastline of Western Australia. Dark blue areas along the coastline represent the position and size of each estuary sampled.

basin of each system (Heap and others 2001; Ryan and others 2003).

Tidal marsh ecosystems within this region are comprised of two main vegetative communities: ecosystems formed by low-lying succulent species, dominated by *Sarcocornia quinqueflora* (Ung.-Sternb.) A.J.Scott; and ecosystems formed by taller rush species, dominated by *Juncus kraussii* Hochst. The variability in tidal marsh soil C_{org} stocks among estuaries was assessed by sampling soil cores in three geomorphic settings within each estuary: marine flood tidal deltas, tidal flats located mid-estuary and upstream areas subject to fluvial influence (hereafter referred to as ‘marine’, ‘intermediate’, and ‘fluvial’ settings, respectively). In each geomorphic setting, three soil cores were sampled, one core at each of three points along a transect that ran perpendicular to the shoreline: water’s edge, middle of the tidal marsh and at the fringing vegetation surrounding the tidal marsh (Figure S3). Within an estuary, sampling was conducted consistently in the same dominant vegetative community (that is, either *S. quinqueflora* or *J. kraussii*). Owing to the geomorphological characteristics of each estuary and the distribution of plant communities, it was not always possible to sample all geomorphic settings within each estuary, which resulted in an unbalanced design (Table 1).

Soil Sampling

Soil sampling was carried out by manually hammering PVC pipes (7.5 cm inner diameter and 1.5 m long). A total of 81 cores were sampled among the 10 estuaries studied. Two types of PVC corers were used. ‘Whole cores’ consisting of entire PVC pipes were used to sample the middle of the tidal marsh across all sites. ‘Port cores’ consisting of PVC pipes with pre-drilled holes (3 cm in diameter) situated at 15 cm depth intervals along the sidewall of the pipe were used to sample near the water’s edge and the landward fringing vegetation. Distances between cores were dependent on the width of the tidal marsh being sampled, ranging between 10 and 50 m. Linear downcore soil compaction was assumed throughout coring and was recorded by measuring the difference in surface soil elevation inside and outside the core (Glew and others 2005). In some operations, the compression of a core was very high ($> 40\%$), which was assumed to be linked to either the nail effect or extreme compaction of unconsolidated fine sediments. In these few such cases, we repeated the coring operation in a nearby location and discarded nail effects by measuring distances inside and outside of a corer at multiple occasions during coring. Furthermore, we successfully diminished compaction by hammering softly and rotating the corer more often. As a result of this approach, the mean compaction of all cores

Table 1. Estuary and Study Site Information for the Tidal Marsh Habitats Sampled in Temperate Western Australia

Estuary	Entrance condition	Dominant Species	Geomorphic Setting	Latitude	Longitude	Hydrological Distance from Ocean (km)
Swan-Canning River	Open	<i>J. kraussii</i>	Intermediate	– 32.0260	115.8127	13.6
			Fluvial	– 32.0286	115.9009	24.1
Peel-Harvey Estuary	Open	<i>S. quinqueflora</i>	Marine	– 32.5434	115.7202	2.8
			Intermediate	– 32.6427	115.6469	5.8
Leschenault Estuary	Open	<i>S. quinqueflora</i>	Fluvial	– 32.5519	115.7586	12.5
			Marine	– 33.3002	115.6795	1.3
Hardy Inlet	Open	<i>J. kraussii</i>	Intermediate	– 33.3019	115.6904	2.0
			Fluvial	– 33.3204	115.6794	2.3
Walpole–Nornalup Inlet	Open	<i>J. kraussii</i>	Marine	– 34.3179	115.1764	1.5
			Intermediate	– 34.2876	115.1618	4.8
Wilson Inlet	Intermittent	<i>J. kraussii</i>	Fluvial	– 34.2613	115.1990	9.5
			Marine	– 35.0231	116.7363	1.3
Oyster Harbour	Open	<i>J. kraussii</i>	Intermediate	– 34.9983	116.7106	4.7
			Fluvial	– 35.0000	116.8118	10.0
Wellstead Estuary	Intermittent	<i>S. quinqueflora</i>	Marine	– 35.0245	117.3267	0.7
			Intermediate	– 35.0085	117.3287	2.1
Gordon Inlet	Intermittent	<i>S. quinqueflora</i>	Fluvial	– 34.9988	117.4789	15.3
			Marine	– 34.9920	117.9413	1.1
Culham Inlet	Intermittent	<i>S. quinqueflora</i>	Intermediate	– 34.9623	117.9750	4.8
			Fluvial	– 34.9385	117.9796	7.4
			Intermediate	– 34.3853	119.3645	3.3
			Fluvial	– 34.3649	119.3656	5.2
			Marine	– 34.2841	119.4926	1.3
			Intermediate	– 34.2836	119.4736	2.9
			Fluvial	– 34.2824	119.4527	5.3
			Marine	– 33.9235	120.0542	1.0
			Fluvial	– 33.8869	120.0917	6.2

Hydrological distance = distance between tidal marsh location and open ocean following the most direct hydrological route (assuming an open estuary mouth).

ranged from 0 to 36% (mean \pm SE; $12 \pm 1\%$), which is typical of unconsolidated wetland soils using similar coring methods (Kelleway and others 2016).

The port cores were subsampled in the field by inserting a 60-mL modified syringe into each port, and the total volume of soils subsampled was recorded (Fourqurean and others 2012b). Samples of macrophytes and surrounding vegetation (genera: *Juncus*, *Sarcocornia*, *Tecticornia*, *Sporobolus*, *Samolus*, *Suaeda*, *Eucalyptus* and *Melaleuca*) were collected where present at the study sites as potential end-members for source attribution using stable isotope approaches. Macrophyte and surrounding vegetation samples comprised fresh stem and leaf biomass. All samples were refrigerated at 4 °C before laboratory analyses.

Analytical Procedures

In the laboratory, whole cores were opened lengthwise and soils were sectioned at 1 cm inter-

vals for the top 20 cm and at 5 cm intervals for the remainder (> 20 cm). This work focused on C_{org} located below-ground because much of the above-ground plant material is not sequestered in the long term, and, most importantly, because the highest proportion of the carbon stock is in the sedimentary, below-ground pool (Serrano and others 2019). All soil samples, including those from the port cores, were dried at 60 °C until constant weight to estimate dry bulk density (DBD; g DW cm^{-3}). The bulk samples were split into subsamples by quartering. Subsamples were individually homogenised and ground to a fine powder for biogeochemical analyses. Approximately 4 g powder of all subsamples was combusted at 550 °C to estimate the proportionate loss of organic matter following the methods reported by Heiri and others (2001).

A subset of samples from whole cores ('compressed' sections 0–1 cm, 3–4 cm, 6–7 cm, 9–10 cm, 14–15 cm, 19–20 cm, 25–30 cm, 40–45 cm,

55–60 cm, 75–80 cm) were analysed for sediment grain size, CaCO_3 , C_{org} and stable isotope composition ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$). Sediment grain size was measured with a Coulter LS230 laser diffraction particle analyser at the University of Barcelona (Spain), after organic matter was digested with 10% H_2O_2 . The grain size data were classified in the following fractions: < 0.063 mm (silt and clay), $> 0.063 < 0.5$ mm (fine and medium sands), $> 0.5 < 2$ mm (coarse sands) and > 2 mm (gravel). Statistical methods focused primarily on the silt and clay sediment fraction as previous research has identified fine-grained sediments to be correlated with enhanced sequestration and preservation of soil C_{org} (Kelleway and others 2017a).

Prior to C_{org} and CaCO_3 analyses, powdered soils underwent the ‘Champagne test’ (Schlacher and Connolly 2014) to determine the presence of inorganic carbon (C_{inorg}). From 266 samples, only 76 bubbled when acidified with 1 M 10% HCl. Where C_{inorg} was identified, about 1 g of powdered sample was acidified with 10% HCl in a pressure calcimeter to estimate % CaCO_3 content (Horváth and others 2005). To ensure accuracy of the visual Champagne test, a subset of samples ($\sim 33\%$) were acidified in the pressure calcimeter to confirm that CaCO_3 was absent in these samples. Pure CaCO_3 was analysed together with the samples to obtain a calibration curve (that is, linear regression) to estimate soil C_{inorg} content.

The C_{org} (%) and stable isotope composition ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) of soil organic matter and macrophyte samples were analysed through isotope ratio mass spectrometry (IRMS) at the University of Hawaii’s Hilo analytical laboratory. All C_{inorg} -free soil and macrophyte samples were encapsulated in tin capsules for analysis, while soil samples containing C_{inorg} were analysed in duplicate after 4% HCl acidification treatment in silver capsules to remove C_{inorg} (Kennedy and others 2005). The content of C_{org} was calculated for the bulk (pre-acidified) samples. The acidification methods used to remove CaCO_3 prior to C_{org} analysis have been shown to result in an underestimation of C_{org} content, compared to the more reliable estimates of C_{org} content in non-acidified and CaCO_3 -free soil samples (Brodie and others 2011). The different analytical procedures conducted in our study could mask the differences in C_{org} storage observed between settings. Isotope ratios are expressed as δ values in parts per thousand (‰) relative to the Vienna PeeDee Belemnite standard ($\delta^{13}\text{C}$) and atmospheric N_2 standard ($\delta^{15}\text{N}$). Replicate assays and standards indicated measurement errors of

0.01‰ for C_{org} content and 0.2‰ for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$. The $\delta^{13}\text{C}$ values of bulk soil organic matter obtained in this study (that is, in acid- and non-acid-treated samples) reassure that CaCO_3 was not present prior to C_{org} analyses; otherwise, little amounts of CaCO_3 would have resulted in shifting $\delta^{13}\text{C}$ values towards 0‰ (Komada and others 2008). Rather, the range of $\delta^{13}\text{C}$ values identified in this manuscript are representative of what would be expected in temperate tidal marsh soils (ranging from -30 to -12 ‰; Peterson 1999). Although the soil cores sampled in this study were not dated, available sedimentology records from WA’s temperate tidal marshes have identified quartz and carbonate sands, Holocene in age, to occur from ~ 30 cm depth (Semeniuk 2000). This suggests that the 1-m-thick soil deposits studied here likely include sediments deposited over the last about 4000 years.

Numerical Procedures

A linear regression between % C_{org} and %LOI was used to estimate % C_{org} for subsamples that were not analysed via IRMS (Fourqurean and others 2012a; Figure S1). Soil C_{org} stocks estimated from the 81 cores were standardized to 1-m-thick soil deposits to allow comparisons with previous studies, by extrapolating linearly consistent C_{org} values (cumulative C_{org} Mg ha^{-1}) with depth (Arias-Ortiz and others 2018). To validate this extrapolation approach, all cores with at least 1-m-thick soil deposits were extrapolated from 60 cm (shortest length of soil sampled across all sites) to 1 m following the procedures described above (Figure S2). Total estimates of soil C_{org} stocks in temperate WA were calculated by multiplying the mean soil C_{org} stock ($\text{Mg C}_{\text{org ha}^{-1}}$) by the total tidal marsh extent in WA (2965 km^2 ; Bucher and Saenger 1991) following the methodology used in Macreadie and others (2017).

To assess differences in tidal marsh soil C_{org} stocks, % C_{org} , DBD, silt and clay contents, $\delta^{13}\text{C}$ values and CaCO_3 contents (independent variables) among geomorphic settings (dependent variable; marine, intermediate and fluvial), univariate permutational analyses of variance (PERMANOVA) were run in Primer 7, using Euclidean distance to produce Fisher’s traditional univariate F statistic (Anderson and others, 2008). Differences in tidal marsh soil C_{org} stocks were also analysed between vegetation type (*J. kraussii* and *S. quinqueflora*), estuary entrance structure (permanently open and intermittently open) and core transect position (water’s edge, middle of tidal marsh and

fringing vegetation). PERMDISP was used to test for homogeneity of multivariate dispersions. Where this assumption was not met, square root transformations were applied. Where homogeneity could not be achieved, variables were interpreted with an increased confidence interval ($P < 0.01$). A principle component analysis (PCA) was also run in Primer 7 to visualise trends between soil %C_{org} content, DBD, silt and clay content, $\delta^{13}\text{C}$ values and CaCO₃ content among geomorphic settings. All data were standardised using z-scores prior to PCA analysis.

Two-isotope, two-source mixing models were run in R to assess the proportional contribution of source material in temperate WA tidal marsh soils. Mixing models were run using *simmr* and *rjags* packages (Parnell and others 2013; Parnell 2019). The isotopes used in this model were $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, and the two-source end-members were broadly classified as 'Depleted in ^{13}C ' and 'Enriched in ^{13}C '. This broad classification distinguished partially autochthonous organic vegetation (tidal marsh halophytes plus supratidal vegetation) as 'Depleted in ^{13}C ' and allochthonous vegetation (seagrass, marine seston and macroalgae) as 'Enriched in ^{13}C '. The reference isotopic signatures (mean \pm SD; Table 2) used in this model comprised weighted mean $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values from this study and published values representative of temperate WA obtained from Smit and others (2005) and Svensson and others (2007; Table S2). Four mixing models were run in total; the first was a broad examination of the total proportional contribution of source material in all temperate WA tidal marsh soils. The remaining three models were structured to explore the differences in source material contribution relative to geomorphic setting (marine, intermediate and fluvial): the first encompassing all tidal marsh, the second *S. quinqueflora*-dominated tidal marsh and the third *J. kraussii*-dominated tidal marsh.

RESULTS

Across all tidal marsh habitats studied, the C_{org} stocks in 1-m-thick soils ranged from 71 to 427 Mg C_{org} ha⁻¹ (mean \pm SE; 194 ± 9 Mg C_{org} ha⁻¹), with mean C_{org} concentration of $5.3 \pm 0.2\%$ and 24.6 ± 0.7 mg C_{org} cm⁻³. On average, soil organic matter contained $48.7 \pm 0.6\%$ C_{org}. The correlations between %C_{org} and %LOI in 1 m soil profiles ($R^2 = 0.96$; Figure S1) and between cumulative soil C_{org} stocks extrapolated to 1 m and C_{org} stocks measured to 1 m ($R^2 = 0.92$) were high (Figure S2). The $\delta^{13}\text{C}$ values in all cores averaged $-24 \pm 0.2\text{‰}$ and ranged from -30 to -12‰ . The mixing model applied to all cores identified that organic matter sources depleted in ^{13}C were prevalent within the soil C_{org} pool in tidal marsh soils in temperate WA (72%), compared to sources enriched in ^{13}C (28%; Table 2).

Overall, the C_{org} stocks in 1-m-thick tidal marsh soils were significantly lower in marine settings (156 ± 12 Mg C_{org} ha⁻¹) than those in fluvial or intermediate settings (209 ± 14 and 211 ± 20 Mg C_{org} ha⁻¹, respectively; $P < 0.05$; Figure 2C). This trend was consistent among all estuaries, with the exception of Culham Inlet that had higher stocks in marine (187 ± 50 Mg C_{org} ha⁻¹) than in fluvial settings (129 ± 5 Mg C_{org} ha⁻¹) and at Hardy Inlet (higher stocks in intermediate settings, 392 ± 28 Mg C_{org} ha⁻¹, than in fluvial and marine settings, 209 ± 31 and 222 ± 17 Mg C_{org} ha⁻¹, respectively; Table S1).

Similarly, the biogeochemical characteristics (%C_{org} content, DBD, CaCO₃ content, silt and clay content, and $\delta^{13}\text{C}$ composition) in the top meter of tidal marsh soils significantly differed between marine and those in fluvial and intermediate tidal marsh habitats ($P < 0.01$, Figure 2). The mean C_{org} concentration was lower in marine tidal marsh ($2.3 \pm 0.2\%$ C_{org} and 19 ± 0.6 mg C_{org} cm⁻³) than in fluvial ($7.1 \pm 0.4\%$ C_{org} and 26.8 ± 0.7 mg C_{org} cm⁻³) and intermediate tidal marsh ($6.2 \pm 0.4\%$

Table 2. Isotopic Reference Signatures (mean \pm SD) of Source End-members Used for Calculations, Compiled from Isotopic Values Measured in This Study and Obtained from Smit and others (2005) and Svensson and others (2007)

Source	Reference signatures		Contribution (%)
	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	
Enriched in ^{13}C	-15.4 ± 4.6	4.5 ± 3.7	27.6 (24.1–28.9)
Depleted in ^{13}C	-26.6 ± 1.5	6.7 ± 3.1	72.4 (68.4–75.9)

Contribution reflects the proportional median input (%) of organic source material in temperate Western Australia's tidal marsh soils as analysed in all cores. Values in parentheses represent the 2.5 and 97.5 confidence percentiles.

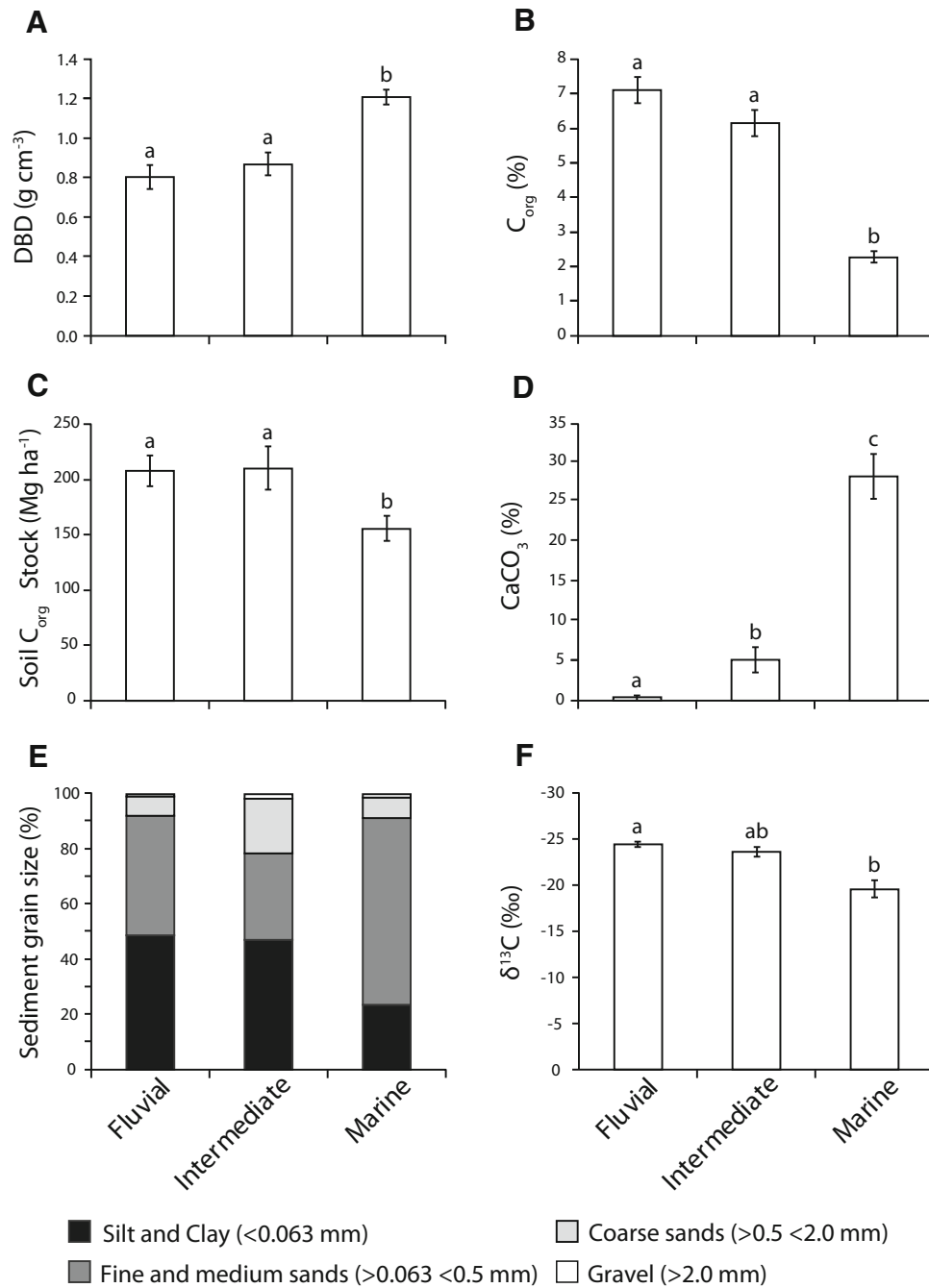


Figure 2. Mean (\pm SE) soil organic carbon stocks (Mg ha^{-1} in 1-m-thick soils) (A); soil organic carbon content (%) (B); dry bulk density (DBD; g cm^{-3}) (C); CaCO_3 (%) (D); sediment grain size fractions (< 0.063 mm; > 0.063 < 0.5 mm; > 0.5 < 2 mm; > 2 mm) (E); and stable carbon isotopes signatures of soil organic matter ($\delta^{13}\text{C}$; ‰) (F) in tidal marsh soils under different geomorphic settings (fluvial, intermediate and marine) in temperate Western Australia. Results of PERMANOVA pairwise tests to assess differences are denoted: different letters (A, B, C) indicate significant differences ($P < 0.01$ in all cases, except for C_{org} stocks ($P < 0.05$)).

C_{org} and $27.0 \pm 0.9 \text{ mg C}_{\text{org}} \text{ cm}^{-3}$; Figure 2B). This trend was reversed for DBD in fluvial, intermediate and marine settings ($0.87 \pm 0.03 \text{ g cm}^{-3}$; $0.93 \pm 0.02 \text{ g cm}^{-3}$; and $1.19 \pm 0.02 \text{ g cm}^{-3}$, respectively; $P < 0.01$, Figure 2A). Fluvial and

intermediate tidal marsh soils contained more than twofold higher silt and clay sediment particles than marine counterparts ($49 \pm 3\%$, $47 \pm 4\%$, and $23 \pm 4\%$, respectively; $P < 0.01$, Figure 2E), while marine tidal marsh had up to 94-fold higher

CaCO_3 content ($28.3 \pm 2.9\%$) than fluvial ($0.3 \pm 0.2\%$) and intermediate ($5.1 \pm 1.6\%$) counterparts ($P < 0.01$, Figure 2D).

The PCA identified two principle components (PC1 and PC2) which explain 49% and 18% of the variability, respectively (Figure 3). Samples were broadly spread by geomorphic settings along PC1 with marine samples clustering at positive values. Intermediate and fluvial samples tended towards more negative values on PC1, though there was little distinction between the two. PCA scores showed that this spread of samples is related to the composition of the mineral substrate (CaCO_3 content vs. silt and clay content), proportion of organic matter and source of C_{org} ($\% \text{C}_{\text{org}}$ vs. DBD and $\delta^{13}\text{C}$).

Marine-situated tidal marsh had significantly depleted ^{13}C values (averaging $-22.7 \pm 0.6\%$) compared to those in fluvial settings ($-24.6 \pm 0.2\%$; $P < 0.01$; Figure 2F). Downcore enrichment of ^{13}C from surface (< 20 cm) to sub-surface (> 20 cm) soils was highest in fluvial and intermediate (2.1% and 1.4% increase in $\delta^{13}\text{C}$ values, respectively) compared to marine tidal marshes (0.6% increase in $\delta^{13}\text{C}$ values; Table S3). The

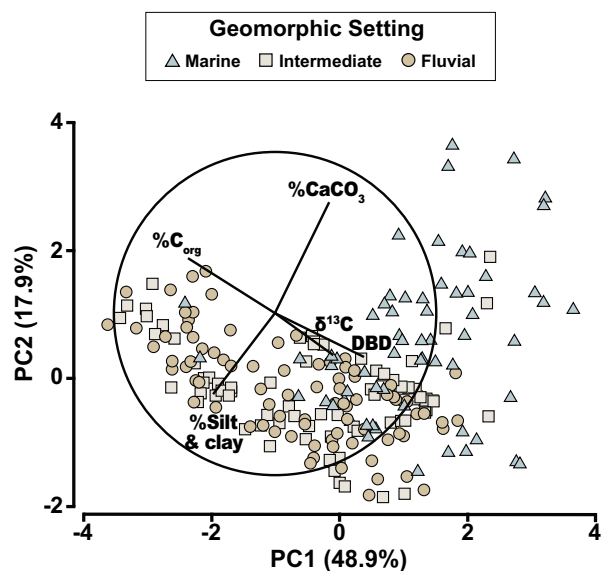


Figure 3. Principal component analysis (PCA) biplot of soil biogeochemical variables as vectors ($N = 236$) and tidal marsh sites colour coded per geomorphic setting. The soil variables included organic carbon content ($\% \text{C}_{\text{org}}$), dry bulk density (DBD), calcium carbonate content ($\% \text{CaCO}_3$), silt and clay content ($\% \text{silt \& clay}$) and the $\delta^{13}\text{C}$ isotopic composition ($\delta^{13}\text{C}$). Opposite vectors are negatively correlated, vectors separated by small angles are positively correlated, and perpendicular vectors are uncorrelated. Higher variability is indicated by longer length of vectors.

mixing models applied indicate that source material depleted in ^{13}C was the dominant contributor of soil C_{org} in fluvial (80%), intermediate (76%) and marine (57%) tidal marsh, except for *J. kraussii*-dominated marine sites where source material depleted in ^{13}C explained a lesser (46%) contribution (Figure 4).

There were no significant differences in soil C_{org} stocks between the two vegetation communities assessed: *J. kraussii* ($202 \pm 13 \text{ Mg C}_{\text{org}} \text{ ha}^{-1}$) and *S. quinqueflora* ($185 \pm 13 \text{ Mg C}_{\text{org}} \text{ ha}^{-1}$; $P > 0.05$, Figure 5A). Furthermore, no significant correlations were found between soil C_{org} stocks and distance to open water ($R^2 = 0.093$), estuary entrance condition ($P > 0.05$; Figure 5B), or core location within tidal marsh habitat (water's edge, middle of tidal marsh and fringing vegetation; $P > 0.05$, Figure 5C).

DISCUSSION

The average soil C_{org} stocks (in 1-m-thick deposits) of temperate WA tidal marsh ($194 \pm 9 \text{ Mg C}_{\text{org}} \text{ ha}^{-1}$) fall above worldwide estimates ($162 \pm 259 \text{ Mg C}_{\text{org}} \text{ ha}^{-1}$; Duarte and others 2013). However, the mean soil C_{org} stock measured in temperate WA tidal marshes is lower than in northeast Canada ($357 \pm 15 \text{ Mg C}_{\text{org}} \text{ ha}^{-1}$; Chmura and others 2003), the contiguous USA (USA; $270 \pm 14 \text{ Mg C}_{\text{org}} \text{ ha}^{-1}$; Holmquist and others 2018) and Europe ($255 \text{ Mg C}_{\text{org}} \text{ ha}^{-1}$, ranging $180\text{--}410 \text{ Mg C}_{\text{org}} \text{ ha}^{-1}$; Chmura and others 2003; Mueller and others 2019), but similar than in China ($189 \text{ Mg C}_{\text{org}} \text{ ha}^{-1}$, ranging $84\text{--}1560 \text{ Mg C}_{\text{org}} \text{ ha}^{-1}$; Zheng and others 2013; Ye and others 2015; Xiao and others 2019) and higher than in the United Arab Emirates (UAE; $80 \pm 18 \text{ Mg C}_{\text{org}} \text{ ha}^{-1}$; Schile and others 2017). Differences between temperate WA's tidal marsh soil C_{org} stocks and the global tidal marsh carbon stocks can be largely attributed to the intensity of relative sea-level rise (RSLR) during the Holocene (Rogers and others 2019). The temperate WA region experienced a mid-Holocene highstand approximately +2 m above present sea levels around six thousand years ago, followed by sea levels declining linearly before returning to present levels approximately 1.5 thousand years ago (Collins and others 2006; Lewis and others 2013). The relative stability of WA's sea level likely reduced the soil C_{org} storage capacity in the region as it limited both vertical and lateral accommodation space, ultimately decreasing the potential for mineral and organic material accumulation and landward encroachment. Conversely, tidal marsh situated along tectonically active coastlines and/or

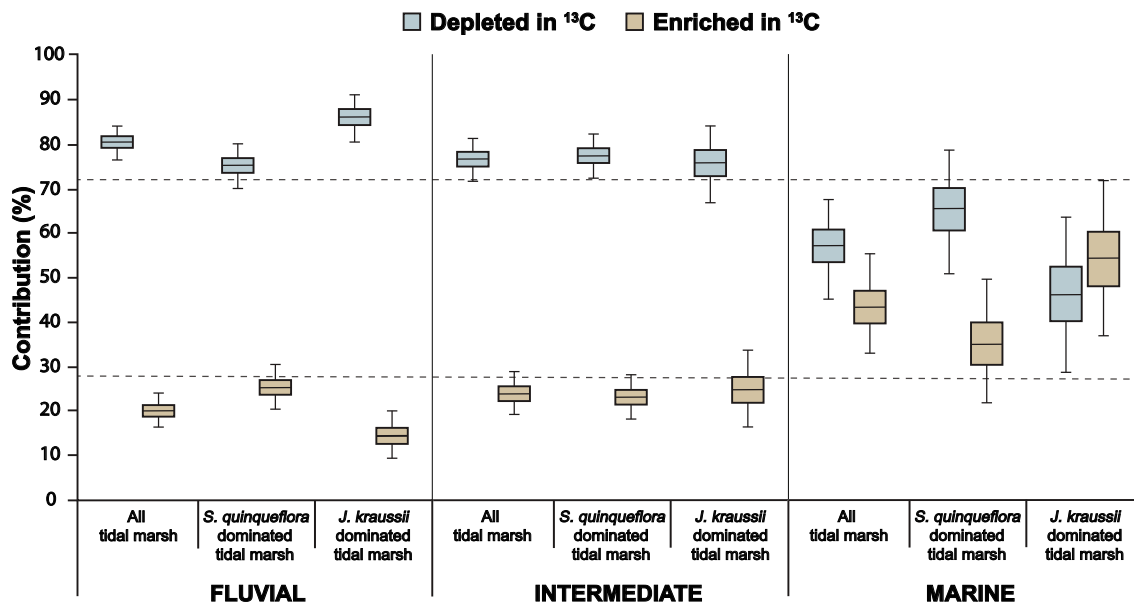


Figure 4. Isotopic mixing model results showing the proportional input (25%, 50% and 75% quantiles) of organic source materials (depleted in ^{13}C and enriched in ^{13}C) in 1-m-thick tidal marsh soils. Three mixing models were run comprising: all sampled tidal marsh, *Sarcocornia quinqueflora*-dominated tidal marsh and *Juncus kraussii*-dominated tidal marsh under different geomorphic settings (fluvial, intermediate and marine) in temperate WA. Dotted lines represent the proportional median input of organic source material as analysed across all cores (depleted in ^{13}C = 72.4% and enriched in ^{13}C = 27.6%).

closer to the maximal ice sheet extent during the last glacial period (that is, regions in Canada, the USA and Europe) were subject to higher rates of RSLR during the Holocene and, therefore, likely experienced an increase in accommodation space and subsequent soil C_{org} sequestration (Rogers and others 2019). The larger soil C_{org} stocks in temperate WA compared to those of the UAE, which maintained a similarly stable sea level during the Holocene, likely stems from multifaceted physical (for example, topography, inundation), chemical (for example, recalcitrance of organic matter) and biological (for example, species composition, productivity) interactions (Campbell and others 2014; Almahsheer and others 2017). For example, the arid climate of the UAE offers higher mean temperatures and lower rates of precipitation than temperate WA, thereby increasing evaporation and promoting hypersaline conditions which reduce tidal marsh plant productivity and increase soil C_{org} decomposition (Laegdsgaard 2006; Schile and others 2017).

The range of soil C_{org} stocks estimated in this study (71–427 $\text{Mg C}_{\text{org}} \text{ ha}^{-1}$) falls within previous estimates of tidal marsh soil C_{org} stocks in temperate Australia (14–962 $\text{Mg C}_{\text{org}} \text{ ha}^{-1}$; Macreadie and others 2017). However, previous estimates of soil C_{org} stocks in WA tidal marshes ($91 \pm 10 \text{ Mg C}_{\text{org}}$

ha^{-1} ; Macreadie and others 2017) were more than twofold lower than the estimates in our study. This is likely a result of the previous estimates stemming from a limited data set from few locations ($N = 10$ cores across two locations) which focused on four seasonally dry (dry during summer months) tidal marsh ecosystems. The seasonally dry ecology of the tidal marsh sampled by Macreadie and others (2017) would likely enhance remineralisation rates of C_{org} and thereby result in relatively lower soil C_{org} stocks compared to the microtidal tidal marshes studied here, which are typical in temperate WA. The seasonally dry nature of tidal marshes reported by Macreadie and others (2017) is linked to floodgate tidal restrictions at the Vasse Wonnerup wetlands (DoW 2010) and the limited water inflow from subterranean oceanic vents and seasonal flooding of the semi-arid Lake MacLeod system located about 20 km inland (DEC 2009). Conversely, the revised assessment of WA temperate tidal marsh soil C_{org} stocks presented here provides a representative and robust estimate of temperate WA, which can be used to further develop blue carbon climate mitigation strategies globally and within Australia under the Emission Reduction Fund (ERF; Kelleway and others 2017a).

Temperate WA tidal marshes situated in fluvial and intermediate settings hold on average 1.3-

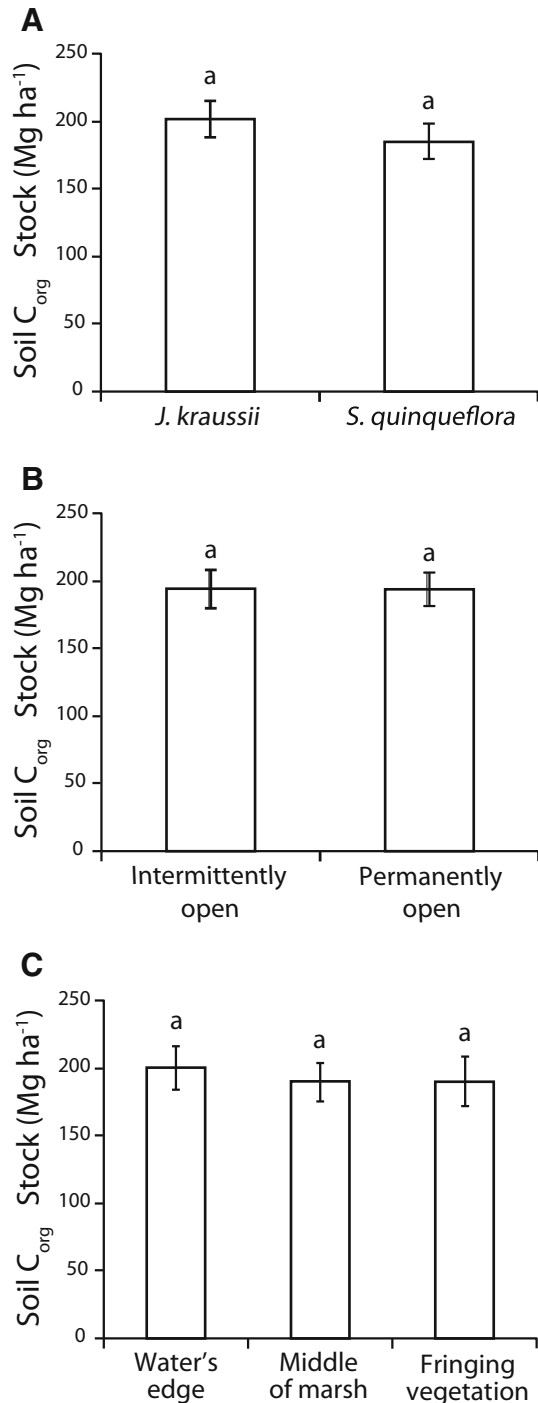


Figure 5. Mean (\pm SE) soil organic carbon stocks (Mg ha⁻¹ in 1-m-thick soils) in *Juncus kraussii*- and *Sarcocornia quinqueflora*-dominated tidal marsh (A); estuary entrance condition (intermittently and permanently open) (B); and core transect position (water's edge, middle of tidal marsh and fringing vegetation) (C) in temperate Western Australia. Results of PERMANOVA pairwise tests to assess differences are denoted: different letters (A, B, C) indicate significant differences ($P < 0.01$).

times greater soil C_{org} stocks than their marine counterparts, in agreement with previous trends described for other Australian and western European tidal marshes (Kelleway and others, 2016; Macreadie and others 2017; Van De Broek and others 2016). The differences in soil C_{org} storage at the within-estuary spatial scale are likely explained by the twofold higher silt and clay contents observed in fluvial and intermediate settings compared to those in marine settings. Fine-grained sediments decrease drainage rates and oxygen exposure time in soils, thereby lowering the redox potential and allowing for higher retention and preservation of C_{org} (Hedges and Keil 1995; Kelleway and others 2016). The two exceptions to this trend were found at Culham Inlet and Hardy Inlet, with relatively higher soil C_{org} stocks in marine and intermediate tidal marsh, respectively, compared to fluvial counterparts. This may be explained by the higher silt and clay contents identified in the marine tidal marsh of Culham Inlet (30% higher than fluvial) and the intermediate tidal marsh of Hardy Inlet (15% higher than fluvial), reflecting higher rates of deposition within these settings. For instance, the Culham Inlet is rarely open to the ocean (once every few decades; Brearley 2006), which could result in the accumulation of terrestrial fine sediments within the lower reaches around our marine sampling site, while potential differences in habitat elevation may influence the depositional characteristics of tidal marsh in the Hardy Inlet. However, further studies (for example, hydrodynamic model, elevation tables) are required to decipher potential causes.

The differences observed in soil C_{org} stocks between fluvial and marine settings in temperate WA are narrower than those identified in estuaries along the temperate east coast of Australia ($>$ twofold differences; Kelleway and others 2016) or in the European Scheldt River ($>$ threefold differences; Van De Broek and others 2016). Sediment deposition on tidal marsh surfaces is reliant on the availability of suspended sediments in the water column and is principally driven by flooding events, including water elevations which barely overflow the shore bank to high spring tide events (Christiansen and others 2000). Owing to the microtidal nature of temperate WA's coastline, which differs from the flooding- and tidal-driven dynamics in estuaries along the east coast of Australia, sediment deposition in upstream marshes may be driven by rainfall events and river inflow. Temperate WA has about 30% lower mean annual precipitation rates compared to those of temperate eastern Australia (824 and 1129 mm, respectively;

Department of the Environment 2015; Bureau of Meteorology 2020), which results in lower river inflow and may explain the lower soil C_{org} stocks in the former. Low levels of precipitation would subsequently reduce the availability of suspended fine-grained mineral and organic materials from upstream sources, as well as decrease the frequency and intensity of inundation events, thereby lowering opportunity for sediment deposition. However, it is important to note that the precipitation gradient along temperate WA's coastline is high (range of 450–1400 mm; Hodgkin and Hesp (1998)) and catchment size varies among estuaries. As such, further research is needed to explore the potential relationship of precipitation rates and the C_{org} storage potential of upstream tidal marsh habitats.

Stable isotope analysis offered insight into the source organic material preserved in tidal marsh soils, as well as the stability of soil C_{org} in temperate WA's tidal marshes. The naturally depleted $\delta^{13}C$ values of surface (< 20 cm) and sub-surface (> 20 cm) soils in fluvial and intermediate tidal marshes are indicative of higher inputs from C3 vascular plants (that is, intertidal halophytes and terrestrial plant litter) compared to marine counterparts. However, as *J. kraussii*, *S. quinqueflora* and the surrounding supratidal vegetation have similarly depleted $\delta^{13}C$ signatures, it is not clear which proportion of these originate from autochthonous (produced within the tidal marsh) or allochthonous C_{org} inputs (imported from adjacent terrestrial ecosystems). Further studies would be required to clarify this question in WA temperate tidal marshes and elsewhere, such as the use of eDNA and molecular techniques (Geraldini and others 2019). The 1–3‰ increase in $\delta^{13}C$ values together with a decrease in % C_{org} with depth in fluvial and intermediate tidal marshes suggests isotopic fractionation during C_{org} decomposition due to selective preservation of organic compounds depleted in ^{13}C (Adame and Fry 2016).

The higher soil C_{org} stocks in fluvial and intermediate compared to marine settings could be partially explained by the higher inputs of tidal marsh and supratidal vegetation in the soil C_{org} pool, which have lower decay rates than those of macroalgae and seagrass (Enríquez and others 1993). Furthermore, the relatively higher inputs of oceanic nutrients expected in marine-situated tidal marsh may reduce root production, thereby lowering soil C_{org} stocks in these settings (Darby and Turner 2008). This is supported by marine-located tidal marsh having accumulated approximately 25% more C_{org} enriched in ^{13}C than their fluvial

counterparts, which likely reflects a proportional increase in the deposition of allochthonous plants with less negative $\delta^{13}C$ values, reflective of the different photosynthetic pathways (that is, seagrasses, many species of macroalgae and marine seston) in marine-situated marshes. Surprisingly, tidal marsh soils dominated by *J. kraussii* had approximately 30% more contribution of C_{org} derived from sources naturally enriched in ^{13}C compared to those dominated by *S. quinqueflora*. This finding contrasts Kelleway and others (2016) who identified higher proportions of allochthonous C_{org} deposits in succulent and grass-dominated marshes as opposed to rush-dominated tidal marsh. This difference can likely be attributed to the microtidal nature of temperate WA's coastline allowing for persistent low-lying tidal marsh habitats which are typically dominated, from shoreline to supratidal vegetation, by a singular plant community. This relative uniformity within sites may also explain the lack of significant difference between soil C_{org} stocks measured along the sampling transect within tidal marsh ecosystems (that is, water's edge, middle of tidal marsh and fringing vegetation) in temperate WA. In contrast, the flooding- and tidal-driven dynamics observed on the east coast of Australia results in increased elevational gradients and heightened habitat zonation, ultimately influencing species assemblages between low and high marshland (Kelleway and others 2017a).

The large increase in $\delta^{13}C$ values with depth (> 3‰) in marine-situated tidal marsh dominated by *J. kraussii* may reflect a change in source material to the modern C3 intertidal halophytes from plant communities such as seagrass and macroalgae (Adame and Fry 2016). Alternatively, this shift may reflect relative contributions from different source material changing systematically with time in response to the balance between available accommodation space and vertical accretion. However, as this downcore enrichment of $\delta^{13}C$ reflects soil profiles of oxic terrestrial sediments (Natelhoffer and Fry 1988), it may be that this enrichment reflects the loss or degradation of proteins and the selective preservation of lignin over time due to pore water exchange and decomposition, thereby resulting in soil organic matter enriched in ^{13}C with depth (Maher and others 2017; Adame and others 2019). Indeed, declining sea levels from 6 to 1.5 thousand years ago (Collins and others 2006; Lewis and others 2013) could explain the increase in marine organic matter sources to soil C_{org} stocks with depth. Further studies are required to determine the age of the sedimentary deposits under-

neath WA' tidal marshes and to estimate C_{org} burial rates.

This data set represents the most extensive estimates of tidal marsh soil C_{org} stocks throughout the western third of Australia. Following this research, the soil C_{org} stocks within the approximately 3000 km² of tidal marsh ecosystems in WA (Bucher and Saenger 1991) were estimated at 57 Tg C_{org} and correspond to about 2% of worldwide tidal marsh soil C_{org} stocks. The inclusion of temperate WA in the national/continental estimate has increased Australia's mean tidal marsh soil C_{org} stocks by 6%, from 165 to 176 Mg C_{org} ha⁻¹, and increased the total Australian blue carbon stock by 2%, that is 30 Tg C_{org} ha⁻¹ (million tonnes; following the spatially unweighted approach of Macreadie and others (2017)). The total C_{org} stored in temperate WA's tidal marshes (in 1-m-thick soils) is roughly equivalent to 5.5 years of total CO₂ emissions by WA from all fossil fuel burning, gas flaring and cement production (WA's emissions estimated at 10.8 Tg C at 2014 rates; CDIAC 2019). However, it is important to note that these estimates stem from a spatially limited and outdated tidal marsh areal data set which does not consider habitat loss or reclamation subsequent to 1989 (Bucher and Saenger 1991). Although this research provides a comprehensive examination of WA's temperate tidal marsh soil C_{org} stocks, it is possible the C_{org} stocks in the arid northwest of Australia reflect the relatively low C_{org} stocks of arid tidal marsh habitats elsewhere (Schile and others 2017). As such, future regional or continental estimates of blue carbon stocks and accumulation rates should focus on including samples from the arid northwest of WA to assess the capacity of Australia's arid tidal marshes to act as viable blue carbon reservoirs. Furthermore, there is a fundamental need to identify and quantify the distribution and coverage of Australia's tidal marshes, particularly within the tidal flats in tropical regions, in order to improve current estimates and facilitate their incorporation into national and international carbon accounting schemes.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

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