

2015

Carbon storage and preservation in seagrass meadows

Mohammad Rozaimi Jamaludin
Edith Cowan University

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CARBON STORAGE AND PRESERVATION IN SEAGRASS MEADOWS

Mohammad Rozaimi Jamaludin

BSc (Life Sciences)

MSc (Marine Biology and Ecology)

This thesis is presented in fulfilment of the requirements for the degree of

Doctor of Philosophy (Environmental Science)

School of Natural Sciences

Faculty of Health, Engineering and Science

Edith Cowan University

March 2015

ABSTRACT

Seagrass meadows are important ‘Blue Carbon’ sinks but many questions remain unaddressed in regards to the organic carbon (OC) sequestration capacity and processes leading to retention and persistence of OC in seagrass sediments. The research summarised in this dissertation examined 37 sediment cores from twelve Australian seagrass meadows (*Posidonia australis* and *Halophila ovalis*) in order to improve our understanding of OC storage and preservation in seagrass sediments. The research: quantified the OC storage in seagrass meadows and the reduction in stores after ecosystem degradation; the rates of OC accumulation; the roles of species composition and the depositional nature of the habitat as factors affecting OC storage; and, characterised the sedimentary organic matter (OM) accumulated over millennia using techniques not previously applied to seagrass sediments.

In Oyster Harbour, Western Australia, *P. australis* had been present over the past 6000 years, as evidenced from radiocarbon analysis of sedimentary matter. Both seagrass- and non-seagrass-derived OM contributed to high sedimentary organic stores (10.79-11.42 kg OC m⁻²; 150 cm sediment depth). The persistence of sedimentary OM over millennial scales indicated that the carbon was well-preserved, thus showing a link between carbon storage and its preservation. By quantifying accumulation rates, and using historical accounts of the highest areal cover (6.1 to 6.7 km²) and recent losses in cover (by 2.8-3.1 km²) due to eutrophication, it was estimated that up to 11.17 Gg OC has been lost from shallow sediments (50 cm depth) following seagrass loss. This carbon was potentially remineralisable and may, therefore, have been liberated back to the atmospheric CO₂ pool.

Nine *Posidonia australis* meadows were then investigated for the effect of the depositional environment on sedimentary OC stores. Based on hydrodynamic differences of meadows categorised as *More Sheltered*, *Less Sheltered*, and *Exposed*, the *More Sheltered* sites had OC stores 6-fold higher (4.57 ± 0.16 to 13.51 ± 0.53 kg OC m⁻²; 140 cm sediment depth) compared to *Exposed* meadows (2.24 ± 0.31 to 3.77 ± 0.85 kg OC m⁻²). The OC stores of *Less Sheltered* meadows were not significantly different to either of the other two categories.

It was concluded that the depositional nature of a seagrass habitat can affect the OC stores, though the affects may be influenced by other site-specific characteristics.

The effect of species composition on OC stores and accumulation rates was subsequently investigated by comparing the stores in estuarine *P. australis* and *H. ovalis* meadows. Comparisons were based on stratigraphic- (OC stores over a set depth) and temporal-based (i.e. accumulation over a set period of time, and as accumulation rates) measures. Organic carbon stores were between 2- (*P. australis*: 10.81 ± 2.06 kg OC m⁻², *H. ovalis*: 5.17 ± 2.16 kg OC m⁻²; 150 cm depth) and 11-fold (*P. australis*: 10.87 ± 2.86 kg OC m⁻², *H. ovalis*: 0.97 ± 0.47 kg OC m⁻²; 2500 yr accumulation) different between meadows of the two species. While the OC stores were different between species, it was also apparent that environmental factors also contributed to the variability, with some *H. ovalis* meadows having stores comparable to some *P. australis* meadows. Thus, both the species and environmental factors needs to be considered for robust predictions of OC storage in seagrass meadows.

The final study reported here investigated the preservation of sedimentary OC in the *P. australis* meadow of Oyster Harbour. A range of biogeochemical variables (age, sediment grain size, anoxia, OM and OC contents, and $\delta^{13}\text{C}$ values) were characterised at increasing depth within a sediment core. Solid-state ¹³C nuclear magnetic resonance was applied to a seagrass core for the first time to characterise the biochemical constituents of the sedimentary OM. There was a 76-80% contribution of seagrass-derived organics (lignin, carbohydrate, and a black-carbon-like OM) into the sediment. The proportion of black-carbon-like material increased with age/depth, indicating that it underwent selective preservation. Carbohydrates decreased with depth/age and lignin showed no changes, indicating that they have undergone non-selective preservation. There was remarkable consistency in the biochemical makeup of the OM with depth, which accumulated over the past 1900 years, indicating a very high preservation potential within seagrass sediments.

Cumulatively, the research presented in this dissertation has highlighted the variability of OC stores in seagrass meadows and how OC may be preserved. The research has indicated that any attempts to estimate regional or global carbon stores must take into account both the species of seagrass that dominate the meadows and the type of depositional environment that

the meadows occur in. It is also clear that *Posidonia* meadows in south-western Australia have the potential to store very large amount of Blue Carbon, comparable in some instances to the highest stores recorded globally, and to preserve these stores over millennia. Modelling future Blue Carbon stores requires an understanding of the fate of the stored carbon following disturbance. It is clear that this carbon can be lost from the meadow, but much of it appears to be in highly recalcitrant forms and it is unclear whether this material is available for subsequent re-mineralisation.

The declaration page
is not included in this version of the thesis

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Many had helped me through this PhD journey. Foremost, I thank Paul Lavery for giving me the opportunity to work with him. A successful student, during the study period and subsequently in future endeavours, is a product of good mentorship; I personally vouch for the exceptional supervision I received from him. My gratitude goes to Oscar Serrano, an avid researcher whom never fails to inspire, lend help and provide insights on many aspects of my research – from the field to the laboratory and down to the office workspace.

I thank my Mom, Jumiah and my Dad, Jamaludin, for their prayers and support for me to succeed in obtaining the degree. My wife, Raja Yana Meleessa, for her patience and care during the many hours spent away from home and the kids. And to my in-laws Esah and Raja Haroon for their understanding and help they provided during my study.

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PREFACE

This dissertation is the culmination of many hours spent in the field and laboratory in search of data relevant to the studies. There are six chapters in the dissertation, with chapters 2, 3, 4 and 5 summarising the main research components. Chapters 3 and 4 share some of the data that are presented within them. Chapter 2 contains data on the organic carbon (OC) stores in a *Posidonia australis* meadow in Oyster Harbour, south-western Australia. This study was undertaken early in my Ph.D. candidature and was written up to allow publication. Subsequently, the study presented in Chapter 3 was undertaken in a variety of locations, including Oyster Harbour to compare the OC stores in *P. australis* meadows from different habitats. In Chapter 4, the OC stores of two different species are compared and to do this, some of the *P. australis* data presented in Chapter 3 are re-analysed in a comparison against *Halophila ovalis* OC stores.

Chapters 2 and 5 have been submitted for publication. All of the research Chapters follow a similar structure except for Chapter 5, which has a combined *Results and Discussion*, compared to separate *Results* and *Discussion* sections in the other chapters. This was to prepare the manuscript in a relevant format for the intended journal.

At the point of submitting this dissertation for publication, Chapters 3 and 4 are in the final stages of preparation for submission to journals. In addition, a sub-set of the data in Chapter 4 was contributed to two papers that have been published:

1. **Rozaimi M.**, O. Serrano, and P. S. Lavery. 2013. Comparison of carbon stores by two morphologically different seagrasses. *Journal of the Royal Society of Western Australia* 96:81-83.
2. P. S. Lavery, M-Á. Mateo, O. Serrano, and **M. Rozaimi**. 2013. Variability in the carbon storage of seagrass habitats and its implications for global estimates of blue carbon ecosystem service. *PLoS ONE* 8:e73748.

I have learnt much on the carbon dynamics of seagrass systems during the years of doing this research. I sincerely hope that this dissertation invigorates your interest in the Blue Carbon ecology of seagrass meadows.

Rozaimi

March 2015

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CHAPTER 1

GENERAL INTRODUCTION

Preamble

In recent decades there has been consistent interest in the relationship between vegetated coastal ecosystems and their relative contributions to the global carbon cycle (e.g. Nellemann et al. 2009, Cebrian and Duarte 1995, Smith 1981). With increasing evidence of global change (IPCC 2013), scrutiny has been directed to these vegetated coastal ecosystems, which potentially play vital roles in mitigating the imbalances of contemporary carbon cycles and the consequent global change (Nellemann et al. 2009). Seagrass meadows are one such ecosystem identified for their important functions as global carbon sinks, yet this important ecosystem service is increasingly compromised due to the losses of seagrass meadows globally (Duarte et al. 2013b). Ecosystem degradation will inevitably reduce carbon sequestration capacity and potentially allow the remineralisation of sedimentary carbon stores (Marbà et al. 2015, Duarte et al. 2013b). It is thus vital to quantify the carbon sequestration capacity of seagrass meadows to emphasise the need for their conservation. Inferences on carbon sequestration potentials for seagrasses, however, are not as robust as they should be. With 60-70 species occurring globally across differing marine environments (den Hartog and Kuo 2006), it is important to establish whether the carbon sink capacities vary among different seagrass species and habitats. This is key to providing reliable estimates of the carbon sink value for seagrasses in general and to underpin modeling of how those values may change in future global or local change scenarios. Understanding the carbon storage capacity of seagrasses also requires a clear understanding on why carbon tends to be

preserved in the sediments of the meadows, a complex outcome resulting from the characteristics of the seagrass, the biogeochemical environment and a range of diagenetic processes.

This thesis attempts to further understand the carbon storage capacity, and the relatively high degree of carbon preservation in seagrass sediments from ecological and biogeochemical perspectives. By linking these two fields, the findings present a more holistic picture of the carbon burial phenomenon in seagrass meadows and contribute new perspectives in understanding the processes leading to carbon sequestration in these ecosystems.

1.1 Global change: Contemporary interests linking the marine carbon cycle with seagrass ecosystems

There have been continual assertions that, in the modern era, incessant and excessive anthropogenic inputs of carbon dioxide to the atmosphere has led to imbalances in the natural carbon cycle, resulting in global change (IPCC 2013). The consensus view is that the imbalance in the atmospheric carbon concentrations may result in global and regional scale changes such as rises in global temperatures, seawater level rises, large scale flooding and ocean acidification (IPCC 2013). Existing natural processes may counter some of this imbalance. Significant amounts of carbon are sequestered through sediment burial in vegetated coastal ecosystems, and in the process, offset anthropogenic outputs of CO₂ (Duarte et al. 2013b, Nellemann et al. 2009). However, much is still unknown with regards to the processes that may capture CO₂ from the atmosphere and its subsequent storage over long time scales. In seagrass meadows, several aspects of carbon cycling have already been established: carbon fixed through photosynthesis during the plants' lifetime may return back to the global carbon cycle when the plant respire, through remineralisation of dead tissues (Mateo et al. 2006, Mateo and Romero 1997, Romero et al. 1992), or through leaching of organic matter (Smith and Penhale 1980). Some other fates of seagrass carbon are:

consumption by herbivores (Valentine and Duffy 2006); export to adjacent ecosystems (Bouillon et al. 2008b, Heck et al. 2008, Duarte and Cebrian 1996) in the form of detritus, or by physical export of whole plants (Short and Wyllie-Echeverria 1996, Hemminga et al. 1991), which may be washed on shores or deep oceans where it accumulates as wrack (Hyndes and Lavery 2005, Orr et al. 2005, Josselyn et al. 1983). Any net productivity after these losses are available for incorporation into seagrass sediments (Duarte and Cebrian 1996). This last fate is of most importance for offsetting anthropogenic increases in CO₂, through carbon sequestration. For sequestration to occur, the buried carbon must be stored in forms and in a biogeochemical environment that allows it to be preserved in the sediments over long timescales. While this phenomenon may be a means through which seagrasses could mitigate some of the imbalance in the contemporary global carbon cycle, the carbon preservation potential of seagrass meadows is poorly explored.

The global carbon cycle is the continuous natural cycling of carbon from the biogeosphere to the atmosphere and back to the biogeosphere (IPCC 2001). Any ecosystem involved in this cycle may be a carbon sink if carbon is accruing there faster than it is released. Conversely ecosystems can be a carbon source if the production of atmospheric carbon from the area exceeds the rate of *in situ* carbon fixation (Trummer et al. 2009). It has been estimated that the world's terrestrial ecosystems contain more than 2,000 Giga tonnes carbon (Gt C) and acts as a net sink of approximately 1.5 Gt C per year (Trummer et al. 2009). This sink is commonly referred to as 'Green Carbon', and is stored as both living vegetation and soil organic matter (OM). However, there have been suggestions that of all the biological carbon in the world, over half (55%) is captured by marine living organisms (Simon et al. 2009, Falkowski et al. 2004). Breaking up this 55% estimate further, 45% is contributed by plankton alone (Arrigo 2005) with the remaining 10% broadly attributed to oceanic carbon sinks of marine plants, i.e. mangroves, salt marshes and seagrasses (Nellemann et al. 2009). These vegetated coastal carbon sinks are termed 'Blue Carbon' sinks (after Nellemann et al. 2009). This 10% may be a relatively small value, but marine plants are extremely efficient carbon sinks, with the carbon buried in their ecosystem about equal to that which is exported

(Bouillon et al. 2008a, Houghton 2007). In addition, these Blue Carbon sinks have a high carbon capture capacity on an areal basis, relative to other vegetated ecosystems (Fourqurean et al. 2012a).

In most of the above commentaries on carbon sinks, the carbon being referred to was organic carbon (OC), i.e. carbon occurring in a multitude of biochemical classes as a result of biogenic processing, including the cyclical formation and destruction of OM (Emerson and Hedges 2008). Carbon also occurs as inorganic forms, notably, as biogenic calcium carbonates in seagrass ecosystems. It had been shown that global inorganic carbon (IC) stores exceed those of OC stores (Hedges and Keil 1995; Table 1.1). However, OC has attracted more interest in understanding the retention and persistence of carbon in sedimentary environments since it is more labile than IC and because the formation and degradation processes for OC are relatively more complex than for calcium carbonate (Emerson and Hedges 1988). In coastal vegetated ecosystems, primary producers contribute OC through photosynthesis. However, many of these ecosystems, including seagrass meadows, can function as IC reactors through the production of IC as a part of the calcification reaction undertaken by calcifying organisms that reside in them (e.g. Marbà et al. 2006). It has been reported that OC production results in a net gain in carbon after all reactions were considered but that biogenic carbonate production results in a net loss of carbon when similar mass balances were considered (Smith 2013). Consequently, there is ongoing debate as to whether seagrass meadows are net carbon sources or sinks when both OC and IC production are considered (Mateo and Serrano 2012). Any consideration of vegetated ecosystems as carbon sinks, then, must take into account OC and IC production. While the potential importance of IC is thus recognised, the focus of research described in this dissertation is, through necessity, limited to the dynamics of OC storage and preservation as one fate for seagrass productivity.

Table 1.1. Major global organic and inorganic carbon pools. Adapted from Hedges and Keil (1995).

Reservoir type	Amount (10^{18} g C)
Sedimentary rocks	
<i>Inorganic</i>	
Carbonates	60000
<i>Organic</i>	
Kerogen, coal, etc.	15000
Active (surficial) pools	
<i>Inorganic</i>	
Marine dissolved inorganic carbon	38
Soil carbonate	1.1
Atmospheric CO ₂	0.66
<i>Organic</i>	
Soil humus	1.6
Land plant tissue	0.95
Seawater dissolved organic carbon	0.6
Surface marine sediments	0.15

1.2 Preservation of organic carbon in seagrass sediments

The IPCC defines carbon sequestration as “the process of increasing the carbon content of a reservoir/pool other than the atmosphere” (IPCC 2007). The carbon sequestration in sediments of vegetated coastal ecosystems is a complex and cumulative process that includes the delivery, production/supply, storage and preservation of the carbon (Nellemann et al. 2009). Studies of carbon stores in seagrass meadows have been pursued (e.g. Serrano et al. 2014, Lavery et al. 2013, Fourqurean et al. 2012a, and others) but there is a dearth of information describing what actually occurs in the sediment that allows carbon to be stored. Contemporary studies of carbon stores in seagrass meadows have quantified the amount of carbon stored as bulk quantities (Serrano et al. 2014, Lavery et al. 2013, Fourqurean et al. 2012a) and possible sources of the carbon (Kennedy et al. 2010), which are important studies on their own right. However, to date, there has been no thorough examination of the biogeochemical processes acting on OC in seagrass sediments or the results of those processes on the nature of that OC. This facet is vital to understanding the processes *leading* to the accumulation in seagrass meadows, and the role these primary producers play in supplying both OM for burial and an ecosystem conducive for burial and preservation.

While a small number of studies allow some limited inferences regarding carbon preservation in seagrass ecosystems, the information available is not sufficient for generalisations to all seagrass meadows. Mateo et al. (2006) provided a temporal perspective on carbon persistence in *Posidonia oceanica* meadows over short (*circa* 1 year) and long residence times (4 years and more) of detrital matter – the longer the OM is retained in the sediment, the higher the preservation potential. Other studies have characterised recalcitrant carbon compounds in seagrasses. Lignin is the main constituent of recalcitrant OM in *P. oceanica* detritus (Klap et al. 2000). Tegelaar et al. (1991) showed that cutans in *Z. marina* is a type of biochemical constituent in its tissue that may be preserved due to its recalcitrant nature, along with recalcitrant carbohydrates in the organic pools of *Z. marina* meadows (Vichkovitten and

Holmer 2005). Although work has been done on the biochemical constituents of seagrass tissues (e.g. Torbatinejad et al. 2007, Touchette and Burkholder 2000), *P. oceanica* and *Z. marina* are the only species studied in the context of sedimentary carbon preservation. With such a scarcity of information, the preservation aspect of carbon sequestration in seagrass meadows is a real unknown, and had actually been pointed out as a distinct knowledge gap in understanding the carbon sink capacities of seagrass meadows (Mateo et al., 2006).

All OM, including those originating from seagrasses, are exposed to different types of diagenetic processing in the sediment, but the degree of preservation may differ based on the relative lability and recalcitrance of the OM. While diagenetic processing is complex, the onset of early diagenesis is influenced by the exposure of OM to oxygen (Wakeham and Canuel 2006) and the presence of microbes (Deming and Baross 1993). These factors affect the rate of OM degradation and preservation, with aerobic bacteria preferentially degrading any labile OM as the most bio-available respiratory substrate (Koho et al. 2013). Consequently, enhanced OM preservation is observed in anoxic and sub-oxic environments (Arndt et al. 2013, Burdige 2007, Hedges and Keil 1995, Cowie and Hedges 1992 and others). Many studies demonstrate that OM buried below the sediment surface is in anoxic conditions, increasing the likelihood of its preservation (e.g. Burdige 2007, Wakeham and Canuel 2006, Hedges and Keil 1995). When availability of oxygen decreases, two overarching diagenetic conditions will then regulate further processing: an absence of factors promoting remineralisation; and/or the presence of factors inhibiting remineralisation (Burdige 2007), both of which can be looked at as component processes in OM preservation.

The OC initially deposited in the marine environment may be classed arbitrarily as detrital biopolymers that undergo different types of diagenetic processing (Figure 1.1). These detrital biopolymers have inherent lability and refractory potentials (Table 1.2). Initial deposition leads to depolymerization of the compounds into low- and high-molecular weight intermediates. In the presence of microfauna, especially sediment microbes, these intermediates can be remineralised. However, some classes of OM in detritus are inherently resistant to degradation and have low rates of remineralisation. This OM tends to be

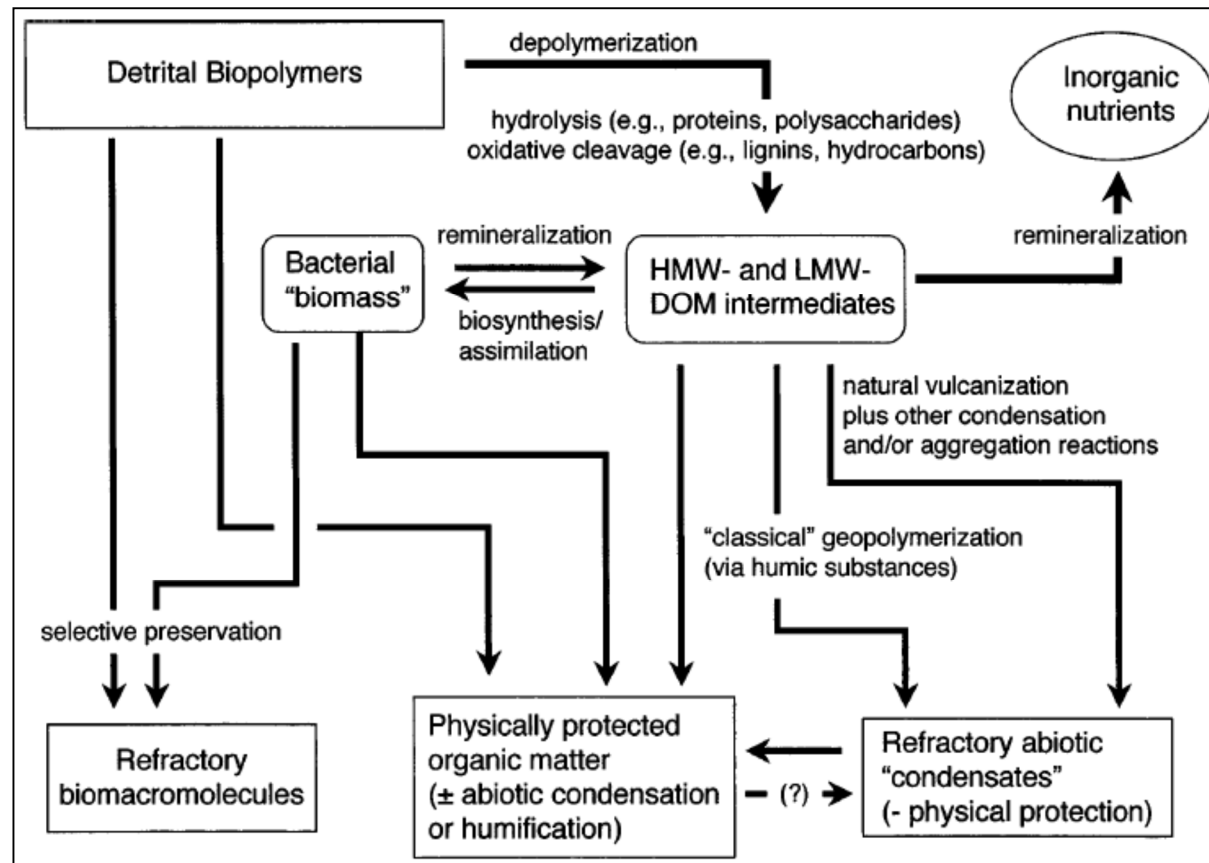


Figure 1.1: Interaction of processes that are involved in diagenesis of pre-processed organic matter into refractory organic matter. Adapted from Burdige (2007).

Table 1.2. List of relative preservation potential of OM categorized according to specific biochemical classes. Preservation potential ranges from – (extensive degradation under depositional conditions) to ++++ (no degradation under any depositional conditions). Adapted from de Leeuw and Largeau (1993).

Biomacromolecules	Occurrence	Preservation potential
Starch	Vascular plants; some algae; bacteria	–
Glycogen	Animals	–
Fructans	Vascular plants; algae; bacteria	–
Laminarans	Mainly brown algae; some other algae and fungi	–
Poly- α -hydroxyalkanoates	Eubacteria	–
Cellulose	Vascular plants; some fungi	– /+
Xylans	Vascular plants; some algae	– /+
Pectins	Vascular plants	– /+
Mannans	Vascular plants; fungi; algae	– /+
Galactans	Vascular plants; algae	– /+
Mucilages	Vascular plants; (seeds)	+
Gums	Vascular plants	+
Alginic acids	Brown algae	– /+
Fungal glucans	Fungi	+
Dextrans	Eubacteria; fungi	+
Xanthans	Eubacteria	+
Chitin	Anthropods; copepods; crustacea; fungi; algae	+
Glycosaminoglycans	Mammals; some fish; Eubacteria	– /+
Proteins	All organisms	– /+
Extensin	Vascular plants; algae	– /+
Mureins	Eubacteria	+
Teichoic acids	Gram-positive Eubacteria	+
Teichuronic acids	Gram-positive Eubacteria	+
Lipoteichoic acids (LTA)	Gram-positive Eubacteria	+
Bacterial lipopolysaccharides (LPS)	Gram-negative Eubacteria	++
DNA, RNA	All organisms	–
Glycolipids	Plants; algae; Eubacteria	+/++
Polyisoprenols (rubber and gutta)	Vascular plants	+
Polyprenols and dolichols	Vascular plants; bacteria; animals	+
Resinous polyterpenoids	Vascular plants	+/++
Cutins, suberins	Vascular plants	+/++
Lignins	Vascular plants	++++
Tannins	Vascular plants; algae	+++ /++++
Sporopollenins	Vascular plants	+++
Algaenans	Algae	+++
Cutans	Vascular plants	++++
Suberans	Vascular plants	++++
Cyanobacterial sheaths	Cyanobacteria	+

selectively preserved when buried in the sediment (de Leeuw et al. 2006, Tegelaar et al. 1989) resulting in increasing concentrations with increasing depth and age (Zonneveld et al. 2010).

Other processes in the geo-chemical environment may result in physical protection of OM from further degradation. Mineral shielding may passively prevent enzymatic attack on the OM (Middelburg and Meysman 2007, Rothman and Forney 2007). Other processes include the reaction of an OM outer layer that encapsulates the inner OM to yield it refractory (Killops and Killops 2005, Hedges 1988). Sub-processes result in intermediate forms of OM during diagenesis and bacteria may process these OM and producing forms that are then selectively preserved (Zonneveld et al. 2010, Ogawa et al. 2001). Other intermediates may also become refractory after undergoing processes such as vulcanization, condensation and/or geopolymerisation with or without bacterial influence (Killops and Killops 2005, Hedges et al. 2000, Tegelaar et al. 1989, Hedges 1988). The above processes, either alone or in combination, result in OM preservation occurring over different timescales, ranging from months to millennia (Mayer 2004). As such, any study involving carbon preservation has to take this temporal context into account in order to provide an informed understanding of the related processes resulting in preservation (Burdige 2006, Emerson and Hedges 1988). It has to be reiterated that the processes described above have never been explored in seagrass sediments. Since long-term retention of OM in seagrass sediments had been recorded for at least one species (i.e. *P. oceanica*; Serrano et al. 2012, Lopez-Saez et al. 2009, Mateo et al. 1997), it is plausible that aspects of the diagenetic processes described above may occur in seagrass sediments.

An approach often used to provide insights into OM preservation is to determine the source and nature (i.e. molecular conformation) of the OM. Knowing the source provides preliminary information on the relative lability/refractory potentials of the OM and can be used to predict the transformations, if any, of the OM as it undergo diagenesis. There are however, analytical hurdles arising from existing methods to elucidate the OM source and characterise the OM. The first challenge posed to identification of the OM source is the

heterogeneity of the sediment matrix, which can include OM from an unknown number of sources (Burdige 2007). Bulk characterization techniques such as using stable isotope signatures and molecular biomarker techniques have been widely used (Altabet 2006, Danovaro et al. 2006), yielding information that provides limited insights into the sources of OM preserved in the sediment. In this regard, one clear advantage for studying seagrass systems is that seagrass-derived OM is naturally enriched in ^{13}C compared to other OM sources (e.g. seston, algal-derived and terrestrially-sourced; Kennedy et al. 2010). Thus, sediments with high seagrass inputs have distinctive $\delta^{13}\text{C}$ values, potentially providing information on the contributions of seagrass-derived OM in the sedimentary OM pool (Table 1.3). Mixing models that allocate the probability of pre-identified potential sources to the organic pool have been employed (Moore and Semmens 2008, Phillips and Gregg 2003), but complications arise if there are too many potential sources within a particular area (Phillips and Gregg 2003). However, if there is a known major source of the OM, such as if the contribution of OM was mainly from a primary producer (e.g. seagrasses, terrestrial plants, mangroves), the elucidation of the potential source becomes less complicated.

The other challenge that arises is that the actual form of the refractory OM in the sediment matrix can be difficult to characterise since non-labile OM in the sediment matrix is insoluble, non-extractable and non-hydrolysable, with a large portion remaining as molecularly uncharacterised OM (Burdige 2007). Indirect inferences can be obtained by examining the conformation of refractory biomacromolecules through pyrolysis (Larter and Horsfield 1993), thermochemolysis (Pulchan et al. 2003), infrared Fourier transform spectroscopy (FTIR; Tremblay and Gagné 2002) and solid-state ^{13}C nuclear magnetic resonance (^{13}C NMR) with cross polarization-magic angle spinning (Nelson and Baldock 2005b, Baldock et al. 2004). While some of these techniques are destructive and tedious, quantitative FTIR and NMR are rapid and non-destructive, though the analytics may only provide data as bulk characterisations (Tremblay and Gagné 2002, Gélinas et al. 2001). More often, a combination of both chromatographic and spectrometric techniques have been used to provide more

Table 1.3. Mean stable carbon isotope values of sediment, seagrass tissues and the sediment-plant matter mixture within meadows of different seagrass species. Adapted in its exact form as appeared in Kennedy et al. (2010), supplementary information. It should be noted that there may be an error in the term *Amphibolis australis*, which more likely could have been *Posidonia australis*.

Community	n	Sediment $\delta^{13}\text{C}$ (‰)		Seagrass $\delta^{13}\text{C}$ (‰)		$\delta^{13}\text{C}$ (‰) seagrass-sediment	
		Mean	SE	Mean	SE	Mean	SE
<i>Amphibolis australis</i>	2	-18.10	0.10	-10.40	0.50	7.70	0.60
<i>Amphibolis griffithii</i>	1	-7.30		-12.70		-5.40	
<i>Cymodocea nodosa</i>	26	-17.50	0.80	-9.40	0.40	8.30	0.80
<i>Cymodocea rotundata</i>	1	-22.20		-12.40		9.80	
<i>Enhalus acoroides</i>	6	-19.90	1.80	-9.80	0.90	10.20	1.40
<i>Halodule uninervis</i>	2	-19.50	0.10	-13.60	1.50	5.90	1.30
<i>Halodule wrightii</i>	7	-15.50	0.30	-9.90	0.00	5.60	0.30
<i>Halophila ovalis</i>	2	-19.50	0.10	-13.10	2.40	6.40	2.20
<i>Heterozostera nigricalis</i>	2	-18.10	2.40	-10.70	0.10	7.40	2.30
<i>Posidonia oceanica</i>	42	-18.50	0.40	-12.20	0.30	5.80	0.50
<i>Posidonia sinuosa</i>	3	-16.10	3.80	-10.80	0.30	5.30	4.10
<i>Ruppia megacarpa</i>	1	-19.60		-14.00		5.60	
<i>Syringodium filiforme</i>	3	-17.50	0.10	-7.10	1.30	10.40	1.30
<i>Thalassia hemprichii</i>	2	-19.30	3.00	-11.80		10.40	
<i>Thalassia testudinum</i>	44	-12.90	0.30	-8.90	0.30	4.00	0.40
<i>Thalassodendron ciliatum</i>	17	-20.40	0.80	-14.50	0.80	5.80	0.60
<i>Zostera japonica</i>	1	-26.40		-12.20		14.20	
<i>Zostera marina</i>	17	-18.40	0.50	-10.90	0.60	7.80	0.60
<i>Zostera noltii</i>	13	-18.20	0.50	-10.30	0.30	7.90	0.50
Mixed community	36	-15.20	0.30	-8.60	0.20	6.40	0.30
Total	228						

detailed biochemical information, by elucidating the compounds occurring within the OM matrix (e.g. De La Rosa et al. 2008, Yamada and Ishiwatari 1999, Stein 1991). However, while existing technology can provide insights into the nature of sedimentary OM, there is a clear and significant trade-off between the time required for analysis and the insights gained. The lack of standard methods to understanding OM preservation in marine sediments may be one reason why there are limited numbers of studies exploring this aspect of carbon sequestration in seagrass meadows. Since almost nothing is known of OM preservation in seagrass meadows, a feasible starting point is to characterise the OM stored in seagrass meadows.

1.3 Seagrass carbon sink and ecosystem services

Recently there has been renewed interest in the carbon sink value of vegetated coastal ecosystems, mainly due to the recognition of the ecosystem services provided, both in terms of economic value and intangible benefits (Costanza et al. 1997), coupled with the knowledge that these services were concurrently being lost through habitat destruction. Seagrass ecosystems are reportedly being lost at a rate of 0.9% per annum since the 1900s, with increased rates of loss in recent times (7% yr⁻¹; Waycott et al. 2009). These global estimates of losses may not reflect region-specific degradation; in the Mediterranean Sea, *Posidonia oceanica* meadows had been estimated to regress by less than 10% cover in the 20th century (Boudouresque et al. 2009). Notwithstanding global or regional estimates, habitat losses still imply a potential loss of carbon sink capacities estimated to range between 6 and 24 Tg C yr⁻¹ (Duarte et al. 2010). Recent studies emphasised the knowledge gap in understanding the reduction of carbon sequestration capacity provided by seagrass meadows as an outcome of habitat loss (e.g. Macreadie et al. 2014, Thomas 2014, Howard et al. 2014, Sifleet et al. 2011). Once ecosystem function is lost, two pertinent consequences may arise (Nellemann et al. 2009): carbon sequestration processes cease; and the stored sedimentary carbon is released to the atmosphere as CO₂ due to remineralisation processes. Such consequences of seagrass

degradation have been reported by Marbá et al. (2015), whom also reported that seagrass meadow created through subsequent transplantation did not attain carbon sequestration capacity similar to a healthy and continuously proliferating seagrass meadow. It is thus vital to quantify the carbon stored in the seagrass sediments, both as a form of baseline reference when the meadow ecosystem health is still intact, and as a means to obtain insights on carbon sink capacity. Robust data can then provide the scientific rationale for conservation of these important ecosystems, especially in threatened seagrass meadows.

With an estimated global carbon store of 4.2 to 8.4 Pg carbon (Fourqurean et al. 2012a), it was inevitable that there would be impetus to either place economic value on these stores or to recommend that a valuation mechanism be developed (e.g. Lau 2013, Pendleton et al. 2012, Siikamäki et al. 2012). Before this can be achieved, a clear understanding of carbon burial trends in seagrass meadows is required, ideally one that can be generalised to all seagrass habitats. However, this aspect is not fully understood (Lavery et al. 2013). To date, the overwhelming number of studies reporting carbon burial in seagrass meadows are for the seagrass *Posidonia oceanica*. Carbon dynamics in *P. oceanica* have been intensely studied over the past few decades, including studies of the detrital stocks (Romero et al. 1992), longevity and stability of those stores (Mateo et al. 2010, Mateo et al. 1997), variability in stores across a depth gradient (Serrano et al. 2014), burial rates across different meadows (Mateo et al. 2006), and organic retention relative to canopy trapping (Hendriks et al. 2008). It was thus inevitable that subsequent meta-analyses on carbon sequestration in seagrass meadows used a high number of *P. oceanica* studies compared to other seagrass species (e.g. Fourqurean et al. 2012a, Kennedy et al. 2010, Duarte et al. 2005). However, the tendency to generalise the carbon storage capacity and characteristics of this one species to all seagrass may result in weak estimates of seagrass carbon burial at the global level (Lavery et al. 2013).

1.4 Carbon stores in seagrass meadows: the case of *Posidonia oceanica*

Posidonia oceanica is endemic to the Mediterranean Sea. Although it is a slow-growing seagrass with a rhizomal growth rate of 1 to 6 cm yr⁻¹ apex⁻¹ (Marbá et al. 1996), it has a relatively high biomass of up to 2112 g dry weight m⁻² (Duarte and Chiscano 1999). Pergent et al. (1994) estimated that up to 30 % of total production was buried below-ground as seagrass mat (i.e. organic-rich deposits consisting of dead sheaths, rhizomes and roots, embedded in a sediment matrix, after Boudouresque and Meinesz 1982). The below-ground rhizomes proliferate as plagiotropic and orthotropic growths (Gobert et al. 2006). This growth strategy results in the rhizomal mat extending vertically away from the sediment surface, and has a two-pronged effect: it enhances the rooting structure of the plant ensuring a three-dimensional sediment stabilisation of the seagrass meadow, which in turn provides a positive feedback mechanism for any detrital matter to remain in a stable sedimentary environment beneath the meadow (Le Hir et al. 2007, De Falco et al. 2000). The organic-rich mat beneath a meadow in Portlligat (Spain) extended as deep as 6 m below the sediment surface (Lo Iacono et al. 2008). At another meadow (Ischia, Italy) the deposits were estimated to be at least 3.2 m below the sediment surface, and as old as 1800 yr (Mateo et al. 1997).

One possible reason for the effective retention of carbon in *P. oceanica* meadows is the low degradation rates of seagrass detrital matter (Mateo et al. 2006). Slow-growing plants have more degradation-resistant or recalcitrant OC compounds (Niemann et al. 1992) and *P. oceanica* is exemplary in this respect. *Posidonia oceanica* has a large proportion of recalcitrant in its tissues, such as lignin and cellulose (Klap et al. 2000). These compounds are mainly structural in function (Klap et al. 2000) but may also deter herbivory (Valentine and Heck 1999). Once buried, there may be limited diagenetic microbial processing of recalcitrant OM due to the exclusion of oxygen in anoxic sediments, and/or the low consumption rates of these substrates as a result of limited microbial enzymatic attack (Burdige 2007, Mateo et al. 2006). Following burial, remineralisation of the more labile

constituent of *P. oceanica* detritus would occur during early diagenesis (Mateo et al. 2006), while the recalcitrant component would contribute to the net accumulation of sediment OM.

With its exceptional carbon sequestration capacities, up to 4 times higher compared to the mean OC stores of Australian seagrasses (Lavery et al. 2013), *P. oceanica* has become the model species to demonstrate the importance of seagrass meadows as Blue Carbon sinks. Some of the most influential Blue Carbon studies have estimated carbon sequestration values from studies of *P. oceanica* meadows: e.g. a burial rate of 27-44 Tg C yr⁻¹, or an areal annual rate of ~83 g C m⁻² yr⁻¹ (Duarte et al. 2005). In an attempt to have broader representation for other species, Kennedy et al. (2010) studied 228 seagrass meadows (42 sites consisted of *P. oceanica* meadows; Table 1.3), and reported that seagrass production (ranging 41-66 g C m⁻² year⁻¹) contributed approximately 50% of the sedimentary OM. In another study of 89 seagrass meadows across different regions (29 sites are Mediterranean meadows), it was estimated that the conservative global carbon stores in seagrass meadows was between 4.2 and 8.4 Pg carbon (Fourqurean et al. 2012a).

While these three studies established the importance of seagrass meadows in sequestering carbon, they all used a disproportionate number of *P. oceanica* meadows in their calculations, primarily due to the absence of data for most other species. As a result, the carbon sink value and carbon sequestration capacity of other seagrass species in other habitats are relatively unknown. While global meta-analyses are valuable tools in accounting for global carbon stores, the resultant estimates are often based on averaged value(s), which potentially masks the contribution of a particular single species or overly-emphasise the contribution of another. Fourqurean et al. (2012a) acknowledged that their data set was “geographically biased” towards data from North America, Western Europe and Australia and with a large proportion from Mediterranean Sea meadows. Ideally, all seagrass species within all these regions should be investigated for its carbon burial potential to have a complete picture on the factor of species on carbon stores. So, the question arises: which should be the next best species to

investigate carbon burial representative of all extant seagrass habitats to compare against the data already provided by *P. oceanica* meadows?

1.5 What about the buried carbon in meadows of other seagrass species?

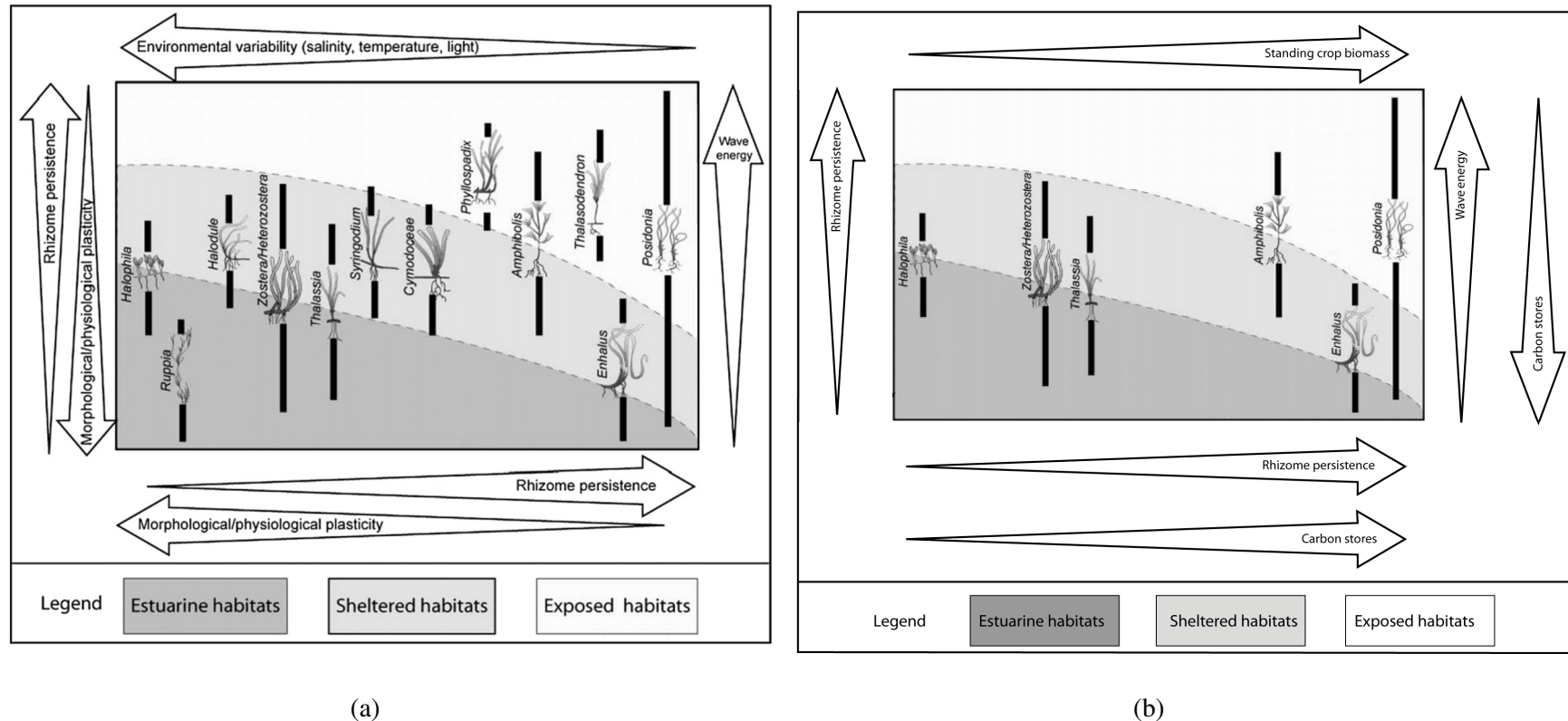
Globally, there are 60-70 species of seagrasses within 12 genera (den Hartog and Kuo 2006). With such inherent diversity, it is inevitable that inter-species variability exist in their biological characteristics. For instance, morphological leaf forms, productivity, biomass and growth strategies differ among species (Lee et al. 2007, Short and Short 2000, Duarte and Chiscano 1999). Carbon sequestration in primary producer habitats is strongly correlated to the plant's net productivity and growth strategies (De Deyn et al. 2008, Catovsky et al. 2002, Cebrian 2002). As such, biological traits may potentially result in variable carbon sequestration potentials among species, notwithstanding the fact that as a functional group, seagrasses comprise a relatively small number of species (den Hartog and Kuo 2006).

To date very few studies have investigated the carbon sequestration potentials of seagrass meadows in relation to their habitat characteristics. Lavery et al. (2013) reported that the OC storage potential varies significantly among different types of seagrass habitats, where habitat was defined by the abiotic environment and seagrass species composition. The authors recommended that these two factors should be taken into account when estimating the OC stores in seagrass meadows. On the basis of species differences, Mateo et al. (2006) commented that other than *P. oceanica*, only *Thalassodendron ciliatum* and *Posidonia australis* may potentially generate significant refractory deposits persisting over decades to millennia. For *T. ciliatum*, the organic deposits could be as high as 100 kg dry weight m⁻², consisting of woody tissue in the rhizomes and vertical stems (Lipkin 1979). For *P. australis* meadows, deposits of “marine fibres” consisting of lignin and cellulose were first reported in the early 1900s, with the sedimentary cellulose being of interest due to its possible applications for industrial purposes (Read and Smith 1919, Winterbottom 1917).

When compared, Lavery et al. (2013) showed that carbon stores of *P. australis* meadows (4.92-20.14 mg C cm⁻³) could be comparable to those of *P. oceanica* (20.16 mg C cm⁻³), though they were variable and many *P. australis* habitats have much lower stores. This comparison demonstrates that while there may be an influence of species on OC stores, it may not necessarily be a consistent one.

In addition to the variability among seagrass species, the abiotic environment in which they occur is also likely to affect their OC storage potential. For example, the OC stores in meadows of the same species can vary due to water depths in which the meadow occurs, as a result of differing net carbon balances (Serrano et al. 2014). Importantly, seagrasses also occur across a range of depositional environments, from estuaries to exposed environments (Carruthers et al. 2007), and this too may affect OC stores. The prevailing depositional conditions as a result of the hydrodynamic energy of an area influences the type of species that occur in that habitat (Carruthers et al. 2007), which may then influence the amount and source of carbon available for burial (Cebrian 2002). In estuaries, which are typically highly depositional, carbon may originate allochthonously from upstream transport in addition to autochthonous production (Aller 1998, Canfield 1994). For sheltered habitats, autochthonous inputs may predominate (Cebrian 2002) while the hydrodynamic conditions in an exposed habitat may result in both export of autochthonous production and the simultaneous import of allochthonous production (Macdonald et al. 1998). In contrast, less OC would be exported from estuarine and sheltered habitats since the energy is lower in these environments compared to exposed sites. Depositional conditions also influence the sediment grain-size and oxygenation of the sediments, both of which have implications on the preservation potentials of sedimentary carbon (Dauwe et al. 2001, Bergamaschi et al. 1997, Keil and Hedges 1993). This complex interaction of local hydrodynamics, with the delivery, production, deposition and preservation of OC suggests that investigation into the role of the depositional environment may provide an improved understanding of carbon storage and preservation in seagrass meadows.

The relationships between seagrass species (expressed as functional types) and the depositional nature of marine habitats has been conceptualised by Carruthers et al. (2007; Figure 1.2). In this model, seagrass genera were categorised according to the variability in rhizome persistence, morphological/physiological plasticity and the depositional environments where they occur: either estuarine, sheltered or exposed habitats. In this dissertation, a modified form of this model is suggested, one that incorporates the three depositional environments to predict likely carbon stores (Figure 1.2). Species biomass correlates with the amount of organic production and may influence the amount of detrital matter supplied by the seagrass. Rhizome persistence may indicate the provision of the seagrass to stabilise the sediments, allowing storage and retention of organic input. The depositional environment then influences the retention of the carbon in the sediments, either influencing net accumulation or export from the meadow. This carbon sequestration functional-form model thus permits a more robust contextualisation of carbon stores in seagrass meadows and has been used to focus the research reported in this dissertation. From the model, it is apparent that if carbon stores in seagrass meadows are to be studied, it should incorporate the two factors: seagrass species and the depositional environment. As such, the seagrasses at opposite extremes of the model (i.e. left to right: species effect; and top to bottom: influence of depositional environment) span the full continuum of conditions in the hypothesised relationship between seagrass species, depositional environment and OC burial. The research presented in this dissertation therefore focuses on *Posidonia australis*, since it has been identified as a species likely to have a high carbon preservation potential (Mateo et al. 2006) and *Halophila ovalis*, because it is at the opposite end of the seagrass functional-form model to *P. australis* and therefore provides a relevant comparison species to test the generalisability of finding to other seagrasses.



(a) (b)

Figure 1.2: Seagrass functional-form diagram showing the habitats of each seagrass genera with respect to its morphology and environmental conditions. (a) The seagrass functional-form suggested by Carruthers et al. (2007); (b) a modified version of the Carruthers et al. (2007) seagrass functional form to include possible relationships between seagrass genera and carbon stores within their respective meadows. In (b), only seagrass genera with occurrences spanning at least two depositional environments are shown.

1.6 Overall rationale of the thesis

There is no doubting the facts that seagrass meadows play an important role in the marine carbon cycle, including as a carbon sink. With more efforts being focused on valuing this ecosystem service, the scientific basis for any carbon accounting must be robust and should include factors that may influence carbon retention and persistence in any particular seagrass meadow. This research has focused on information gaps that can help to improve our understanding of, and ability to predict, carbon storage and preservation potential in seagrass ecosystems. At this point, there are a number of key information gaps that need to be addressed: the a lack of clarity on methods to account for ecosystem services losses with loss of seagrass cover; the relative importance of species identity and the depositional environment in determining carbon stores in seagrass meadows; the carbon sequestration potentials of seagrasses other than *P. oceanica*; and a fundamental understanding of why seagrass meadows are able to preserve carbon in their sediments – accounting for factors such as the biochemical class of OM that was buried in addition to the biogeochemical environment may further explain some aspects of the diagenesis within seagrass meadows. This thesis therefore bridges the fields of seagrass ecology and marine biogeochemistry to address these gaps and allow further insights into carbon storage and preservation in seagrass meadows.

1.6.1 Aims and structure of the thesis

The aims of the research reported in this dissertation were to:

- Account the losses in sedimentary carbon stores as a consequence of meadow losses, using the *Posidonia australis* meadows of Oyster Harbour (Albany, Western Australia) as a case study;

- Determine whether the depositional environment affects the sedimentary carbon stores in *Posidonia australis* meadows;
- Determine if seagrass species identity influences the sedimentary carbon stores and carbon burial rates by comparing *P. australis* and *Halophila ovalis* meadows; and
- Determine the biochemical class and the geochemical conditions allowing carbon preservation in seagrass sediments by characterising the OM buried in a *Posidonia australis* meadow.

This thesis is structured into 6 Chapters. Chapter 2 reports on a study that quantified the carbon buried in a *Posidonia australis* meadow and subsequently quantifying the possible losses in sedimentary carbon due to losses in seagrass cover. This chapter also sets the scene for subsequent chapters by describing the techniques used to sample seagrass sediments, the laboratory protocols to analyse OM and carbon, and the application of chronostratigraphic models to determine carbon burial rates, all of which are applied in the subsequent chapters. In Chapter 3, the effect of the depositional environment on carbon stores in *Posidonia australis* meadows is examined. Chapter 4 reports on how the species identity influences carbon stores and carbon burial rates. Chapter 5 then summarises the results of an investigation into the biogeochemical conditions, and the forms of OM preserved beneath seagrass meadows using solid-state ^{13}C nuclear magnetic resonance with cross polarisation/magic angle spinning analysis (^{13}C CP/MAS NMR). The final chapter is a general discussion of the implication of the findings of the thesis as a whole, and what they mean for our understanding of carbon storage and preservation in seagrass meadows in a general context.

CHAPTER 2

LONG-TERM CARBON STORAGE AND ITS RECENT LOSS IN A TEMPERATE ESTUARINE *POSIDONIA AUSTRALIS* MEADOW

Abstract

Oyster Harbour, on the south coast of Western Australia, supports 3.6-3.9 km² of seagrass meadows, following the loss of approximately 2.8-3.1 km² in the 1980s. This small area of prevailing meadows hold significant carbon stores accumulated over the past 3000 years. In this study, we sampled three sediment cores from a *Posidonia australis* meadow and analysed organic matter (OM), organic carbon (OC) and inorganic carbon (IC) contents in bulk sediments, and $\delta^{13}\text{C}$ signatures of OM. The OM and OC contents (mean \pm SE) in the cores were $9.07 \pm 0.36\%$ and $2.24 \pm 0.05\%$, respectively. The mean IC content was $3.16 \pm 0.17\%$. $\delta^{13}\text{C}$ signatures of the sedimentary OM ranged from -10.01‰ to -13.28‰ . Using a Bayesian isotopic mixing model, it is estimated that 57-67% of the OM in the seagrass sediments was derived from *P. australis* detrital matter. The total carbon (TC) stores in 150 cm-thick seagrass sediments averaged 27.92 kg TC m⁻² (10.79 kg OC m⁻² and 17.13 kg IC m⁻²). Based on radiocarbon dating, the mean sediment accumulation rate was 0.0494 cm yr⁻¹, which led to a long-term TC accumulation rate of 8.92 g TC m⁻² yr⁻¹ (3.45 g OC m⁻² yr⁻¹ and 5.47 g IC m⁻² yr⁻¹). Based on historical seagrass cover (3.6-6.7 km² during the 1960s to 1980s), the estimated TC stores in 150 cm-thick seagrass sediments at Oyster Harbour would have been 101-187 Gg TC. The eutrophication-driven loss of seagrass during the 1980s resulted in the absence of OC accumulation capacity amounting to 280-310 Mg OC (over 29 years). The

loss of seagrass area could also have resulted in the release of 15-17 Gg CO₂, assuming that all recently accumulated OC in the sediment was remineralised.

2.1 Introduction

There is a constant flux of carbon between oceans and the atmosphere through a variety of organic and inorganic carbon forms. The component processes are complex, but the critical intermediaries are the formation and destruction of organic matter (OM) and calcium carbonate (Emerson and Hedges 2008), with the reversible capture and release of CO₂. Less than 0.5% of the total organic carbon (OC) produced in the oceans enters long-term carbon storage in marine sediments (Hedges and Keil 1995). Organic matter degrades at varying rates depending on ambient conditions and its inherent biogeochemical characteristics. For instance, the recalcitrant OM buried in anoxic sediments has low rates of remineralisation (Burdige 2007). Calcium carbonate is less labile and natural coastal settings are often unfavourable for its dissolution resulting in inorganic carbon (IC) being permanently sequestered as carbonates (Smith 2013, Millero 2007).

The carbon storage capacities (OC and IC) of seagrass meadows have been recognised since the early 1980s (e.g. Smith 1981) but interest has only recently been renewed (e.g. Lavery et al. 2013, Fourqurean et al. 2012a). Due to their high productivity coupled with low rates of OM remineralisation, seagrass meadows have been recognised as potentially significant Blue Carbon sinks (Nellemann et al. 2009). The OC that accumulates in seagrass sediments is derived not only from seagrass production (i.e. recalcitrant tissues of seagrass plants) but from the trapping of other organic particles, since seagrass canopies enhance sedimentation and resuspension (Marbà et al. 2015, Kennedy et al. 2010, Gacia et al. 1999). Seagrass meadows occur in a variety of depositional environments, including estuarine, exposed and sheltered areas (Carruthers et al. 2007). Of the three, estuaries have relatively high amounts of buried OM, either from the deposition of allochthonous OM transported through riparian systems

(Cai 2011, Abril et al. 2002, Bianchi et al. 1999), or from accumulation of autochthonous OM production production (Couto et al. 2013).

The recognition of seagrass meadows as intense carbon sinks, and that conserving seagrasses offer potentials for climate change mitigation, has led to interests in evaluating seagrass ecosystem services for their role as carbon sinks (Duarte et al. 2013a). This interest has been intensified by policy-making decisions moving towards carbon pricing economies (Lavery et al. 2013). Yet of all the natural carbon sinks on Earth, seagrass meadows rank among the highest in terms of global loss rates (with net decline of global area of 0.9-7% y⁻¹, Waycott et al. 2009). Consequently, there will be an inevitable loss of all the ecological services they provide, including carbon sequestration (Nellemann et al. 2009).

Posidonia australis is a dominant seagrass species found in many of the estuarine systems throughout sub-tropical and temperate Australia, where it is endemic (Green and Short 2003). Its meadows are highly productive, with estimates as high as 1.7-2.6 g dry weight (DW) m⁻² day⁻¹ (Marba and Walker 1999, Cambridge and Hocking 1997, Walker and McComb 1988). Estuaries vegetated with this seagrass species may thus potentially have relatively high carbon stores compared to other seagrasses (e.g. Couto et al. 2013, Lavery et al. 2013, Flindt et al. 1999). Furthermore, long-term storage has been described for *Posidonia oceanica* meadows (e.g. Serrano et al. 2014, Serrano et al. 2012, Pergent et al. 2012, Mateo et al. 2006) but not for *P. australis*. While there have been studies that included estimates of carbon in other *P. australis* sites by either quantifying the carbon stores (2.74 ± 2.29 kg C m⁻² in Lavery et al. 2013) or carbon content (0.17-0.95% dw OC in Fourqurean et al. 2012a, supplementary information), little is known about the long term carbon accumulation rates and the longevity of these carbon stores. This had been pointed out as a distinct knowledge gap in understanding the carbon cycle in seagrass ecosystems (Mateo et al. 2006).

In this study, we quantified the long-term stores and accumulation rates of sedimentary carbon in a temperate *P. australis* meadow in Oyster Harbour, an estuary in south-western Australia. Eutrophication of the estuary during the 1980s reduced the meadow cover from

6.1-6.7 km² to 3.6 km² (Bastyan 1986). Recent studies confirm that this loss of seagrass cover simultaneously led to a reduction of their carbon storage capacity in this area (Marbà et al. 2015). In addition to quantifying the stores and accumulation rates we also characterise the sources of carbon entering the sedimentary organic pool and estimate the loss of carbon storage ecosystem service as a consequence of the seagrass loss within the estuary (i.e. loss of OC sequestration capacity and potential remineralisation of sedimentary stores).

2.2 Materials and Methods

2.2.1 Study site

Sediment cores ($n = 3$) were collected in 2012 within mono-specific meadows of *P. australis* (water depth of 1.5-2.0 m) in Oyster Harbour (Albany, Western Australia, S 34°58'58.0" E 117°58'29.9", Figure 2.1). This naturally protected embayment has an area of 15.6 km², with freshwater inputs supplied mainly by the Kalgan and the King Rivers (Hodgkin and Clark 1990). The only marine exchange is through a channel at the south of the embayment. It is a marine-dominated estuary with seagrasses recorded to a maximum depth of 5 m. *Posidonia australis* dominates the system and is common to depths of 2.5 m while *Posidonia sinuosa* occurs between 2.5 and 5 m depth (Bastyan and Cambridge 2008). There is little area of hard substrate in the system and so the dominant algae are free-floating species, such as *Cladophora* or those epiphytic on seagrasses. Sediments are medium-coarse to fine grain, silty sands occurring together with biogenic carbonates (Hodgkin and Clark 1990).

2.2.2 Coring and core processing

The sediment cores were sampled randomly within an area of 100 m² by manually hammering sharpened aluminium pipes (46.8 mm inner diameter; 180 cm long) into the seafloor. Compression of unconsolidated sediment during coring was inevitable and corrections were applied (i.e. linear regression; Serrano et al. 2012, Glew et al. 2001) to decompress the

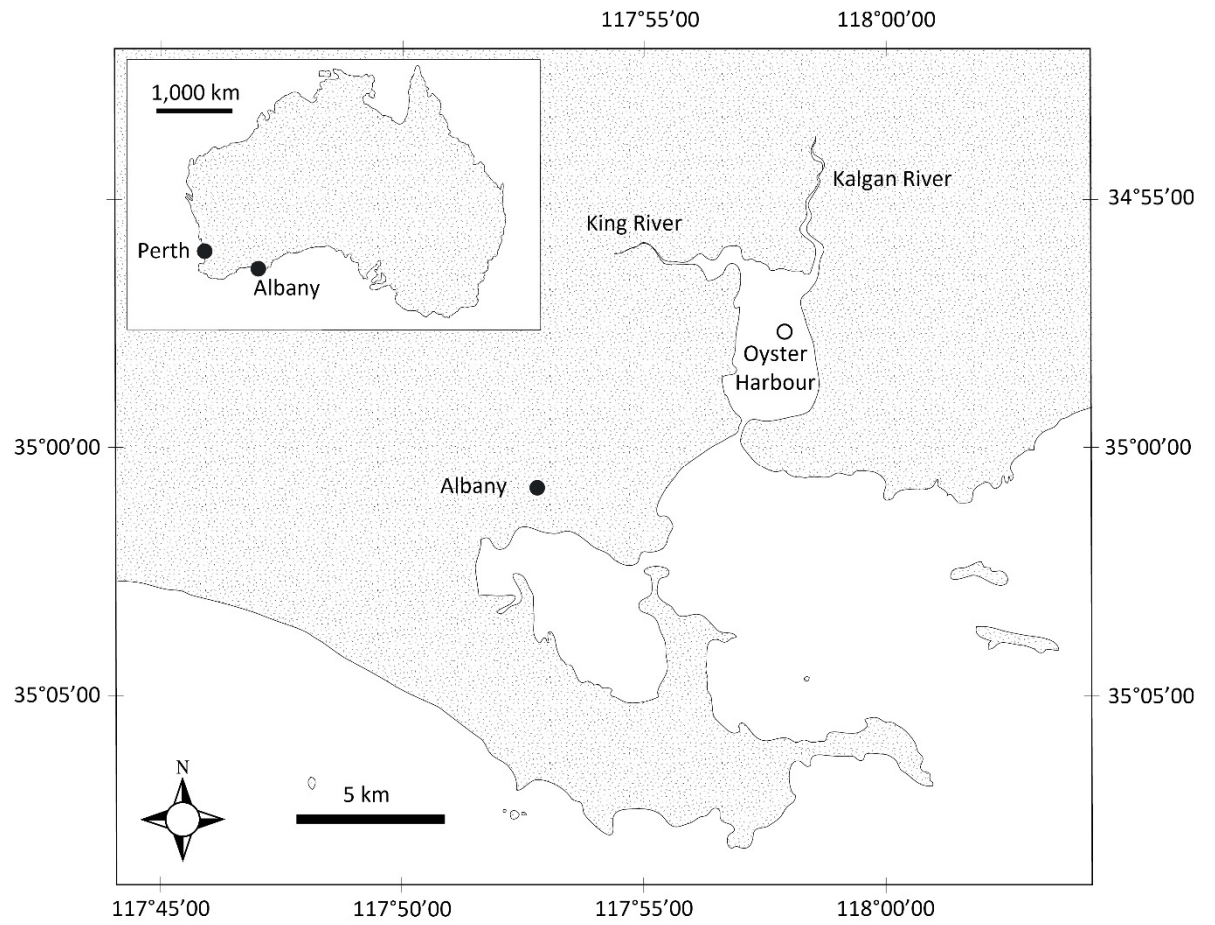


Figure 2.1. Location of the study site at Oyster Harbour (Albany, Western Australia). The open circle denotes the sampling site within the Harbour.

sediment sequence and obtain the corrected core lengths. These lengths then ranged from 119 cm to 150 cm among the three cores. All analysed variables were plotted against these corrected lengths. After transport to the laboratory, the sediment cores were sub-sampled into 1 cm-wide slices. The sub-samples were oven-dried at 60°C to constant weight to calculate the sediment dry bulk density (in g DW cm⁻³). Alternate slices (15-27 samples per core at regular intervals of 5 cm for the top 50 cm and 10 cm for core length beyond 50 cm) were ground to fine powder using a mortar and pestle, and further processed for biogeochemical analysis.

2.2.3 *Biogeochemical analysis of Posidonia australis sediments*

Ground sub-samples were combusted in a muffle furnace at 550°C (5 hours) to determine the OM content, and then for 950°C (2 h) to determine the CaCO₃ content through loss on ignition (Heiri et al. 2001). The difference between pre-combustion and re-combustion weights provided the proportional weight of the non-CaCO₃ mineral fraction. The inorganic carbon (IC) content of the CaCO₃ was calculated through stoichiometry using the mass of carbon ($A_r = 12$) and the molecular weight of CaCO₃ ($M_r = 100$). Both values of IC CaCO₃ contents were reported to present the proportion of each variable in bulk sediment.

Another set of ground sub-samples (0.5 g) were used for organic carbon (OC) and stable isotope ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) analysis. The sediment samples were acidified (1 M hydrochloric acid) to remove all IC. When effervescence ceased after 12-18 h, the mixture was centrifuged (3400 revolutions min⁻¹ for 4.5 min) and pipetted to remove the supernatant with acid. Deionised water (10 ml) was added to wash off residual acid, centrifuged, and the supernatant removed by pipetting. The acidified sample was then oven-dried at 60°C until constant weight was attained. Then, 9-10 mg of the acidified sample was encapsulated in tin capsules and combusted in a continuous flow isotope ratio mass spectrometer analyser (Delta V Plus: Thermo-Finnigan) at the West Australian Biogeochemistry Centre (The University of Western Australia). The OC contents reported by the analytical facilities were corrected to

account for the weight of pre-acidified bulk sediment samples. The sum of OC and IC contents (the latter obtained in the step described earlier) constituted the total sedimentary carbon (TC) content. The $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values (in ‰) were reported relative to the Vienna Pee Dee Belemnite (VPDB) standard and nitrogen from atmospheric air, respectively. Since there are disagreements in reports that acidification of OM will alter $\delta^{15}\text{N}$ values (Schlacher and Connolly 2014, Mazumder et al. 2010, Jaschinski et al. 2008) we ran preliminary analyses to gauge such effects on the samples. $\delta^{15}\text{N}$ values of acidified and non-acidified samples (ranging 0.98‰ to 1.97‰ and 0.69‰ to 1.44‰, respectively) were not statistically significant (t-tests, $p>0.05$), and thus we used both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values from acidified sediments.

2.2.4 Isotope mixing model

The dual $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ signatures obtained from the cores together with isotopic data from potential OM sources in Western Australia coastal waters were incorporated into a Bayesian mixing model (MixSIR version 1.0.4; Semmens et al. 2009, Moore and Semmens 2008) to account for the relative contribution of potential OM sources to the sedimentary OM pool in *P. australis* meadows. The software employed a graphical user interface using MATLAB with 10^8 iterations to produce a useable model function. The output summarises the results in the form of source contribution to the mix, indicating the probability of a given source contributing to the sedimentary OM pool. The isotopic signatures of potential sources (i.e. *Posidonia* plant matter, seagrass epiphytes, *Cladophora* algae, phytoplankton and benthic microalgae; Table 2.1) were obtained from Prado et al. (2008) and Hindell et al. (2004). These reference signatures were results of sample analyses from Cockburn Sound, Western Australia (Prado et al. 2008) and Oyster Harbour (Hindell et al. 2004). Reference signatures of *Posidonia* plant matter and seagrass epiphytes were similar (Table 2.1) but the software could discriminate the two sources due to the differences in $\delta^{15}\text{N}$ signatures. Since there was a relatively small difference in the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotopic values of *P. australis* and *P. sinuosa* (1-2‰ for the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of both above-ground and below-ground tissues), isotopic

Table 2.1. Proportional median contribution of potential organic sources to bulk OM in the sediment based on the MixSIR Bayesian mixing model. The values in parentheses are the 5% and 95% confidence percentiles. Reference isotopic signatures used for calculations (mean \pm SD) were obtained from Prado et al. (2008) and Hindell et al. (2004).

Source	Reference signatures		Contribution
	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)	
<i>Posidonia</i> plant matter	-8.69 ± 1.94	2.75 ± 0.76	0.608 (0.565 – 0.665)
Benthic microalgae	-14.37 ± 0.81	2.11 ± 0.52	0.377 (0.301 – 0.417)
<i>Cladophora</i>	-12.18 ± 0.75	2.57 ± 0.31	0.006 (0.002 – 0.043)
Phytoplankton	-22.67 ± 1.94	4.03 ± 0.70	0.004 (0.000 – 0.024)
<i>Posidonia</i> epiphytes	-8.65 ± 2.57	3.53 ± 0.76	0.003 (0.000 – 0.017)

signatures were pooled to a single *Posidonia* value in the mixing model. The selection of sources were based on relative biomass abundance and most likely source in Oyster Harbour (Hillman et al. 1990, Bastyan 1986) while limiting the number of sources to increase resolution in the mixing model output (see Phillips and Gregg 2003). The available data set used ($n = 49$) was sufficiently large to render prior information as uninfluential and was thus not applied to the model (Semmens and Moore 2008). Fractionation was set to a null value assuming diagenetic effects were negligible for both carbon and nitrogen isotopes (Prokopenko et al. 2006, Fogel and Cifuentes 1993, Hayes 1993, Sweeney and Kaplan 1980).

2.2.5 Calculations of chronostratigraphy, carbon stores and accumulation rates

Plant fibres, at 31 cm, 48 cm and the distal end (150 cm) of one core were dated by ^{14}C Accelerator Mass Spectrometry (AMS). These samples were rinsed in ultrapure MilliQ water, placed in a sonic bath (5 min) to dislodge any attached sediment particles, inspected under a microscope, and sent to the Beta Analytic Radiocarbon Dating Laboratory and Direct AMS for a further acid-base-acid treatment and subsequent ^{14}C analyses. Radiocarbon data was corrected for the marine reservoir effect (i.e. subtracting 71 yr from the uncorrected radiocarbon ages; Ulm 2006). The radiocarbon dates obtained were then plotted as a best-fit smooth-spline function using CLAM.R software (Blaauw 2010) to produce the chronostratigraphic model (Figure 2.2).

Carbon stores per unit area were calculated by multiplying the sediment dry bulk density (in g cm^{-3}) by the carbon content (in %C) to obtain the carbon density (in g C cm^{-3} and kg C m^{-3}). The cumulative mass (in g C m^{-2}) was computed to account for the OC, IC and TC stores in 150 cm-thick sediments. To allow comparisons with other contemporary studies (i.e. Fourqurean et al. 2012a), stores in 100 cm-thick sediments were also estimated. Carbon accumulation rates (in $\text{g C m}^{-2} \text{ yr}^{-1}$) were calculated by dividing the carbon stores in the 150 cm-thick sediments by the age at that depth (i.e. 3132 cal yr BP). The OC stores multiplied by the *P. australis* areal cover at Oyster Harbour in the 1980s (6.1-6.7 km^2 ; Bastyan 1986, McKenzie 1962) provided an estimate of the OC stores beneath the *P. australis* meadows at

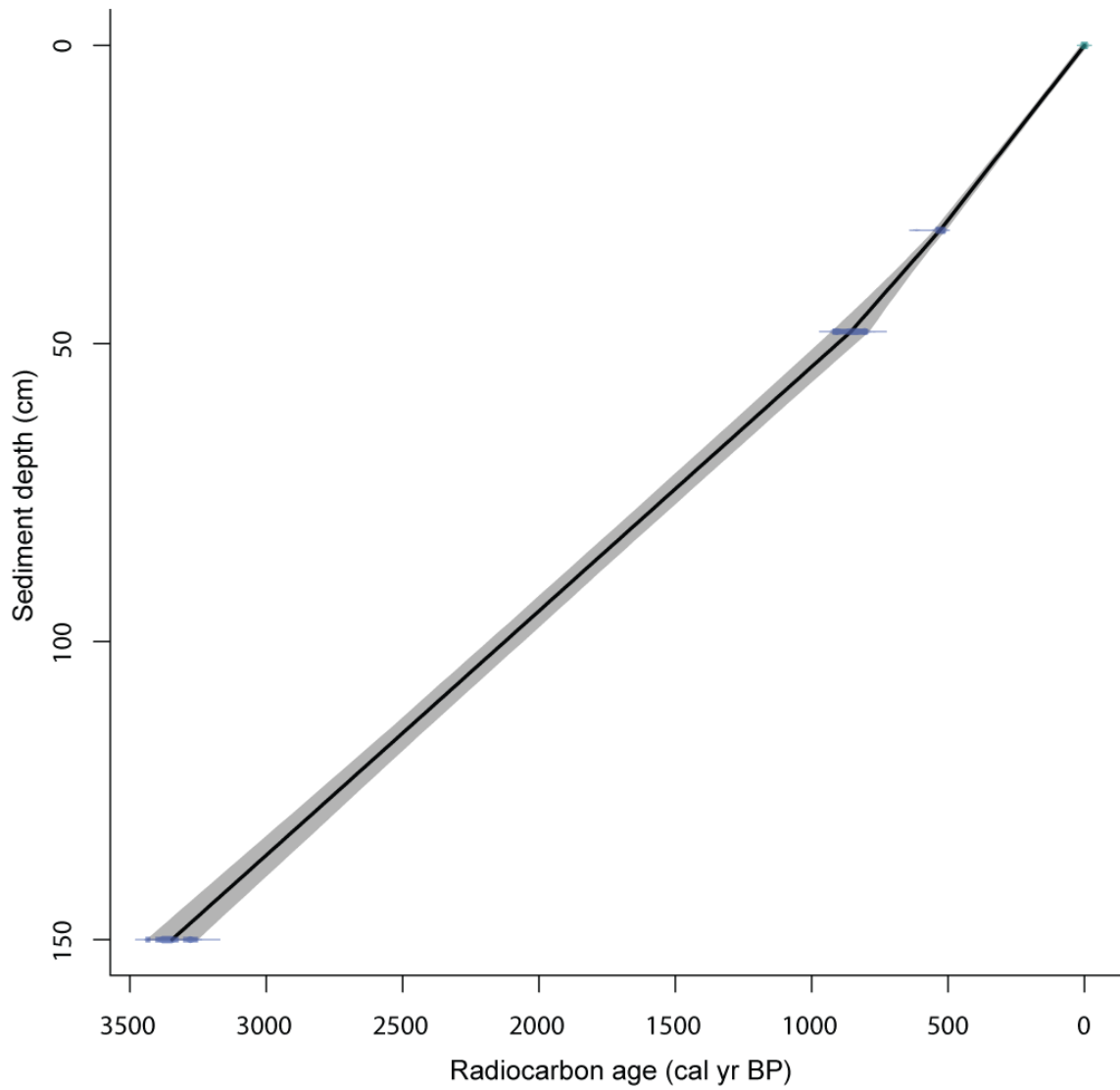


Figure 2.2. Chronostratigraphic framework for the studied core. The solid line represents a best fit (a smooth-spline model; Blaauw, 2010) through three radiocarbon dates calibrated and corrected for the reservoir effect.

Oyster Harbour within this period. The empirical value of 1500 g OC m⁻², obtained from Marba et al. (2015), was multiplied by the area of seagrass cover lost (2.8-3.1 km²) from the 1980s until present (Department of Water 2008) to account for the potential loss of OC stores in Oyster Harbour (i.e. assuming that the OC in shallow sediments was remineralised after meadow disturbance, after Marba et al. 2015). We used a factor of 3.67 to convert the estimates of cumulative OC mass (in Mg OC ha⁻¹) to equivalent units of CO₂ (i.e. the molar ratio of CO₂ to C, after Howard et al. 2014). The loss of seagrass cover also represented an absence of OC sequestration (Marba et al. 2015), which was estimated by multiplying the average OC accumulation rates (in g OC m⁻² yr⁻¹) by the seagrass area loss (2.8-3.1 km²) and the period in which seagrasses have been absent (i.e. 29 years).

2.3 Results

2.3.1 Biogeochemical characteristics of *P. australis* sediments

The top 15 cm of sediment had dense clumps of live *P. australis* above- and below-ground organs consisting of sheaths, roots and rhizomes. The sediment colour (Munsell 2000) from 15 cm to 25 cm depth was greyish-brown (Gley1 4/N). At depths greater than 25 cm, it progressively became lighter brown (10YR 6/3) and then to a consistent brown (10 YR 6/4) towards the distal core end. Localised dense agglutination of fine strands of *Posidonia* organs (sheaths, root and rhizome detritus) were found throughout the cores. Whole and broken shells were dispersed within these agglutinations.

The estimated radiocarbon age of the dated core after corrections, and calibrated to years before present (cal yr BP, OxCal 4.2; ShCal13 curve; present taken as AD 2012) were 511 ± 21 cal yr BP, 946 ± 30 cal yr BP and 3132 ± 30 cal yr BP for the 31 cm, 48 cm and 150 cm samples, respectively. The mean accumulation rate calculated through this model was 0.0494 cm yr⁻¹ (150 cm-thick sediment; resolution of 20.26 yr cm⁻¹). It was assumed that the other two replicate cores (sampled in relative close proximity) had the same chronostratigraphy.

The OM and OC contents in all three cores showed little variation across depth in the sediment (Pearson correlation, $p > 0.05$; Figure 2.3). The mean OM content was $9.07 \pm 0.36\%$ (mean \pm SE), with a higher OM content in the top 15 cm (10-15%) compared to 15 cm to 150 cm layer (6-12%; Figure 2.3). Organic carbon content ranged between 1.0% and 3.5%, with a mean value of $2.24 \pm 0.05\%$ in 150 cm long cores (Table 2.2 and Figure 2.3). The lack of a decrease in OC and OM content towards the distal core ends is consistent with the observation that there were visual presence of coarse OM at these ends. Thus, the interpretations of carbon stores in the sediment is limited to this core depth only (i.e. up to 150 cm sediment depth).

The CaCO_3 content was relatively consistent from the sediment surface to 132 cm depth (20-35%) for the three cores. In the longest core, the decline thereafter was to a minimum of 10% at the distal end (Figure 2.3). Mean CaCO_3 and IC contents were $26.40 \pm 1.70\%$ and $3.16 \pm 0.17\%$, respectively (Table 2.2). Across the whole core, $\delta^{13}\text{C}$ signatures ranged from -10.0‰ to -13.3‰ (Figure 2.3). The $\delta^{13}\text{C}$ signatures of the OM in the top 5 cm of the cores were relatively low (-13.0‰ to -13.5‰), and increasing thereafter to -11.7‰ at 40 cm. From 40 cm until the distal core end, the $\delta^{13}\text{C}$ signatures ranged from -10.5‰ to -12.8‰ with no obvious trends. The MixSIR output indicated a median contribution of *Posidonia* plant matter to the OM sedimentary pool of 61% (ranging 57-67%; Table 2.1). Benthic microalgae were the second highest contributor (30-41%). Other sources included in the model (i.e. *Cladophora*, phytoplankton and *Posidonia* epiphytes) had low (< 1%) likelihood of contributions.

2.3.2 Carbon stores and long-term carbon accumulation rates

The average TC stores in the 150 cm-thick seagrass sediments at Oyster Harbour were estimated to be $27.92 \text{ kg TC m}^{-2}$ ($10.79 \pm 0.12 \text{ kg OC m}^{-2}$ and $17.13 \pm 1.74 \text{ kg IC m}^{-2}$; Figure 2.3). This value is also equivalent to $279 \text{ Mg TC ha}^{-1}$ ($108 \text{ Mg OC ha}^{-1}$ and $171 \text{ Mg IC ha}^{-1}$; Table 2.2). Total carbon stores in the 100 cm-thick seagrass sediments were $188 \text{ Mg TC ha}^{-1}$ (73 Mg OC ha^{-1} and $115 \text{ Mg IC ha}^{-1}$; Table 2.3). The long-term carbon stores (in 150 cm-thick sediment, ~3132 yr period) beneath the *P. australis* meadows at Oyster Harbour was

Table 2.2. Carbon content (in %), carbon density (kg m⁻³), carbon accumulation rates (kg m⁻²) and cumulative mass (g m⁻² yr⁻¹, based on varying sediment depths) in the *Posidonia australis* mats studied (mean ± SE). The values in parentheses refer to the equivalent carbon stores in Mg ha⁻¹.

Variable	Sediment mat thickness (cm)	Organic carbon (OC)	Inorganic carbon (IC)	Total carbon (TC)
Content in bulk sediment (%)	150	2.24 ± 0.05	3.16 ± 0.17	5.40
Density (kg m ⁻³)	150	14.65 ± 0.98	21.61 ± 1.93	36.26
Cumulative mass (kg m ⁻²)	100	7.31 ± 0.11 (73)	11.47 ± 0.70 (115)	18.78 (188)
	150	10.79 ± 0.09 (108)	17.13 ± 1.44 (171)	27.92 (279)
Accumulation rate (g m ⁻² yr ⁻¹)	150	3.45	5.47	8.92

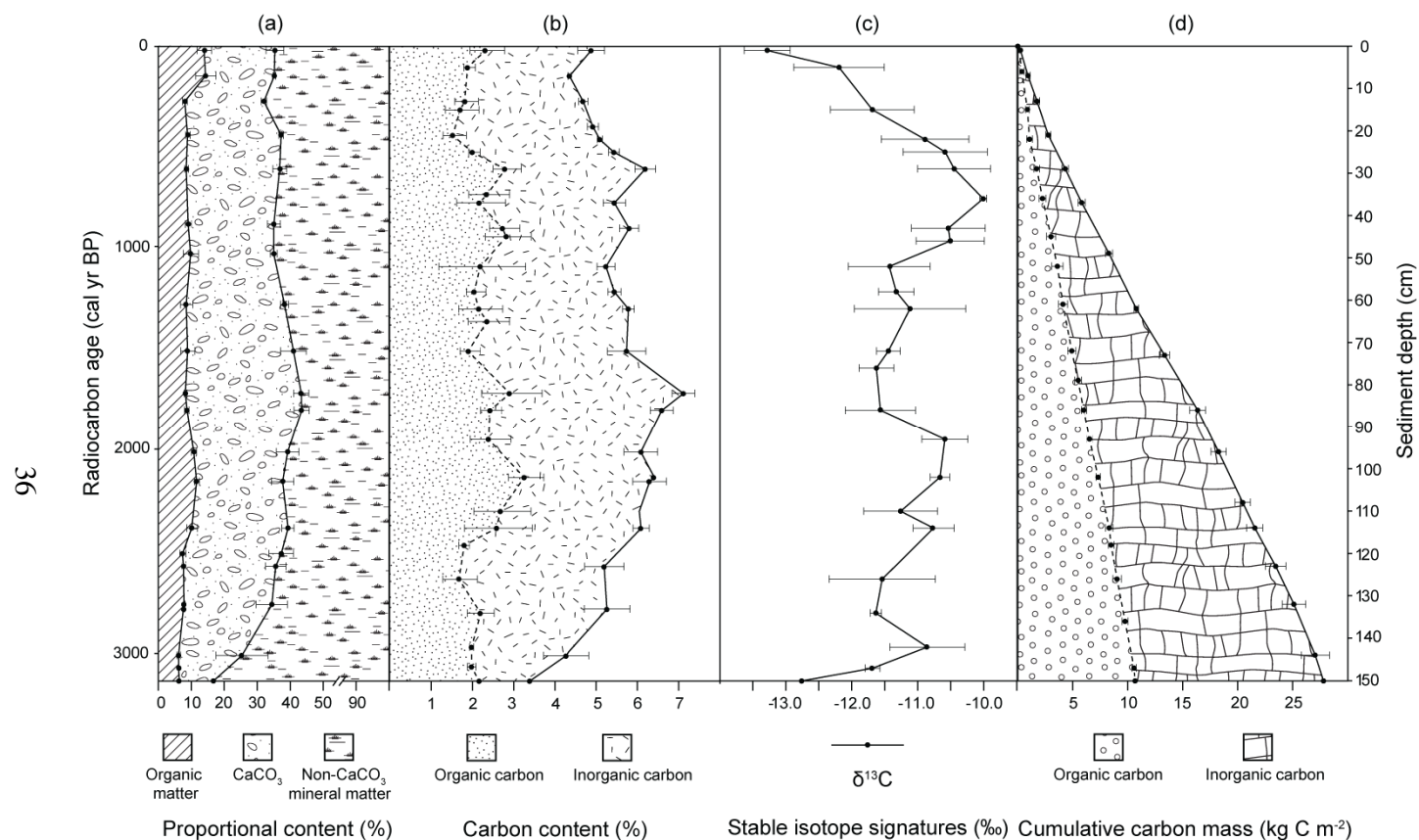


Figure 2.3. Chronostratigraphic profile of sediment characteristics based on the three *P. australis* mat cores. (a) Proportion of organic matter, CaCO_3 and non- CaCO_3 mineral contents in bulk sediment; (b) Percentage of total carbon as mean organic carbon and inorganic carbon contents in bulk sediment; (c) stable carbon isotope ($\delta^{13}\text{C}$) signatures of organic matter; and (d) cumulative carbon mass. Data points are mean \pm SE.

estimated to be in the range of 101-187 Gg TC (39-72 Gg OC and 62-115 Gg IC; 6.1-6.7 km², Table 2.3). The estimated carbon accumulation rate in the seagrass mat calculated over a period of 3132 yr was 8.92 g TC m⁻² yr⁻¹ (3.45 g OC m⁻² yr⁻¹ and 5.47 g IC m⁻² yr⁻¹; Table 2.2). Due to the loss of cover, it is estimated that the *P. australis* meadows of Oyster Harbour had remineralised 4.20 to 4.65 Mg OC, which is equivalent to the potential release of 15-17 Gg CO₂ (Table 2.3).

2.4 Discussion

2.4.1 Long-term carbon storage in *P. australis*

The *Posidonia australis* meadows at Oyster Harbour accumulated significant carbon stores, at least in the past 3000 yr when considering the cumulative mass of sedimentary OC and IC contents. The accumulated OC may be subject to more variability in quantities compared to IC due to its relative lability in marine sediments. Inorganic carbon, in the form of calcium carbonate, tends to remain permanently sequestered in natural oceanic settings. Organic carbon content in this study ($2.24 \pm 0.05\%$) is higher than those *P. australis* meadows included for global estimates of carbon stores (i.e. 0.26-1.05% OC; supplementary information in Fourqurean et al. 2012a). In comparison, IC stores (115 Mg IC ha⁻¹; this study) are within the range of existing global estimates (3-1660 Mg IC ha⁻¹; Mazarassa et al. 2015). Organic carbon stores estimated in this study for shallow sediments (15 cm depth; 2-4 kg OC m⁻²; Figure 2.3) were in the same range as those obtained by Marba et al. (2015) in the same study area (i.e. 2.7 kg OC m⁻²). In contrast, the estimated OC stores for the top 100 cm (73 Mg OC ha⁻¹) are below the median estimate in seagrass meadows globally (median 140 Mg OC ha⁻¹; range 9-628 Mg OC ha⁻¹; 100 cm depth; Fourqurean et al. 2012a). These global estimates were strongly influenced by OC stores in *P. oceanica* meadows in the Mediterranean Sea (Fourqurean et al. 2012a), which have an unusual ability to capture OC. The larger OC accumulation capacity of *P. oceanica* (17-75 kg OC m⁻³ and 6-175 g OC m⁻²

Table 2.3. Estimate of long-term carbon stores (OC, IC and TC) within the top 150 cm of the seagrass mats at Oyster Harbour. Potential losses in carbon sequestration by seagrass meadows between 1984 and 2012 was due to the loss of seagrass cover. It was assumed that the current cover (in 2012) was the same as that in 2008. We also assumed that there was no carbon accrual in the seagrass lost area, and that all OC stores in shallow sediments accumulated over the past 60 yr had been remineralised to CO₂ (Marba et al. 2015).

Estimated area (km ²) and reference	Estimated OC (Gg)	Estimated IC (Gg)	Total carbon (Gg)	Potential absence in OC accumulation (Mg)	Potential remineralisation of OC (Gg)	CO ₂ emissions (Gg)	Remarks
6.1-6.7 Mckenzie (1962); Bastyan (1986)	72.3	114.8	187.1	NA	NA	NA	Percent cover value used as baseline reference (i.e. loss = 0)
3.6 Bastyan (1986)	38.8	61.7	100.5	310	4.65	17	Carbon loss estimates after seagrass cover loss (meadow cover in 1980s)
3.9 Department of Water (2008)	42.1	66.8	108.8	280	4.20	15	Carbon loss estimates after seagrass cover loss (meadow cover at 2008)

yr⁻¹; adapted from Serrano et al. 2012, Serrano et al. 2014) compared to *P. australis* (14.7 kg OC m⁻³ and 3.5 g OC m⁻² yr⁻¹; this study, Table 2.2) could be related to the higher production of below-ground biomass of the Mediterranean species (1610 g DW m⁻² and 658 g DW m⁻², respectively; Paling and McComb 2000, Duarte and Chiscano 1999) resulting in higher organic inputs into the sedimentary OC pool. In the same regard, the IC accumulation rate estimated from this study (i.e. 5.47 g IC m⁻² yr⁻¹) is much lower compared to that reported for *P. oceanica* meadows (47-138 g IC m⁻² y⁻¹; De Falco et al. 2008), which could also be attributed to the higher CaCO₃ production with increasing seagrass productivity (Gacia et al. 2003). The dense and relatively high canopy of *P. oceanica*, which enhances sediment deposition and reduces re-suspension, together with its higher substrate accumulation rates (0.6 to 10 mm yr⁻¹; see review in Serrano et al. 2012) compared to *P. australis* at Oyster Harbour (0.494 mm yr⁻¹) may also partly explain the variability in the TC accumulation rates observed among *Posidonia* species. It has to be noted that the OC accumulation rates in this study (3.5 g OC m⁻² yr⁻¹) is lower than those estimated globally (45–190 g OC m⁻² yr⁻¹; Mcleod et al. 2011). It is plausible that vegetation losses or discontinuity in *P. australis* growths may result in the lower accumulation rates. This possibility, however, remain hypothetical and thus warrants further investigations into such occurrences within the accumulation period.

Estimates of OC stores and sediment accumulation rates (1.8-7.4 kg OC m⁻³ and 0.6-1.3 mm yr⁻¹; Serrano et al. 2014), and below ground productivity (468 g DW m⁻²; Paling and McComb 2000) in *Posidonia sinuosa* meadows demonstrate the high productivity and OC storage capacity of *Posidonia* meadows. *Posidonia* species differ in the formation of their rhizomal mat and their rates of substrate accretion, which influences the rate of OC accumulation. Plagiotropic and orthotropic rhizomal growths are both dominant morphological features of *P. oceanica*, while *P. australis* and *P. sinuosa* are mainly horizontal spreaders with little vertical shoot production (Gobert et al. 2006). *Posidonia* species contain a relatively high proportion of recalcitrant OM in their tissues compared to other seagrass species, consisting of large macromolecular OM such as lignin, cellulose and hemi-cellulose (Torbatinejad et al.

2007, Klap et al. 2000). In addition, the larger below-ground biomass of *Posidonia* species compared to most other seagrasses (Duarte and Chiscano 1999) may constitute a key factor contributing to its large OC stores. Although further studies are required to fully understand the phenomenon of OC accumulation in seagrass meadows, the differences observed among seagrass species are sufficiently large to conclude that inter-species variation must be taken into account when attempting to model global carbon burial estimates in seagrass habitats, supporting previous findings by Lavery et al. (2013).

Long-term storage of OC in *Posidonia australis* meadows is likely due to a combination of key biotic factors and biogeochemical processes. First, *P. australis* meadows has productivity levels resulting in the accumulation of recalcitrant below-ground material as high as 658 g DW m⁻² (Paling and McComb 2000). In Oyster Harbour, living rhizomes were more commonly observed growing 25-50 cm beneath the sediment surface but may grow up to 50-100 cm beneath the meadow (Kuo and Cambridge 1978). A high amount of this below-ground production is buried *in situ* as detrital matter (Campbell and Paling 2003) consistent with the output of the isotope mixing model in this study, which indicated that the sedimentary OM had a median of 61% comprising of *Posidonia* origin. This material is not only a direct addition to the sedimentary OM but it also helps to stabilise the sediments, facilitating the ongoing accumulation of the sedimentary OM. Second, the retention and preservation of the seagrass sedimentary OM may be augmented by the sheltered nature of Oyster Harbour (Hodgkin and Clark 1990), which may reduce sediment upheaval. The relatively low hydrodynamic energy would be further reduced by the dense and relatively high canopy of *P. australis*, which, in other species, has been shown to enhance sediment deposition and reduce re-suspension (Marbà et al. 2015, Zhuang and Chappell 1991). The geomorphology of Oyster Harbour and the depositional conditions within the estuary may ensure concurrent burial of both autochthonous and allochthonous OM and increase the likelihood of OC being preserved. Finally, the anoxic conditions found in seagrass sediments (Borum et al. 2006) reduce microbial degradation (Pedersen et al. 2011, Burdige 2007) and

contribute to the millenary preservation of OC stores in *Posidonia* meadows (Serrano et al. 2012, Mateo et al. 2006).

The carbon stores estimated here are likely to be an under-estimate of the actual stores in the estuary. Our estimates are limited to 150 cm, the maximum depth to which we could core. However, it is likely that the OC stores extend beyond this depth. At the distal core end (150 cm-depth, ~3132 cal yr BP), the OM content was still 6-7% OM (2.0-2.2% OC). According to the curve of the Holocene sea level change predicted along the Australian coasts and estuaries, the sea level rose between 0 and 3 m during the last 6000-7000 years (Lambeck 2002, Hodgkin and Hesp 1998). Thus, assuming that the study area did not experience significant geomorphological alterations for the last 6000 yr and that the seagrass was present over that period, the potential depth of *P. australis* mats beneath the sediment surface may be up to 300 cm. If this is the case, then the OC stores could potentially be up to 22 kg OC m⁻². As such, our estimates of the carbon stores estimated from this study are conservative at best. Sampling beyond the 150 cm depth is necessary to capture the full extent of buried OC within the meadow since the last sea level rise (before 6000 yr BP).

The consistent OM content towards the distal core end (Figure 2.3) indicates that, after burial, the OM was preserved over millennia with little decomposition. In most seagrass sediments, the OM and OC contents decrease with depth and aging (e.g. Serrano et al. 2012; Fourqurean et al. 2012b), but in others the OM content remains stable with age, leading to exceptionally high OC stores, such as those found in *Posidonia oceanica* meadows at the Balearic Islands (Serrano et al. 2014). This comparison illustrates the unique preservation of OC in the *P. australis* deposits of Oyster Harbour compared to other seagrass habitats but further investigations are needed to provide more insights into the factors controlling OM preservation (e.g. geomorphology, sediment grain-size and OM composition; Burdige 2007, Bergamaschi et al. 1997, Hedges and Keil 1995).

2.4.2 Characteristics and sources of organic carbon

The preserved sedimentary OM of the *Posidonia australis* meadow was dominated by seagrass-derived OM, as indicated by the stable isotope mixing model and further supported by the visual observation of *P. australis* roots, rhizomes and sheaths along the cores. In a global review of seagrass sedimentary OM, Kennedy et al. (2010) found that the OM accumulated in seagrass meadows is derived not only from seagrass production but also from the trapping of allochthonous matter. The iterations from MixSIR indicated that *Posidonia* detritus contributed about 61% of the OC pool, significantly more than in other seagrasses worldwide (~50%; Kennedy et al. 2010). This disparity could be partially explained by the high productivity of temperate *Posidonia* meadows (Duarte and Chiscano 1999), but also to differences in the sedimentary depths studied; Kennedy et al. (2010) investigated the upper 5–10 cm compared to 150 cm in this study. It seems probable that shallow sediments may have more heterogeneous OM compared to those at greater sediment depths, for two key reasons. First, seagrasses contain a high proportion of recalcitrant OM in their tissues compared to other OM sources (Torbatinejad et al. 2007, Klap et al. 2000), which may lead to a higher preservation of seagrass-derived OM with aging (Rozaimi et al. *submitted*). Second, human impacts in coastal areas has led to the degradation of seagrass ecosystems (e.g. reduced meadow cover and density, algal blooms and increased run-off after land clearance; Short and Wyllie-Echeverria 1996) causing a decline in seagrass OM production and a relative increase in non-seagrass matter inputs added to surficial and sub-surficial sediments (Macreadie et al. 2012, Bratton et al. 2003).

While seagrass matter dominated the contribution to OM in the Oyster Harbour meadow, the variability in the $\delta^{13}\text{C}$ values indicates a reduced proportion of seagrass material in the upper 5–10 cm of the cores. At the surface, the $\delta^{13}\text{C}$ value was approaching -13.0‰ , significantly lower than the $\delta^{13}\text{C}$ signatures of *Posidonia* rhizomes ($-10.82 \pm 0.37\text{‰}$; Prado et al. 2008) and indicative of more heterogeneous inputs of OM possibly related to human impacts in the study area (e.g. land clearance and coastal eutrophication; Brearley 2005, Bastyan 1986).

With depth, the $\delta^{13}\text{C}$ signatures of bulk sediment ($-11.1 \pm 0.06\text{‰}$) were only slightly depleted compared to *Posidonia* $\delta^{13}\text{C}$ values, reflecting either less input of allochthonous matter or better preservation of the seagrass matter over time, or a combination of both. A change in $\delta^{13}\text{C}$ values of organic matter with age had been shown in other studies to be small or negligible (Galimov et al. 1995; Fogel and Cifuentes 1993, and Hayes 1993). Therefore it was assumed that there were negligible variation in the $\delta^{13}\text{C}$ values of seagrass-derived OC buried in the sediments of Oyster Harbour after millennia of burial.

2.4.3 Evaluation of the carbon sequestration ecosystem service provided by *P. australis* meadows at Oyster Harbour

The estimated TC stores in 150 cm-thick seagrass sediments at Oyster Harbour based on seagrass cover in the 1980s (6.1-6.7 km²; Bastyan, 1986) and assuming this species had historically been the dominant seagrass of Oyster Harbour (Department of Water 2008, Bastyan 1986, McKenzie 1962), was 101-187 Gg TC (39-72 Gg OC and 62-115 Gg IC). The capacity of a healthy seagrass meadow allows the capacity for both IC and OC accumulation. Decreases in seagrass cover results in decreases in OC accumulation and also concomitant with increases of OC remineralisation (Pergent et al. 2012). Declines in seagrass ecosystem health due to eutrophication in Oyster Harbour led to a loss of 2.8-3.1 km² in *P. australis* cover from 6.1-6.7 km² in 1980s to 3.6 km² in 1984, and 3.9 km² in 2008 (Table 2.3; Department of Water 2008, Bastyan 1986); the small difference between the two most recent estimates of seagrass cover (i.e. 0.3 km²) is possibly due to different survey techniques used or a slight increase in cover due to revegetation. The loss of seagrass vegetation results in no further contributions of seagrass-derived OM to the carbon storage pools (Marbà et al. 2015). It could be estimated that the loss of seagrass cover represented an absence of 280-310 Mg OC accumulation over a 29 yr period (Table 3). Additionally to the absence of sequestration, the exact fate of the OC in shallow sediments is unknown. It is likely that much of the sedimentary OC from degraded seagrass meadows was released back into the ocean-atmosphere CO₂ pool over the last 29 years (Marbà et al. 2015; Fourqurean et al. 2012a). By

mass balances, we estimate that the loss of seagrass area could hypothetically result in the release of up to 15-17 Gg of CO₂, assuming that all of the OC in the top half meter of sediment (i.e. 10.09-11.17 Gg OC; Table 3) is remineralised. Shallow sediments (up to 1 m as indicated by Fourqurean et al. 2012a, or up to 60 yr accumulation period as estimated by Marba et al. 2015 in Oyster harbour) are most susceptible to disturbances and thus prone to subsequent losses of any stored OC. This potential loss relates to a relatively small area of seagrass cover and emphasises the potential loss of ecosystem services, including CO₂ sequestration, associated with the global loss of seagrasses, currently estimated at 7% of the total area per annum (Waycott et al., 2009).

2.5 Conclusion

Significant stores of organic and inorganic carbon are found in the *P. australis* meadow at Oyster Harbour, which have been accumulated, at least, over the last 3000 years. Seagrass primary production contributes more than 60% to the sedimentary OC pool. The ultimate significance of the *P. australis* ecosystem, in the context of mitigation of global climate change, is related to the massive long-term carbon stores accumulated over millennia, and therefore, efforts should be concentrated on conserving the meadows to keep this reservoir intact. Seagrass meadows are at risk worldwide, and it is likely that much of the OC stored in sediments under degraded seagrass meadows is being released back into the environment, entailing significant ecological consequences.

CHAPTER 3

INFLUENCE OF THE DEPOSITIONAL ENVIRONMENT ON ORGANIC CARBON STORES IN SEAGRASS MEADOWS

Abstract

Meadows of the seagrass *Posidonia australis* accumulate significant amounts of organic carbon in their sediments. While it is known that *P. australis* occurs in estuaries and oceanic environments, inter-habitat variability has never been considered in accounting for carbon stores in its meadows. From a study of 27 sediment cores from nine *P. australis* meadows, sites were categorised as either *More Sheltered*, *Less Sheltered* or *Exposed* habitats based on exposure indices and grain size analyses. There was up to 6-fold differences in carbon stores among *P. australis* meadows. Higher carbon stores were found in the *More Sheltered* sites (4.57-13.51 kg OC m⁻²) compared to *Exposed* sites (2.24-3.77 kg OC m⁻²). Carbon stores in the *Less Sheltered* environments were intermediate (4.31-8.00 kg OC m⁻²), with no significant differences to either the *More sheltered* or *Exposed* habitats ($p > 0.05$). While there was a trend in carbon stores across the three depositional environments, there was no clear distinction in carbon stores within sheltered *P. australis* meadows consisting of estuarine and non-estuarine sites. Variations observed in carbon stores among different depositional environments can be explained by differences in seagrass primary production and biomass, and differences in allochthonous and autochthonous organic matter burial and preservation. The results provide new insights into the intra-species variation in carbon storage potential

with regards to different depositional environments and emphasise the need to factor this aspect into regional and global estimates of seagrass Blue Carbon stores.

3.1 Introduction

The sedimentary organic carbon (OC) stores of seagrass meadows can differ significantly, with reports of up to 18-fold differences among seagrass meadows of different species and in different habitats (Lavery et al. 2013). Uncertainty remains in the relative importance of the species identity and the inherent habitat characteristics in contributing to this variability. Seagrasses occur in a variety of coastal habitats, ranging from shallow estuaries, which are naturally depositional, through to highly exposed offshore waters, which are much less depositional (Carruthers et al 2007). Since seagrasses occur in a variety of habitat types, this raises the question of whether it is possible to generalise the likely carbon stores for a particular species across all the habitats where it occurred, or whether the habitat type will significantly influence the OC stores even within a single species.

A major determinant of the OC stores of any coastal habitats, including seagrass meadows, is the depositional nature of the environment. For example, carbon accumulates more in the highly depositional environment of estuaries compared to the open oceans (estimated at 81 Tg C y⁻¹ and 45.2 Tg C y⁻¹, respectively; Nellemann et al. 2009). If species identity is the major determinant of sedimentary OC stores, relatively little variation among meadows of the same species is expected. On the other hand, if the depositional nature of the environment is important, then we might expect large variation in OC stores among meadows of the same species depending on the nature of the habitat in which they occur. To date, comparisons of the sedimentary OC stores of seagrass meadows of the same species across a range of depositional habitat types has not been undertaken.

In seagrass meadows, depositional conditions may affect the net accumulation of OC in sediments from autochthonous accretion as well as allochthonous depositional processes (Hendriks et al. 2010, Mateo et al. 2006, Cebrian 2002). The net result can be a heterogeneous mixture of accumulated organic matter (OM) derived from seagrasses, seagrass epiphytes, microphytobenthos, algae, seston or terrestrial OM (Kennedy et al. 2010). Seagrass meadows in environments conducive for depositional processes also accumulate higher amounts of fine-grained sediments that originate from adjacent ecosystems (de Boer 2007). These sediments are typically more anoxic and facilitate OM preservation (Bergamaschi et al. 1997). Comparatively, exposed marine environments accumulate more coarse-grained sediments, allowing oxygenation of the sediment interstitial spaces (Dauwe et al. 2001), potentially allowing higher rates of OM degradation compared to sheltered sites.

At the global level, seagrass habitats have been classified into three major depositional environments: *Estuarine*, *Sheltered* and *Exposed* habitats (Carruthers et al. 2007). This classification, in the form of a seagrass functional-form model, further differentiates seagrasses based on morphological plasticity and rhizome persistence, among other traits (Figure 1.2). One implication of this model for OC accumulation is that there may well be a two-way interaction between the habitat's hydrodynamic characteristics and the seagrass community. The prevailing hydrodynamic energy of an area influences the type of species that occur in that habitat. Once established, the seagrass community may in turn, influence the amount and source of organic accumulation through detrital plant inputs. In addition, the seagrass canopy may retain any OM produced *in situ* and also trap any imported particles, directing it to the canopy base and enabling subsequent burial (Gacia et al. 1999). In estuaries, OC originating from autochthonous production may dominate the OM pool and be supplemented by allochthonous deposition from upstream transport (Aller 1998, Canfield 1994). For sheltered habitats, autochthonous inputs may dominate (Macdonald et al. 1998) while the inherent hydrodynamic energy in exposed habitats may result in the simultaneous

export of autochthonous production and import of allochthonous materials (Macdonald et al. 1998).

The interaction of local hydrodynamics, carbon delivery and deposition described above may trigger the formation of organic-rich sediments within seagrass beds (Cebrian 2002). Following deposition, initial OM processing (i.e. cycling and remineralisation) may result in the loss of labile OC through diagenesis (Burdige 2007). More recalcitrant biochemical components will be available for burial and contribute to long term OC storage (Chapter 2, Mateo et al. 2006). With many possible sources and fates of OM in seagrass meadows, there is the potential for differences in the magnitude and nature of OC stores in seagrass meadows occurring in different depositional environments (i.e. estuarine, sheltered and exposed habitats).

This study aimed to investigate the amount of sedimentary OC stored in the meadows of one seagrass species, *Posidonia australis*, as a function of its depositional environment. The focus on *P. australis* was because: it occurs across a wide range of habitat types, including estuarine and oceanic environments (Carruthers et al. 2007); it has been shown to have large but variable OC stores (Lavery et al. 2013); it is an ecologically important species, being a major habitat-forming seagrass in temperate regions of Australia (Gobert et al. 2006); and, based on initial estimates, is a significant contributor to the total sedimentary OC stores of seagrasses in the Australian continent (Lavery et al. 2013). It was hypothesised that OC stores would be greatest in *Estuaries*, intermediate in *Sheltered*, and least in *Exposed* habitats.

3.2 Materials and methods

Nine *P. australis* meadows were studied, three each in what were *a priori* classifications as *Estuarine*, *Sheltered* and *Exposed* habitats based on their geomorphology and exposure to prevailing wind and swell (Table 3.1 and Figure 3.1). Oyster Harbour, Waychinicup Inlet

Table 3.1: Site coordinates and depositional environment discriminations of the studied *P. australis* meadows. Values in parentheses indicate the relative exposure index (REI) of the respective sites.

Meadow site	Coordinates	Depositional environment discrimination		
		Geomorphological setting of meadow	Relative exposure category and REI Index	Sediment grain-size categorisation
Waychinicup Inlet Estuary	34°53'35.9"S 118°19'57.7"E	Estuary	Estuarine and Sheltered (0.1)	More sheltered
Oyster Harbour	34°58'58.3"S 117°58'29.9"E	Estuary	Estuarine and Sheltered (7.8)	More sheltered
Monkey Mia - Faure Island	25°50'51.18"S 113°50'06.83"E	Oceanic	Exposed (87.6)	More sheltered
Faure Island	25°46'18.00"S 113°49'21.00"E	Oceanic	Exposed (154.8)	More sheltered
Princess Royal Harbour	34°04'31"S 117°55'14"E	Oceanic	Sheltered (9.2)	Less sheltered
Frenchman's Bay	35°01'57.70"S 117°56'24.38"E	Oceanic	Exposed (47.8)	Less sheltered
Botany Bay	34°00'33.4"S 151°11'16.91"E	Estuary	Estuarine and Sheltered (1.6)	Less sheltered
Peron Point	32°16'27.94"S 115°41'01.06"E	Oceanic	Exposed (57.5)	Exposed
Lal Bank	31°48'41.01"S 115°43'04.35"E	Oceanic	Exposed (127.2)	Exposed



Figure 3.1: Location of study sites. Three cores were sampled from each site, represented by the yellow dots as the coring points. Sites with one dot (i.e. Waychinicup Inlet Estuary, Frenchman's Bay and Botany Bay) consisted of three cores sampled close to each other and indistinguishable due to the resolution of the available map.

Estuary and Botany Bay are estuaries while the other sites are oceanic *P. australis* meadows with varying degrees of exposure to oceanic swells and waves. While the oceanic sites were *a priori* classified as *Sheltered* or *Exposed*, the sites were further ranked according to a Relative Exposure Index (REI, after Garcon et al. 2010, Fonseca and Bell 1998) and by sediment grain size analysis to provide an indication of the likely degree of exposure (methods provided below). From each of the nine meadows, three sediment cores were collected in 2012 and 2013 at water depths of 1.5-2 m. This small range was selected for all sites to minimise the effect of water depth on OC stores (after Serrano et al. 2014).

3.2.1 *Coring and core processing*

Three randomly located cores were sampled within each of the nine meadows (distance of 10 to 100 m between cores, depending on the meadow) by manually hammering PVC pipes into the seafloor. The pipes were 50 mm wide (inner diameter) and with distal ends cut transversely at an angle of about 45° to provide a ‘hypodermic design’. This design enhanced penetration into the sediment floor, allowing the core barrel to sample deeper sediments up to the point where no further penetration was possible. Compression of unconsolidated sediment during coring was inevitable and corrections were applied to obtain the presumed actual length of the core. By linear proportions (after Serrano et al. 2012, Glew et al. 2001), the sampled core was decompressed to occupy the length of the core barrel embedded within the sampling point and thus providing the corrected core length. Among all meadows, variable core lengths and degree of compression were obtained. After corrections, these were the maximum core lengths obtained at each meadow: Oyster Harbour (OHarb) - 254 cm; Waychinicup Inlet Estuary (WInlet) - 179 cm; Botany Bay (BBay) - 150 cm; Princess Royal Harbour (PRHarb) - 147 cm; channel between Monkey Mia and Faure Island (MMia-FIsla) – 323 cm; Faure Island (FIsla) - 269 cm; Frenchman’s Bay (FBay) - 181 cm; Peron Point (PPoint) - 154 cm; and Lal Bank (LBank) - 140 cm. All variables relating to the stratigraphy of the sediment core were then plotted against these corrected core lengths. Analysed variables (see below) were taken as the mean of three cores from each meadow. After

transport to the laboratory, the core barrel was cut length-wise to expose the core log. Then, the sediments were sub-sampled into 1 cm-wide slices. Alternate slices (17 to 32 samples per core, depending on the length of the sampled core, at regular intervals of 5 cm for the top 50 cm and 10 cm for core length beyond 50 cm) were selected for biogeochemical analysis and oven-dried at 55°C to constant weight, to calculate the sediment bulk density. These slices were then ground to a fine powder using a ball-mill grinder (Retsch).

3.2.2 *Organic matter analyses*

The ground sub-samples were combusted in a muffle furnace at 550°C (5 hours) to obtain an estimate of the OM weight, through loss on ignition (after Heiri et al. 2001). Organic matter mass was reported as the percent content in bulk sediment.

3.2.3 *Organic carbon elemental composition and stable isotope signatures*

Another set of ground sub-samples (0.5 g) were used for OC and $\delta^{13}\text{C}$ analyses by firstly acidifying them (1 M hydrochloric acid) to remove all inorganic carbon. When effervescence ceased after 12-18 h, the mixture was centrifuged (3400 revolutions min^{-1} for 4.5 min) and pipetted to remove the acid. Deionised water (10 ml) was added to wash off residual acid, centrifuged, and the supernatant removed by pipetting. The sample was then oven-dried and weighed. For elemental composition and isotope analysis, 9-10 mg of the acidified sample was encapsulated in tin capsules and combusted in a continuous flow isotope ratio mass spectrometer analyser (Delta V Advantage: Thermo-Finnigan) at the LIENSs Stable Isotope Facility (University of La Rochelle, France). The OC content reported by the analytical facilities was corrected to account for the weight of the pre-acidified bulk sediment samples. $\delta^{13}\text{C}$ values were reported relative to the Vienna Pee Dee Belemnite (VPDB) standard.

3.2.4 *Calculations of organic carbon stores across stratigraphy*

Due to the variability in core lengths sampled among the nine meadows studied, OC stores were standardised to stratigraphic depths of 140 cm, 100 cm and 30 cm to allow comparisons.

The interpretations of carbon stores in the sediment is limited to this core depth only (i.e. up to 140 cm sediment depth). The limit of 140 cm sediment thickness was based on the shortest core sampled among the meadows (i.e. at LBank), 100 cm adopted to compare values with other studies (i.e. Fourqurean et al. 2012a), and 30 cm to obtain values in shallow sediments. Organic carbon stores ($Store_{OC}$; g OC cm⁻²) were calculated as:

$$Store_{OC} = \rho_b \times \frac{OC}{100} \times L$$

(Equation 3.1)

where ρ_b is the sediment dry bulk density (g cm⁻³); OC is the mean organic carbon content of the dry sediment (%) for the length of core being considered; and L is the length of core over which the $Store_{OC}$ is being calculated (cm). The units of OC content, obtained as g OC cm⁻², were then standardised to kg OC m⁻². After values were obtained, OC stores were normalised to the lowest stores recorded among the meadows and within each respective depositional environment category (see below) to obtain relative values of lowest to highest stores.

3.2.5 Characterising the depositional environment among *P. australis* meadows

Initially, the nine meadows were classified as *Estuarine*, *Sheltered* or *Exposed* sites. *Sheltered* and *Exposed* were classified according to the exposure of those sites based on the Relative Exposure Index (REI; after Garcon et al. 2010, Fonseca and Bell 1998). To allow comparisons, the REI was also calculated for the *Estuarine* sites.

To verify the exposure categorisations, a further discrimination was based on sediment granulometry (i.e. grain sizes) as a proxy for hydrodynamic sorting (Molinaroli et al. 2009, Bianchi et al. 2007, Bergamaschi et al. 1997). While there may be interactions between sediment grain size, hydrodynamic conditions and the seagrass canopy (e.g. Herkül and Kotta 2009, Madsen et al. 2001), any confounding effects were minimised by sampling cores in growth patches with similar canopy heights and shoot densities.

Relative exposure index

Relative exposure index was calculated based on long-term wind data and effective fetch length of each site. Data on annual wind velocity (in m s^{-1}) and directional percentage frequency (P_i) of the wind direction (in %) occurring from the i th compass direction was taken from the Bureau of Meteorology, Australia. Effective fetch of the seagrass meadows (i.e. distance between each site relative to the nearest wave-blocking obstacle along a given compass direction) was calculated as:

$$F_i = \sum_{n=1}^5 (y_n \times \cos x_i)$$

(Equation 3.2)

where y_n is the length of the radiating lines as direct fetch in the N, E, S, W, and perpendicular directions (i.e. $n=5$) measured through Google Earth distance ruler, and x_i is the angle of departure from the i th compass heading ($i=8$, i.e. from N, NE, E, to W and SW in 45° increments).

The REI for each site was then calculated as:

$$\text{REI} = \sum_{i=1}^8 (V \times P_i \times F_i)$$

(Equation 3.3)

where i is the i th compass heading, V the mean wind velocity (m s^{-1}), P_i the wind direction frequency (%) and F_i the effective fetch (km).

Sediment grain size analyses

Sediment grain size was determined on dried bulk samples from one core of each meadow, which was assumed to be broadly representative of the three cores from the same site.

Between 5 and 13 samples were analysed per core depending on the core length, and selected at every 10th cm along the core. About 1-2 g of the sediment samples were treated by hydrogen peroxide to eliminate all OM followed by an ultrasonic agitation (1 min) with sodium polyphosphate solution as a dispersing agent. Treated sediment samples were then run through a Beckman-Coulter LS 230 particle analyser at the Laboratori de Sedimentologia (Universitat Autònoma de Barcelona). Results for each sample were provided as the proportion of sediment fractions across six grain size classes (<4 µm, 4-63 µm, 63-125 µm, 125-250 µm, 250-500 µm, and 500- 1000 µm fractions), but only three fractions (i.e. 63 µm, 63-125 µm and 250-500 µm fractions) were used for subsequent analysis (see below).

3.2.6 Statistical analysis

The sediment grain size distribution data for the nine meadows was analysed using principle component analysis (PCA, Primer ver. 6). An initial PCA indicated that three sediment grain size categories (i.e. <63 µm, 63-125 µm and 250-500 µm fractions) explained 97.8% of the total variability of PC1 and PC2 (66.4% and 31.4% for, respectively). Therefore, this PCA was conducted using only these three grain size classes. The clustering of meadows according to sediment grain size was used to categorise the sites as *More Sheltered* (greater proportion of <63 µm sediments), *Less Sheltered* (larger proportion of 63-125 µm sediments) and *Exposed* (larger proportion of coarse-grained sediments, 250-500 µm).

The groupings of sites were then compared with the REI classification to check if the two discrimination methods yielded comparable classifications. After categorising the sites according to similar depositional environments, PERMANOVA (Primer ver. 6) was used to test for statistically significant differences in the mean OC stores among *P. australis* meadows in each depositional environment (two factor: Depositional Environment, and Site nested in Depositional Environment). PERMANOVA pairwise tests were performed to test for statistical differences in OC stores among sites within each nested category.

3.3 Results

3.3.1 Categorisation of seagrass meadows based on depositional environment

Ranking of the nine meadows showed that the three estuarine sites (WInlet, OHarb and BBay) had the lowest REI (all <8). For the oceanic sites, PRHarb (REI = 9) had an REI value only ~1 unit higher than the highest estuarine site of OHarb (REI = 8). FBay and PPoint had the next highest REI values (48 and 58, respectively) while MMia-FIsla, LBank and FIsla had the highest REI values (88, 127 and 155, respectively; Table 3.1). While the REI has been used to differentiate sites in other studies (e.g. Garcon et al. 2010, Fonseca and Bell 1998) there is no clear guidance on the REI value that corresponds to either *Sheltered* or *Exposed* categories. For this study, REI values < 10 was considered as *Sheltered* (WInlet, OHarb and BBay and PRHarb) while > 10 as *Exposed* (MMia-FIsla, FIsla, FBay, PPoint, and LBank) since the value of REI at this interval provided a clear separation limit among the sites.

At a broad level, there was reasonable consistency in the ranking of sites according to REI and grain size distribution-based PCA (Table 3.1 and Figure 3.2). Eigenvector outputs showed that the samples distributed along the positive axis of PC1 according to grain size fraction of 63-125 μm (PRHarb, FBay and BBay) and along the negative axis of PC1 based on the 250-500 μm fraction (PPoint and LBank). Principal Component 2 was strongly correlated with the proportion of sediment in the <63 μm size class and four sites separated out along this axis (WInlet, OHarb, MMia-FIsla and FIsla). Thus, OHarb, WInlet, grouped as the most sheltered sites (i.e. highest fraction of small grain sizes), while BBay and PRHarb as less sheltered. Peron Point and LBank grouped with the exposed sites (characterised by

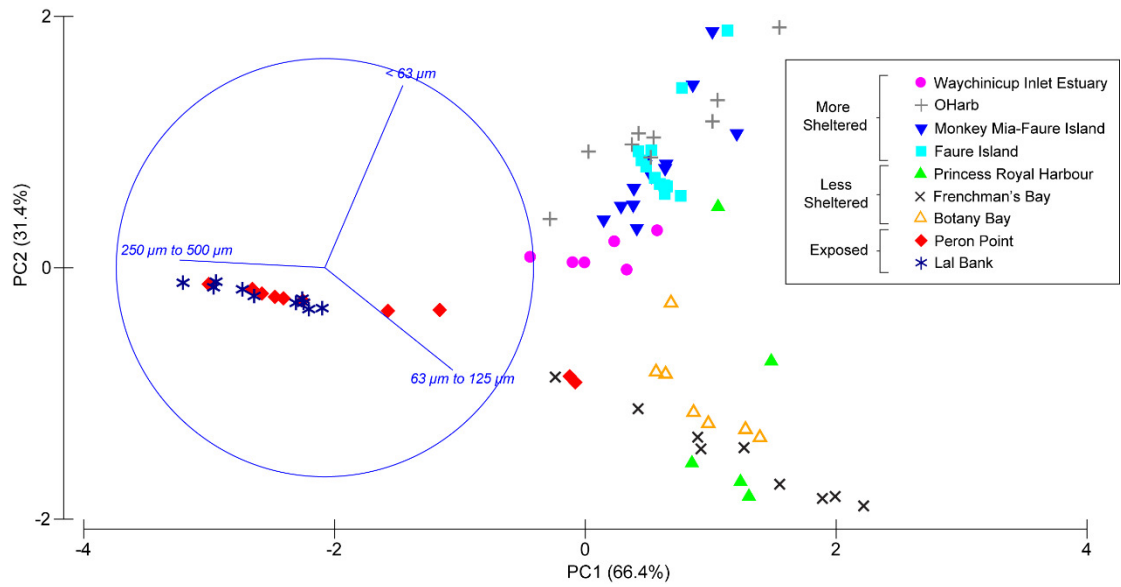


Figure 3.2: Principal component analysis of the nine studied meadows based on granulometry and classification of each meadow into each respective depositional environment category.

greater proportion of large size classes). The remaining sites showed different rankings according to the REI and grain size approaches. The Shark Bay sites (MMia-FIsla and FIsla) were classified as exposed using REI but had grain size distributions that clustered them with the most sheltered sites. FBay was grouped with the *Less Sheltered* oceanic sites in the grain size PCA, rather than with the *Exposed* sites in the REI analysis.

The use of REI is a relatively simple approach to hydrodynamic classification of sites and does not take into account currents, tides and other factors that may influence hydrodynamics. In contrast, grain size distributions reflect the overall depositional nature of a site (de Boer 2007). Since the two approaches were broadly in agreement, the grain size classification was used to *a posteriori* classify the sites as ‘*More Sheltered*’ (i.e. sites with a higher proportion of grain size <63 μm : OHarb, Winlet, MMia-FIsla, FIsla), ‘*Less Sheltered*’ (grain size 63-125 μm : FBay, PRHarb, BBay) and ‘*Exposed*’ (PPoint, LBank) sites. As such, these *a posteriori* categorisations, which led to an unbalanced design for the number of sites within each category, were used to compare the OC stores and characteristics of the *P. australis* meadows. For consistency, the terms *More Sheltered*, *Less Sheltered* and *Exposed*, are used hereafter to indicate relative increases in the hydrodynamic energy of the sites. When the term ‘*Sheltered*’ is used, it denotes a grouping of the *More Sheltered* and *Less Sheltered* sites as a single category (i.e. no specification of the site’s hydrodynamic energy within these two sub-categories).

3.3.2 Organic matter and organic carbon content across stratigraphy

Generally, the OM content of the sediments declined with increasing depth and was typically between 2 and 4% higher in the top 20 cm than in the rest of the core, though the trend was more extreme for PRHarb, which had 10-22% OM content in the upper layers (Figure 3.3). The %OM of the deeper core layers (>20cm depth) was generally highest in the *More Sheltered* sites and lowest in the *Exposed* sites. The *Exposed* sites (PPoint and LBank) had 2-

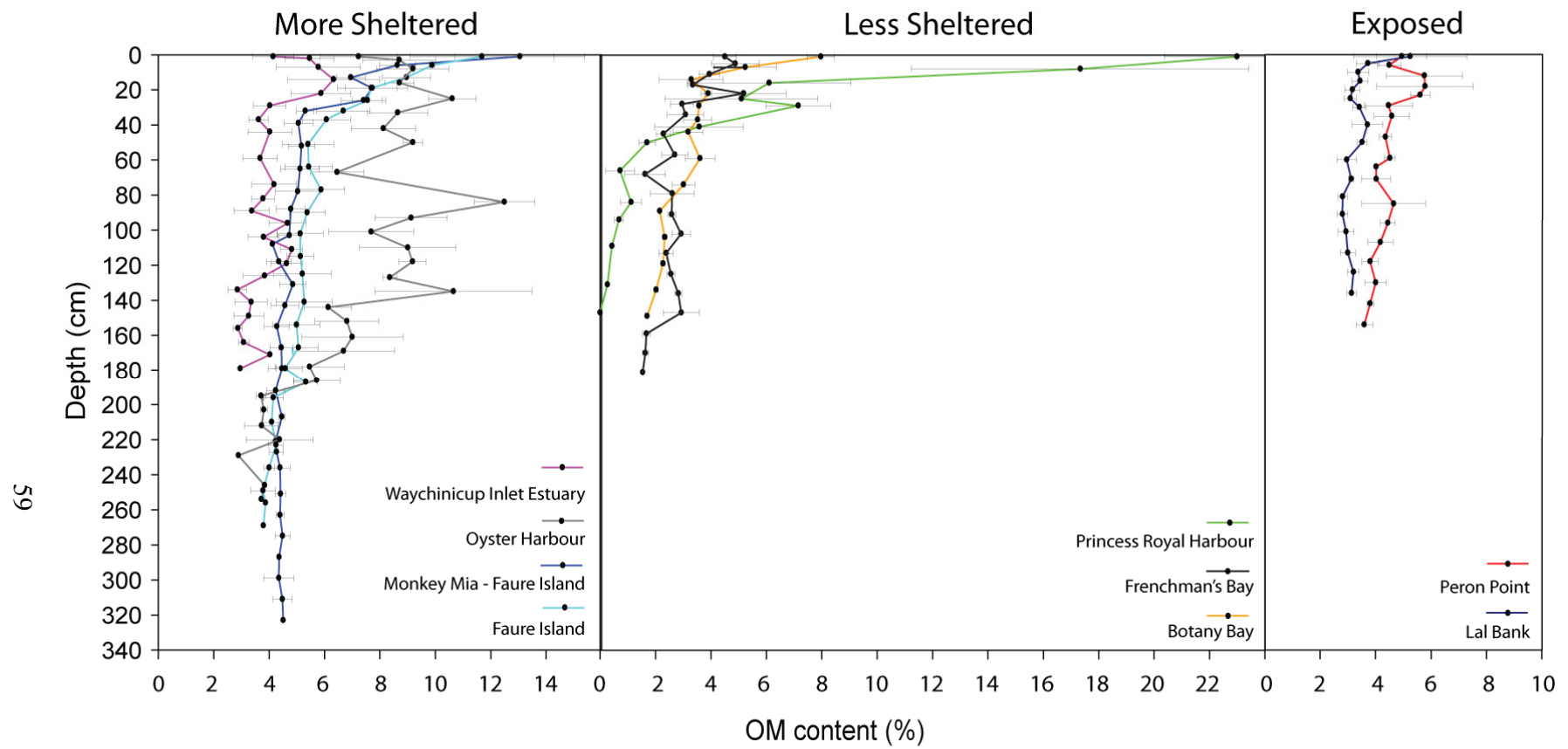


Figure 3.3. Organic matter content across stratigraphy (mean \pm SE) from the studied meadows.

4% OM content at depths greater than 20 cm (Figure 3.3). Apart from OHarb, the *More Sheltered* sites had 2-6% OM across the deeper layers (in OHarb this trend was only observed at depths greater than 160 cm). The *Less Sheltered* sites had highly variable OM content at deeper layers with FBay and BBay approaching the low levels of the *Exposed* sites while PRHarb, which had the highest OM content in surface sediments, showed a large decline in OM content with depth and generally had less than 2% OM in the deeper layers.

Organic carbon content showed similar vertical variation as OM, with high values in the surface layers declining with depth (Figure 3.4). The differences in OC content among exposure categories became most apparent at depths below 40 cm. The *Less Sheltered* sites had intermediate stratigraphic OC content, with two sites (FBay and BBay) having OC content (0.5-1% OC, 40 cm to 140 cm thickness; Figure 3.4) similar to the *More Sheltered* sites while PRHarb (having <0.5% OC) was more similar to the *Exposed* sites (<0.5% OC; Figure 3.4). The highest mean OC content (in 140 cm sediment thickness; Table 3.2) was in the *More Sheltered* sites (WInlet and OHarb; $2.18 \pm 0.13\%$ and $1.20 \pm 0.1\%$ OC, respectively), though the other two *More Sheltered* sites (MMia-FIsla and FIsla) had only a mean of 0.68-0.76% OC and were similar to that of the *Less Sheltered* sites (0.71-1.77% OC; Table 3.2). The *Exposed* sites (PPoint and LBank) had the lowest mean OC content (0.16-0.38% OC).

3.3.3 $\delta^{13}\text{C}$ values across stratigraphy

Generally, *Exposed* sites had lower $\delta^{13}\text{C}$ values compared to *More* and *Less Sheltered* sites (Figure 3.5). For the top 40 cm of sediment (i.e. more recently accumulated), the *More Sheltered* sites had $\delta^{13}\text{C}$ values that ranged between -14.0‰ to -9.0‰, while the *Exposed* sites ranged from -11‰ to -20‰. The *Less Sheltered* sites showed variation in the $\delta^{13}\text{C}$ values, with two sites (i.e. FBay and PRHarb) having values similar to the *More Sheltered* sites, and BBay being more similar to the *Exposed* sites. Botany Bay had the most apparent difference in $\delta^{13}\text{C}$ trends compared to all other sites. Values increased between the surface to

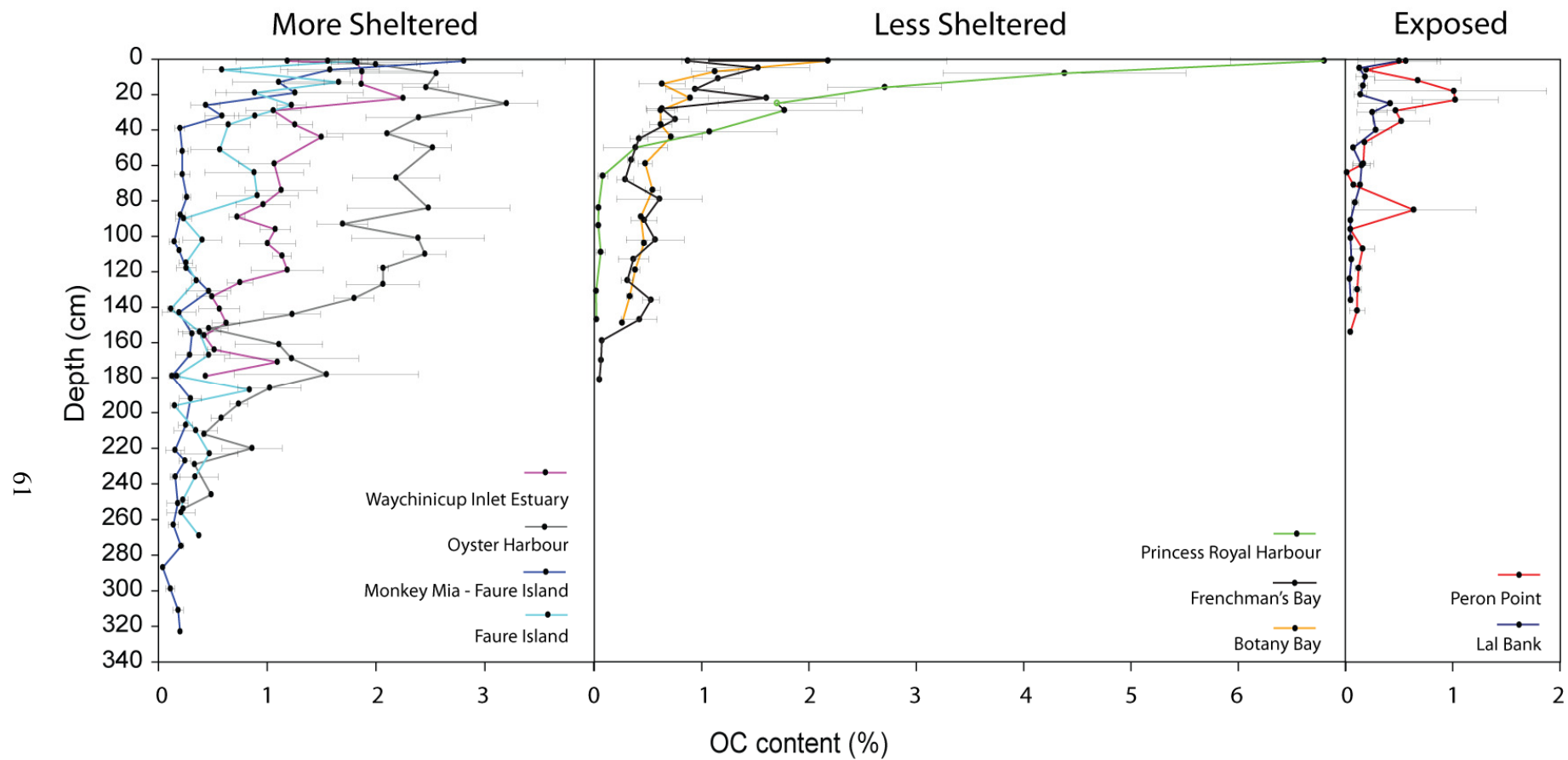


Figure 3.4. Organic carbon content across stratigraphy (mean \pm SE) from the studied meadows

Table 3.2. Organic matter characteristics in sampled cores within top 140 cm sediment thickness. Values are mean \pm SE.

Site category	Meadow site	Sediment dry bulk density (g DW cm ⁻³)	OM (%)	OC (%)
More sheltered	Waychinicup Inlet Estuary	1.18 \pm 0.03	4.34 \pm 0.14	1.20 \pm 0.09
	Oyster Harbour	0.6 \pm 0.03	8.76 \pm 0.51	2.18 \pm 0.13
	Monkey Mia - Faure Island	0.97 \pm 0.04	6.22 \pm 0.41	0.68 \pm 0.05
	Faure Island	1.04 \pm 0.06	6.75 \pm 0.6	0.76 \pm 0.15
Less sheltered	Princess Royal Harbour	1.10 \pm 0.05	6.43 \pm 1.39	1.77 \pm 0.40
	Frenchman's Bay	1.27 \pm 0.01	3.15 \pm 0.24	0.71 \pm 0.08
	Botany Bay	1.28 \pm 0.07	3.88 \pm 0.54	0.72 \pm 0.10
Exposed	Peron Point	1.38 \pm 0.06	4.60 \pm 0.34	0.38 \pm 0.19
	Lal Bank	1.32 \pm 0.03	3.34 \pm 0.19	0.16 \pm 0.00

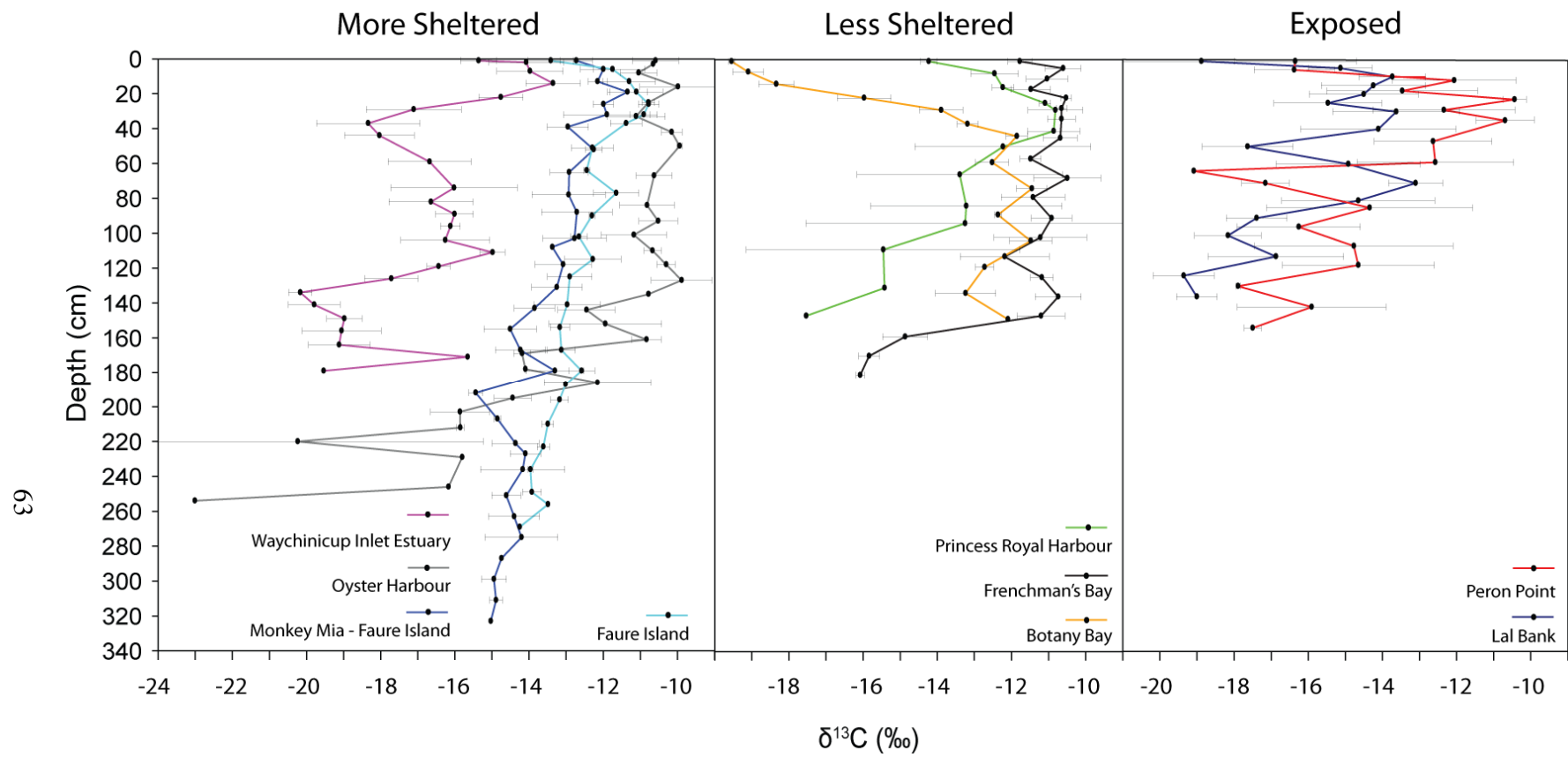


Figure 3.5 Stable carbon isotope values carbon content across stratigraphy (mean \pm SE) from the studied meadows.

the 40 cm depth (from -20.0‰ to -14.0‰). At depths greater than 40 cm at this meadow, values were at a constant range of -13.0‰ to -11.0‰. For the other sites, $\delta^{13}\text{C}$ values generally decreased with increasing depth (Figure 3.5). Oyster Harbour showed the largest range in $\delta^{13}\text{C}$ values between the surface and the distal core end (-9.89‰ to -23.01‰, respectively).

The results of $\delta^{13}\text{C}$ values and OC content across stratigraphy presented as a bi-plot showed further relationships in the organic characteristics among sites based on the depositional environment categories (Figure 3.6). There were discernible trends obtained from the plots; low OC content is generally associated with lower $\delta^{13}\text{C}$ values but the predictor varied from strong to weak relationships. There were weak (MMia-Fisla and FIsla, $R^2 < 0.29$) to moderate (Winlet and OHarb, $R^2 = 0.42$) relationships for the *More Sheltered* meadows but strong (FBay: $R^2 = 0.71$) to weak (BBay and PRHarb: $R^2 < 0.29$) for the *Less Sheltered* meadows. Both sites in the *Exposed* category had moderate ($R^2 = 0.43$ - 0.55) relationships between OC content and $\delta^{13}\text{C}$ values. Data points generally spread in a wider range for *More Sheltered* meadows (0 to 4.0% OC and -8‰ to -25‰), intermediate for the *Less Sheltered* meadows (0 to 4.5% OC and -8‰ to -20‰, discounting data points showing very high OC content, which may be a result of eutrophication for PRHarb), and had the smallest spread for the *Exposed* sites (0 to ~2% OC and -9‰ to -25‰).

3.3.4 Organic carbon stores

The *More Sheltered* sites had 1.3 to 3.0-times higher OC stores across all three stratigraphic thicknesses (30 cm, 100 cm and 140 cm) than the *Less Sheltered* and *Exposed* sites (Table 3.3 and Figure 3.7). Though there was a weak correlation between the variables of OC stores and sediment proportion $< 63\ \mu\text{m}$ ($R^2 = 0.18$, $p > 0.05$; Figure 3.8), the OC stores in 140 cm sediment depth decreased with decreasing proportion of sediment grain sizes ($< 63\ \mu\text{m}$ fraction). This trend is generally consistent when categorising the study sites with less proportion of fine sediments while concurrently being more exposed. When the stores were

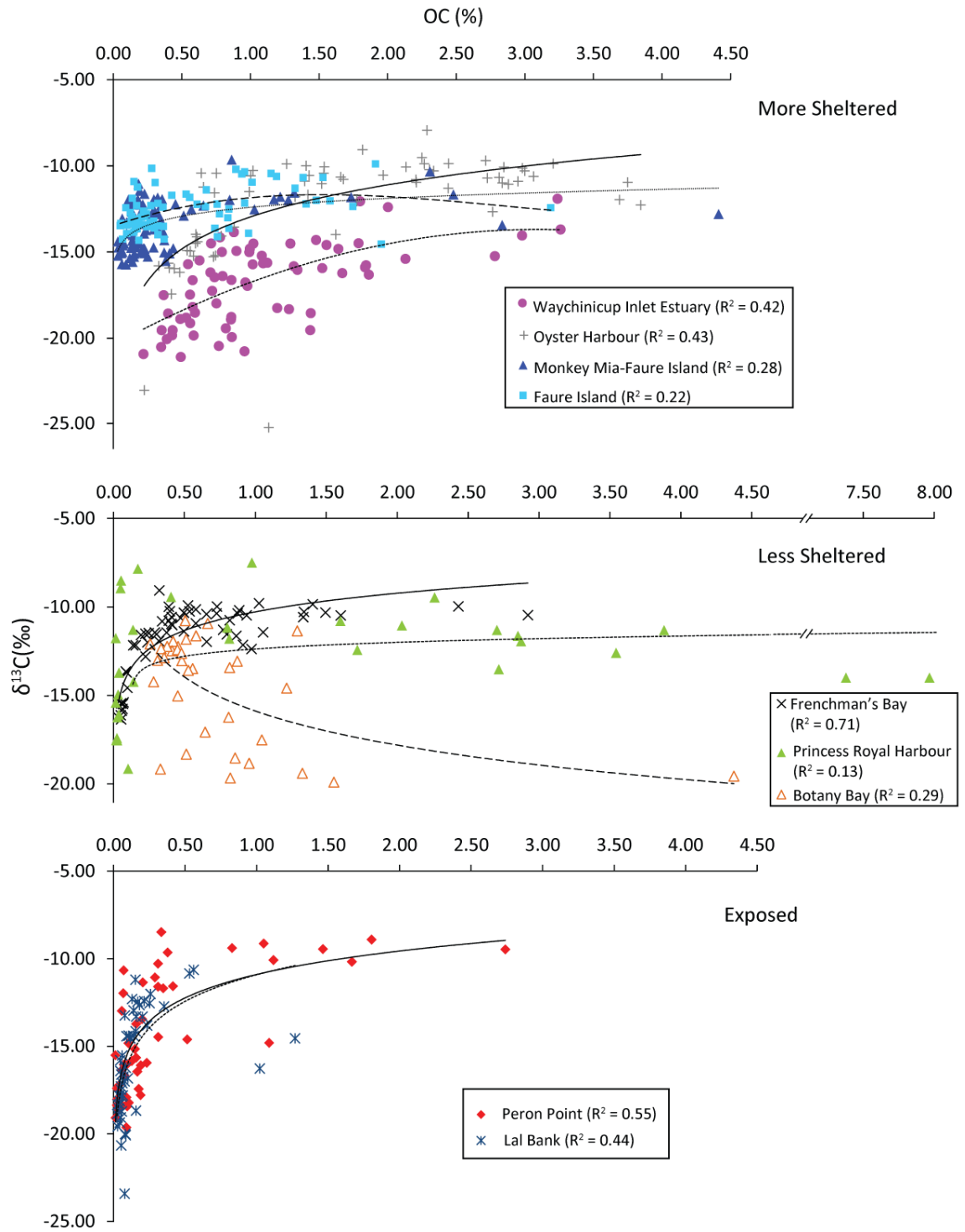


Figure 3.6 Bi-plot of organic carbon content against $\delta^{13}\text{C}$ for the studied meadows.

Table 3.3: Carbon stores in the top 140 cm, 100 cm and 30 cm of sediments at the studied seagrass meadows. Values in parentheses are normalised stores, which refer to the stores at a given site normalised to the lowest stores recorded at any of the sites and within each respective depositional environment category.

Site category	Meadow	OC stores in top 140 cm		OC stores in top 100 cm		OC stores in top 30 cm	
		Mean (\pm SE) OC stores (kg OC m ⁻²)	Normalised stores	Mean (\pm SE) OC stores (kg OC m ⁻²)	Normalised stores	Mean (\pm SE) OC stores (kg OC m ⁻²)	Normalised stores
More sheltered	Waychinicup Inlet Estuary	13.51 \pm 0.53	6.0	10.77 \pm 0.28	5.3	3.52 \pm 0.95	4.2
	Oyster Harbour	10.90 \pm 0.21	4.9	8.07 \pm 0.05	4.0	2.16 \pm 0.25	2.6
	Monkey Mia - Faure Island	4.57 \pm 0.16	2.0	3.42 \pm 0.43	1.7	2.11 \pm 0.47	2.5
	Faure Island	6.80 \pm 1.17	3.0	6.27 \pm 1.37	3.1	2.30 \pm 0.16	2.7
	Mean OC stores	8.94	(3.0)	7.13	(2.4)	2.52	(1.9)
Less sheltered	Princess Royal Harbour	4.31 \pm 1.11	1.9	4.20 \pm 1.11	2.1	2.63 \pm 0.60	3.1
	Frenchman's Bay	8.00 \pm 0.51	3.6	6.96 \pm 1.01	3.4	3.12 \pm 0.56	3.7
	Botany Bay	6.98 \pm 0.38	3.1	5.61 \pm 0.29	2.8	1.85 \pm 0.31	2.2
	Mean OC stores	6.43	(2.1)	5.59	(1.9)	2.53	(1.9)
Exposed	Peron Point	3.77 \pm 0.85	1.7	3.89 \pm 0.48	1.9	1.88 \pm 0.67	2.2
	Lal Bank	2.24 \pm 0.31	NA (relative lowest value)	2.03 \pm 0.31	NA (relative lowest value)	0.84 \pm 0.04	NA (relative lowest value)
	Mean OC stores	3.01	(NA: relative lowest value)	2.95	(NA: relative lowest value)	1.36	(NA: relative lowest value)
Mean (\pm SE) stores within <i>P. australis</i> meadows (kg OC m ⁻²)		6.79 \pm 0.69		5.65 \pm 0.52		2.27 \pm 0.20	

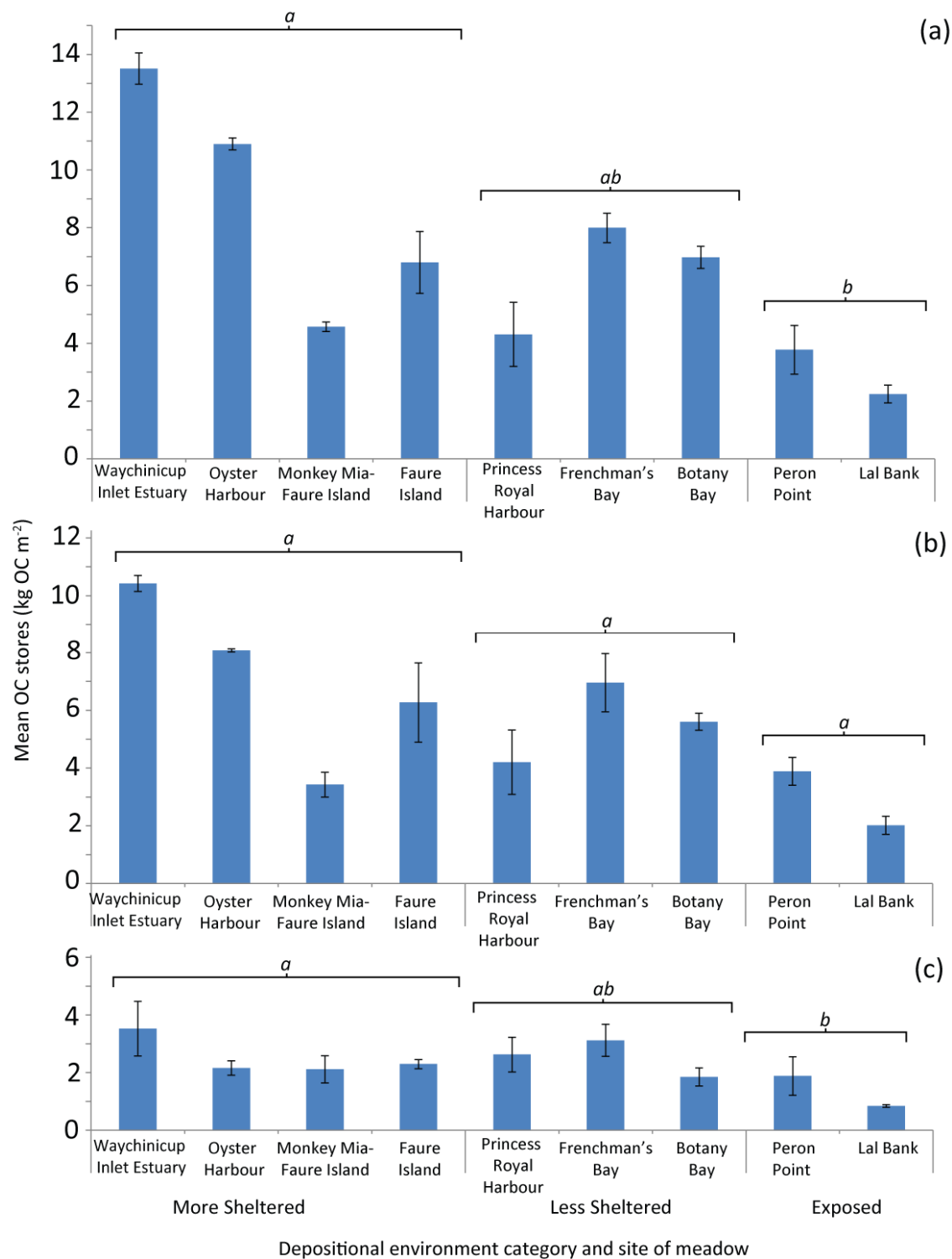


Figure 3.7 Organic carbon stores (mean \pm SE) in the nine *Posidonia australis* meadows based on cumulative stratigraphic accumulation within sediment thickness of: (a) 140 cm; (b) 100 cm; and (c): 30 cm. Similar letter above bars indicate no statistical differences.

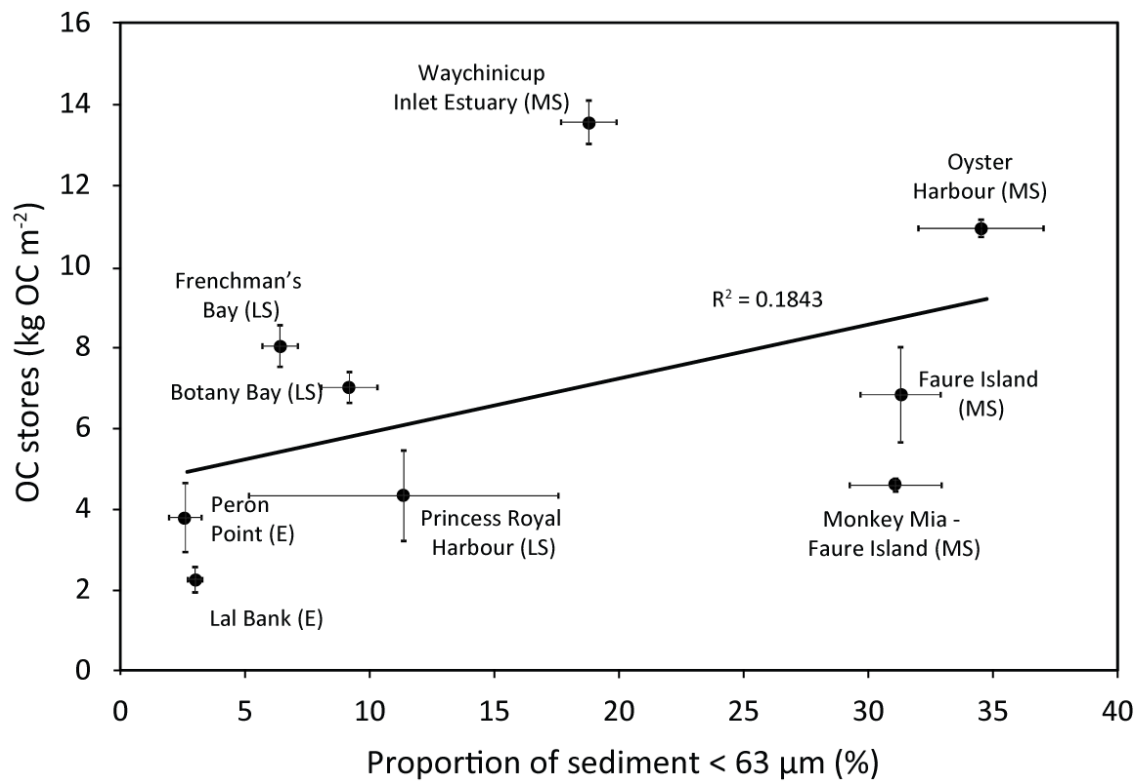


Figure 3.8. Scatter-plot relationship between OC stores of all nine meadows and proportion of sediment with grain size less than 63 μm. Letters in parentheses refer to the depositional environment categories: MS – More Sheltered; LS – Less Sheltered; and E – Exposed.

Table 3.4 Summary results for nested PERMANOVA for differences in mean OC stores among *P. australis* meadows (site) in each depositional environment (dep envt).

Sediment thickness	Source of variation	df	Sum of Squares	Mean Square	F	P
140 cm	dep envt	2	7,891.8	3,945.9	3.37	0.066
	site (dep envt)	6	7,027.8	1,171.3	7.07	0.002
100 cm	dep envt	2	5,237.3	2,618.6	2.28	0.16
	site (dep envt)	6	6,881.3	1,146.9	6.62	0.001
30 cm	dep envt	2	4,420.6	2,210.3	4.25	0.036
	site (dep envt)	6	3,117	519.5	1.61	0.182

Table 3.5 Summary results for PERMANOVA pairwise test for differences in mean OC stores in the *P. australis* meadows among depositional environments

Sediment thickness	Groups	t	P
140 cm	More sheltered x Less sheltered	0.79	0.50
	More sheltered x Exposed	2.36	0.049
	Less sheltered x Exposed	2.28	0.091
100 cm	More sheltered x Less sheltered	0.6	0.64
	More sheltered x Exposed	1.83	0.12
	Less sheltered x Exposed	1.91	0.13
30 cm	More sheltered x Less sheltered	0.13	0.98
	More sheltered x Exposed	2.62	0.039
	Less sheltered x Exposed	2.14	0.1

integrated over the top 140 cm, *More Sheltered* sites were significantly (3 fold) higher than the *Exposed* sites ($p < 0.05$), though the *Less Sheltered* sites did not differ to either of the other two categories (Tables 3.4, 3.5 and Figure 3.6). The same trends were observed when stores were integrated over the top 30 cm; OC stores in *More Sheltered* sites were significantly higher than in *Exposed* sites, but neither differed from the *Less Sheltered* sites. When stores were integrated over the top 100cm, there were no significant differences among any categories of exposure.

Among individual meadows, there was a 6-fold difference between sites with the highest and the lowest OC stores in the top 140 cm of sediment (WInlet: 13.51 ± 0.54 kg OC m⁻², and LBank: 2.24 ± 0.31 kg OC m⁻²), decreasing to a 4-fold difference in the top 30 cm of sediment (Table 3.3). Although MMia-FIsla and FIsla were categorized as *More Sheltered* based on sediment grain size characteristics, OC stores (e.g. 4.57-6.80 kg OC m⁻²; 140 cm sediment depth) were more similar to those of the *Less Sheltered* sites (4.31-8.00 kg OC m⁻²; 140 cm depth).

3.4 Discussion

3.4.1 Organic carbon stores in *P. australis* meadows as influenced by the depositional environment

Across the nine *P. australis* meadows that were compared, the *More Sheltered* meadows had significantly higher OC stores compared to *Exposed* meadows. The *Less Sheltered* sites had intermediate OC stores, but with no significant differences in stores compared to the *More Sheltered* sites and the *Exposed* sites. These findings assumes that the categorisation of the meadows into levels of exposure is valid. The REI and grain size analysis produced generally consistent rankings of sites from least to most exposed, and this method categorised the depositional environment among the meadows. While there were some differences in the

rankings produced by the two classification approaches, this probably reflects intrinsic conditions unique to each meadow (i.e. meadow structure and their capacity to retain fine-grained sediment particles) that could result in the REI modelling being inconsistent to the categorisations based on granulometric distribution of the sediments. For example, it has been shown that tidal currents and water level can strongly influence the sediment grain sizes of marine environments (e.g. Green and Coco 2014, Malvarez et al. 2001, Madsen et al. 2001) and this could well be independent of the fetch at a site (which is the only factor considered in the REI). Sediment grain size may be a more valid indicator of the depositional environment of the studied meadows since the accumulation of the granulometric distribution is the net result of the ambient depositional setting of each site (Madsen et al. 2001, Viana et al. 1998), assuming a similar supply of particles is available to each site and that the meadow canopy characteristics are similar within sites.

The results are indicative of interactions between the depositional nature of a habitat and the role of the seagrass meadow (i.e. trapping allochthonously transported OM, and contributing seagrass-derived OM into the sedimentary OC pool) in driving the OC storage capacity of *P. australis* meadows. The mechanism that accounts for this link is likely complex and also may include other interactive effects beyond the direct effect of hydrodynamic regimes on sediment accumulation. These include factors such as the effects of hydrodynamics on meadow establishment, seagrass productivity, degradation/remineralisation processes acting on the OC after burial.

The lower OC stores found in *Exposed* sites can be explained by the negative effects of water motion affecting the ability of seagrasses to establish due to the stresses imposed on the plant by limiting photosynthesis (Fonseca and Kenworthy 1987) and also due to the scouring of meadow sediments that simultaneously uproots live plants (Short and Wyllie-Echeverria 1996). Water motion can similarly affect the ability of propagules to persist at a meadow leading to low rates of shoot density increments (Orth et al. 2006). Upon successfully forming meadows, water movement is known to affect seagrass productivity (Fonseca and Kenworthy

1987) and thus the OC supply for burial may further vary. At the same time, the seagrass canopy exerts a negative feedback on local hydrodynamics and can act to facilitate the capture and burial of organic matter (Agawin and Duarte 2002, Gacia et al. 1999). Post-burial, sediment oxygen exposure may affect the preservation of OC stores (Burdige 2007). The prevalence of coarse grained sediments in *Exposed* sites can enhance oxygenation into the interstitial sedimentary spaces (Dauwe et al. 2001), providing conditions favourable for OM degradability (Hedges and Keil 1995), compared to the finer grained sediments of sheltered sites, which typically have less exposure to oxygen (Bergamaschi et al. 1997). In this study, all sites had established *P. australis* meadows and so it is likely that the differences in sedimentary OC stores is driven by post-recruitment influences of hydrodynamics. These could relate to the productivity of the meadows and the density of the canopies, which affected the production and retention of organic matter. Though these parameters were not measured, some indications on the productivity and OC retention may be provided by the organic characteristic (i.e. carbon richness and $\delta^{13}\text{C}$ values; see below) of the sediments. Since the *Sheltered* meadows had a higher proportion of fine sediment particles and seagrass inputs into the sedimentary pools, it is also possible that reduced oxygen exposure and higher sediment accumulation rates favoured OM preservation at these meadows (Serrano et al. *in preparation*).

While there was a 3-fold difference in the OC stores in the top 140 cm of sediment among the most sheltered and the most exposed *P. australis* meadows studied, the differences were less strong with decreasing stratigraphic thicknesses (i.e. 140 cm to 30 cm). The differences among sediment depths observed in this study may reflect the influence of living seagrass material (i.e. below-ground biomass) in the upper layers of the sediment, while deeper layers are also reflecting historical variation in the meadows, including the productivity of the meadows and diagenetic processes. This finding highlights the importance of standardising the sediment thickness before making comparisons of OC stores among meadows. Other studies of OC stores in seagrass meadows usually estimated carbon content across the

stratigraphy within a standardised sediment depth (e.g. 25 cm in Lavery et al. 2013, and 100 cm in Fourqurean et al. 2012a, 2012b) to provide a broad understanding of the quantity of OC in sediments among a number of seagrass meadows. In 100 cm depth, the OC stores in this study (2.03-10.77 kg OC m⁻², equivalent to 2-108 Mg OC ha⁻¹), are below the median estimate in seagrass meadows globally (median 140 Mg OC ha⁻¹; range 9-628 Mg OC ha⁻¹; 100 cm depth; Fourqurean et al. 2012a). These global estimates were strongly influenced by OC stores in *P. oceanica* meadows in the Mediterranean Sea (Fourqurean et al. 2012a), and which have a high capacity to capture OC (17-75 kg OC m⁻³ and 6-175 g OC m⁻² yr⁻¹; adapted from Serrano et al. 2014, Serrano et al. 2012) compared to *P. australis* meadows (14.7 kg OC m⁻³ and 3.5 g OC m⁻² yr⁻¹; Chapter 2, this thesis).

The $\delta^{13}\text{C}$ values indicated that both *P. australis*-derived OM and non-seagrass-derived OM were buried in the sediments of all studied meadows, but are consistent with a model of increasing capture of seagrass-derived OM at *Sheltered* sites relative to *Exposed* sites. Seagrass-derived organic matter is enriched in ¹³C compared to algal- and terrestrial-derived OM (Kennedy et al. 2010). *Posidonia australis* plant matter has $\delta^{13}\text{C}$ values in the range of -6.3‰ to -9.9‰ (Hyndes and Lavery 2005; Hindell et al. 2004). At all meadows, the sedimentary $\delta^{13}\text{C}$ signatures showed lower values relative to the seagrass values, as has been reported for many seagrass sediments (Table 1.3; Kennedy et al. 2010), likely due to a contribution of OM from non-seagrass sources (i.e. seston-, algal-, or terrestrial-based) in the sediments (Kennedy et al. 2010) or fractionation of OC as a result of remineralisation (McNichol et al. 1991). While fractionation is theoretically possible, it had been shown that changes in carbon isotopic abundances due to diagenetic processes were negligible in sediments (Fogel and Cifuentes 1993, Hayes 1993). Thus, it is plausible that the lower $\delta^{13}\text{C}$ values of the sediments relative to *P. australis* values resulted from increasing contributions of non-seagrass material to the sedimentary OM pool.

The relationship between the two variables of sedimentary OC content and $\delta^{13}\text{C}$ values may be an indicator of seagrass-derived OM in the sediments (Figure 3.6). The trends are

reasonably consistent; there were stronger influences of seagrass derived OM (i.e. higher OC content and higher $\delta^{13}\text{C}$ values) in the *Sheltered* sites, but decreased influence of seagrass-derived OM (i.e. lower OC content and lower $\delta^{13}\text{C}$ values) in the *Exposed* sites. The absence of clear trends of OC content corresponding to $\delta^{13}\text{C}$ values indicates that other site-specific modifiers may have influenced the relative proportions of seagrass-derived and allochthonous OM in the sediments. Nonetheless, the trends increases confidence in categorising the depositional environment sites. Organic matter in the *More Sheltered* sites were likely composed of a wider variety of OM retained in a more depositional environment (varying both in OC content and $\delta^{13}\text{C}$ values) compared to relatively lower quantity and variety of OM in the *Exposed* sites (smaller range in OC content and $\delta^{13}\text{C}$ values).

Most of the *Sheltered* sites (with the exception of Waychinicup Inlet Estuary) had sediment $\delta^{13}\text{C}$ signatures within 2-3‰ of *P. australis* across a relatively large stratigraphic layer (more than 100 cm-thickness), indicating that *P. australis* detrital matter was a major contributor to the sediment OM pool. In contrast, *Exposed* sites had $\delta^{13}\text{C}$ values more than 2-3‰ from the *P. australis* value, and in some cases as much as 10‰ less (Figure 3.5), indicating a larger influence of seston- and algal-derived OM compared to *Sheltered* sites. The exception to this was the 20-40 cm sediment layer at both *Exposed* sites, which showed $\delta^{13}\text{C}$ values similar to *P. australis* tissue and which probably reflects the predominance of live below-ground organs occurring at these depths; core sampling typically revealed live rhizomes at about 30-50 cm beneath the sediment surface (*personal observation*). The apparent link between the amount of seagrass-derived OM and the exposure of the meadows suggests that *in situ* seagrass production of OM tends to remain in *Sheltered* sites while mixing with non-seagrass derived OM is a feature of the studied *Exposed* sites.

3.4.2 Site-specific variations in OC stores: deviations from clear trends among *P. australis* meadows

While there was a clear difference in OC stores between the *More Sheltered* and the *Exposed* sites, the *More Sheltered* and the *Less Sheltered* categories contained meadows that were both estuarine and oceanic, and there were no clear differences in the OC stores of estuarine and non-estuarine sites or, for that matter, of *More Sheltered* and *Less Sheltered* sites (Figure 3.7). These results indicate that site-specific aspects of those meadows, other than seagrass species and broad depositional classification, potentially affected the OC stores. This is despite the standardisation of the sampled cores to similar water depths of 1.5-2 m among all sites. The standardisation was made since there it was shown that productivity values of seagrasses relates to the depth of the water column and hence the potential for OC storage in its sediments will differ according to water depths of the meadow (Serrano et al. 2014). Of the nine meadows, four meadows showed significantly different trends in OC stores to the others in their exposure category and may be useful case studies to consider the site-specific factors that can influence OC stores. These meadows were the two Shark bay sites (Monkey Mia-Faure Island and Faure Island), Princess Royal Harbour and Waychinicup Inlet Estuary; these instances are considered below.

Monkey Mia-Faure Island and Faure Island

Based on sediment grain size, Monkey Mia-Faure Island and Faure Island meadows were categorized as *More Sheltered*. However, these two meadows had OC stores more similar to the *Less Sheltered* sites than the other two *More Sheltered* sites (i.e. Waychinicup Inlet Estuary and Oyster Harbour). Net Primary production rates are known to influence sedimentary OC stores (Cebrian and Duarte 1995). The low OC stores of the sites may reflect the results of factors other than direct hydrodynamic effects upon the productivity of *P. australis* meadows in Shark Bay. *Posidonia australis* meadows in Shark Bay have reported productivity of 967 g dry weight m⁻² yr⁻¹ (Walker and McComb 1988) compared to other *P.*

australis meadows (e.g. Botany Bay and Cockburn Sound), which are as high as 1153-2331 g dry weight m⁻² yr⁻¹ (Paling and McComb 2000, West and Larkum 1979). Since these were the most northerly of the nine meadows studied, there may be a latitudinal effect resulting in differences in productivity. Water temperatures in Shark Bay are in the range of 16-26°C (Davies 1970), above the reported optimum temperatures for production (19-23°C; Lee et al. 2007). In contrast, water temperatures in the other meadows (12-24°C: Cambridge and Kendrick 2009, Lisbjerg and Petersen 2000) are more similar to the reported optimum temperature range. The productivity of Shark Bay *P. australis* meadows may also be phosphorus-limited (Fourqurean et al. 2012b). These hypotheses in regards to the probable lower productivity of the the Shark Bay sites is indicated by the smaller spread of OC content and $\delta^{13}\text{C}$ values (0-2.5% OC and -9‰ to -16‰) compared to the carbon-rich OM (0-4% OC) in the other *More Sheltered* sites though more studies are needed to confirm this possibility.

Princess Royal Harbour

The Princess Royal Harbour site was anomalous in that the meadow had comparatively high OC content (up to 7%) in the top 40 cm of sediment relative to the other *P. australis* meadows. The exponential decline in OC with depth in these cores is indicative of recent OM accumulation, if depth is taken as a surrogate of time. This is consistent with eutrophication in the lagoon during the 1980s (Bastyan 1986). Both Oyster Harbour and Princess Royal Harbour underwent eutrophication during this period. However, Princess Royal Harbour was more heavily impacted, with an estimated accumulation of 11650 tonnes dry weight of macroalgae in the lagoon compared to 1200 tonnes in Oyster Harbour (Hillman et al. 1990). This may explain the high OM content observed in the top sediments of Princess Royal Harbour compared to Oyster Harbour, and confirmed by earlier studies in the area (Talbot 1990). The $\delta^{13}\text{C}$ values of sediments in Princess Royal Harbour supports this eutrophication hypothesis; in the top 20 cm, the values were lower relative to seagrass signatures, but consistent with the inputs of algal contribution within this depth reported by Talbot (1990), while in deeper layers (20-60 cm) they became increasingly similar to *P. australis* tissue

values. These observations are consistent with a previously seagrass-dominated system being exposed to high inputs of algal matter following eutrophication that accumulated in the sediments above the seagrass rhizome layer (e.g. McGlathery et al. 2007).

Waychinicup Inlet Estuary

The estimated OC stores in Waychinicup Inlet Estuary were the highest among the nine *P. australis* meadows. However, the $\delta^{13}\text{C}$ signatures across the stratigraphy indicated a dominance of non-seagrass OM. Despite being categorised as a *More Sheltered* site, which would be expected to facilitate the accumulation of *P. australis* detritus, the $\delta^{13}\text{C}$ signatures were up to 8‰ lower than *P. australis* tissue values. They were also highly variable through the stratigraphic layers. At the other *More Sheltered* sites, the $\delta^{13}\text{C}$ values either stayed within a constant range, or steadily increased/decreased by 0-3‰ with increasing depth. The large variation in $\delta^{13}\text{C}$ signatures within a relatively small stratigraphic thickness at Waychinicup Inlet may be indicative of a more dynamic sedimentary environment with flux events resulting in allochthonous sediment deposition. Other estuaries show similar oscillating shifts in $\delta^{13}\text{C}$ signatures coinciding with events that modified the existing hydrology, due to flood events or changes in vegetation structure (e.g. Masiello and Druffel 2001, Byrne et al. 2001, DeLaune 1986). In those estuaries, sedimentary $\delta^{13}\text{C}$ values vary more than 4‰ between specific stratigraphic layers identified as pre- and post-event periods (Masiello and Druffel 2001, Byrne et al. 2001, DeLaune 1986). The hydrodynamic and historical depositional processes that occurred in Waychinicup Inlet Estuary are not currently known. However, there is radiocarbon dating evidence (see Chapter 4 for further details) that showed the stratigraphy of the seabed has been heavily modified historically through erosion of sediment, possibly leading to major scouring of the sediment in the estuary. Such scouring could well contribute to highly variable isotopic signatures and OM stores. Furthermore, Waychinicup Inlet is a small system (~ 1 km in length) and while relatively sheltered, it does experience a high amount of exchange with the ocean due to its geomorphological orientation, which contrasts with the other *Estuarine* and *More Sheltered* sites in the study (Brearley 2005). This

ocean exchange would facilitate the input of sestonic and algal production to the meadows where it is likely to be trapped and, together with inputs of terrestrial OM from the catchment, could account for the lower $\delta^{13}\text{C}$ values of the sedimentary OC.

3.4.3 *Broader context and implications of habitat variability in P. australis sedimentary OC stores*

While some seagrass species exist in only one type of habitat, most span across *Estuarine* and *Sheltered* conditions (Carruthers et al. 2007). *Posidonia* is relatively unique, occurring in all three habitats. It is reasonable to ask, therefore, whether the findings on variation in OC stores of *P. australis* meadows provide us with confidence in predicting the OC stores of other seagrass species based on the depositional environment in which they occur. The results for *P. australis* clearly indicate that at the more extreme ends of the hydrodynamic exposure continuum, there are significant differences in the OC stores of seagrass sediments. These differences are comparable to the inter-species differences in OC stores that have been reported (Lavery et al 2013; Fourqurean et al. 2012a). Thus, there is clear evidence that environmental factors, in addition to the depositional environment, play a significant role in determining the OC stores of seagrass meadows and, in very broad terms, we can expect greater stores in sheltered areas than exposed areas, particularly estuaries. One implication in regards to this habitat variability is that any disturbance of *Sheltered* meadows will potentially release higher amount of CO_2 than in *Exposed* sites in view of the high OC stores contained in sediments of *Sheltered* meadows.

While hydrodynamic exposure may be a general indicator of OC stores, no two meadows are exactly the same, even if composed of the same species. Within-species variation in terms of productivity, oxygen exposure and the delivery of allochthonous organic matter, both terrestrial and marine, are among the factors, which might explain significant variation in the OC stores of meadows in relatively similar exposure conditions. In this regard, other species of seagrasses may show similar inter-habitat variability in sedimentary OC stores. Regional

or global estimates of seagrass sedimentary OC stores (Serrano et al. 2012, Nellemann et al. 2009) have previously been forced to rely on limited data for seagrasses without including variability in environmental influences or species composition. However, this study clearly indicates that those sorts of estimates should take into account both the habitat characteristics and species identity when predicting those stores. Further studies are thus needed to identify other influences on OC storage when attempting to obtain robust estimates of OC stores in seagrass meadows.

3.5 Conclusion

This study showed that there was significant variability in OC stores among the *P. australis* meadows, but it did not strictly conform to the initial hypothesis posed. Categorisations of depositional environments shifted away from the meadows being *Estuarine* or *Oceanic* sites to *More Sheltered*, *Less Sheltered* or *Exposed* sites. Organic carbon stores in the most sheltered meadows (i.e. the *More Sheltered* meadows category) were significantly higher than the most exposed meadows (i.e. *Exposed* category). There were less clear differences in the OC stores of *More Sheltered* and *Less Sheltered* meadows, likely due to other site-specific modifiers. The results showed that the depositional nature of the habitat, as a single variable, is not a perfect predictor of the seagrass sedimentary OC stores; those site-specific modifiers in the form of biological and biogeochemical factors may play a key role in driving variability in OC stores among seagrass meadows.

CHAPTER 4

THE ROLE OF SPECIES COMPOSITION ON ORGANIC CARBON STORES AND ACCUMULATION RATES IN SEAGRASS MEADOWS

Abstract

The organic carbon stores beneath seagrass meadows vary significantly among different species. This effect was investigated by comparing the stores in 18 sediment cores collected from *Posidonia australis* and *Halophila ovalis* meadows. Comparisons were based on stratigraphic- (OC stores over a set depth) and temporal-based (i.e. accumulation over a set period of time, and as accumulation rates) measures. Organic carbon stores were between 2-11-fold (*P. australis*: 10.81 ± 2.06 kg OC m⁻², *H. ovalis*: 5.17 ± 2.16 kg OC m⁻²; 150 cm depth) and 11-fold (*P. australis*: 10.87 ± 2.86 kg OC m⁻², *H. ovalis*: 0.97 ± 0.47 kg OC m⁻²; 2500 yr accumulation) between meadows of the two species. $\delta^{13}\text{C}$ signatures for *P. australis* and *H. ovalis* sediments were in the range of -9.89 to -23.01 ‰ and -16.64 to -21.41 ‰, respectively. The $\delta^{13}\text{C}$ signatures of organic matter in these sites indicated a range of potential sources: from seagrass-derived to allochthonous origins. While the OC stores were different between species, it was also apparent that site-specific factors also contributed to the variability. Thus, both the species and environmental factors need to be considered for robust predictions of OC storage in seagrass meadows.

4.1 Introduction

The concerns for human and environmental well-being generated by global change have intensified the research focus on carbon sequestration by seagrass ecosystems (Nellemann et al. 2009). Lavery et al. (2013) reported that organic carbon (OC) storage potential varies significantly among different types of seagrass habitats, where habitat was defined by the abiotic environment and seagrass species composition. In the previous chapter, the role of one abiotic environmental factor (the depositional nature of the meadow) on OC stores was explored. Another study showed the variation of OC stores of seagrass meadows with water column depth (Serrano et al. 2014). However, despite this known variability and the presumed importance of species identity, there is a dearth of studies that explicitly examine the contribution of species composition to variation of OC stores among meadows in similar abiotic environments. Almost all efforts to estimate regional or global stores of seagrass Blue Carbon require the scaling up of OC stores determined in individual meadows (Fourqurean et al. 2012a, Nellemann et al. 2009). One important assumption for the prediction in OC stores is that the estimates obtained for a species of seagrass at one site are characteristic of other sites dominated by that same species; that is, species identity is major determinant of the stores in seagrass habitat. In this chapter, the effect of species identity on organic carbon (OC) stores is explored.

High OC stores in *Posidonia oceanica* meadows have been reported (Mateo et al. 2006, Duarte et al. 2005, Mateo et al. 1997, Cebrian and Duarte 1995) and have led to the perspective that seagrass habitats do have high amounts of OC in their sediments (Nellemann et al. 2009). It has been suggested, however, that the high OC stores in meadows of this species is the exception rather than the norm (Lavery et al. 2013). Thus, other studies attempted to address the variability of OC stores among multiple seagrass habitats (e.g. Lavery et al. 2013, Rozaimi et al. 2013, Fourqurean et al. 2012a). Among mono-specific Australian seagrass meadows, 18-fold differences in OC stores was observed (Lavery et al. 2013). Fourqurean et al. (2012a) studied OC stores among 89 meadows, forming the first and

latest comprehensive account for OC stores on a meadow-wide scale. While this study provided estimates of average OC stores in seagrass meadows at the global level, there was no exploration of the effect of seagrass composition on OC stores (Fourqurean et al. 2012). Furthermore, the data summarised by Fourqurean et al. (2012) were dominated by mainly two species (*P. oceanica* and *Thalassia testudinum*), with considerably less data being available to them for other seagrass species.

There are 60-70 species of seagrasses globally, spanning 12 genera (den Hartog and Kuo 2006). These species are highly variable in terms of morphological forms, primary production rates and photosynthetic capacities (Lee et al. 2007, Short and Short 2000, Duarte and Chiscano 1999), resulting in differing above- and below-ground biomass, growth strategies, turn-over rates and their capacity to trap allochthonous OC (Agawin and Duarte 2002, Gacia et al. 1999, Duarte and Chiscano 1999). This is especially relevant to the quantity of sedimentary OC that they may store, since carbon sequestration by primary producers is strongly correlated to their productivity and growth strategies (De Deyn et al. 2008, Catovsky et al. 2002). Sedimentary OC derived from seagrass tissues mainly originate from below-ground organs (Chapter 2), but the quantity may vary among species; large-sized seagrass species tend to develop high below-ground biomass compared to smaller species (Duarte and Chiscano 1999). Furthermore, there is a large range of below-ground production among species ($0.01\text{-}10.5\text{ g DW m}^{-2}\text{ day}^{-1}$; Duarte and Chiscano 1999). The amount of recalcitrant tissue, which provides the bulk of OM available for burial (Mateo et al. 2006), can also vary among species. Further variation can arise from the relative contribution of seagrass-derived versus non-seagrass-derived OM in the sediments (Chapter 2, Kennedy et al. 2010).

The potential for OC sequestration by different seagrasses can be hypothesised from a functional-form model of seagrasses developed by Carruthers et al. (2007; see Figure 1.2). This model differentiated seagrasses based on morphological plasticity, biomass, rhizome persistence, and the depositional nature of the environments in which species occur. These same factors may influence the OC stores when comparing meadows. Studying species

located towards different ends of this continuum, and therefore with contrasting plant traits and habitat geomorphology, may present a generalisable picture of OC stores in seagrass sediments. Meadows of smaller, ephemeral seagrasses with low standing crop are likely to have less OC storage capacity compared to larger and more persisting seagrasses with higher biomass. In this respect, the seagrass genera *Halophila* and *Posidonia* are placed at opposite ends of the continuum. *Halophila* has a small biomass with less persistent rhizomes compared to *Posidonia*, which has the opposite traits, and that these two seagrass species may occur in sheltered, exposed or estuarine habitats (Carruthers et al. 2007).

In this study, estuarine seagrass habitats dominated by *Halophila ovalis* and *Posidonia australis* were studied to compare the OC stores per unit area (cumulative mass from stratigraphic accumulation), per unit of time (temporal accumulation), and OC accumulation rates, restricting differences in habitat type confounding the comparison. It was hypothesised that meadows of *Halophila* will have lower OC stores than *Posidonia*. The characteristics of sediment OC and stable isotope signatures were also explored to elucidate the relative contribution of seagrass-derived and non-seagrass derived OM to the sedimentary organic pool within each species and estuarine habitats.

4.2 Methods

Organic carbon stores were compared in three *Posidonia australis* and three *Halophila ovalis* meadows (Figure 4.1) within Australian estuaries to minimise any confounding effect of the depositional environment on OC stores (see Chapter 3). Three sediment cores were collected from each meadow during 2012 to 2013 at a water depth of 1-1.5 m. This small range was selected for all sites to minimise the effect of water depth on OC stores (after Serrano et al. 2014). Cores for *P. australis* meadows were sampled from Oyster Harbour (OHarb), Waychinicup Inlet Estuary (WInlet), Botany Bay (BBay) and cores for *H. ovalis* were collected from the Swan River Estuary (SRiver), Harvey Estuary (HEst) and Leschenault



Figure 4.1. Location of study sites. Three cores were sampled from each site, represented by the yellow dots as the coring points. Sites with one dot (i.e. Waychincup Inlet Estuary, and Botany Bay) consisted of three cores sampled close to each other and indistinguishable due to the resolution of the available map.

Inlet (LInlet). Despite existing geomorphological and hydrological differences among sites (Table 4.1), they all share a similar geological history of formation resulting from Holocene marine transgression within the past 6000-8000 years, which flooded riverine basins and shaping the present estuarine systems (Lewis et al. 2013, Hodgkin and Hesp 1998).

4.2.1 Coring and core processing

At each of the six meadows, three randomly located sediment cores were sampled by either manually hammering core barrels (PVC pipes) or by vibracoring (Vibecore-D, SDI) into the sediments. Compression of unconsolidated sediments during coring was inevitable and corrections were applied to obtain the real length of the sediment sampled. By linear proportions (Serrano et al. 2012, Glew et al. 2001), the sediment cores were decompressed to occupy the total length of the core barrel embedded in the substrate. The corrected average core lengths recovered at each site were: OHarb, 254 cm; WInlet, 179 cm; BBay 150 cm; SRiver - HEstuary, 216 cm; and LInlet, 219 cm. All biogeochemical variables studied in the sediment cores were plotted against the corrected core lengths. After transport to the laboratory, the core barrel was cut length-wise to expose the core log and the sediments were sub-sampled into 1 cm-wide slices. Alternate slices (14 to 32 samples per core, depending on the length of the core at regular intervals of 5 cm for the top 50 cm and 10 cm for core length beyond 50 cm) were selected for biogeochemical analysis and oven-dried at 60°C until constant weight to calculate the sediment bulk density. The samples were then were ground to a fine powder by a ball-mill grinder (Retsch) before further analysis.

4.2.2 Organic matter analyses

The ground sub-samples were combusted in a muffle furnace at 550°C (5 hours) to obtain the OM weight, through loss on ignition. Organic matter mass was then calculated as percent content in bulk sediment.

Table 4.1. Geomorphological and physical characteristics of the studied estuarine seagrass meadows

Seagrass meadow	Estuary	Coordinates	Geo-morphology	Geological setting	Freshwater input	Dominant sediment grain-size characteristic	Hydrodynamic influence	Reference for background information
<i>Posidonia australis</i>	Waychinicup Inlet Estuary	34°53'35.9"S 118°19'57.7"E	Permanently open estuary; rocky headland serve as opening to ocean	Flooded river gorge, which was carved through fault in Precambrian granite	Waychinicup River	Mix of sediment classes (fine to coarse sand) biogenic carbonates	Tide and swell dominated	Geoscience and Australia 2013, Hodgkin and Hesp 1998, Phillips and Lavery 1997)
	Oyster Harbour	34°58'58.3"S 117°58'29.9"E	Sheltered lagoonal embayment	Drowned river valley system located in inundated hilly terrain of Precambrian rock and undulating sand plain	Kalgan River and the King River	Medium-coarse to fine grain, silty sands together with biogenic carbonates	Wave dominated	Geoscience and Australia (2013), Pen et al. (2000), Hodgkin and Clark (1990)
	Botany Bay	34°00'33.4"S 151°11'16.91"E	Inter-barrier system	Drowned river valley system	Georges River and Cooks River	Very fine sand to fine sand	Tide dominated	Geoscience and Australia (2013), Lee and Patterson (2002), Albani et al. (1976)
<i>Halophila ovalis</i>	Swan River Estuary	32°00'46.1"S 115°47'43.5"E	Permanently open estuary	Drowned river valley system inundated valley tract of the Swan and Canning Rivers, traversing a range of coastal plain geomorphic units	Swan River and Canning River	Sandy mud to fine sand	Wave dominated	Geoscience and Australia (2013), O'Callaghan (2004), Pen et al. (2000)
	Harvey Estuary	32°38'00.3"S 115°38'51.7"E	Barrier estuary	Depression between dunes, flooded by rising sea levels located at junction between limestone ridge and Bassendean Dune terrain	Harvey River	Sandy mud to fine sand	Wave dominated	Geoscience and Australia (2013), Pen et al. (2000), McComb et al. (1998)
	Leshenault Inlet	33°15'41.2"S 115°42'02.6"E	Barrier estuary	Formed between old dune lines in an inter-barrier depression located behind high Holocene dune barrier, and encroaching on high sandy Mandurah-Eaton Ridge	Collie-Brunswick River; Preston-Ferguson Rivers; ground-water seepage	Mud and sandy mud	Historically tide dominated; presently wave dominated	Geoscience and Australia (2013), Semeniuk (2000), Pen et al. (2000)

4.2.3 *Organic carbon elemental composition and stable isotope signatures*

A separate set of ground sub-samples (0.5 g) were used for OC and $\delta^{13}\text{C}$ analyses. These samples were acidified (1 M hydrochloric acid) to remove all inorganic carbon. When effervescence ceased, after 12-18 h, the mixture was centrifuged (3400 revolutions min^{-1} for 4.5 min) and the supernatant pipetted off to remove the acid. Deionised water (10 ml) was then added to wash off residual acid. The sample was re-centrifuged and the supernatant removed by pipetting. The acidified sample was then oven-dried and weighed. For elemental composition and isotope analysis, 9-10 mg of the acidified sample was encapsulated in tin capsules and combusted in a continuous flow isotope ratio mass spectrometer analyser (Delta V Advantage: Thermo-Finnigan) at the LIENSs Stable Isotope Facility (University of La Rochelle, France). The OC content in the sediment samples reported by the analytical facilities (as %OC) was corrected to account for the weight of pre-acidified bulk sediment samples. $\delta^{13}\text{C}$ values were reported relative to the Vienna Pee Dee Belemnite (VPDB) standard. The result obtained (i.e. OM and OC contents, $\delta^{13}\text{C}$ values) were plotted either relative to depth (stratigraphic-based) or age (temporal-based).

4.2.4 *Calculations of organic carbon stores, chronostratigraphy, and accumulation rates*

Selected samples (Table 4.2) were dated by ^{14}C Accelerator Mass Spectrometry (AMS). These samples were rinsed in ultrapure MiliQ water, placed in a sonic bath (5 min) to dislodge any attached sediment particles, inspected under a microscope for visual observation of sample integrity, and sent to the AMS lab (ANSTO and Direct AMS) for further pre-treatment. Samples (10-100 mg) comprising of plant OM underwent an acid-base-acid treatment while shells were etched before ^{14}C analysis. Two samples of one selected core from BBay, SRiver, HEst and LInlet were ^{14}C dated, while all three cores from OHarb and WInlet were dated (Table 4.2). For Oyster harbour and WInlet, two cores had two depths each that were dated while the last core had only one dating done (Table 4.2). In those cases where only one replicate core per meadow was dated, it was assumed that the

Table 4.2. Radiocarbon ages and calculated sediment accretion rates for *Posidonia australis* and *Halophila ovalis* meadows. Sample depth was corrected for post-coring sediment compression. Sediment accretion rate obtained from Clam.R was obtained as the rates between the two dated points from the respective analysed cores. The numbers in parentheses for mean sediment accretion rate refer to the rate as yr cm^{-1} .

Seagrass meadow	Meadow	Replicate core ID	Sample depth (cm)	Type of material dated	^{14}C age (yr)	Corrected conventional ^{14}C age (cal yr BP)*	Sediment accretion rate from Clam.R (yr cm^{-1})	Mean sediment accretion rate (cm yr^{-1})
<i>Posidonia australis</i>	Waychinicup Inlet Estuary	WInlet 1	74	Organic matter (fibres)	1875 ± 30	1322 ± 30	na**	
		WInlet 1	164	Organic matter (fibres)	2485 ± 45	2022 ± 45	12.09	0.083 (12)
		WInlet 2	74	Organic matter (fibres)	755 ± 40	307 ± 40	na**	
		WInlet 2	180	Organic matter (fibres)	2900 ± 45	2542 ± 45	14.54	0.069 (15)
		WInlet 3	168	Organic matter (fibres)	2700 ± 45	2277 ± 45	13.47	0.074 (13)
	Oyster Harbour	OHarb 1	254	Organic matter (fibres)	7310 ± 40	7677 ± 40	33.35	0.030 (33)
		OHarb 2	84	Organic matter (fibres)	2195 ± 45	1677 ± 45	18.91	0.037 (27)
		OHarb 2	212	Organic matter (fibres)	6640 ± 40	7057 ± 40	49.21	
		OHarb 3	84	Organic matter (fibres)	2110 ± 45	1577 ± 45	17.5	0.039 (26)
		OHarb 3	225	Organic matter (wood)	7330 ± 35	7697 ± 35	49.73	
	Botany Bay	BBay 1	73	Organic matter (fibres)	1242 ± 26	1234 ± 26	16.04	0.066 (15)
		BBay 1	149	Organic matter (fibres)	2265 ± 31	2257 ± 31	14.19	
<i>Halophila ovalis</i>	Swan River Estuary	SRiver 1	50	Shells	4251 ± 27	4243 ± 27	96.3	0.016 (64)
		SRiver 1	98	Shells	6203 ± 38	6195 ± 38	47.49	
	Harvey Estuary	HEst 1	43	Organic matter (wood)	3981 ± 38	3973 ± 38	103.42	0.010 (102)
		HEst 1	66	Organic matter (wood)	5956 ± 34	5948 ± 34	101.3	
	Leshenault Inlet	LInlet 1	70	Shells	4069 ± 33	4061 ± 33	65.18	0.038 (26)
		LInlet 1	176	Shells	5088 ± 27	5080 ± 27	16.55	

**na = possible sediment mixing (see section 4.4.2 for elaborations)

chronostratigraphy of the undated cores was similar to the dated cores within the same meadow. The estimated radiocarbon age of all samples after calibration to calendar years before present (Cal yr BP; <https://c14.arch.ox.ac.uk/oxcal/OxCal.html>; present taken as AD 2013) and corrected for marine reservoir effect (i.e. subtracting 71 yr from the uncorrected radiocarbon ages of Western Australian meadows and 60 yr from the Eastern Australian meadow of BBay; Ulm 2006, 2002) is reported in Table 4.2. These radiocarbon dates were plotted as a best-fit smooth-spline function using CLAM.R software (Blaauw 2010) to produce a chronostratigraphic model for each meadow (Figure 4.2). The mean sediment accumulation rates for all meadows were between 0.009 cm yr⁻¹ to 0.082 cm yr⁻¹ (resolution of 115 yr cm⁻¹ and 12 yr cm⁻¹, respectively: Table 4.2).

Due to the variability in core lengths sampled among the six study meadows, OC stores and accumulation rates were standardised to stratigraphic depths of 150 cm, 100 cm and 30 cm to allow comparisons. The limit of 150 cm sediment thickness was based on the shortest core sampled among the meadows (i.e. at BBay), 100 cm adopted to compare values with other studies (i.e. Fourqurean et al. 2012a), and 30 cm to obtain values in shallow sediments. Organic carbon stores ($Store_{OC}$; g OC cm⁻²) were calculated as:

$$Store_{OC} = \rho_b \times \frac{OC}{100} \times L$$

Equation 4.1

where ρ_b is the sediment dry bulk density (g cm⁻³); OC is the mean organic carbon content of the dry sediment (%) for the length of core being considered; and L is the length of core over which the $Store_{OC}$ is being calculated (cm). The units of OC content, obtained as g OC cm⁻², were then standardised to kg OC m⁻².

For temporal-based accumulations, OC stores were determined as in the stratigraphic-based method described above, but within the thickness of the core corresponding to a ¹⁴C age of 2500 Cal yr BP. The normalisation procedure was based on the estimated youngest age (at

the distal core end) sampled among the meadows (i.e. at BBay, see Table 4.2). As a result, all meadows had variable sediment thicknesses based on similar periods of accumulation, which ranged from 21 cm to 170 cm (HEst and Winlet, respectively: Table 4.3).

The long-term accumulation rates of OC within the cores of each meadow ($Accum_{OC}$; g OC $m^{-2} yr^{-1}$) were then calculated for each meadow as:

$$Accum_{OC} = \frac{Store_{OC}}{t}$$

Equation 4.2

Where t = age of the sediment (cal yr BP) at the sampled depth.

4.2.5 Statistical analysis

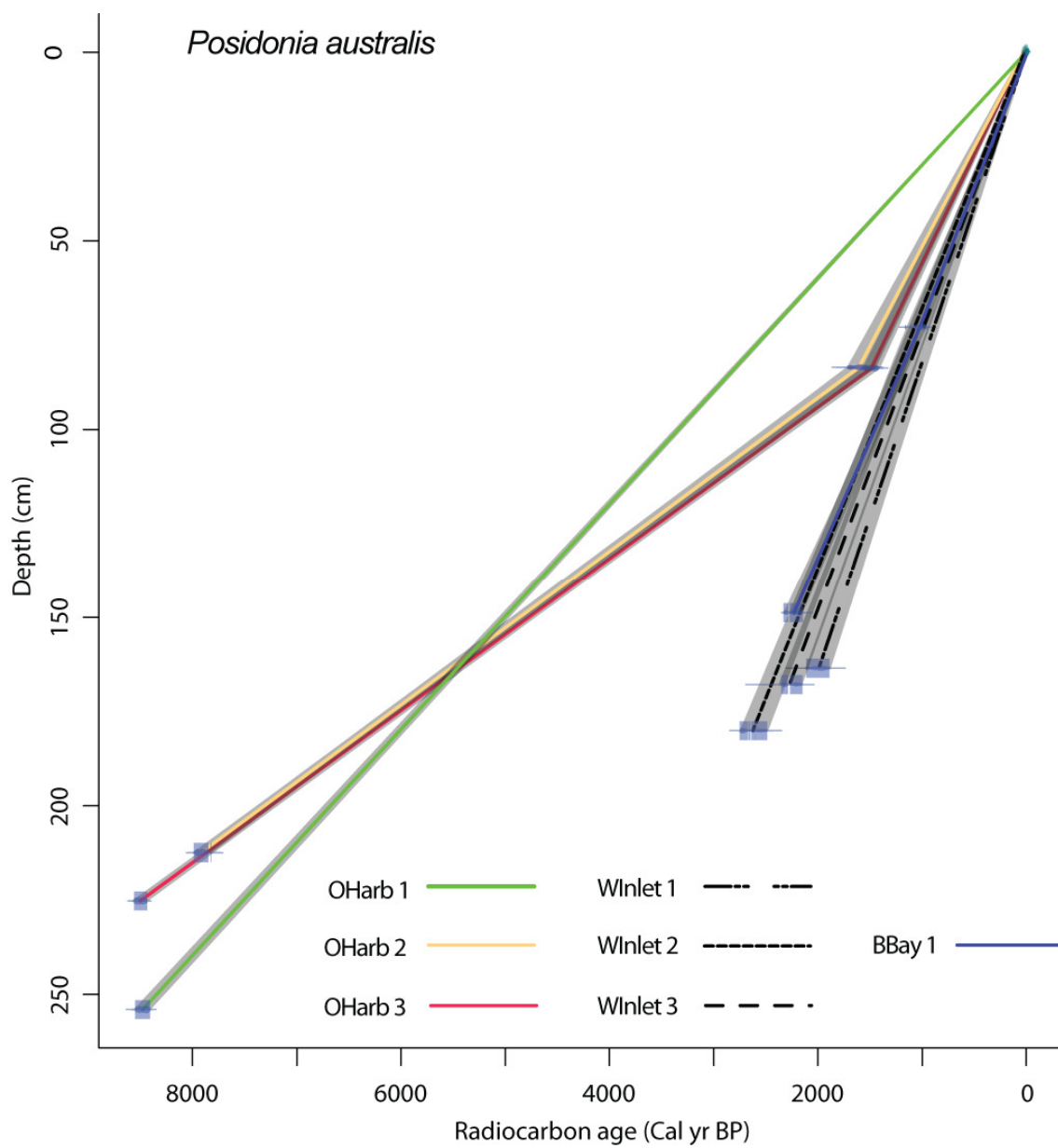
For testing the differences in OC stores within similar sediment thicknesses (i.e. top 150 cm, 100 cm and 30 cm) and similar accumulation period (past 2500 Cal yr BP) between the two seagrass species and meadows, a two-way nested PERMANOVA (Primer version 6) was applied (species as fixed factor, meadow nested within species). A PERMANOVA pairwise test was applied to assess differences in OC stores among the individual meadows.

4.3 Results

4.3.1 Organic matter and organic carbon content

In the top 150 cm of sediment, the mean organic content for the *P. australis* meadows ranged from 3.83-8.65% OM and 0.71-1.17% OC compared to 1.01-7.71% OM and 0.16-1.52% OC for *H. ovalis* meadows (Table 4.3 and Figure 4.3). The distribution of OM within the top 150 cm was not consistent, either within cores or among cores. For the *H. ovalis* meadows, within the top 10 cm (accumulated over the last 1100 years; Figure 4.4), OM content ranged from

(a)



(b)

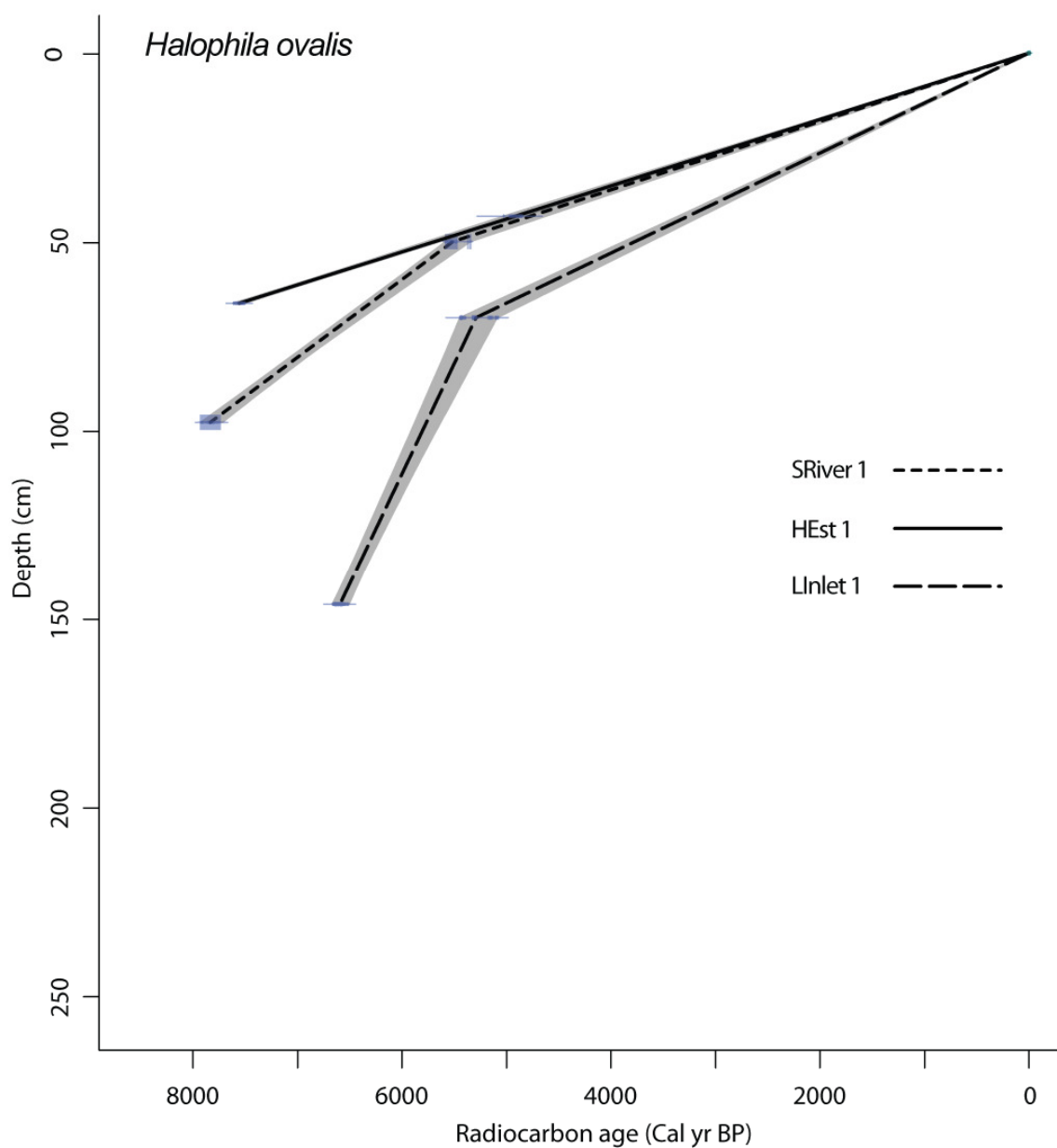


Figure 4.2. (Opposite and facing page) Interpolation of the radiocarbon data from the CLAM.R software and plotting as a smooth-spline function for all meadows. (a) Replicates cores from *P. australis* meadows consisting of Waychinicup Inlet Estuary, Oyster Harbour and Botany Bay; and (b) Cores from *H. ovalis* meadows consisting of Swan River Estuary, Harvey Estuary, and Leschenault Inlet.

Table 4.3. Sedimentary organic matter characteristics of *Posidonia australis* and *Halophila ovalis* meadows

Species	Site	Sediment dry bulk density (g DW cm ⁻³)	SE	Mean OM (%)	SE	Mean OC (%)	SE
<i>Posidonia australis</i>	Waychinicup Inlet Estuary	1.18	0.04	4.29	0.11	1.17	0.08
	Oyster Harbour	0.61	0.03	8.65	0.53	2.12	0.16
	Botany Bay	1.28	0.07	3.83	0.56	0.71	0.10
<i>Halophila ovalis</i>	Swan River Estuary	1.39	0.04	1.01	0.07	0.16	0.03
	Harvey Estuary	1.27	0.44	2.23	1.08	0.35	0.92
	Leshenault Inlet	1.30	0.13	7.71	3.97	1.52	0.16

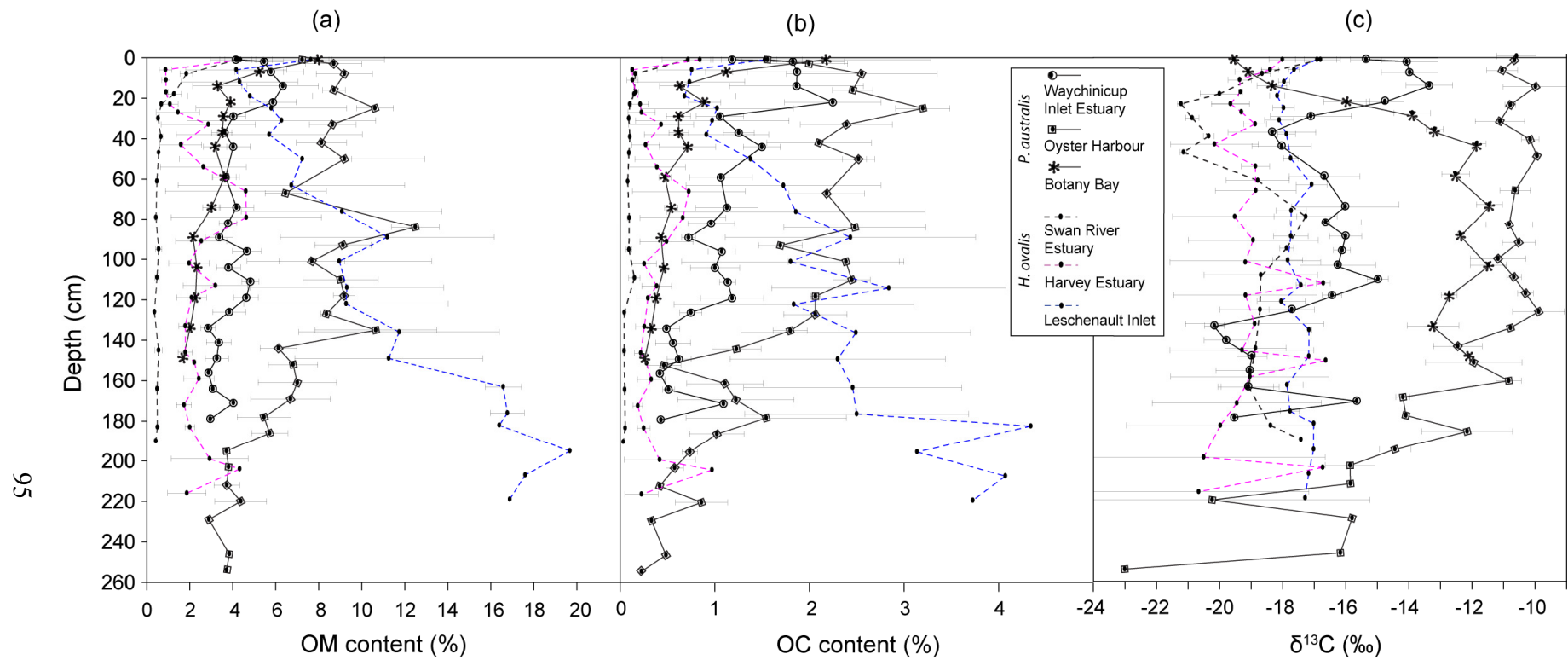


Figure 4.3. Characteristics of sedimentary organic matter (mean \pm SE) plotted across stratigraphy (i.e. sediment depth, in cm) in *Posidonia australis* and *Halophila ovalis* meadows: a) organic matter content; b) organic carbon content; and c) stable carbon isotope values.

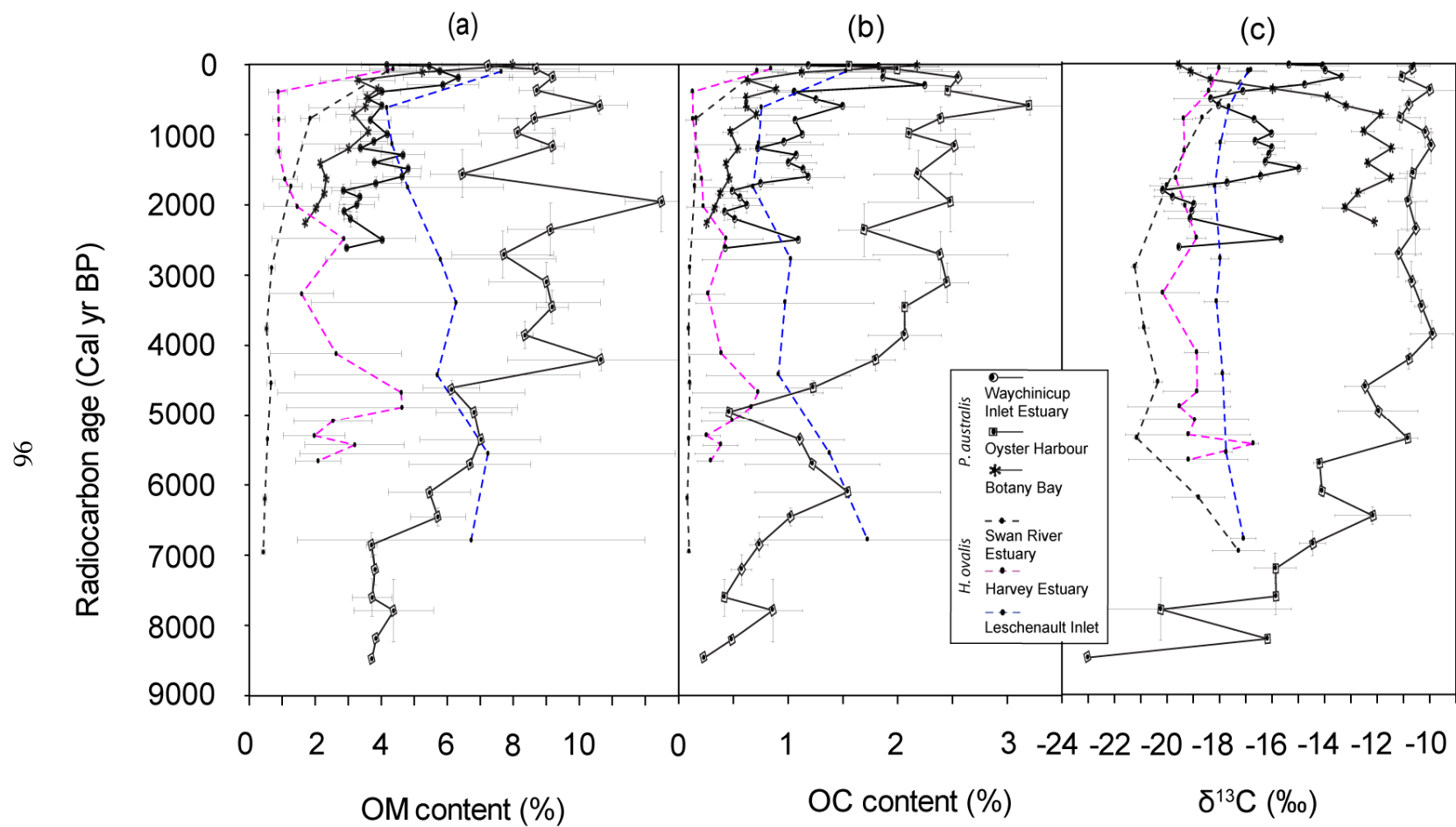


Figure 4.4. Characteristics of organic matter (mean \pm SE) plotted as a function of temporal accumulation (i.e. sediment ages, in Cal yr BP) in *Posidonia australis* and *Halophila ovalis* meadows: a) organic matter content; b) organic carbon content; and c) stable carbon isotope values.

4-8% (corresponding to OC of 0.5-2.0%). At depths beyond 10 cm, the three meadows showed different trends in organic content: at SRiver, OM declined to 0.4 to 1% (< 0.15% OC); HEst had a constant OM content through sediment depth and age ranging from 1-5% (0.1-0.8% OC); and LInlet had progressively increasing OM, ranging from 4-20% (1.0-4.5% OC). For the *P. australis* meadows, OM content in the top 10 cm (dated as present to 300 Cal yr BP) ranged 4-8%, similar to the *H. ovalis* meadows, but the OC content was 1.0-2.5%, higher than in *H. ovalis* meadows (0.5-2.0%). At depths beyond 10 cm, OM content for WInlet and BBay declined to about 4.0% until approximately 30 cm depth (400 - 450 Cal yr BP) and remained constant (2.0-4.0%) towards the distal end of the cores. OHarb had the highest OM content among the three *P. australis* meadows. At depths beyond 10 cm, OM content was in the range of 6.0-13.0% (2.0-3.5% OC) to about the 180 cm level (6000 – 6400 Cal yr BP), where it then began to decrease to between 2.0 and 4.0% (0.25-1.5% OC).

4.3.2 Organic carbon stores in *P. australis* and *H. ovalis* meadows

There were more evident differences in OC stores in meadows of the two species when estimated as temporal accumulations (i.e. normalised to a set time period) rather than stratigraphic accumulation (i.e. normalised to a set depth). Based on OC accumulation across a temporal frame of ~2500 Cal yr BP for all meadows (Figure 4.5), *P. australis* meadows had significantly higher OC stores than *H. ovalis* meadows ($p < 0.01$). Across all meadows, *P. australis* meadows had a mean OC store of 10.87 kg OC m⁻² compared to 0.97 kg OC m⁻² in *H. ovalis* meadows, approximately an 11-fold difference (Table 4.4).

The mean OC stores in sediment thicknesses of 150 cm, 100 cm and 30 cm were 2-3 fold higher in *P. australis* compared to *H. ovalis* meadows (Table 4.4 and Figure 4.6). Over the top 150 cm, the greatest store was in the *P. australis* meadows of WInlet (14.03 kg OC m⁻²) while the *H. ovalis* meadows at SRiver had the lowest stores (1.44 kg OC m⁻²). Despite the difference in mean OC stores, there were no overall statistical differences ($p > 0.05$: Table

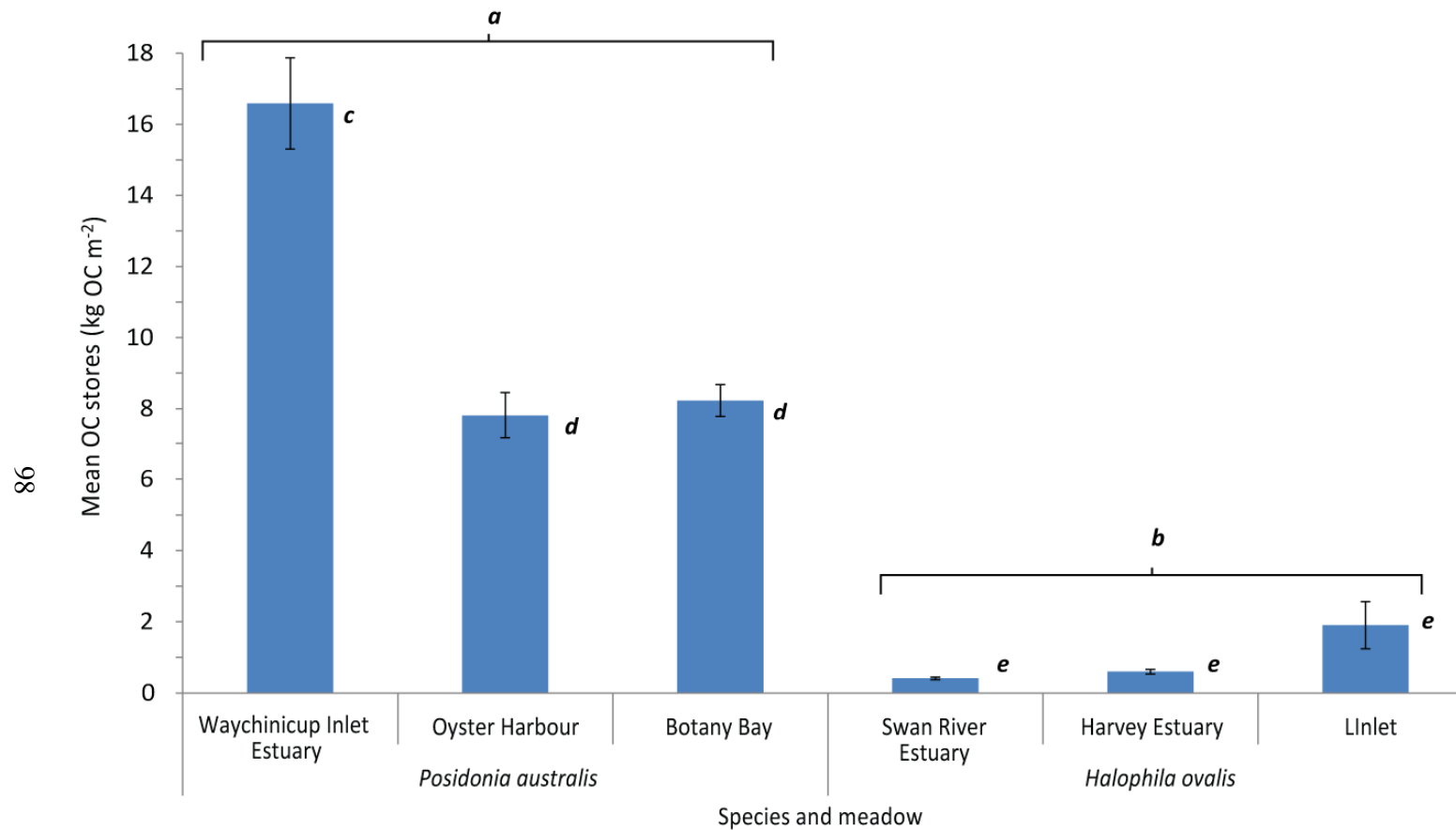


Figure 4.5. Organic carbon stores (mean \pm SE) in *Posidonia australis* and *Halophila ovalis* meadows based on temporal accumulation across the 6 studies sites within sediment age of 2500 Cal yr BP. Shared letters on horizontal lines indicate no significant difference between species. Shared letters beside bars indicate no statistical differences within species.

Table 4.4. Organic carbon stores in *P. australis* and *H. ovalis* sites across three categories of sediment thicknesses (top 150 cm, 100 cm and 30 cm), and within a temporal accumulation period of 2500 Cal yr BP. For OC stores based on stratigraphy, the results of Leschenault Inlet are presented for means from three and two cores (related calculations based on the two Leschenault Inlet cores are in parentheses).

Method for estimating OC stores	Standardisation	Species and meadow site	<i>Posidonia australis</i>			<i>Halophila ovalis</i>			
			Waychinicup Inlet Estuary	Oyster Harbour	Botany Bay	Swan River Estuary	Harvey Estuary	Leshenault Inlet	
Stratigraphic	Top 150 cm	Cumulative OC stores (kg OC m ⁻²)	14.03	11.43	6.98	1.44	5.14	8.92 (5.70)	
		Mean of OC stores within species (kg OC m ⁻²)	10.81 ± 2.06			5.17 ± 2.16 (4.10 ± 1.33)			
		Fold difference	2.09 (2.64)						
	Top 100 cm	Cumulative OC stores (kg OC m ⁻²)	10.77	8.07	5.61	1.00	3.45	5.17 (2.98)	
		Mean of OC stores within species (kg OC m ⁻²)	8.14 ± 1.48			3.24 ± 1.18 (2.51 ± 1.07)			
		Fold difference	2.51 (4.30)						
	Top 30 cm	Cumulative OC stores (kg OC m ⁻²)	3.52	2.16	1.85	0.47	0.90	1.53 (1.04)	
		Mean of OC stores within species (kg OC m ⁻²)	2.51 ± 0.51			0.97 ± 0.31 (0.82 ± 1.30)			
		Fold difference	2.60 (3.11)						
	Temporal	2500 Cal yr BP	Sediment thickness (cm)	170	95	166	26	21	38
			Cumulative OC stores (kg OC m ⁻²)	16.58	7.81	8.23	0.41	0.60	1.9
			Mean of OC stores within species (kg OC m ⁻²)	10.87 ± 2.86			0.97 ± 0.47		
Fold difference			11.22						

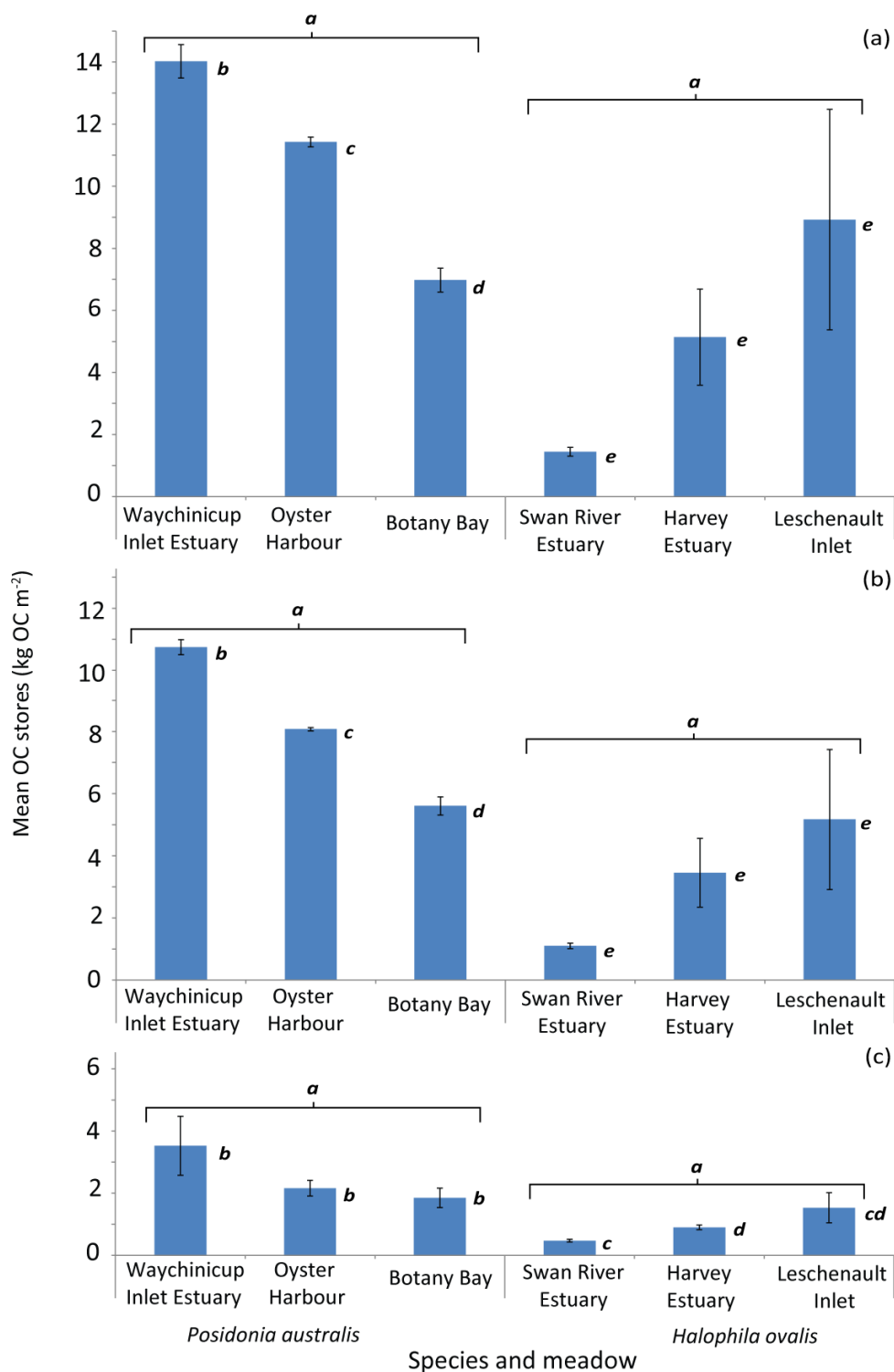


Figure 4.6. Organic carbon stores (mean \pm SE) in *Posidonia australis* and *Halophila ovalis* meadows based on stratigraphic accumulation across the 6 studies sites within sediment thickness of: (a) 150 cm; (b) 100 cm; and (c): 30 cm. Shared letters on horizontal lines indicate no significant difference between species. Shared letters beside bars indicate no statistical differences within species.

4.5) between OC stores in *P. australis* and *H. ovalis* meadows. This lack of statistical difference was largely due to one core from the *H. ovalis* meadows at LInlet. This core had relatively high OC stores (15.37 kg OC m⁻², compared to 3.12 kg OC m⁻² and 8.30 kg OC m⁻² in the other two replicate cores), which increased the mean OC stores of the *H. ovalis* meadows (from 4.10 kg OC m⁻² to 5.17 kg OC m⁻²; Table 4.3). When this carbon-rich core was excluded from the analysis, OC stores in *P. australis* were significantly higher than in *H. ovalis* meadows ($p < 0.05$). Similar to the results for OC stores in 150 cm sediment thickness, there were no significant differences ($p > 0.05$; Table 4.5) for OC stores between *P. australis* and *H. ovalis* meadows in the 100 cm and 30 cm sediment thickness.

4.3.3 Organic carbon accumulation rates

The radiocarbon results showed a coherent chrono-stratigraphy (i.e. age versus depth) for all cores, except for WInlet, where there was an inconsistency of sediment ages between two dated cores (Table 4.2). At the same depth of 74 cm, the WInlet 1 core was dated as 1677 Cal yr BP, while WInlet 2 core was 307 Cal yr BP, indicative of sediment mixing or erosion within these layers for either or both cores at WInlet, as had been reported for other estuaries (e.g. Colman et al. 2002). As such, this result was not taken into account in the chrono-stratigraphic model; only the means of radiocarbon dates obtained at the distal end of the WInlet cores were used for estimating the sediment accretion and OC accumulation rate at this meadow. All the other meadows had at least one core dated at two levels with subsequent estimations of sediment accretion and OC accumulation rates calculated accordingly.

Posidonia australis meadows had significantly higher OC accumulation rates than *H. ovalis* meadows ($p < 0.01$; Table 4.5 and 4.6). The mean accumulation rate for *P. australis* meadows was 4.15 ± 1.36 g OC m⁻² yr⁻¹, with WInlet having the highest OC accumulation rate (6.80 g OC m⁻² yr⁻¹) and OHarb the lowest (2.35 g OC m⁻² yr⁻¹; Table 4.6). For the *H. ovalis* meadows, OC accumulation rates ranged from 0.13-1.29 g OC m⁻² yr⁻¹, with a mean of 0.57 ± 0.36 g OC m⁻² yr⁻¹.

Table 4.5 Summary results for nested PERMANOVA for differences in mean OC stores and accumulation rates between *P. australis* and *H. ovalis* (species) based on each respective meadow site (meadow). The stratigraphic method marked with (*) denotes the statistical test done for the *P. australis* cores against 8 *H. ovalis* cores (the excluded core was the single core from Leschenault Inlet with exceptionally high OC stores – see section 4.3.2 for elaborations).

Method for quantifying OC stores	Source of variation	df	Sum of Squares	Mean Square	F	P
Stratigraphic (top 150 cm)	species	1	1.5086×10^8	1.5086×10^8	4.01	0.113
	meadow (species)	4	1.504×10^8	3.7601×10^7	4.85	0.016
* Stratigraphic (top 150 cm)	species	1	2.1137×10^8	2.1137×10^8	8.67	0.0451
	meadow (species)	4	9.5661×10^7	2.3915×10^7	8.55	0.002
Stratigraphic (top 100 cm)	species	1	1.0809×10^8	1.0809×10^8	6.68	0.0647
	meadow (species)	4	6.4725×10^7	1.6181×10^7	5.01	0.0131
Stratigraphic (top 30 cm)	species	1	1.0726×10^7	1.0726×10^7	6.6391	0.064
	meadow (species)	4	6.4625×10^7	1.6156×10^6	2.4742	0.106
Temporal (2500 Cal yr BP)	species	1	4.4151×10^8	4.4151×10^8	11.705	0.028
	meadow (species)	4	1.5087×10^8	3.7718×10^8	27.76	0.001
Accumulation rate	species	1	57.695	57.695	6.51	0.001
	meadow (species)	4	35.47	8.8675	23.52	0.001

Table 4.6. Mean sedimentary OC accumulation rates of and *Halophila ovalis* meadows. Similar superscripted letters beside rate values indicate no statistical differences.

Species	Meadow	Replicate core ID	Sediment accretion rate (cm yr ⁻¹)	OC accumulation rate (g m ⁻² yr ⁻¹)	Meadow mean OC accumulation rate (g m ⁻² yr ⁻¹)	Species mean OC accumulation rate (g m ⁻² yr ⁻¹)	
<i>P. australis</i>	Waychinicup	WInlet 1	0.083	8.103	6.80 ^c	4.15 ^a	
	Inlet Estuary	WInlet 2	0.069	5.929			
		WInlet 3	0.074	6.383			
	Oyster	OHarb 1	0.030	2.243	2.35 ^d		
	Harbour	OHarb 2	0.037	2.333			
		OHarb 2	0.039	2.470			
	Botany Bay	BBay 1	0.066	3.084	3.30 ^e		
		BBay 2		3.647			
		BBay 3		3.155			
<i>H. ovalis</i>	Swan River	SRiver 1	0.016	0.128	0.13 ^f	0.57 ^b	
	Estuary	SRiver 2		0.121			
		SRiver 3		0.152			
	Harvey	HEst 1	0.010	0.225	0.28 ^f		
	Estuary	HEst 2		0.155			
		HEst 3		0.458			
	Leshenault	LInlet 1	0.038	2.248	1.29 ^f		
		Inlet	LInlet 2				0.454
			LInlet 3				1.180
OC accumulation rate fold-difference between species						7.29	

4.3.4 $\delta^{13}\text{C}$ values

The two species of seagrass showed contrasting patterns in $\delta^{13}\text{C}$ values across stratigraphy (Figure 4.3) and age (Figure 4.4). Overall, the *H. ovalis* meadows had similar ranges of $\delta^{13}\text{C}$ values (-21.5‰ to -17‰). Surface sediment (top 10 cm) values for these meadows were between -17.0‰ and -18.5‰ that declined with depth and age, less noticeably at Linlet (where it was generally between -17.5‰ and -18.5‰) and more so at HEst and SRiver, where values initially declined to around -20‰ to -22‰ and fluctuated between -17‰ and -21‰ afterwards. In contrast to *H. ovalis*, the three *P. australis* meadows had highly contrasting $\delta^{13}\text{C}$ profiles. At the surface (top 10 cm), the $\delta^{13}\text{C}$ values of these meadows ranged from -11‰ at OHarb to -15.5‰ at Winlet and -19‰ at Bbay, thus showing greater variability and a wider range than surface values in *H. ovalis*. The subsequent changes in $\delta^{13}\text{C}$ values with depth and age in the sediment were also different among the three *P. australis* meadows. OHarb had a consistent value of -10‰ to -11‰ from the surface to about 120 cm (3200 – 4000 Cal yr BP; Figure 4.4) and, thereafter, declined to below -20‰ at a depth of 240 cm (8000 Cal yr BP). Winlet had surface values of -15.5‰ that initially increased with depth (-13.5‰ at 20 cm cf. 250 – 300 Cal yr BP) before declining from -15‰ to -18‰ below 40 cm (450 – 600 Cal yr BP) and then to -19‰ to -21.5‰ below 140 cm (1700 – 2450 Cal yr BP). BBay had the most depleted surface values, around -19.5‰, which increased with depth (reaching -12‰ by 50 cm (700 Cal yr BP) and then remained at -11‰ to -13‰ to the bottom of the core at 140 cm (2100 Cal yr BP).

4.4 Discussion

4.4.1 Differences in organic carbon stores between *P. australis* and *H. ovalis* meadows

This study used two different ways to consider the OC storage capacity of seagrass meadows. When estimated for a defined period of time, in this case 2500 yr of accumulation, the mean

OC stores in the three *P. australis* meadows were significantly higher (11-fold) than those in the *H. ovalis* meadows, and the OC accumulation rates were up to 7-fold higher. When stores were compared over a standardised depth of sediment (i.e. stratigraphic-based), the differences were not statistically significant, though the mean of the three *P. australis* meadows was up to 2.6-fold higher than in the *H. ovalis* meadows. However, a single core of *H. ovalis* strongly influenced this outcome and when it was omitted from the analysis, the difference between species was significant (see further discussion below). Based on these comparisons, the difference in OC stores is consistent with the hypothesis that the species have different OC stores, with this being most evident when they are compared over similar periods of accumulation.

Although previous studies comparing OC stores among seagrass meadows were based on the thickness of sediment deposits (e.g. Fourqurean et al. 2012a), the results obtained in this study demonstrated that temporally-based comparisons are likely most useful since they will reflect the amount of OC sequestered in different habitats over a comparable time period and thus the ecosystem service provided by the meadows being studied (Duarte et al. 2013b). Stratigraphic-based comparisons may be useful in situations where meadows may be disturbed to a particular depth and the potential loss of stored carbon needs to be estimated – for example, following a dredging event, flow-induced scouring or cyclonic disturbances (e.g. Loneragan et al. 2013, Carlson et al. 2010, Boudouresque et al. 2009). The stratigraphic comparisons for OC stores in this study were made based on several core lengths, with the differences between species being greater, and closer to being statistically significant as the depth of the cores increased. This highlights the need for comparisons to be made across standardised stratigraphic thicknesses. Deeper profiles capture longer time periods, introducing the possibility for shifts in both species and abiotic characteristics at the sites (Macreadie et al. 2012, Raniello and Procaccini 2002) and including the variability in diagenetic processes (i.e. remineralisation rates of the OC with aging) within habitats. While the meadows studied here were *P. australis* or *H. ovalis* this may not always have been the

case and it is possible there may be depths where the contribution of seagrass detritus to the organic pool is negligible since these layers may be devoid of seagrasses back in time (Reich et al. 2015). Smaller sediment thicknesses allow comparisons of more recent accumulation, thus providing insights on the influence of the contemporary seagrasses on OC stores.

To date, there are no studies that have compared OC stores in seagrass meadows based on temporal accumulation. Existing studies tend to compare the OC accumulation capacity among seagrass meadows by quantifying OC stores for a certain sediment thickness e.g. 140 cm sediment-thickness (Chapter 3, this thesis); 100 cm (Fourqurean et al. 2012a); or 25 cm (Lavery et al. 2013). Here, estuarine meadows were selected to reduce confounding effects due to the depositional environment (see Chapter 3 for further details). Comparisons of OC stores of the two species over 3 different depth strata (150, 100 and 30 cm) found the stores in *P. australis* to be 2.1- to 2.5-fold higher than in *H. ovalis*. Lavery et al. (2013) found a similar difference, with a temperate *P. australis* meadows having 1.3-fold higher OC stores than a tropical *H. ovalis* meadows, though it is known that ambient sediment temperatures can influence OC stores (Canuel et al. 2012) and may have contributed to the difference. More generally, the range of OC stores among the meadows studied here (100 cm depth; 1.10-10.75 kg OC m⁻², equivalent to 1-107 Mg OC ha⁻¹) is within the range reported by Fourqurean et al. (2012a), i.e. 6-628 Mg OC ha⁻¹, but lower than the *P. australis* meadows studied in Fourqurean et al. (2012b) i.e. 115-335 Mg OC ha⁻¹ (11.5-33.5 kg OC m⁻²). This latter range were from offshore Shark Bay meadows and fundamentally different from the estuarine meadows studied in this chapter. Fourqurean et al. (2012a; supplementary information) also studied a mixed meadow of *H. ovalis* co-occurring with *Halodule uninervis* in the Swan River Estuary and reported that OC stores were 2.03 to 25.39 mg mL⁻¹ (50 cm depth; equivalent to 0.002-0.025 g cm⁻³), similar in order of magnitude to the OC stores in this study at the same estuary.

Within any given estuary, the variability in OC stores among cores was relatively low, with the exception of Leschenault Inlet. There, one core had high carbon-richness (15.37 kg OC m⁻²) compared to the other two (3.11- 8.30 kg OC m⁻²) and this introduced sufficient variability to mask what would otherwise have been statistically significant differences between *P. australis* and *H. ovalis* meadows based on the same stratigraphic depth. Removal of this core then confirmed that OC stores in the nine *P. australis* cores (as the mean of three *P. australis* meadows) were significantly higher compared to the remaining eight *H. ovalis* cores. This carbon-rich core was sampled approximately from the middle of the estuarine lagoon, while the other two cores were sampled along the periphery of the western shore. Wind-induced processes that transport organic rich sediments into the central basin of the estuary (Semeniuk 2000) probably account for the high OC stores and the site-specific OC accumulation variability in this *H. ovalis* meadow. Such spatial variation in OC stores within the same site may not necessarily apply to all seagrass meadows, however the identification of significant variability among meadows of the same species within one estuary indicates that factors other than species identity can affect OC stores even within a broadly similar geomorphological habitat. So, while overall, the results clearly indicate that species composition may be used to generalise likely differences in the OC stores of seagrass habitats, other habitat factors may also contribute to differences, even within similar geomorphological habitat types.

The 11-fold higher OC stores in *Posidonia australis* compared to *Halophila ovalis* habitats (accumulated over 2500 years) noted in this study are consistent with the plants' morphologies and growth strategies. *Posidonia australis* meadows are highly productive, generating below-ground biomass as high as 286-658 m⁻² (Holmer and Kendrick 2013, Paling and McComb 2000) with a significant portion retained in the meadow due to its low turnover rates (3.86 yr⁻¹: Duarte 2001). Its rhizomes, which contain recalcitrant tissues (Kuo and Cambridge 1978), tend to be buried *in situ* upon plant senescence (Campbell and Paling 2003) and this material tends to be preserved in place due to the rooting structures, which stabilises

the sediments. Furthermore, due to the depths in the sediment (typically >30 cm) that the roots and rhizomes grow, the OM input are more likely to enter reducing conditions, which lowers OM decomposition rates by limiting microbial degradation (Burdige 2007), thus increasing preservation potential (Borum et al. 2006). Finally, the sediment accretion rate was relatively high in the *P. australis* meadow (0.030-0.083 cm yr⁻¹), probably due to its large canopy structure, which facilitates the trapping of both allochthonous and autochthonous matter (Marbà et al. 2015, Zhuang and Chappell 1991). This results in more rapid burial of OM and its introduction to an anoxic environment, thus likely enhancing their preservation.

In many respects, the opposite set of characteristics and conditions typify *H. ovalis* meadows. Its low biomass and high turnover rates (60-120 g DW m⁻² and 20.85 yr⁻¹, respectively: Hillman et al. 1995, Duarte 1991) potentially result in less seagrass detritus available for burial. Its ephemeral below-ground organs grow within 1-5 cm below the sediment surface (*pers. observ.*), making OM inputs prone to scouring and export (Cabaco et al. 2008), accentuated by the relatively low sediment accumulation rate (0.016-0.038 cm yr⁻¹). Despite also having recalcitrant tissues (i.e. lignin; Baydoun and Brett 1985), remineralisation of any detrital matter is likely due to higher oxygen exposure within the shallow sediment depths (Harrison 1989). In addition, plants with fast growth rates, such as in *H. ovalis*, likely has high decomposition rates (Enriquez et al. 1993) and is thus another factor that may account for the lower organic stores in its sediments. The results obtained demonstrated that both the higher contribution of recalcitrant tissues and higher accumulation rates of these tissues in *P. australis* meadows are key factors triggering the OC storage in seagrass meadows.

4.4.2 Variation in OM sources between *P. australis* and *H. ovalis* meadows

The $\delta^{13}\text{C}$ data indicate that both seagrass- and non-seagrass-derived OM were preserved in the sediments of all the meadows studied. *Posidonia australis* tissues have $\delta^{13}\text{C}$ values in the range of -6.3‰ to -9.9‰ (Prado et al. 2008, Hindell et al. 2004) while signatures for *H. ovalis* range from -6.4‰ to -15.5‰ (Hemminga & Mateo 1996). Reported $\delta^{13}\text{C}$ values for seston,

algal and terrestrial OM in the region range from -13‰ to -29‰ (Smit et al. 2005, Loneragan et al. 1997).

The bulk sedimentary OM $\delta^{13}\text{C}$ values in the *P. australis* sediments was -9.9‰ to -23.0‰ (mean of -14.7‰) while it was -16.6‰ to -21.4‰ (mean of -18.5‰) in *H. ovalis*. Apart from two *P. australis* sediment samples that had $\delta^{13}\text{C}$ signatures exactly at the lower end of *Posidonia australis* tissues signatures (i.e. -9.9‰) all other sediment samples for both species, consistently showed lower sedimentary $\delta^{13}\text{C}$ signatures relative to seagrass values, as has been reported for many seagrass sediments (Kennedy et al. 2010). This is likely due to a contribution of OM from non-seagrass sources (i.e. seston-, algal-, or terrestrial-based) in the sediments (Kennedy et al. 2010). Alternatively, fractionation of the sedimentary OC as a result of remineralisation may have occurred (McNichol et al. 1991). While fractionation is theoretically possible, it had been shown that changes in carbon isotopic abundances due to diagenetic processes were negligible in sediments (Fogel and Cifuentes 1993, Hayes 1993). Studies addressing diagenetic changes in isotopic signatures of seagrass organic matter during decomposition are based on short term (yearly or monthly basis) periods (e.g. Fourqurean and Schrlau 2003). A change in $\delta^{13}\text{C}$ values of sedimentary organic matter with age had been shown in other studies to be small or negligible (Galimov et al. 1995; Fogel and Cifuentes 1993, and Hayes 1993). Therefore it was assumed that there were negligible variation in the $\delta^{13}\text{C}$ values of seagrass-derived OC buried in the sediments of Oyster Harbour after millennia of burial.

The relative consistency in sediment $\delta^{13}\text{C}$ values throughout the *H. ovalis* cores (-17‰ to -21‰) indicates consistency in the sources of OM to the sediment over the accumulation period, assuming minimal fractionation during diagenesis (Fogel and Cifuentes 1993, Hayes 1993).

This contrasts the findings for *P. australis*, where there were differences in sediment $\delta^{13}\text{C}$ values between meadows and vertically through the sediment profiles. The source of this

apparently higher variability in the source of OC cannot be definitively identified from this study, though a number of site-specific factors could be contributing, as discussed below.

In Botany Bay, surface sediments (top 30 cm, ~500 Cal yr BP) had lower $\delta^{13}\text{C}$ values (minimum of -19.6‰) than the other two meadows (minimum of -17.1‰). This may relate to the expansion of European settlement in Sydney from the early nineteenth century and the subsequent eutrophication of the estuary (Mitchell and Adam 2009, Larkum and West 1990). Greater algal inputs into the sediments, as a consequence of this eutrophication (e.g. Macreadie et al. 2012, Bratton et al. 2003), may explain the lower $\delta^{13}\text{C}$ values occurring within the past 200 Cal yr BP. Mixing of the surface sediments could account for the apparently earlier onset (i.e. 300-500 Cal yr BP) of the eutrophication (Blaauw et al. 2007), as could the uncertainties associated with dating modern samples with radiocarbon techniques (Griffith et al. 2010). Eutrophication has also occurred in Oyster Harbour in the 1980s, though a similar $\delta^{13}\text{C}$ signal was not observed in those sediments. This may be because of the much more recent onset of eutrophication in that system and the subsequent rehabilitation of the estuary (Cambridge et al. 2002, Bastyan 1986).

In Waychinicup Inlet, the vertical variation in $\delta^{13}\text{C}$ values through the core stratigraphy was the most pronounced of all *Posidonia* meadows, with oscillating shifts in $\delta^{13}\text{C}$ values, varying as much as 8‰. Waychinicup Inlet Estuary is a relatively pristine meadow, isolated from major human settlements (Western Australian Planning Commission 2007) and so eutrophication is unlikely to explain these variations. Importantly, there was a large difference in the sediment age at 74 cm for two sampled cores, one being dated at 1322 Cal yr BP and the other at 307 Cal yr BP. This inconsistency indicates that major reworking of the sediment may have occurred (e.g. Colman et al. 2002). A major but as yet unknown disturbance, possibly a large flood, plausibly explains the discrepancy in ages among cores and the variations in $\delta^{13}\text{C}$ values. Such events are common in estuaries and coastal lagoons leading to sediment scouring (e.g. Cooper 2002), which would simultaneously remove seagrass sediments and introduced allochthonous OM, resulting in shifts in $\delta^{13}\text{C}$ values.

4.5 Conclusion

This study had demonstrated that OC stores in seagrass meadows is indeed a function of the species composition but trends in OC stores can be modified by site-specific environmental variables. Results of the current study are consistent with the hypothesis that there are less OC stores in meadows of a seagrass species with lower biomass and higher turnover rates relative to a species with high biomass and low turnover rates. The species chosen for this study are positioned at the extreme ends of Carruthers et al.'s (2007) model, and present the most polarised differences in biological characteristics among seagrass species. An exploration of the species factor on OC stores using species at intermediate positions along the Carruthers et al. (2007) model may well yield OC stores intermediate to those values presented in this study, and less significant differences among species. However, site-specific abiotic modifiers upon seagrass meadows prevents a simple generalisable view of OC stores based on species composition alone. Inevitably, interactive effects between the species and the abiotic environment upon OC stores will occur. The implication in accounting for OC stores is that both the species and its abiotic environment need to be considered since meadow-specific determinants drives further variation in estimates.

CHAPTER 5

PRESERVATION OF ORGANIC CARBON IN A *POSIDONIA AUSTRALIS* MEADOW

Abstract

The high organic carbon (OC) stores in seagrass meadows have led to their recognition as significant Blue Carbon sinks, though the diagenetic conditions that enable OC retention in seagrass sediments remain unclear. In this study, seagrass sediments were sampled from a *Posidonia australis* meadow in Oyster Harbour (Albany; south-western Australia) to investigate the preservation of sedimentary OC. We analysed sediment characteristics (colour, grain size and redox potential), radiocarbon age, and characterised the organic matter (OM) using solid state CP/MAS ^{13}C NMR spectroscopy to examine the preservation of OM down the sediment profile. There was minimal change in organic composition over 1900 years of accumulation, indicating long-term OM preservation. Primarily, this preservation appears to be driven by the recalcitrance of seagrass detritus buried in the deeper, anoxic sediments. The majority (70-83%) of total sedimentary OM comprised components directly attributable to seagrass origins (lignin, carbohydrate and a black carbon-like matter), while the remainder consisted mostly of protein, some of which may have been present in seagrass biomass, along with likely contributions from algae and/or microbes. The chronostratigraphic content increase of a biochemical component identified as black carbon-like matter suggests its selective preservation. Carbohydrate significantly decreased with age and depth (i.e. it appeared to be selectively decomposed), while lignin and protein did not show any

quantitative changes. The persistence of these three components is consistent with non-selective preservation. The findings demonstrate the exceptional preservation of seagrass-derived OC that lead to its long-term storage and retention in the seagrass sediments.

5.1 Introduction

The organic carbon (OC) sequestration capacity of seagrass meadows has led to their recognition as important 'Blue Carbon' sinks (Nellemann et al. 2009). In the meadows, sequestration of OC in sediments results from a combination of factors, including the high productivity of seagrass meadows (Cebrian and Duarte 1995), the large supply of seagrass detritus coupled with its low degradation rate (Harrison 1989), and the capacity of seagrass canopies to trap and retain organic particles (Hendriks et al. 2010, Peralta et al. 2008). The burial of organic matter (OM) in these habitats results in a net accumulation of OC that can persist in the sediment over millennial timescales (Mateo et al. 2010, Mateo et al. 2006). Organic carbon stores in seagrass meadows can be as high as 115-829 Mg OC ha⁻¹, significantly broader than the range of 150-200 Mg OC ha⁻¹ reported for soils of terrestrial forests (Fourqurean et al. 2012a). A major factor contributing to the high OC sequestration in seagrass meadows is the high degree of OM preservation (Pedersen et al. 2011). However, the biogeochemical processes in seagrass sediments that lead to large OC stores remain poorly understood.

The concentration of OM in seagrass sediments is a function of physical, biological, and chemical factors. Physical factors primarily relate to geomorphology of the environment in which seagrass meadows occur. Seagrasses typically inhabit sheltered areas (Carruthers et al. 2007) whose depositional nature is conducive for the retention and burial of both autochthonously produced OM and imported allochthonous OM (Kennedy et al. 2010). Furthermore, the predominance of fine sediments reduces oxygen exchange and results in low sediment redox potentials just below the sediment surface (Dauwe et al. 2001, Argese et

al. 1992). Anoxic sediments are known to enhance OM preservation, mainly because aerobic bacterial respiration, and therefore OM consumption, is greatly reduced (Burdige 2007, Wakeham and Canuel 2006). Biological factors influence the amount and type of OM that is available for burial. Seagrasses are generally highly productive (Mateo et al. 2006), though meadow cover and the compartmentalisation of the productivity to below-ground organs varies (Bell et al. 2006, Duarte 1991). This suggests that there may be differences in the quantity and quality of OM supplied for burial among seagrass meadows. There are also variations in the proportions of seagrass-derived and non-seagrass-derived OM buried in the sediment (Kennedy et al. 2010). This may be important to OM preservation because seagrasses contain relatively high amounts of degradation-resistant organic compounds in their tissues (Mateo et al. 2006), often referred to as lignic components (e.g. Torbatinejad et al. 2007, Klap et al. 2000), compared to other potential OM sources. Species of *Posidonia* have relatively high tissue concentrations of lignic compounds while *Zostera marina* contains cutans and tannins, which have similar preservation potential to lignin (Tegelaar et al. 1989). In contrast, non-seagrass-derived OM, consisting of seston and algae deposited in seagrass sediments contains more labile biomolecules that undergo more rapid microbial cycling and remineralisation during early diagenesis (Laursen et al. 1996).

Conformational biochemical changes occur after OM deposition and burial due to a number of diagenetic processes in marine sediments. Organic matter in surficial sediments is usually more reactive than OM in deeper sediments due to greater exposure to physical processes and microbial degradation, which leads to high decomposition rates (Henrichs 1992). However, a fraction of the OM escapes early diagenesis due to its recalcitrant nature (Burdige 2007). Upon deeper sediment burial, decomposition under anoxic conditions may occur but is limited by slow rates of microbial activity (Wakeham and Canuel 2006). The OM that survives early diagenesis can be further preserved through processes such as vulcanization, condensation and/or geopolymerization with or without bacterial influence (Killops and Killops 2005, Hedges et al. 2000, Tegelaar et al. 1989, Hedges 1988). For detrital matter

originating from recalcitrant plant matter, selective preservation and mineral shielding are thought to be the major processes leading to OM preservation (Hedges et al. 2001, Briggs 1999). These diagenetic processes may also work together rather than as distinct processes (Burdige 2007). As such, considerable difficulty arises in elucidating individual processes responsible for OM preservation.

Identifying changes in the biochemical forms of OM through millenary sedimentary deposits in seagrass sediments may help elucidate diagenetic mechanisms responsible for its preservation. However, structural elucidation of buried OM is made difficult by the heterogeneity of its biochemical constituents. At present, there are no direct or standardised approaches to characterise the molecular biochemical components of buried OM (Burdige 2007). Characterisation is further hampered because the resolution provided through conventional analysis (such as hydrolysis, solvent extraction or chromatographic methods) is insufficient to decipher intricate bonding linkages of the various compounds that occur within the OM matrix (Burdige 2007). In addition, conventional analytical methods do not provide quantitative detection of all OM naturally present in samples and can suffer from the synthesis of artefacts during procedural steps (Nelson and Baldock 2005a). These conventional methods are more applicable as biomarker techniques that identify specific compounds or components to a high degree of resolution, rather than for bulk sediment characterisation (Kögel-Knabner 2000). On the other hand, methods have been developed employing solid-state ^{13}C nuclear magnetic resonance with cross polarisation/magic angle spinning analysis (^{13}C NMR CP/MAS; Nelson and Baldock 2005, Baldock et al. 2004) for biochemical characterisation of OM from fresh plant matter as well as marine sediments (e.g. Krull et al. 2009, Dickens et al. 2006, Benner et al. 1990). These methods are limited to detection of major biochemical components rather than specific molecules (Nelson and Baldock 2005a). Despite this limitation, the more exhaustive and quantitative nature of this approach compared to conventional analytical methods (Conte et al. 2004) could provide insights on the stability and sources of persistent forms of OM in marine sediments.

Posidonia australis meadows contain significant quantities of OC, ranking among the highest of all seagrasses (Lavery et al. 2013). Bulk characterisation of living and detrital *P. australis* tissues confirmed the presence of recalcitrant OM (i.e. lignic matter; Torbatinejad et al. 2007). However, such characterisation has not been carried out on *P. australis* sediments, which accumulate over millennia. Occurrences of buried fibre-like detrital matter in the seagrass meadows of Oyster Harbour (southern Western Australia) have been consistently observed over recent decades (e.g. Marbà et al. 2015, Hindell et al. 2004, Bastyan 1986, McKenzie 1962) and to depths of 150-200 cm with ages of several thousands of years (Rozaimi et al. *submitted*). However, the biochemical structural composition of the OM, and the extent of changes through depth/aging after burial (i.e. the preservation mechanisms) remains unknown.

In this paper, we investigate the extent of OM preservation in a *Posidonia australis* meadow by studying the characteristics and changes with aging of the OM stored in seagrass sediments at Oyster Harbour. In this regard, we studied the link between the chronostratigraphic profile and sediment biogeochemical attributes. This approach can provide new insights into the pathway for OM preservation beneath seagrass meadows that triggers the accumulation of Blue Carbon in these ecosystems.

5.2 Materials and methods

5.2.1 Study site

Oyster Harbour is a naturally protected estuary with freshwater inputs from the Kalgan and King Rivers in the north and marine exchange through a narrow channel in the south (Figure 5.1). It is a marine-dominated estuary with water depth reaching 12 m (D'Adamo 1991). Seagrasses have been recorded growing to maximum depths of 5-8 m (Hodgkin and Clark 1990, McKenzie 1962). The sediments are medium-coarse to fine grained silty sands together

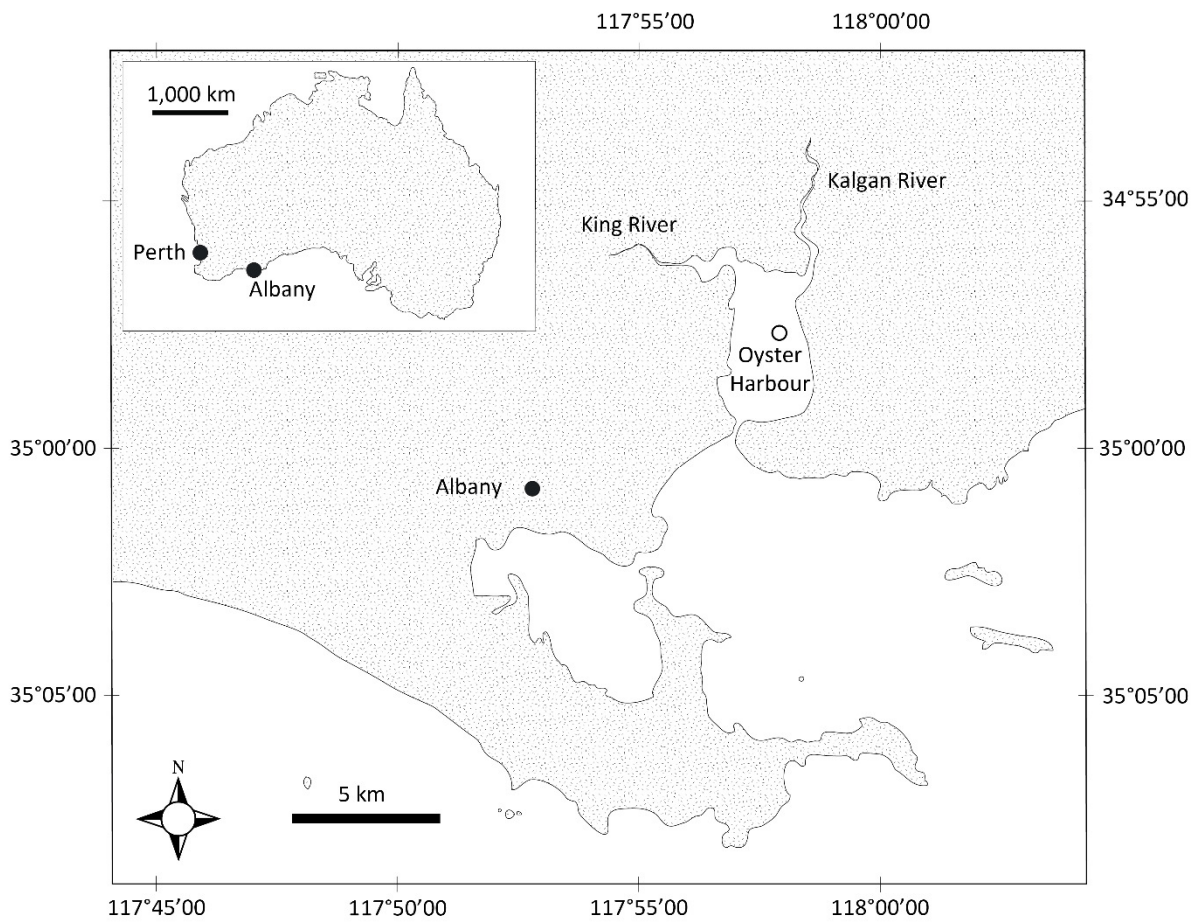


Figure 5.1. Location of the study site at Oyster Harbour (Albany, south-western Australia). The open circle denotes the sampling site within the estuary.

with biogenic carbonates (McKenzie 1962). In winter months, fine sediments enter the estuary from the rivers following heavy rain. For most of the year, intermittent river flows result in typical marine salinities (35 ppt) within the estuary (D'Adamo 1991). The estuary has had a continuous presence of *Posidonia australis* meadows since the 1960s but had lost significant cover between the 1960s to the 1980s (Bastyan 1986, McKenzie 1962). Nonetheless, *P. australis* has likely been present in the system over several millennia (Rozaimi et al. *submitted*).

5.2.2 Coring and core processing

In May 2012, a sediment core was taken from a large mono-specific *Posidonia australis* meadow located on the eastern margin of the estuary (S 34°58'58.0" E 117°58'29.9"; water depth of 1.5-2.0 m). A pre-drilled PVC pipe (89 mm outer diameter) with 10 transverse sampling ports (after Fourqurean et al. 2012b) along the coring barrel was hammered manually into the seafloor. The ports were sealed with PVC tape both on the inner and outer walls to prevent sample loss and alteration of sediment integrity during coring and core extraction. The core barrel was sharpened at the distal end to assist with penetration. Within ten minutes of core retrieval, the PVC tape covering each sampling port was removed sequentially from the top to the distal core end to measure redox potential by inserting a platinum electrode (WTW SenTix ORP) into each sampling port. Following that, a 50 ml tube (inner diameter of 30 mm) was inserted into the ports to sample the sediment.

Compression of unconsolidated sediment during coring is an inevitable phenomenon and resulted in the recovery of a 100 cm core out of the 150 cm core barrel used (33% compression). By linear proportions, the core was decompressed to occupy the length of the core barrel embedded within the sampling point and thus providing the corrected core length (Serrano et al. 2012, Glew et al. 2001), which was 150 cm. The 10 sampling ports corresponded to the following corrected depths for the sampled sediment layers: 2-4 cm, 7-9 cm, 12-14 cm, 17-19cm, 22-24 cm, 28-30 cm, 34-36 cm, 51-53 cm, 91-93 cm, and 131-133 cm. All variables relating to the stratigraphy of the sediment core were plotted against these corrected core lengths.

In the laboratory, the bulk sediment samples were oven-dried at 60°C until constant weight was attained and divided by quartering for subsequent analyses. Two sets of sub-samples were used for sediment grain size and ^{14}C analysis. The other sets were ground using a mortar and pestle to a fine powder. The powdered sub-samples were then separately pre-treated and used for the analysis of bulk sediment organic characteristics (i.e. OM, OC and N contents, $\delta^{13}\text{C}$ signatures and NMR spectroscopy).

5.2.3 *Sediment chronostratigraphy*

Plant fibres were identified and isolated from the 22-24 cm, 51-53 cm and 131-133 cm layers for dating by ^{14}C accelerator mass spectrometry (AMS). These samples were rinsed in ultrapure MilliQ water, placed in a sonic bath (5 min) to dislodge any attached sediment particles and inspected under a microscope to ensure minimal presence of extraneous particulate contaminants in the samples. The cleaned samples were then sent to DirectAMS (Seattle; USA) for a further acid-base-acid treatment prior to ^{14}C analysis (Brock et al. 2010) and subsequent ^{14}C measurements. Radiocarbon data were calibrated to years before present (cal yr BP, www.radiocarbon.org/IntCal09.htm; present taken as AD 2012) and corrected for the marine reservoir effect by subtracting 71 yr from the uncorrected radiocarbon ages (Ulm 2006). These radiocarbon dates were then plotted as a best-fit smooth-spline function using CLAM.R software (Blaauw 2010) to produce the chrono-stratigraphic model (Figure 5.2).

5.2.4 *Sediment grain size analysis*

Sediment aliquots from the 10 samples were digested with hydrogen peroxide to remove all OM. The samples were then subjected to ultrasonic agitation in a sodium polyphosphate solution as a dispersing agent and subsequently introduced to a Mastersizer particle analyser (Malvern) at the Centro de Estudios Avanzados de Blanes (Centre for Advanced Studies of Blanes) for granulometric analysis. Results are provided as the proportion of sediment size fractions (in %) across 5 category ranges (< 63 μm , 63-250 μm , 125-250 μm , 250-500 μm , 500- 2000 μm , and > 2000 μm fractions).

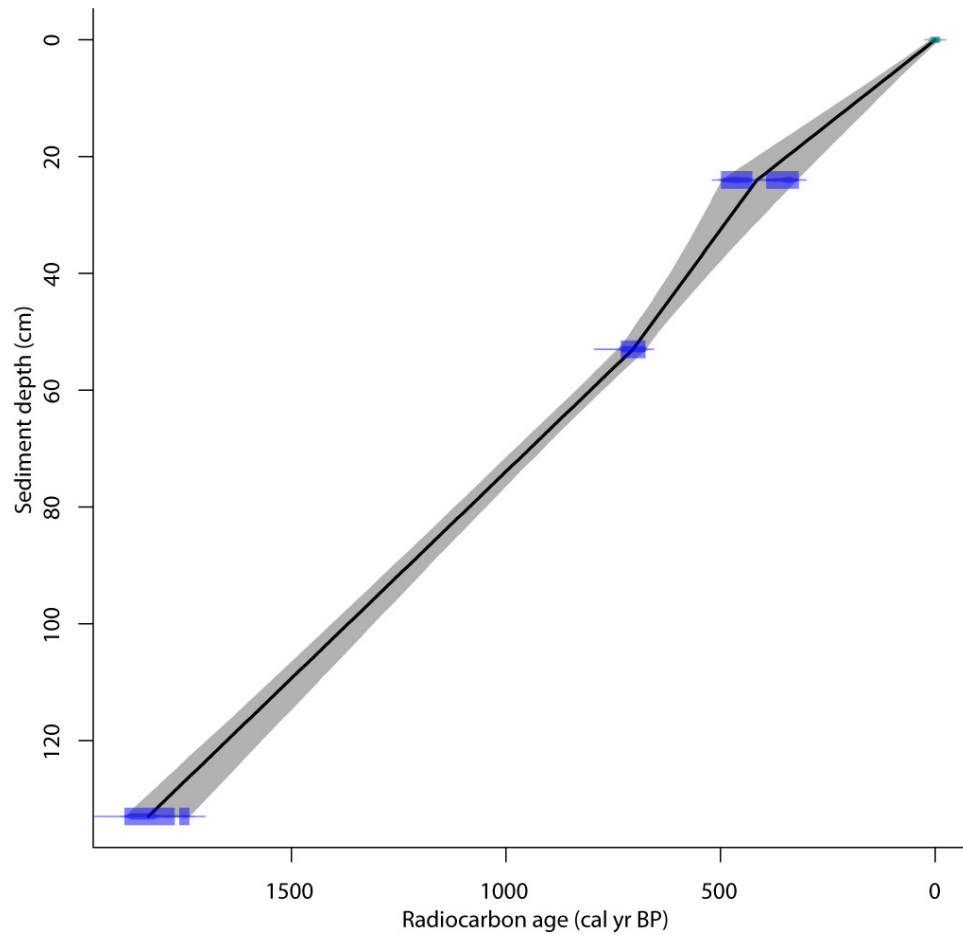


Figure 5.2. Chronostratigraphic framework for the Oyster Harbour *Posidonia australis* core constructed using CLAM.R software (Blaauw 2010). The solid line represents a best fit (smooth-spline model) using three radiocarbon dates calibrated and corrected for the reservoir effect. The age-depth relationship is consistent with an average sediment accretion rate of $0.076 \pm 0.012 \text{ cm yr}^{-1}$ or $13.8 \pm 2.11 \text{ yr cm}^{-1}$ (mean \pm SE).

5.2.5 *Organic matter, organic carbon, and $\delta^{13}\text{C}$ analyses*

For each sediment sample, a ground sub-sample was combusted in a muffle furnace at 550°C for 4 hours to obtain the weight loss on ignition (Heiri et al. 2001), used here as a measure of OM content. Another sub-sample was used for OC and N content, and $\delta^{13}\text{C}$ analysis. To remove all inorganic carbon, 0.5 g of ground sample was acidified in 1 M hydrochloric acid. When effervescence ceased, after 12-18 h, the mixture was centrifuged (3400 revolutions min^{-1} for 4.5 min) and the acid removed by pipette. Deionised water (10 ml) was added to wash the sample of residual acid, the mixture centrifuged and the supernatant removed by pipette. Each acidified sample was then oven-dried and weighed. Then, 9-10 mg of acidified sample was encapsulated in a tin capsule and combusted in a continuous flow isotope ratio mass spectrometer analyser (PDZ Europa: Sercon) at the UC Davis Stable Isotope Laboratory, yielding the sample OC and N content (in %), and $\delta^{13}\text{C}$ value. The OC and N contents reported by the analytical facility were corrected for OC and N contents in pre-acidified bulk sediment samples. $\delta^{13}\text{C}$ values are reported relative to the Vienna Pee Dee Belemnite (VPDB) standard.

5.2.6 *^{13}C NMR spectroscopy*

Prior to NMR analysis, the sediment samples were pre-treated based on a modification of methods outlined by Skjemstad et al. (1994) and Schmidt et al. (1997). The pre-treatment aimed to digest the mineral fraction and concentrate the OM fraction, thereby improving NMR sensitivity (Skjemstad et al. 1994). In addition, the pre-treatment removed sedimentary paramagnetic constituents, which would otherwise decrease signal intensity and peak resolution (Smernik and Oades 2000, 1999, Skjemstad et al. 1994). Ground sediment samples (5 g) were placed in centrifuge tubes and 50 ml of 10% (v/v) hydrofluoric acid was added. This mixture was shaken end over end for 5 h. After standing for a further 12 h, samples were centrifuged (2000 revolutions min^{-1} for 20 min). The supernatant was discarded through a Millipore 5 μm Durapore membrane filter that retained any light sediment fraction. Another

cycle of this pre-treatment step was performed with the residue subsequently washed three times with 50 ml of Milli-Q water. Washed samples were combined with any light fraction obtained through the membrane filter and dried at 75°C.

Solid-state ^{13}C cross polarization (CP) NMR spectra were acquired with magic angle spinning (MAS) at a ^{13}C frequency of 50.33 MHz on a Bruker 200 Avance spectrometer. Samples were packed in a 7 mm diameter cylindrical zirconia rotor with Kel-F end-caps, and spun at 5 kHz. Spectra were acquired using a ramped-amplitude cross polarization (CP-ramp) pulse sequence, in which the ^1H spin lock power was varied linearly during the contact time. A 1-ms contact time and a 1-s recycle delay were used and 4000-20000 transients were collected for each spectrum. All spectra were processed with a 50 Hz Lorentzian line broadening. Chemical shifts were externally referenced to the methyl resonance of hexamethylbenzene at 17.36 ppm.

All spectral processing was completed using Bruker TopSpin 3 software. Empty rotor background signals were subtracted and the resultant spectra were integrated across the following chemical shift limits to provide estimates of broad carbon types after Baldock and Smernik (2002), i.e. amide/carbonyl (210–165 ppm), O-aryl (165–145 ppm), aryl (145–110 ppm), di-O-alkyl (110–95 ppm), O-alkyl (95–60 ppm), *N*-alkyl/methoxyl (60–45 ppm), and alkyl (45–10 ppm). The proportional detections of each region (in %) were then used in a molecular mixing model from eight equations to assign the detections into six components of organic biochemical families (i.e. carbohydrate, lignin, protein, lipid, carbonyl and black carbon [BC]; after Nelson and Baldock 2005a, Baldock et al. 2004). The assignments provided the proportion of the six components as relative abundances (in %) within each analysed sample.

5.3 Results and discussion

5.3.1 Physical attributes and chronostratigraphy of *P. australis* sediments

Visual observations showed the existence of seagrass mat along the core sample. Seagrass mats (after Boudouresque and Meinesz 1982) are an agglomeration of below-ground seagrass detritus (i.e. rhizome, sheaths and roots) and biogenic carbonates buried within a sediment matrix. Samples from the top 14 cm of the sediment had live *Posidonia australis* below-ground organs (i.e. roots, leaf sheaths, and rhizome tissues). The sediment colour within the 24 cm depth-layer was greyish- brown (Gley1 4/N, after Munsell 2000; Table 5.1). Below this depth, localised dense agglutination of coarse strands of plant matter, likely to be *P. australis* fibres, existed throughout all samples. Whole and broken shells were dispersed within these agglutinations. Sediment colour progressively changed from lighter brown (10YR 6/3) to a consistent brown (10 YR 6/4) in the deepest sample (131-133 cm layer). These physical attributes are visual indicators of continued presence of seagrass meadows over the period reconstructed, with OM persisting over millennial timescales (see below).

The colour of the seagrass mat (i.e. greyish-brown to consistent brown) indicates the presence of reduced iron (Giosan et al. 2002, Phillips and Marion 2001), and thus the presence of a reducing environment that is favourable for OM preservation (DeLaune and Reddy 2005). Direct visual observations of sediment colour has been utilised as a rapid assessment informing the primary mineralogy and diagenetic changes with depth (e.g. Giosan et al. 2002). The diffuse changes in sediment colour across core stratigraphy rather than distinct laminations indicates the stability of the sediment bed, which is consistent with the retention of fibres and sediments within seagrass meadows (Serrano et al. 2012, Gacia and Duarte 2001).

The mineral matrix within the mat sequence had a predominance of fine sediment particles (i.e. grain sizes < 250 µm). This sediment fraction accounted for $42.9 \pm 2.86\%$ (Mean \pm SE) of the total bulk composition (Table 5.1). McKenzie (1962) reported that the sediment of the

Table 5.1: Sediment physical characteristics (colour, redox potential and grain size fraction) and nitrogen content. Ages marked with (*) correspond to samples dated by AMS ^{14}C ; all other sample ages are estimates modelled through Clam.R software (Blaauw 2010). The codes in parentheses refer to the corresponding Munsell Colour Chart classifications (Munsell 2000).

	Depth layer (cm)	Estimated age (cal yr BP)	Sediment colour	Redox (mV)	N (%)	Sediment grain size fraction (%)				
						< 63 μm (silt and clay)	63-250 μm (fine sand)	250-500 μm (medium sand)	500-2000 μm (coarse sand)	> 2000 μm (gravel)
125	2-4	69	Greyish-brown (Gley1 4/N)	-25	0.26	5.67	50.29	17.48	13.53	13.04
	7-9	155	Greyish-brown (Gley1 4/N)	-100	0.19	4.63	47.72	16.19	9.6	21.87
	12-14	242	Greyish-brown (Gley1 4/N)	-228	0.15	3.57	34.38	23.26	18.82	19.97
	17-19	328	Greyish-brown (Gley1 4/N)	-336	0.17	6.91	42.68	17.96	11.29	21.16
	22-24	357*	Greyish-brown (Gley1 4/N)	-378	0.22	4.5	38.15	19.67	14.81	22.87
	28-30	475	Lighter brown (10YR 6/3)	-426	0.15	1.76	23.45	18.18	21.08	35.53
	34-36	535	Lighter brown (10YR 6/3)	-522	0.20	7.14	35.88	22.57	20.01	14.4
	51-53	767*	Consistent brown (10 YR 6/4)	-572	0.18	4.13	41.94	28.43	18.57	6.93
	91-93	1269	Consistent brown (10 YR 6/4)	-505	0.15	3.23	30.37	22.11	16.49	27.79
	131-133	1899*	Consistent brown (10 YR 6/4)	-520	0.21	3.55	39.55	25.91	19.5	11.5

estuary was a composite of terrigenous and organogenic components – the former transported from the catchment into Oyster Harbour as fine inorganic matter. The prevalence of fine sediment is consistent with the sheltered nature of the estuarine environment and with the high retention capacity of fine-grained particles by seagrasses (Hendriks et al. 2010, Peralta et al. 2008). Furthermore, the seagrass canopy and the mat that underlies the meadow provides a positive feedback mechanism for enhanced sediment stabilisation while reducing erosion and increasing sedimentation (Le Hir et al. 2007, De Falco et al. 2000).

Redox potentials were negative throughout the sediment sequence, though samples in the top 9 cm layer were comparatively less reducing (-25 to -100 mV; Table 5.1) than the samples at greater depth (-228 mV at the 12-14 cm layer, and further decreasing to *ca.* -500 mV towards the distal core end). Although seagrass rhizosphere sediments are known to have higher redox potentials due to the physiological transport of oxygen towards below-ground organs (Marba et al. 2010), the anchoring depth of *P. australis* rhizomes results in its proliferation in anoxic sediments. Any available oxygen due to physiological activities may have been readily taken up by sediment microbes (Koho et al. 2013) resulting in the observed redox potentials. In addition, the high abundance of fine sediments is conducive to negative redox potentials; the greater tortuosity of the interstitial spaces in fine sediments can reduce oxygenation resulting in the onset of anoxia in the first few centimetres below the sediment surface (Dauwe et al. 2001, Argese et al. 1992). Anaerobic processes begin to occur in moderately reducing conditions around +300 mV and are especially pronounced in highly reducing conditions below -200 mV (DeLaune and Reddy 2005). In the *Posidonia australis* mat, burial in sub-surficial sediment (i.e. within the top 10 cm layer) would promote OM preservation due to the onset of moderate reducing conditions at these depths. Preservation would be further enhanced by the highly reducing sediment environment at greater depths (e.g. Hedges and Keil 1995, Harrison 1989).

The deepest sample (i.e. at 131-133 cm layer) had an age of 1899 ± 26 Cal yr BP (Table 5.1 and Figure 5.2). From the ^{14}C dating, there was no apparent mixing in the sediment, which

would result in the absence of discernible trends with depth. The chronostratigraphic relationship did not vary markedly along the core sequence, indicating that sediment accretion rates in the meadow have not varied dramatically over 1900 years of accumulation (Figure 5.2, mean \pm SE: 0.076 ± 0.012 cm yr⁻¹). The presence of coarse seagrass debris in the deepest sample indicates that seagrass meadows have been present in the area for at least 1900 years and likely much longer (Rozaimi et al. *submitted*). Seagrass mats can have an age of up to 6000 Cal yr BP (Lo Iacono et al. 2008) and further diagenesis may occur within the mat with aging. Since age is an important factor in diagenetic processing and the subsequent preservation of OC (e.g. Kusch et al. 2010, Blair et al. 2003, Meyers 1994), the inferences for OM preservation drawn from the age in the studied *Posidonia australis* mat should be limited to the sampled ages/depth and different preservation potentials may hold for older seagrass sediment deposits.

5.3.2 Sediment organic content and $\delta^{13}C$ values

Organic matter content in the sediment ranged from 8.6% to 22.3% while OC content was 3.0% to 6.8% (Figure 5.3). The highest OC content was recorded for the 2-4 cm sediment layer (6.8% OC) while the mean was $4.0 \pm 0.3\%$ OC at depths greater than this layer. There was no significant association between age and either OM or OC content (Pearson correlation, $p > 0.05$, $R^2 < 0.001$ in both cases). The OC/OM ratios ranged from 2.8 to 3.3 indicating similar carbon richness of the OM with depth (Figure 5.3). The sediment layer at 2-4 cm contained the largest amounts of OM presumably because it was the most recently deposited (70 cal yr BP) and contained both autochthonous and allochthonous inputs (Marbà et al. 2015). Surficial sediment layers may contain larger amounts of labile OM compared to deeper sediments. These layers are typically less reducing than deeper sediments (Table 5.1) and remineralisation of the more labile OM compounds may range from 30-99% during early diagenesis (Henrichs 1992). The small range in OC/OM ratio among the sediment layers suggests that after initial decomposition of the labile OM (after *ca.* 70 cal yr BP), the remainder is preserved with no major degradation with aging, which would otherwise have

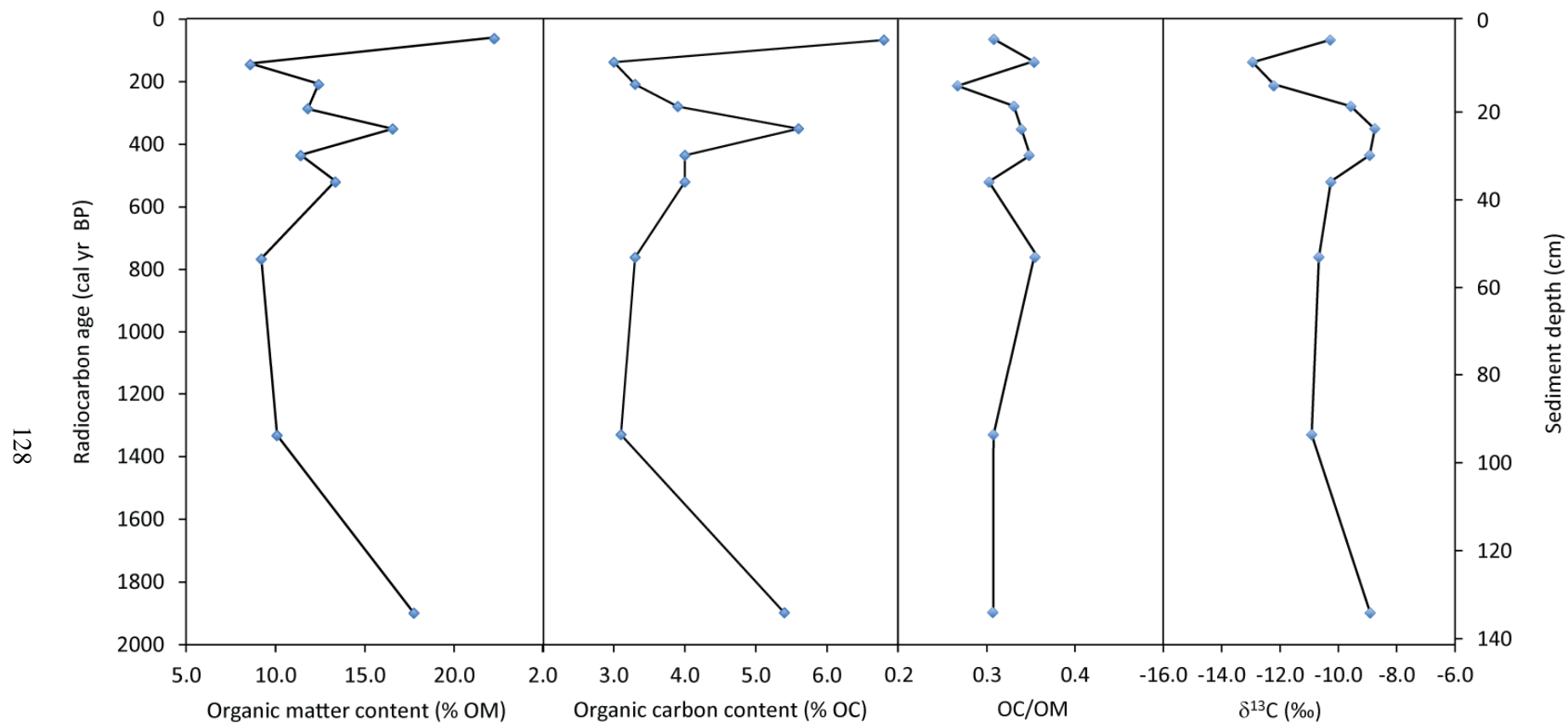


Figure 5.3. Chronostratigraphic profile of organic matter characteristics in the sampled core: (a) organic matter content; (b) organic carbon content; (c) OC/OM ratio; and (d) $\delta^{13}\text{C}$ isotope signature.

decreased the OM content and increased the carbon content of the OM (i.e. increase of OC/OM ratio; e.g. Emerson and Hedges 1988). In some seagrass sediments, the OM and OC contents decrease with depth and aging (e.g. Serrano et al. 2012, Fourqurean et al. 2012b) but in others they remain stable, resulting in high OC stores (e.g. Serrano et al. 2014). This highlights the differences in OM preservation with aging among different seagrass meadows, and the capacity to preserve significant amounts of OM in the meadows of Oyster Harbour.

The organic characteristics (i.e. %OC, N/C ratios and $\delta^{13}\text{C}$ signatures) of bulk seagrass sediments provide preliminary information on the possible source and preservation of the buried OM. Combined with the NMR results (see below), the evidence strongly suggests that *Posidonia australis* was a dominant contributor to the sediment OM pool in the meadow. *Posidonia australis* has high productivity (Marba and Walker 1999, Walker and McComb 1988) resulting in carbon-rich but nitrogen-poor tissues (Duarte 1990). Across the core sequence, nitrogen content was comparatively low (< 0.3%: Table 5.1) compared to OC content (3.0% to 6.8% OC; see above). The resultant N/C ratios in the sediment (ranging from 0.038-0.050, the corresponding range of reciprocal values for C/N ratios is 16-26) show a similarity to the N/C ratios in seagrass tissues (Figure 5.4; Supplementary Information 5.1). In contrast, C/N ratios lower than 20 indicate a contribution of sestonic- and algal-derived OM (Meyers 1994). $\delta^{13}\text{C}$ values of the bulk OM ranged from -8.94‰ to -13.08‰ (Figure 5.3), and showed no consistent changes with age (Pearson correlation, $p > 0.05$; $R^2 = 0.11$). These values are similar to those of fresh *P. australis* tissues (-9.9‰ to -11.9‰; Hyndes and Lavery 2005, Hemminga and Mateo 1996). In contrast, other sources of OM likely to be present in Oyster Harbour (i.e. seston, algae and terrestrial OM) have lower $\delta^{13}\text{C}$ values (-13‰ to -29‰; Smit et al. 2005, Hindell et al. 2004, Loneragan et al. 1997). Over the past 1900 yr of accumulation, it is plausible that there are changes in the quantity of non-seagrass OM into the sediments. However, due to the visual presence of fibres along the core profile without any clear breaks in the sediment layers and given the similarity of the sedimentary

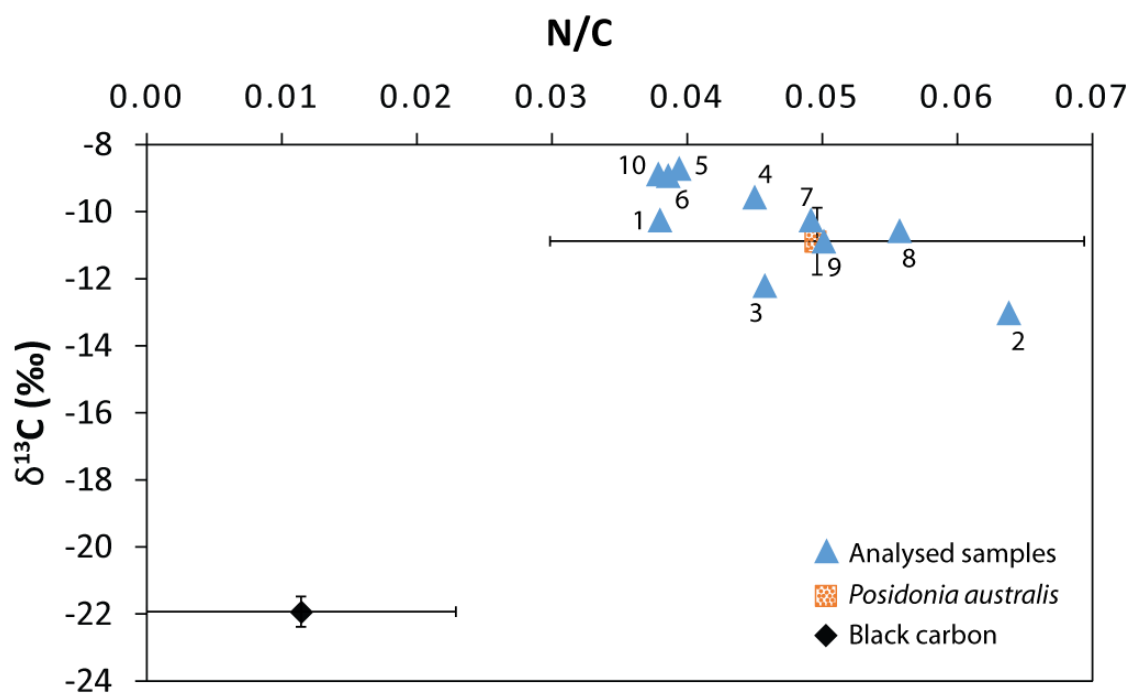


Figure 5.4: Scatterplot of N/C ratio against $\delta^{13}\text{C}$ values. Symbols with numbers are the 10 analysed samples that correspond to consecutive depth layers (1: 2-4 cm; 2: 7-9 cm; 3: 12-14 cm; 4: 17-19cm; 5: 22-24 cm; 6: 28-30 cm; 7: 34-36 cm; 8: 51-53 cm; 9: 91-93 cm; and 10: 131-133 cm). *Posidonia australis* values were taken as the average (mean \pm SE) obtained from Hyndes and Lavery (2005) , Hemminga and Mateo (1996) and Duarte (1990). Average black carbon values (mean \pm SE) were obtained from Masiello & Druffel (2003), Nelson and Baldock (2005), Yu et al. (2010) and Song et al.(2002).

OM $\delta^{13}\text{C}$ values to those of *P. australis* tissue, it is likely that seagrass-derived OM is the main source of the sedimentary OM in the core within the accumulation period.

5.3.3 ^{13}C NMR spectroscopy

The ^{13}C NMR spectra of the ten sediment samples (Figure 5.5) are remarkably similar, indicating low variation in the quality of the OM with depth and aging. Quantitative analysis of the spectra, via integration across seven broad chemical shift regions that can be assigned primarily to broad C-types, confirm this similarity (see Supplementary Information 1 for NMR integral data). The majority of the NMR signal was detected in the aryl (145-110 ppm; 21-26%) and O-alkyl (95-60 ppm; 27-38%) regions, indicative of the prevalence of lignin and carbohydrate in the OM, respectively (Figure 5.5). The NMR integral data was converted to estimates of biochemical components using the molecular mixing model of Nelson and Baldock (2005a). This analysis confirmed the generally similar composition of the sediments, but also some trends with age. In particular, the relative abundance of carbohydrate (21-40%) appeared to decrease exponentially with sediment age ($R^2 = 0.87$; Figure 5.6), the only component to show a clear declining trend. In contrast, the relative abundance of black carbon (BC) increased linearly with age ($R^2 = 0.71$) and had the second highest relative abundance (19-28%). Lignin and protein accounted for 17-25% and 9-19% of the assigned biochemical component, respectively, with no significant variation in abundance with age ($R^2 < 0.14$, $p > 0.05$). There was an almost negligible proportion of carbonyl (relative abundance $< 2\%$) and comparatively little detectable lipid (relative abundance of 3-11%) in the sediments.

The predominance of carbohydrate and lignin indicate a predominance of plant matter within the sediment OM. While carbohydrate is present in both plant and non-plant matter (Richmond 1991), the presence of lignin in sediments usually indicates a dominant contribution by terrestrial-based angiosperms (Baldock et al. 2004, Hedges et al. 1997). Lignin occurs uniquely in vascular plant tissues and is generally associated with cellulose and hemicellulose (Burdige 2007). Seagrasses are also vascular plants and represent a unique

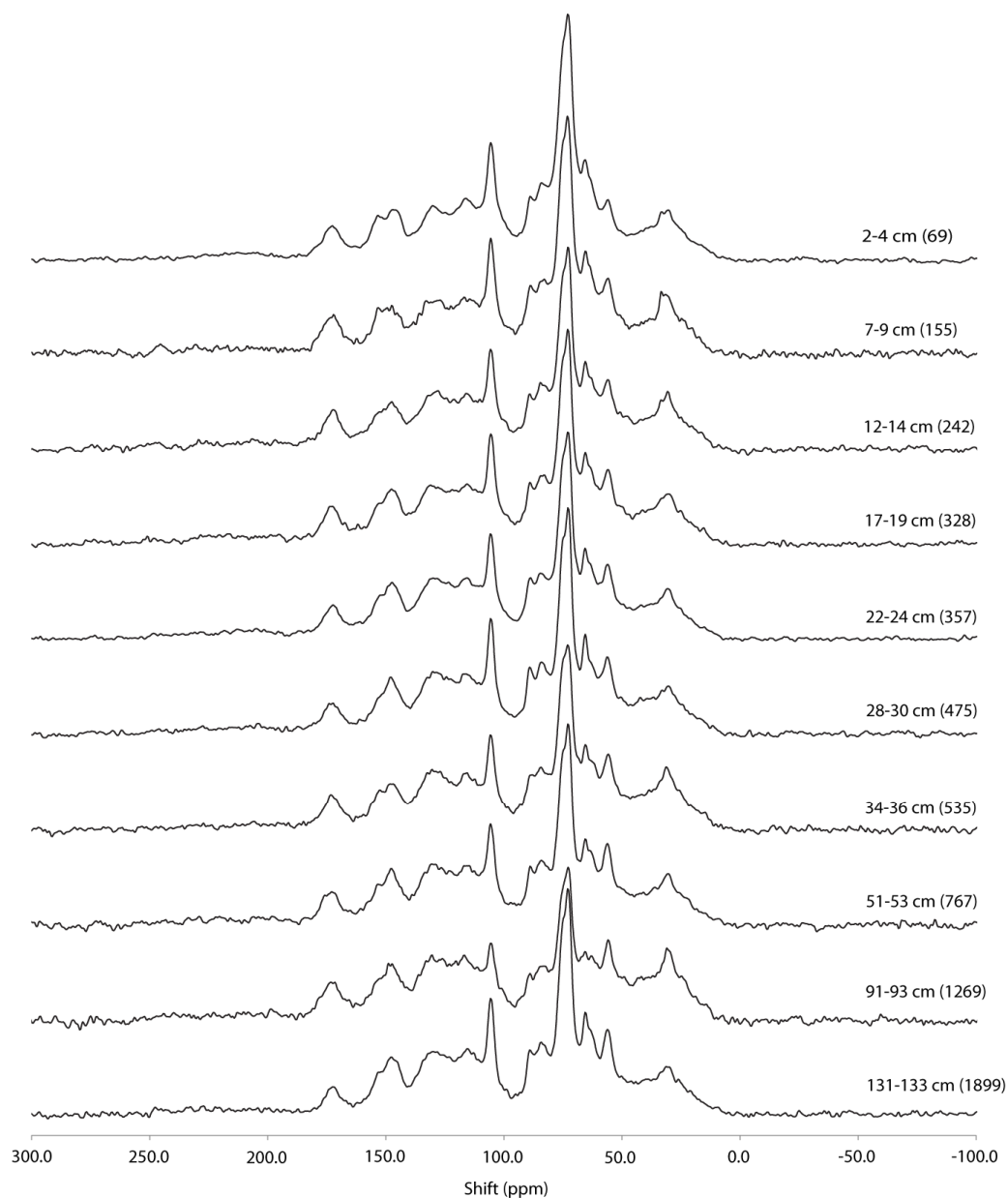


Figure 5.5. Solid-state ^{13}C nuclear magnetic resonance (NMR) with cross polarisation/magic angle spinning spectra of the ten sediment samples. Numbers in the right column indicate the sampled depth layers. Values in parentheses refer to the corresponding estimated radiocarbon age in calendar years before present (cal yr BP).

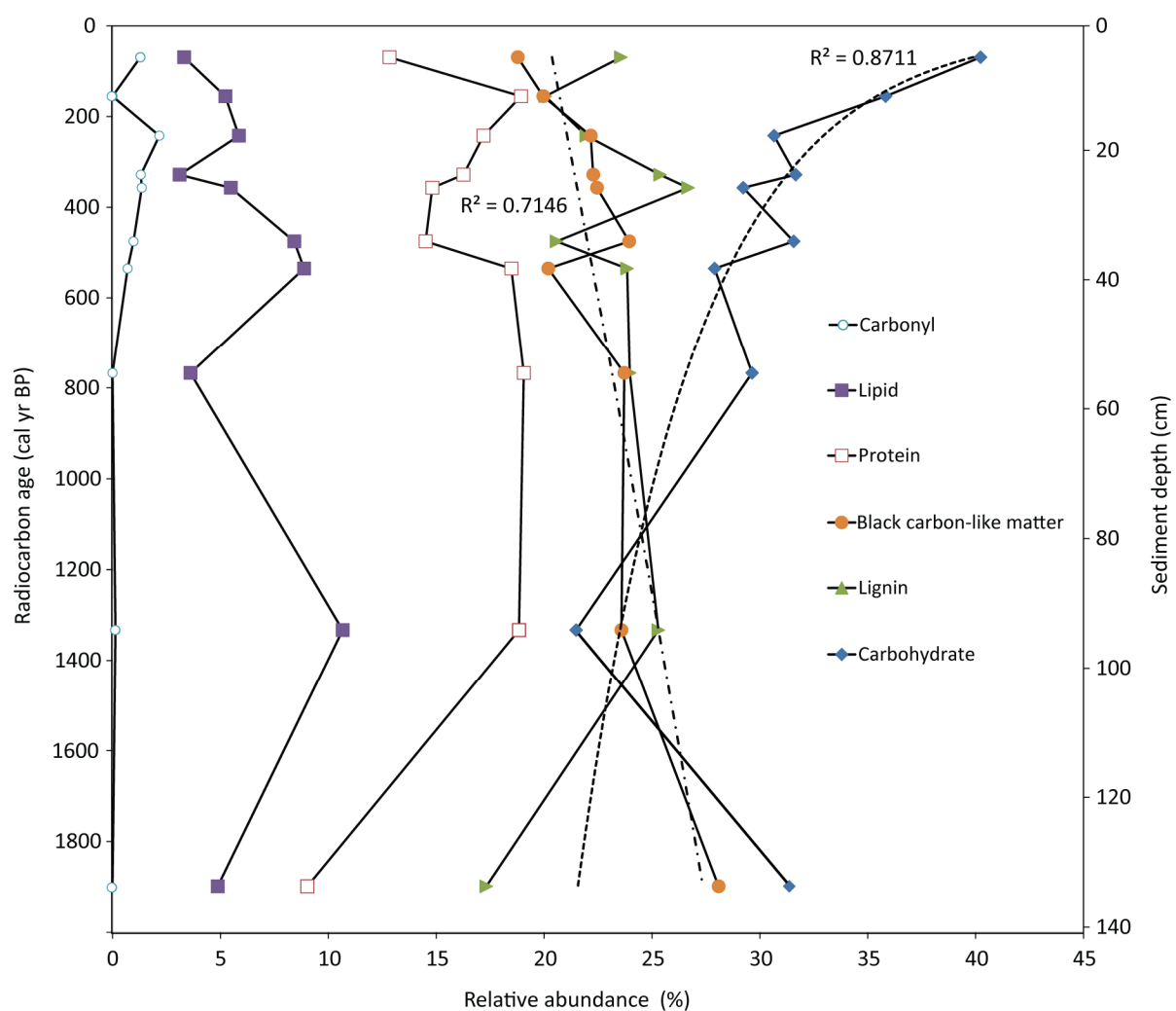


Figure 5.6. Chronostratigraphic profile of the sampled core showing relative abundances of the six biochemical components estimated from NMR spectra using the molecular mixing model of Nelson and Baldock (2005). R^2 values are only shown for carbohydrate and black carbon-like matter proportions, as a function of radiocarbon age.

exception to the rule that lignin indicates a terrestrial origin of sediment OM. *Posidonia australis* contains large amounts of lignin and carbohydrate (as cellulose) in bulk tissues and in its rhizome cells (Torbatinejad et al. 2007, Kuo and Cambridge 1978). The similarity of the sedimentary OM N/C ratios and $\delta^{13}\text{C}$ values with those of *P. australis* tissue (Figures 5.3 and 5.4; see discussion above), indicate that the lignin found in the sediment was seagrass-derived rather than allocthonously exported into the seagrass meadow.

Following burial, cellulose is comparatively more degradable than lignin (de Leeuw and Largeau 1993, Tegelaar et al. 1989) and this is consistent with the observation that the relative abundance of carbohydrate (cellulose) decreased with age compared to lignin (Figure 5.6).

Degradation of cellulose proceeds in anoxic sediments, likely due to the activities of anaerobic micro-organisms working together, but not as efficiently as occurs in aerobic systems (Leschine 1995). Low rates of decomposition and the lack of total remineralisation resulted in the persistence of cellulose in the sediment even after millennia of diagenesis; this has also been observed in terrestrial peat sediments (Bourdon et al. 2000), where anaerobic conditions similarly limit OM degradation rates. In comparison, lignin is one of the most recalcitrant biomolecules in plant tissues (Lewis and Yamamoto 1990) and burial reportedly enhances its longevity in sediments (e.g. Burdige 2007, Derenne and Largeau 2001, de Leeuw & Largeau 1993).

Although results from the the molecular mixing model analysis of the NMR data (Figure 5.6) indicate significant amounts of BC in the sediments, there is some uncertainty regarding the interpretation of its biochemical character. The occurrence of BC has never been investigated in detail for seagrass sediments although it has been observed that old detrital rhizomes in seagrass mat can have a texture similar to char (e.g. Babcock et al. 2007). In Oyster Harbour, the detrital rhizomes in deep sediment layers were a dark shade of brown-black (5YR 2/2 to 5YR 2/1) with a brittle texture that disintegrated easily (*personal observation*). Conventionally, BC is composed of diverse OM types but all originates from incomplete

combustion of fossil fuels or those OM types which may be allochthonously transported to marine systems (e.g. Coppola et al. 2014). $\delta^{13}\text{C}$ signatures of BC in naturally occurring marine sediments are lower than those of seagrass tissues, usually less than -21.50‰ (Masiello and Druffel 2003, Middelburg et al. 1999)(Masiello and Druffel 2003, Middelburg et al. 1999). Black carbon samples with high $\delta^{13}\text{C}$ signatures have been reported but these have mostly been through experimentally-induced charring of fresh OM (e.g. Bird and Ascough 2012). In addition, there is generally little or no elemental nitrogen in BC isolated from marine sediments (Nelson and Baldock 2005a, Song et al. 2002), although it is possible that there were allochthonous inputs of BC into Oyster Harbour. A plot of N/C ratios against $\delta^{13}\text{C}$ values shows the overlap of *Posidonia australis* tissue samples with the sediment samples, while the BC was far removed from this overlap (Figure 4). This suggests that the detected biochemical component was unlikely to be only BC (i.e. char, as commonly identified in terrestrial ecosystems).

It would thus appear likely that the molecular mixing model is identifying a biochemical component with a ^{13}C NMR signature similar to BC but that is not actually BC. It should be noted that other studies have reported that BC can be overestimated or mis-identified using a variety of BC quantification approaches (Glaser and Knorr 2008, Simpson and Hatcher 2004). In the case of the molecular mixing model used here, there are some plausible explanations for overestimation of BC. First, the majority of signal for both lignin and BC occur in a similar region of the NMR spectrum, i.e. in the 145-110 ppm aromatic region (Figure 5.5). Lignin produces signals centred at 130 and 150 ppm (as well as at 55 ppm) while BC produces a broad signal at approximately 130 ppm and thus there can be uncertainty in attributing aromatic signal to BC or to lignin and other non-pyrogenic aromatics (Simpson and Hatcher 2004). Degradation of lignin that results in the loss of the methoxyl group, as demonstrated by Simpson and Hatcher (2004), is likely to result in identification of this degraded lignin as BC. Alternatively, there may be a diagenetic process that produces OM with BC-like character from the buried detrital matter, as has been reported in terrestrial systems (Glaser

and Knorr 2008). A more definitive characterisation of this BC-like biochemical component and identification of the processes leading to its formation are beyond the scope of this study. However, it can be concluded that this OM component is non-pyrogenic, but condensed and aromatic in nature (after Glaser and Knorr 2008) and that it probably originates mainly from *P. australis* detritus since visual observations showed large amounts of seagrass detritus along the core, with no clear indications for the presence of BC.

The molecular mixing model analyses indicated protein comprised a maximum of 20% of OM in the samples with little variation with age and persisting in the sediment over 1900 yr (Figure 6). The presence of proteinaceous substances in marine sediments is usually attributed to planktonic inputs (Hedges et al. 1997), though protein from terrestrial sources have also been identified in seagrass sediments (i.e. glomalin; Adame et al. 2012). Seagrasses are generally nitrogen poor (1-3% dry weight; Duarte 1990) and may thus supply relatively small quantities of protein to the sediments. Other studies suggest that organic nitrogenous compounds undergo recycling by the seagrass or are consumed by microbes within the meadow (Vonk et al. 2008), with up to 8% of total sedimentary OC being bacterial biomass in *Posidonia* sediments (Danovaro et al. 1994). Notwithstanding the protein source, Burdige (2007) stated that this component may degrade in the presence or absence of oxygen during early diagenesis. The reducing conditions in the samples may encourage preservation of protein by limiting aerobic processes (Wakeham and Canuel 2006). With the onset of anoxic conditions, anaerobic microbial activity proceeds but rather than remineralising the protein, studies show that they undergo preservation due to microbial polymerisation and condensation, forming refractory proteinaceous matter (Derenne and Largeau 2001). The protein content of the *P. australis* sediment may, therefore, reflect both microbial biomass and proteinaceous by-products of microbial diagenesis. Further preservation may then be initiated by mineral shielding but *in situ* formation of these new compounds further complicates the identification of the protein source (Burdige 2007). Without further characterisation, the nature of the protein in the samples cannot be definitively described other

than accounting for bulk quantities of protein in the samples; the origins of the protein detected by the NMR analyses is, potentially, diverse.

5.3.4 Organic carbon preservation in seagrass sediments

This study provides quantitative analyses (i.e. sediment OC content and stable isotope signatures of carbon), semi-quantitative compositions (sediment biochemical component abundances) and qualitative descriptions of the abiotic conditions under which high amounts of OM retention appear to have occurred in *Posidonia australis* sediments. Multiple factors appear to have worked in concert to create a favourable setting for OM preservation in the Oyster Harbour *P. australis* meadow over at least 1900 years. The persistence of the coarse *P. australis* fibres accounts for much of the organic richness of the sediment beneath the meadow during this period of accumulation and this recalcitrant OM supplies the bulk of the OC sequestered in the studied meadow. Cumulative detection of lignin, carbohydrate and the BC-like matter (having a total relative abundance of 70-83%), taken in concert with bulk OM characteristics (i.e. N/C ratios and $\delta^{13}\text{C}$) suggested that *P. australis* matter was a major contributor to the sedimentary organic pool at Oyster Harbour. This contrasts with studies of non-seagrass sediments. Dickens et al. (2006) found that terrestrial- and planktonic-derived OM constituted the bulk of the OC in marine sediments. In other estuarine sediments of recent deposition but bereft of seagrasses, there were relatively higher proportions of lipid, protein and carbohydrate with negligible detection of lignin and BC (Krull et al. 2009). In other parts of that estuary, lignin detected was taken as evidence that the site was once vegetated by the macrophyte *Ruppia megacarpa* (Krull et al. 2009). In this study, the contribution by *P. australis*-derived OM to the sediment is significantly higher than the estimates in other seagrass meadows worldwide (~50%; Kennedy et al. 2010) demonstrating that our findings are not common to all seagrass communities. Only established meadows of certain seagrass species (e.g. *Posidonia*), which partition a comparatively high amount of production to large, below-ground organs, tend to form organic-rich seagrass mats. Even within the same seagrass species, sediment organic content may vary among meadows (Serrano et al. 2014). As such,

the carbon sequestration potential, and likely preservation processes described in this study may not necessarily be representative of all seagrass meadows.

The profiles of OM constituents in the sediment core provide insights into the processes leading to preservation of OM in *Posidonia australis* sediments, and indicate that different processes may be acting on different components of the OM. The retention of the OM over millennial scales indicated protective mechanisms enabling preservation of the seagrass detritus. One process often associated with the preservation of recalcitrant plant matter is selective preservation (e.g. Zonneveld et al. 2010, Burdige 2007). Selective preservation conventionally refers to the tendency for biomacromolecules that are intrinsically non-soluble, non-hydrolysable, and resistant to biological degradation to comprise increasing proportions of OM with increasing diagenetic maturity (de Leeuw and Largeau 1993, Tegelaar et al. 1989). Therefore, if selective preservation were occurring in the Oyster Harbour meadow, there would have been an increasing proportion of these components in the deeper sediments. The BC-like matter was the only OM component that exhibited an increase in relative abundance across the sediment chronostratigraphy (Figure 5.6), consistent with selective preservation of this highly recalcitrant component. The NMR results showed that the chronostratigraphic trends in the other biochemical components show little or no shift along the degradation gradient; this is most apparent for lignin and protein, both of which remained constant through the chronostratigraphic sequence while carbohydrate showed a highly significant decline (Figure 6). The lack of a degradation gradient essentially precludes selective preservation as the main process contributing to the OM preservation for these three biochemical components. It was more likely that these components were subject to physical protection, also termed non-selective preservation (after Hedges et al. 2001). Non-selective preservation is defined as OM that should have undergone remineralisation but remained refractory due to organic or inorganic matrices protecting them and potentially resulting in longer post-burial residence times than their chemistries would predict (Hedges et al. 2001). Such protective shielding would allow preservation of lignin, carbohydrate and protein from

physical and microbial degradation (Burdige 2007, Hedges et al. 2001) and may be an important process leading to the preservation of these components in the *P. australis* sediments.

It is concluded that two different processes of OM preservation in the seagrass meadow lead to sequestration and long-term retention of carbon in *Posidonia australis* sediments: non-selective preservation for lignin, carbohydrate and protein, and selective preservation for the BC-like matter. While carbon sequestration in seagrass sediments is inherently complex when considered in terms of carbon input, accumulation and remineralisation, this study provides some biogeochemical basis for the persistence of OM with depth and aging in the meadow. The findings are consistent with accepted processes of OC sequestration, whereby multiple diagenetic processes work in concert with physical, chemical and biological factors, resulting in OM storage and preservation. It is likely that other diagenetic mechanisms may also contribute to OM sequestration and warrant further investigations in these, and other seagrass sediments.

Supplementary Information 5.1: Characteristics of the analysed organic matter based on N/C ratio and proportion of chemical shift spectral regions from NMR C-detections. Numbers in parentheses indicate reciprocal values (i.e. C/N values).

Depth layer (cm)	N/C ratio	Proportion of biochemical component (%)						
		Amide/Carboxyl (210-165 ppm)	O-Aryl (165-145 ppm)	Aryl (145-110 ppm)	O ₂ -Alkyl (110-95 ppm)	O-Alkyl (95-60 ppm)	N-Alkyl/Methoxyl (60-45 ppm)	Alkyl (45-10 ppm)
2-4	0.0380 (26.33)	7.30	8.06	20.93	9.93	37.84	6.17	9.77
7-9	0.0638 (15.67)	7.62	7.67	21.39	8.97	36.49	7.50	10.37
12-14	0.0457 (21.86)	9.63	9.51	23.32	8.41	31.83	6.91	10.40
17-19	0.0450 (22.23)	8.55	9.08	24.05	8.87	32.83	7.19	9.44
22-24	0.0394 (25.37)	8.42	9.34	24.69	8.59	32.27	7.37	9.33
28-30	0.0386 (25.91)	7.96	8.41	24.09	8.55	33.61	7.53	9.84
34-36	0.0492 (20.34)	8.60	8.44	23.07	7.98	31.23	7.95	12.73
51-53	0.0557 (17.94)	8.03	9.09	24.87	8.51	31.65	7.91	9.94
91-93	0.0501 (19.96)	8.55	9.32	25.95	7.25	26.78	8.52	13.63
131-133	0.0379 (26.40)	5.55	8.42	24.89	8.60	34.45	8.20	9.89

CHAPTER 6

GENERAL DISCUSSION

The research presented in this dissertation has significantly progressed our understanding of OC sequestration in seagrass meadows and has addressed a number of significant knowledge gaps, leading to further insights into the Blue Carbon ecology of seagrass meadows (Table 6.1). The insights into OC storage and preservation in seagrass meadows has been improved by examining the biological, abiotic and biogeochemical aspects of these ecosystems. This study, together with previous research (e.g. Lavery et al. 2013, Fourqurean et al. 2012a, Kennedy et al. 2010, Mateo et al. 2006) shows that seagrass meadows contain variable quantities of buried OC in their sediments as a result of many interactive and simultaneously-working processes. These processes, as sub-components in carbon sequestration, include the delivery of OC from multiple sources, the storage and retention of the OC, and its eventual preservation in the sediment. Seagrass habitats, however, are highly variable, with the species composition and abiotic environment unique to any given meadow. Since both the plant characteristics and the habitat characteristics affect the processes critical to carbon storage, it is unsurprising that OC stores differ among meadows. This variability in OC stores is apparent not only in meadows of different species (Chapter 4) but also among meadows of the same species (Chapter 3), confirming earlier studies that had measured stores in different seagrass ecosystems but less systematically or only over shallow sediment depths (Lavery et al 2013; Fourqurean et al 2012a). Comparatively lower stores are found in meadows of those species with low net primary productivity and low retention capacity of seagrass-derived OC

(Chapters 3 and 4, Fourqurean et al. 2012a). In contrast, OC stores are relatively higher in meadows of species with higher supplies and retention capacity of detrital matter (Chapters 3 and 4). When the OC is retained upon burial in the sediment, the longevity of the stores can be up to millennial scales, showing that the OC is well-preserved (Chapters 2 and 5, Mateo et al. 2006, Mateo et al. 1997). In the sediment, the constituent biochemical components undergo different fates. There are compounds that decompose, increase in content, or remain constant with depth and aging (Chapter 5). The pathways that these biochemical components undertake depends on the relative recalcitrance of the OM and the diagenetic processes that occur with sediment depth and age. In addition, the abiotic geochemical environment influences the diagenesis of OM in the seagrass sediments.

6.1 Organic carbon storage in seagrass meadows

The importance of seagrass meadows as valuable OC sinks had been of consistent interest during the past decades (Nellemann et al. 2009, Cebrian and Duarte 1995, Smith 1981). More recently, awareness on the significance of OC stored in seagrass meadows has increased (e.g. Lavery et al. 2013, Fourqurean et al. 2012a) with the recognition that seagrass meadows are experiencing a net decline in global area at an annual rate of 7% (Waycott et al. 2009). Losses of cover simultaneously entail losses in ecosystem services, including OC sequestration capacities (e.g. Marbà et al. 2015). This ecological aspect of ecosystem functioning, however, is poorly understood for the majority of the existing seagrass meadows. Chapter 2 showed that with certain base knowledge of the seagrass meadow, such as highest recorded seagrass cover and areal losses at the studied site, quantification of OC stores and its potential losses, either from absence of sequestration due to vegetation loss or OM remineralisation within shallow sediments, can be estimated. In this sense, the *Posidonia australis* meadow of Oyster Harbour is a unique site. Baseline data on meadow characteristics (such as the area of cover and sediment characteristics) are available as far back as the 1960s to more recent times

Table 6.1. Summary of knowledge gaps addressed and contribution to further understanding carbon storage and preservation in seagrass meadows as an outcome of this thesis production.

Knowledge gap	Studies that identified the gap	How this thesis addressed the knowledge gap	Contributions to existing state of knowledge	Relevant chapter(s)
How is carbon sequestration, as a form of ecosystem service, reduced due to habitat degradation?	Macreadie et al. (2014) Thomas (2014) Howard et al. (2014) Sifleet et al. (2011) Nellemann et al. (2009)	Provided methods and estimates to quantify the ecosystem service from OC losses	Using <i>P. australis</i> as the studied species: <ul style="list-style-type: none"> Confirm that long-term storage contributes to high stores in seagrass sediment; Long-term storage is linked to OC preservation; Stratigraphic quantification of stores is more appropriate for comparing losses of OC due to ecosystem disturbance/degradation. 	Chapter 2
What is the role of habitat (species composition and abiota) in influencing variability of OC stores?	Lavery et al. (2013) Forqurean et al. (2012a)	Compared OC stores in: <ul style="list-style-type: none"> meadows of different species within the same habitat; and meadows of the same species but in different habitats. 	The interaction of both species composition and the abiotic environment contribute to the variability in OC stores among seagrass meadows. Using <i>Halophila ovalis</i> and <i>P. australis</i> as the studied species: <ul style="list-style-type: none"> For OC stores in seagrass habitats: within-species differences exist, between-species differences exist, and within-meadow differences may also exist; Quantification of temporal-based accumulation of OC stores is the method to use for comparing OC content among different seagrass meadows; Stratigraphic comparisons of stores may not be the most appropriate method for comparing OC content among meadows 	Chapter 3 Chapter 4
Does <i>Posidonia australis</i> have unrepresentatively high OC stores and long term carbon reservoirs?	Mateo et al. (2006)	Compared OC stores in nine <i>P. australis</i> meadows from different habitats;	Confirmed that OC deposits can be buried over thousands of years in some <i>P. australis</i> meadows but there is also less capacity for retention in other meadows. OC stores in <i>P. australis</i> meadows are variable, ranging from 2.0 to 10.4 kg OC m ⁻² (stratigraphic stores; 100 cm depth), and are in the lower range of OC stores in <i>P. oceanica</i> meadows.	Chapter 2 Chapter 3 Chapter 4
What is the contribution of seagrass-derived OM to the sedimentary organic pool?	Kennedy et al. (2010)	Employed stable isotope and nuclear magnetic resonance (NMR) techniques to estimate the proportion of seagrass source contributions in <i>P. australis</i> meadows.	Confirmed that seagrass-derived OC is contributing to the sedimentary OC pool, along with allochthonously-derived OM. Seagrass-derived OM may be higher than the global estimate of ~50%. <i>Posidonia australis</i> contribution to the sedimentary OM pool can be as high as 80%.	Chapter 2 Chapter 5
Which biochemical component of seagrass-derived OM is preserved, and how does it persist in sediments?	Mateo et al. (2006)	Outlined a specific analytical method that can be used to characterise the organic constituents contained in the sediment matrix by using existing biomarker techniques (i.e. solid state ¹³ C NMR)	Confirmed that lignin is a dominant contributor to preservation, thus storage of OC in seagrass sediments In addition to lignin, carbohydrate is also the dominant seagrass OM source of leading to OC preservation, using <i>P. australis</i> as the studied species; Identified the biogeochemical conditions and diagenetic processes that led to sedimentary OC preservation	Chapter 5

(Department of Water 2008, Hillman et al. 1990, Bastyan 1986, McKenzie 1962). Furthermore, the *P. australis* meadow here is the longest monitored seagrass site in the world in terms of its restoration in ecological function through transplantation following meadow losses in the 1980s (Marbà et al. 2015, Cambridge et al. 2002). Unfortunately, meadows with these types of baseline data are very scarce and thus complicates the understanding of its carbon sink capacity. For most seagrass meadows, quantification of OC stores are estimates of the current (or recent) stores, with minimal insights on the potential changes in stores that have occurred from the past to contemporary times. The data in Chapters 2, 3 and 4 encompass 39 cores from 12 sites, including both *P. australis* and *H. ovalis* meadows. In future scenarios involving changes in meadow structure, the amount of stored OC may be different to the values presented in this dissertation depending on the health of the meadow (Nellemann et al. 2009). Estimates of stores for those studied sites will be a valid reference on whether the ecosystem function in terms of OC storage is altered, which is indicated by the capacity to accumulate OC (Duarte et al. 2013a).

Understanding the OC sequestration relationship with seagrass cover is evidently important but knowing the existing OC stores and how it may vary among habitats are equally important. Among the 12 meadows studied, there were contrasting differences in OC stores (Chapters 2, 3 and 4). In addition to between-species variation (Chapter 4) or within-species differences (Chapter 3), there can also be variability in OC stores within the same meadow. In the case of Oyster Harbour, there are far more homogenous OC stores with sediment depth of the *P. australis* cover compared to the *Halophila ovalis* meadows of Leschenault Inlet Estuary (Chapters 2, 3 and 4). For the latter, there are distinct areas in the estuary with higher stores than others, demonstrating within-meadow variability in OC stores. These observations substantiates findings from earlier studies (Lavery et al. 2013) that showed the habitat factor was indeed important in accounting for OC stores that led to a large range in OC stores among meadows. Forqurean et al. (2012a) rightly concluded that the variability in OC stores among meadows was very large: from 9-628 Mg OC ha⁻¹ in sediment depth of 100 cm. Estimation

of stores presented in this dissertation fell within the range of those estimates (i.e. 11-107 Mg OC ha⁻¹: Chapters 2, 3 and 4) and thus corroborates the estimates by Fourqurean et al. (2012).

The research presented here used two approaches to estimate OC stores and accumulation in seagrass meadows: a temporal-based (or chronostratigraphic) approach, which assessed the stores accumulated over given time periods; and a stratigraphic approach which assessed the stores over a given depth of sediment, the latter approach commonly used in many studies which lack sediment dating (Lavery et al. 2013, Fourqurean et al. 2012a, da Silva et al. 2009, Vichkovitten and Holmer 2005). Using both approaches has provided insights into the relative merits of the approaches. In Chapter 4, it was shown that quantifying the accumulated OC normalised to an equivalent time period (i.e. temporal-based accumulation) revealed up to an 11-fold variation in OC stores of different species, compared to a 3-fold difference using the stratigraphic approach. Both stratigraphic-based and temporal-based comparisons are valid but the context for its relevance and the insights obtained ultimately differ. The use of stratigraphic-based studies is more applicable for comparing losses at a given depth, while temporal-based comparisons are appropriate when comparing stores quantitatively among meadows.

There are significant advantages in using temporal-based comparisons to determine OC stores, as demonstrated from results of Chapters 2, 4 and 5. Primarily, the OC accumulation rates can be derived based on the attributed age of the sediment. In addition, it reveals the behaviour of the buried OC with age. At present, only some studies addressed this aspect of OC sequestration in seagrass meadows (e.g. Marba et al. 2015, Serrano et al. 2014) and thus far had been insufficiently discussed in the literature. Especially with regards to the nature of OC with age, this aspect is important to understand the preservation aspect of OC sequestration. If for any reason a study is unable to attribute a coherent chronosequence to the sediments (e.g. cost-constraints, mixing of sediments, unsuitability of sediment material for dating), the alternative is to analyse OC in shallow sediment depths (e.g. standardised to 30 cm depth in Chapters 3 and 4; 25 cm depth in Lavery et al. 2013) to minimise the variability

that deep cores (e.g. > 30 cm depth) may capture From the same cores, OC stores in both shallow (< 50 cm) and deep (> 50 cm) sediments can be estimated, as was the approach taken in Chapters 2, 3 and 4. Organic carbon stores in shallow sediments reveal the nature of contemporary stores (i.e. recent accumulation) on the premise that there are small ranges in sediment accumulation rates within these depths (Sadler 1999, Sadler 1981). In contrast, thicker sediment deposits show the cumulative effect of multiple processes in the past (including environmental and biological influences) that led to the stores observed at present (Sadler 1999). In this respect, deep cores (> 100 cm) introduce greater variability to the OC store estimates since the studied meadows may have dissimilar sediment accumulation rates, due to site-specific processes (e.g. de Boer 2007). Therefore if any study employs only stratigraphic-based measures, the limitations mentioned above needs to be considered in interpreting the results, especially if the design entail sampling deep cores.

6.2 Preservation of organic carbon in seagrass sediments

Understanding OC sequestration in seagrass meadows invariably includes investigating the phenomenon of OM preservation to explain the persistence of OC stores in deep sediments. Although identified as a distinct knowledge gap since the mid-2000s (Mateo et al. 2006), the OC dynamics of OC preservation in seagrass meadows are still poorly understood. Long-term storage is an indicator that OC is well-preserved (Chapters 2 and 5). In Chapters 2, 4 and 5, it was shown that OC can persist in the sediment over millennial scales, but with varying amounts with age and depth. The Oyster Harbour *Posidonia australis* meadow is worth focusing on to explore the preservation potential of seagrasses. Here, suitable abiotic conditions coupled with diagenetic processes appear to account for the high OC stores in the sediment (Chapters 2 and 3) compared to other habitat types. With respect to abiotic conditions, Oyster Harbour is a sheltered seagrass meadow, and thus the OM tends to be buried *in situ* rather than exported out of the estuary. In addition to this sheltering, other

physical aspects such as the stability of the mat structure and sediment anoxia enhance OC preservation (Chapter 2 and 5). Subsequent long-term storage is then the result of the intrinsic biogeochemical behaviour of the OM with age and depth (e.g. Kusch et al. 2010, Blair et al. 2003). Primarily, limited decomposition of the buried OM led to long-term storage. At least one of the biochemical constituent was selectively preserved (i.e. the non-pyrogenic aromatic compound) while lignin, carbohydrate and protein underwent non-selective preservation. This emphasises the interactive effects of the biogeochemical environment, the biological aspects and the abiota in contributing to OC preservation in the seagrass sediments.

6.3 Prediction of OC stores in seagrass meadows: between-habitat and within-habitat differences

Forqurean et al. (2012) provided estimates of OC storage in seagrass meadows at the global level but pointed out that their study excluded the factor of species. Assuming that comparison parameters (i.e. whether stratigraphic-based or temporal based) were standardised among meadows, it is possible to have a generalisable view of the amount of OC in seagrass sediments but it must be site-specific to a particular seagrass meadow. Essentially, meadows with the highest stores are those with high supplies of degradation-resistant detrital matter available for burial while having relatively high OC retention capacity in its sediments; and *vice versa*. Whether the stores are high or low, variability in the stores among habitats is due to the interactive effect of biological and abiotic aspects (Chapters 3 and 4). Biological aspects comprise the inherent biological characteristics of the seagrass, such as the proportion of lignin in the tissues, the growth depth of below-ground organs, and the longevity of the preserved OM (Chapters 2 and 5). Chapter 4 clearly showed that small seagrasses (i.e. *H. ovalis*) has lower stores compared to *P. australis*. However, stores in the *H. ovalis* meadows of Leschenault Inlet can be up to 2.6-fold higher (100 cm depth) than in some *P. australis* meadows studied in Chapter 3 (i.e. Peron Point and Lal Bank) indicating

that the seagrass abiotic environment, other than species composition may influence OC stores of a seagrass meadow. Recent studies provide further evidences on the abiotic influences on OC stores in the form of water column depths; shallow seagrass meadows tend to have higher stores than deep meadows (Serrano et al. 2014). The implication in predicting stores among seagrass meadows (either within-species or between-species comparisons) is thus complicated by the interactive effects between the species and the abiotic environment.

Based on the findings in this research, it is recommended that OC stores are evaluated on an individual meadow basis, rather than based on a functional form model (i.e. Figure 1.2). A model generalising OC stores in a particular site can be simplified to two overarching elements: the amount of OM available for burial (cumulatively either from autochthonous production or allochthonous input) and the capacity for OM retention (Figure 6.1). Broadly, biological and abiotic factors influence OC storage and preservation in sediments. The former can be any biotic aspects in OM accrual that encompass variability in OC supply (e.g. seagrass productivity: Chapters 2, 3, 4) and quality (i.e. recalcitrance of biochemical constituents: Chapter 5). On the other hand, abiotic factors are drivers that result in either transience or longevity of the OC upon sediment burial (e.g. depositional environment: Chapter 3). Though the factors of microbial activity, temperature and oxygen exposure were not explored in this study, these factors may be important in diagenetic processing of buried OM (e.g. Burdige 2007) and thus included in this model but clearly, more investigations are warranted to substantiate this hypothesis.

6.4 The question of *Posidonia* OC stores

This dissertation had explored the OC stores of *P. australis* in detail as a lead from Mateo et al. (2006, pg 184), whom stated that “...*P. oceanica*, and possibly *P. australis*, seem to be the only seagrasses that form such thick long-term organic reservoirs...”. *Posidonia australis*

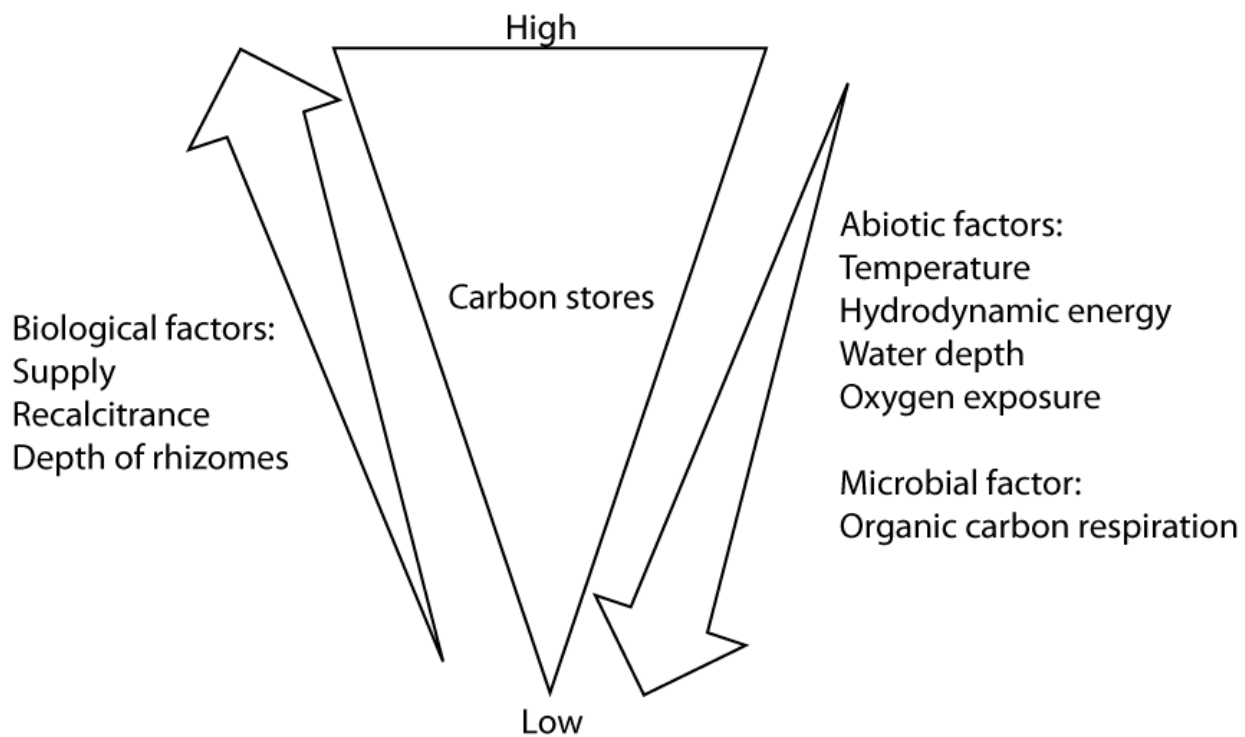


Figure 6.1. Conceptual model for the prediction of OC stores in seagrass sediments.

does deposit significant amounts of OC in its sediments after burial over thousands of years (Chapters 2, 3 and 4) but the OC stores vary among different *P. australis* habitats. Averaged among *P. australis* meadows, OC stores in this species are up to four times lower than in *P. oceanica* meadows (Lavery et al. 2013). It is apparent that *P. oceanica* is a unique seagrass in terms of the quantity of OC that can be stored in its sediments. In all likelihood, this species may be the most exemplary species to demonstrate that there are high OC stores in seagrass sediments, warranting further discourse on the matter.

Studies that reported on high seagrass OC stores (averaged 37 kg OC m⁻² and identified as Mediterranean seagrass meadows; Fourqurean et al. 2012a) obtained estimates from localised *P. oceanica* meadows, situated mainly in Spain (Portlligat and the Balearic Islands) and Italy (Ischia) (e.g. Serrano et al. 2012, Lopez-Saez et al. 2009, Mateo et al. 2006, Mateo et al. 1997). The question on whether these values are representative of the stores in other *P. oceanica* meadows is posed and one which can be addressed by the conceptual model suggested earlier (Figure 6.1). *Posidonia oceanica* can vary in its productivity, ranging from 130 to 1284 g DW m⁻² yr⁻¹ based on a study of 25 meadows from varying habitats (Pergent et al. 1997). In a separate work that the author was involved in simultaneous to the production of this dissertation (Serrano et al. 2014), water column depth was shown to influence OC stores of *P. oceanica*. Therefore based on Figure 6.1, which hypothetically states that OC stores are a positive function of productivity and a negative function of meadow depth, it is thus likely that there are variable OC stores in *P. oceanica* meadows depending on the habitat. It is worth pointing out, too, that the low OC stores measured in *P. oceanica* meadows at their depth-limit distribution (32 m depth, 4.7 kg OC m⁻², 100 cm sediment thickness; Serrano et al. 2014), is intermediate to the range of OC stores reported in this dissertation for *P. australis* meadows (2.03-10.77 kg OC m⁻², 100 cm sediment thickness; Chapters 3 and 4). It is thus evident that the OC stores in *P. oceanica* are in the upper ranges of seagrass meadows globally. This species is an exceptional seagrass in its carbon sink capacity that was aptly pointed out by Lavery et al. (2013). Thus, global Blue Carbon estimates using data from *P.*

oceanica would inevitably over-represent estimates leading to high OC storage values. The alternative in the form of regional estimates including habitat and species variability (after Lavery et al. 2013 and Fourqurean et al. 2012a) may be more robust in presenting data on the OC storage potential of seagrass meadows.

In that same study (i.e. Serrano et al. 2014), *P. sinuosa* was also shown to have significant OC stores, though relatively lower than in *P. oceanica* sediments. High OC stores in *P. sinuosa* was anticipated due to its similar growth strategy to *P. australis* (Gobert et al. 2006). The impulse will then be to generalise the genus *Posidonia* as having high OC stores, but it is premature at this stage to predict such phenomenon for all *Posidonia* species. The genus *Posidonia* contains nine species (den Hartog and Kuo 2006), though there is still debate on the exact number (Waycott et al. 2006). Nonetheless, *Posidonia* can be grouped into three main categories based on its meadow-forming traits (Gobert et al. 2006). *Posidonia oceanica*, having both vertical and horizontal rhizomes, is a solitary member in one group. *Posidonia australis* and *P. sinuosa* belong to the same group, being horizontal rhizome spreaders, while species in the *P. ostenfeldii* group only grow vertically and forms vegetation clumps and not “meadows” in its strictest sense. Meadow-forming species, notably *P. oceanica*, *P. australis* and *P. sinuosa*, incidentally are also the species that had been the most investigated for OC stores. Since the *P. ostenfeldii* group is not similar in its growth strategy to the other two groups, it is unlikely to have similar OC storage capacity. Therefore, due to the difference in biological traits, the current extent of knowledge on OC stores trends cannot be applied to all *Posidonia* species without further studies.

6.5 Recommendations for future Blue Carbon research

Since the publication of the first Blue Carbon report (Nellemann et al. 2009), many knowledge gaps in the Blue Carbon ecology of seagrass meadows were identified (e.g. Macreadie et al. 2014, Thomas 2014, Howard et al. 2014, Sifleet et al. 2011, Nellemann et

al. 2009). These gaps can be summarised into a few key areas: information on global extent of seagrass cover and relating it to losses in OC sequestration; information on OC sequestration in regions with different climates; and information on the remineralisation of the stored OC in both undisturbed and disturbed conditions. This dissertation contributed to the understanding of habitat influences and the biogeochemical basis of OC storage and preservation in seagrass meadows. On the effect of environmental influences, many questions remain unanswered and need further studies to understand their role in OC sequestration, and hence the Blue Carbon ecology of seagrass meadows. The research presented in this dissertation was restricted to the selection of meadows that are mono-specific meadows in temperate regions of Australia, with the most northerly sites being a sub-tropical *P. australis* meadow. This approach minimised any confounding effects of species composition, ambient temperatures on OC stores. In tropical regions (and thus warmer waters), mixed-species meadow are more commonly found than in temperate waters. The effect of a mixed-species composition on within-meadow variability of OC stores is not understood. Seagrass meadows are also found in colder waters (such as in the northerly waters of the northern hemisphere) than in the sites studied in this dissertation. It is possible that the ambient water temperatures of those meadows influence both storage and preservation in sediments (Pedersen et al. 2011). Similarly, sediment instability, such as that due to bioturbation and erosion, may export the OM or expose the OM to oxygen resulting in increased microbial activities of recycling and/or respiration of the OC substrates (Koho et al. 2013). Such remineralisation aspects in understanding the Blue Carbon ecology of seagrass meadows are especially pertinent to quantify and account for the fate of both buried detrital matter and exported leaf litter as a result of sediment disturbances.

A significant knowledge gap in Blue Carbon ecology is our understanding of diagenetic aspects of OC sequestration. Biogeochemical research on OM preservation has mainly focused on non-seagrass sediments (e.g. Burdige 2007, Dickens et al. 2006, Hedges and Keil 1995) where the sedimentary OM is primarily derived from planktonic inputs (Hedges et al.

1997). Seagrass sediments are different; the buried OM consists of both autochthonously-produced OM from seagrass production in addition to capturing allochthonous inputs. Chapter 5 has provided some initial exploration of factors that lead to OC preservation in seagrass sediments, though it is by no means an exhaustive study of the diagenetic processes. Organic carbon retention is not limited to selective preservation and non-selective preservation; the buried OM may undergo different pathways such as such as vulcanization, condensation and/or geopolymerisation with or without bacterial influence that led to its preservation (Killops and Killops 2005, Hedges et al. 2000, Tegelaar et al. 1989, Hedges 1988). As the contrast to preservation, the role of OM decomposition in seagrass sediments, which may lead to degradation or retention (through transformation of the OM into degradation-resistant forms) of the OM still needs further investigation. Since microbial activities can lead to diverging fates of buried OC – either remineralisation or preservation (Koho et al. 2013), studies in microbial ecology may be key to understanding the diagenesis of OC in seagrass sediments.

Another fundamental aspect for understanding OC storage and preservation in seagrass sediments is the identification of the OM source. The results from bulk characterisations from $\delta^{13}\text{C}$ analyses showed that the buried OM, which was accumulated and preserved over millennia constitutes both seagrass-derived OM and allochthonous sources. The nuclear magnetic resonance technique employed (Chapter 5) is advantageous in attributing the proportion of seagrass-derived OM in the sediment. Simultaneously, the apportioning of the OM constituents through the molecular mixing model identified those constituents according to major biochemical classes. However, this method, at least as demonstrated by the results in Chapter 5, does not provide insights on the sources of non-seagrass derived OM. In comparison, the Bayesian mixing model used in Chapter 2 is useful in the sense that it models the relative contribution of the possible sources of OM in the sediment. The caveat of course, is that this requires knowledge of the stable isotope values of the sources most likely to contribute to the sediment organic pool. While this dissertation provides insights into the

contribution of seagrass-derived OM for burial, the characteristics of allochthonous inputs of OM still need to be addressed. Globally, these OM inputs constitute at least 50% of the OC in seagrass sediments (Kennedy et al. 2010) and thus may be preserved simultaneously with seagrass-derived OM in the sediments. Furthermore, knowing the OM source has major implications on determining whether the seagrass species composition of a meadow in the past was the same as at present. The use of biomarker techniques may be a way forward to address this matter and is suggested as a pertinent aspect in understanding OC sequestration in seagrass meadows.

This dissertation had addressed major knowledge gaps in understanding the OC storage and preservation potentials of seagrass meadows. Fundamentally, the outcomes of this dissertation led to insights on OC sequestration in these habitats while highlighting further research to improve our knowledge of the Blue Carbon potentials of these ecosystems. The research has significantly progressed our understanding of the processes of OC delivery, retention in the sediment, and the amount accumulated using *P. australis* and *H. ovalis* meadows as species covering extremes of seagrass functional types. Based on the results from *P. australis*, there will invariably be a link among the quantity of stored OC, the longevity of these stores, and how well the sedimentary OC is preserved upon burial. Furthermore, the work had clarified that both species composition and the abiotic environment are important factors in OC accumulation and preservation; inclusion of both factors in models will provide more robust estimates of current and projected OC stores in seagrass sediments.

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APPENDIX 1

PUBLICATION STATUS OF PAPERS FROM THIS DISSERTATION

The following is a summary of the direct and indirect outputs for publications from data contained in this dissertation:

CHAPTER 2

LONG-TERM CARBON STORAGE AND ITS RECENT LOSS IN A TEMPERATE ESTUARINE *POSIDONIA AUSTRALIS* MEADOW

Submitted for publication in March 2015 as:

Rozaimi M., P. S. Lavery, O. Serrano, D. Kyrwood. (submitted). Long-term carbon storage and its recent loss in a temperate estuarine *Posidonia australis* meadow.

Part of the data presented in this chapter was used to answer a separate research question to those in this dissertation and was submitted in March 2015 as:

Serrano O, G. Davis, P. S. Lavery; C. M. Duarte; A. Martinez-Cortizas; M. A.- Mateo; P. Masque; A. Arias-Ortiz; **M. Rozaimi**; G. A. Kendrick. (submitted). Reconstruction of millennial-scale fluxes of metals in the Australian coastal environment using seagrass archives.

CHAPTER 3

INFLUENCE OF THE DEPOSITIONAL ENVIRONMENT ON ORGANIC CARBON STORES IN SEAGRASS MEADOWS

After submission of this dissertation for publication, the following work will be slightly modified for submission as:

Rozaimi M., P. S. Lavery, O. Serrano. (in preparation). Influence of the depositional environment on organic carbon stores in seagrass meadows.

CHAPTER 4

THE ROLE OF SPECIES COMPOSITION IN ORGANIC CARBON STORES AND ACCUMULATION RATES IN SEAGRASS MEADOWS

Part of the data used in this Chapter was used:

- a) in preliminary work leading to the dissertation output as –

Rozaimi M., O. Serrano, and P. S. Lavery. 2013. Comparison of carbon stores by two morphologically different seagrasses. *Journal of the Royal Society of Western Australia* 96:81-83.

- b) to answer a separate research question to those in this dissertation. It was published as –

Lavery P. S., M-Á. Mateo, O. Serrano, and **M. Rozaimi.** 2013. Variability in the carbon storage of seagrass habitats and its implications for global estimates of blue carbon ecosystem service. *PLoS ONE* 8:e73748.

After submission of this dissertation for publication, the following work will be slightly modified for submission as:

Rozaimi M., P. S. Lavery, O. Serrano. (in preparation). The role of species composition in organic carbon stores and accumulation rates in seagrass meadows.

CHAPTER 5

PRESERVATION OF ORGANIC CARBON IN A *POSIDONIA AUSTRALIS* MEADOW

Submitted for publication in March 2015 as:

Rozaimi M., P. S. Lavery, R. J. Smernik, O. Serrano. (submitted). Preservation of organic carbon in a *Posidonia australis* meadow.

Part of the data presented in this chapter was used to answer a separate research question to those in this dissertation. It is currently being prepared as a manuscript as:

Säwström C., O. Serrano, **M. Rozaimi,** P. S. Lavery. (in preparation). High metabolic diversity of heterotrophic bacteria in Western Australian millenary seagrass sediments.

