Characterising blue carbon:

An assessment of

soil carbon in

temperate coastal wetlands

A thesis submitted in fulfillment
of the requirements for the degree of Doctor of Philosophy

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Declaration

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in my name, in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. In addition, I certify that no part of this work will, in the future, be used in a submission in my name, for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint-award of this degree.

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Thesis Abstract

Vegetated coastal wetlands (mangrove forests, tidal marshes and seagrass meadows) provide a suite of ecosystem services and are some of the most productive habitats in the world. As part of their ecosystem services, vegetated coastal wetlands store a significant amount of carbon in their above and below ground biomass and soils; and it is known as blue carbon. These ecosystems, despite their small global footprint of just 2% of the Earth's surface, store significant amounts of carbon and act as significant carbon sinks. A majority (>50%) of the carbon stored by these environments is accounted for as soil organic carbon (SOC). The saline and saturated nature of blue carbon soils results in slower turnover rates of organic matter, promoting long-term storage of carbon. The capture and retention of carbon in these natural environments is, in part, contributing to climate change mitigation.

The work presented in this thesis focuses on the quality of blue carbon, specifically the chemical composition of carbon, to better understand some of the mechanisms that underpin the long-term stability of carbon in mangrove and tidal marsh soils of temperate coastal wetlands. The focus of current blue carbon research has been on understanding the drivers of SOC accumulation and the quantifying SOC stocks within and across blue carbon habitats at both a regional and global extent but does not address its quality. The quality of SOC, for example its chemical composition, is one of the controlling factors of its long-term stability in the environment. However, in the same way the quantity of carbon should not be the sole focus, the quality of SOC in any ecosystem should not be assessed without first quantifying current carbon storage.

In this thesis I explore both aspects of blue carbon systems, as follows: In *Chapter Two* mangrove and tidal marsh surface soil (top 10 cm) carbon and nitrogen stocks and their (within

site) spatial variability were quantified and compared, across nine selected case study sites in temperate coastal wetlands. This led investigations to *Chapter Three* and *Four (Part 2),* that assessed if combined infrared resonance spectroscopy and partial least squared regression analyses (IR/PLSR) could successfully predict carbon and nitrogen stocks and the allocation of SOC to size fractions, that were quantified in *Chapter Four (Part 1),* in blue carbon soils. Then in *Chapter Five,* the chemical composition of temperate blue carbon in temperate coastal wetland soils were investigated.

Overall, it was found that differences in surface soil (top 10 cm) carbon and nitrogen stocks in temperate coastal wetlands were driven by the characteristics of a site and its inherent environmental conditions rather than the vegetation. Although, vegetation did effect surface soil (top 10 cm) carbon and nitrogen stocks at some sites and sampling highlighted significant within site spatial variability of the stocks. The highest proportion of OC was allocated to the humus pool (58 % and 53 % for mangrove and tidal marsh samples, respectively), supporting the longevity notion of blue carbon. However, a dominance of labile carbon forms (O-alkyl) in the surface soils (top 10 cm), irrespective of vegetation type, suggests SOC in the blue carbon environment is vulnerable to rapid decomposition should environmental conditions of the soil change. Overall, the summation of this work provides a comprehensive assessment of SOC chemistry in temperate blue carbon ecosystems. Additionally, the application of robust IR/PLSR predictive algorithms developed in this thesis can provide rapid and cost-effective estimates of carbon and nitrogen stocks that will improve future estimates and can account for the variability of stocks in blue carbon soils.

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Presentations

Asanopoulos, C.H., Baldock, J.A., Macdonald, L.M., Cavagnaro, T.R., 2017, *'Carbon storage potential in vegetated coastal sediments'*, Post graduate symposium, University of Adelaide, Australia.

Asanopoulos, C.H., Baldock, J.A., Macdonald, L.M., Cavagnaro, T.R., 2017, *'Investigating SA's coastal blue soil carbon'*, Young researcher science day, CSIRO Adelaide, Australia.

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Asanopoulos, C.H., Baldock, J.A., Macdonald, L.M., Cavagnaro, T.R., 2018, *'At the interface: investigating the soil organic carbon in South Australian mangrove and tidal saltmarsh soil'*, Australian Mangrove and Saltmarsh Network conference, Sydney, Australia.

Asanopoulos, C.H., Baldock, J.A., Macdonald, L.M., Cavagnaro, T.R., 2019, *'Quantifying the vulnerability of blue carbon in temperate wetlands'*, Australian Mangrove and Saltmarsh Network conference, Melbourne, Australia.

Asanopoulos, C.H., Baldock, J.A., Macdonald, L.M., Cavagnaro, T.R., 2019, *'Investigating the relationship between SOC and age in temperate blue carbon ecosystems'*, International Soil organic matter conference, Melbourne, Australia.

Presenting author in **bold*

Preface

This thesis is presented as a series of manuscripts prepared to be submitted for publication.

At the outset of this research I intended to investigate the impacts of anthropogenic activity, including land use change and coastal eutrophication, on soil carbon storage in vegetated coastal wetlands. However, throughout the course of my research it became apparent knowledge pertaining to the fundamental understanding of the controls on carbon storage in vegetated coastal wetlands required more information.

To provide context to the overall thesis, *Chapter One* provides an overview of the literature on vegetated coastal wetlands, dynamics of soil carbon storage and methods for the characterisation of organic carbon in soils, prepared at the start of the project in February 2016. This chapter also includes the proposed objectives of this research. Introductory material relevant to the succeeding research chapters is not presented in detail in the literature review because it appears in the introduction of each chapter.

Chapter Two is a research chapter prepared for publication in *Soil Science.* It provides a quantitative assessment of surface soil carbon and nitrogen stocks and their spatial variability in temperate coastal wetlands of South Australia.

Chapter Three is a co-authored research chapter. In this chapter, the capability of the combined infrared spectroscopy and partial least squares regression analyses (IR/PLSR) approach to

predict total (TC), organic (OC) and inorganic (IC) carbon and total nitrogen (TN) concentrations in blue carbon soils are assessed.

Chapter Four is a research chapter prepared for publication in *Science of the Total Environment.* In this chapter, the allocation of soil OC across soil carbon storage pools with different rates of decomposition are quantified. It also describes the development and application of IR/PLSR models for predicting organic carbon (OC) distribution across these pools in vegetated coastal wetland soils.

Chapter Five is a research chapter prepared for publication in *Organic Geochemistry*. It describes the chemical composition of soil organic carbon, and its particle size fractions in vegetated coastal wetland soils.

Chapter Six is a synthesis of the findings contained in this thesis and gives recommendations for future work.

Chapter One

An introduction to blue carbon

The carbon cycle

Carbon is naturally cycled on Earth between the land, oceans and atmosphere (Figure 1.1) in a balanced exchange of atmospheric carbon dioxide (CO_2) to carbon storage in above and belowground biomass, living and decomposing organic matter, and soils (Bloom 2010; Horwath 2007). Under natural conditions, the global carbon cycle results in no net change of atmospheric CO_2. However, when CO_2 enters the atmosphere through anthropogenic activity, for example, via the extraction of carbon from sedimentary rocks and subsequent burning of fossil fuels (i.e., coal, oil, gas and petroleum) or cement production, there is an unbalanced net release of CO_2 into the atmosphere (Bloom 2010; McLeod et al. 2011; Pendleton et al. 2012). In addition, when land use changes and natural carbon sinks are removed (i.e. removal of the above and below ground biomass) the carbon they have stored becomes a source of CO_2 emissions (Pendleton et al. 2012; Siikamäki et al. 2013). The removal of natural vegetation therefore increases CO_2 emissions in the short term, but also results in the loss of potentially significant carbon storage pools (McLeod et al. 2011; Pendleton et al. 2012). The main implication of this is increased atmospheric CO_2, a direct cause of climate change.
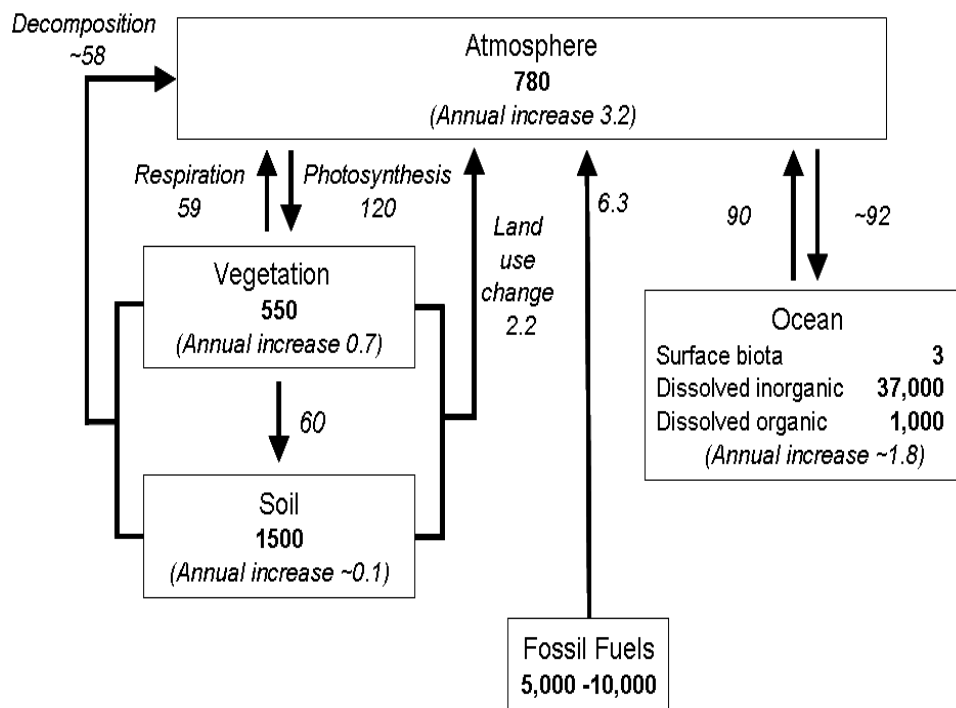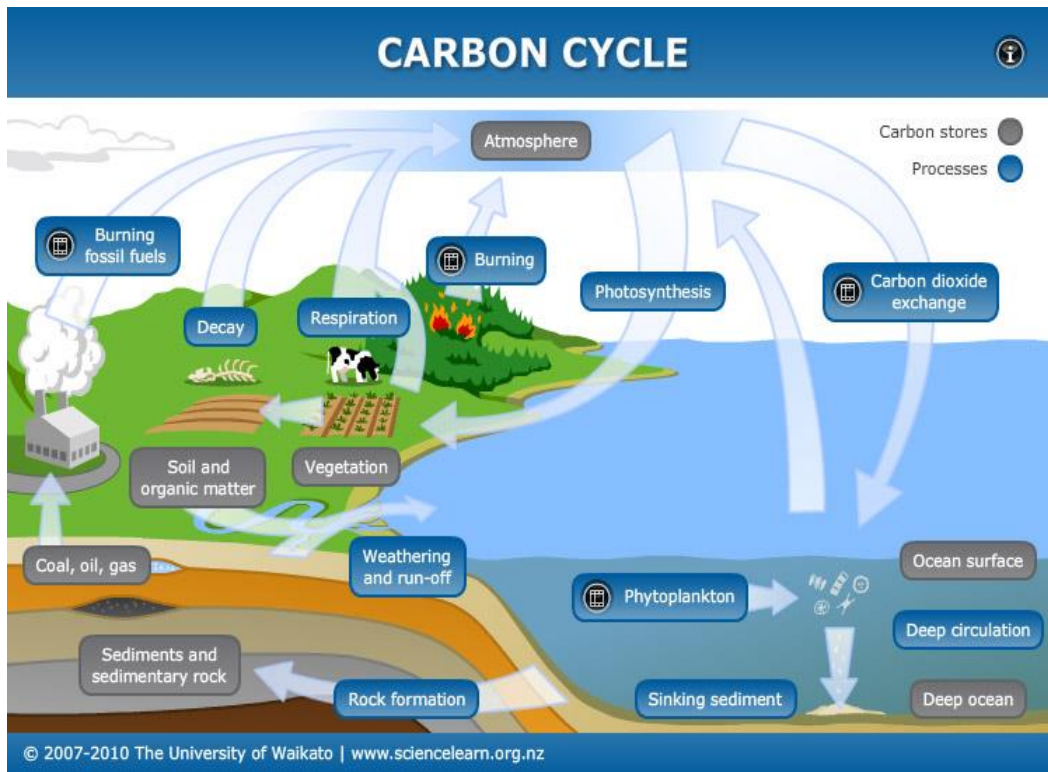
Figure 1.1 Schematic of the global carbon cycle. All carbon pool units represent Pg C and all fluxes are given in units of Pg C yr-1. Sourced (Baldock & Broos 2012; University of Waikato 2007-2016).

Increasing the concentration of greenhouse gasses (GHGs) in the atmosphere results in increasing temperatures on earth. In the natural cycling of GHGs, shortwave solar radiation from the sun enters earth's atmosphere and warms the troposphere (Figure 1.2). The lower atmosphere, land and oceans absorb the suns short wave energy heating the earth to sustain life. The long wave radiation, i.e. heat, that was absorbed by the troposphere is subsequently radiated back towards space. Small fractions of heat are absorbed by atmospheric GHGs, such as water vapor (H_2O), CO_2, methane (CH_4), nitrous oxide (N_2O) and ozone (O_3), and re-radiated back to earth; or completely leave the atmosphere. An increase in GHGs concentrations in the atmosphere causes a larger fraction of the re-radiated heat to be absorbed by Earth's atmosphere. This heat is dispersed with greater intensity back to the Earth's surface and absorbed by the earth. Less heat is therefore escaping into space and the Earth's lower atmosphere, land and oceans are increasing in temperature (Horwath 2007). The process is known as radiated forcing.
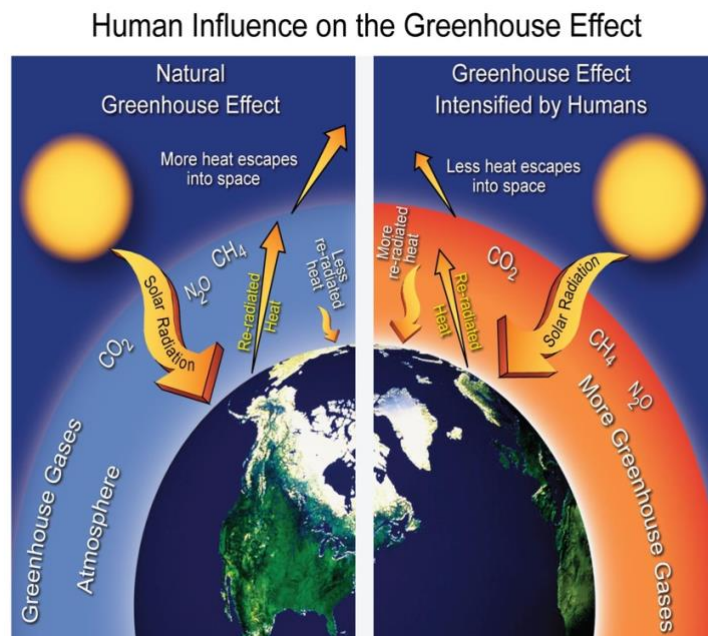
Figure 1.2 Schematic of the natural and intensified greenhouse effect. (BRACE Illinois 2016).

Anthropogenic activity is a key contributor to the increase of atmospheric GHGs and increased emissions of CO_2, CH_4 and N_2O are key drivers of climate change (Pendleton et al. 2012). Burning of fossil fuels and land use change are the leading cause of increased atmospheric CO_2 concentration, whilst increased CH_4 and N_2O concentrations are largely attributed to agriculture (IPCC 2007; Pendleton et al. 2012). The potential to mitigate global climate change via the capture and sequestration of atmospheric CO_2 in natural ecosystems is being widely investigated (Atwood et al. 2017; Kauffman & Bhomia 2017; Macreadie et al. 2017; Pendleton et al. 2012). Investigations include a strong emphasis on the potential to store and sequester carbon in natural ecosystems including terrestrial and marine habitats (Macreadie et al. 2019).

The coastal zone

The coastal zone forms a critically important eco-tone between terrestrial and marine systems. They are dynamic environments that can be characterised into four broad classes (deltas, tidal systems, lagoons and fjords) where the deposition of river-born sediments create platforms suitable for the establishment of vegetated coastal wetlands (Crossland et al. 2005; Dürr et al. 2011; Twilley et al. 2018). Their formation is controlled by the transportation and accumulation of sediments derived from rivers, tides and waves (Dürr et al. 2011; Rovai et al. 2018; Twilley et al. 2018). The rate of sediment accumulation in the coastal zone is driven by the frequency of tidal inundation and wave energy of their environmental setting (Alongi 2014; Dittmann et al. 2013; Twilley et al. 2018). Where wave energy is limited, large particles are incorporated into the soil matrix and begin to accumulate. However, in shallow waters where wave energy can reach the sea floor and orbital velocity is generated, nutrient rich soils can be resuspended and transported to adjacent locations (Segar 2007).

Coastal soils are accumulated continuously over time by the deposition of sediments on top of previously deposited layers that results in the vertical accretion of soils (Twilley et al. 2018). The vertical accretion of sediments causes buried soils to have different properties than the recently deposited soils as a result of changes in environmental conditions and the depletion of oxygen as depth increases (Chmura et al. 2003; Kelleway et al. 2017b; McLeod et al. 2011; Pendleton et al. 2012). In the absence of mixing, older sediments are found deeper in the soil profile with younger sediments on top (Kelleway et al. 2016; Twilley et al. 2018). Transportation of sediments, therefore, results in the lateral and vertical deposition and accumulation of fine particle soils in lower energy margins of an estuary (Saintilan et al. 2013).

The coastal zone contains some of the world's most productive ecosystems that provide a variety of ecosystem services (Edyvane 1999; Millennium Ecosystem Assessment 2005; Nellemann et al. 2009). For example, the coastal vegetation, including mangroves, tidal marshes and seagrasses and the abundant nutrient rich soils of the coastal zone provide feeding grounds, spawning grounds and nursery habitats for terrestrial and marine organisms (Edyvane 1999; Himes-Cornell et al. 2018). Their extensive below ground and dense above ground biomass also help to maintaining water quality by filtering nutrients and trapping sediments; reduce coastal erosion and flooding of urbanised coastal regions; prevent sediments entering adjacent marine environments (i.e., coral reefs); filter pollution from waterways; reduce coastal erosion and buffer coastal communities from extreme weather (Alongi 2002; Howard et al. 2014; Millennium Ecosystem Assessment 2005; Nellemann et al. 2009; Pendleton et al. 2012; Siikamäki et al. 2013).

In addition to the ecosystem services the coastal zone provide they also support a significant pool of global carbon stores (Breithaupt et al. 2012; Chmura et al. 2003; Duarte et al. 2005;

Millennium Ecosystem Assessment 2005; Nellemann et al. 2009). Coastal soils account for 50 % of the carbon stored in the ocean despite their small spatial footprint of just 2 % of the Earth's surface (Duarte & Cebrián 1996; Duarte et al. 2005; Nellemann et al. 2009). The primary source of carbon generated in the coastal zone is produced by the current *in situ* vegetation (Owers et al. 2020).

Mangroves

Mangroves are woody trees and shrubs that occur on depositional coastlines. They are predominant in tropical and subtropical latitudes but also occur in temperate regions (Craft 2016b; Siikamäki et al. 2013). Mangroves are recognised as one of the most important marine habitats by the United Nations Environment Programme (UNEP) for the ecosystem services they provide (Kaiser et al. 2005; Segar 2007). In addition, they are considered to be the most carbon rich forests on earth (Atwood et al. 2017; Donato et al. 2011). The global extent of mangroves is estimated to be 139,170 km2 (Siikamäki et al. 2013). A total of 73 mangrove species exist worldwide and forests can contain a variety of tree species (Craft 2016b). Species diversity is highest in the tropics with 24 genre and 58 species of mangroves present but gradually decreases towards the sub-tropics to five genera and 12 species (Craft 2016b). In Australia, they occupy approximately 11,500 km2 of the Australian coastline and the sub-tropical grey mangrove, *Avicennia marina*, is the only species distributed along the South Australian coast, covering 211 km2, figure 1.3 (Robertson & Alongi 1995). In temperate regions *Avicennia marina* is a stunted version of the *Avicennia* genera and a species that is more tolerant to colder temperatures than other species (Craft 2016b; Ewers Lewis et al. 2018).
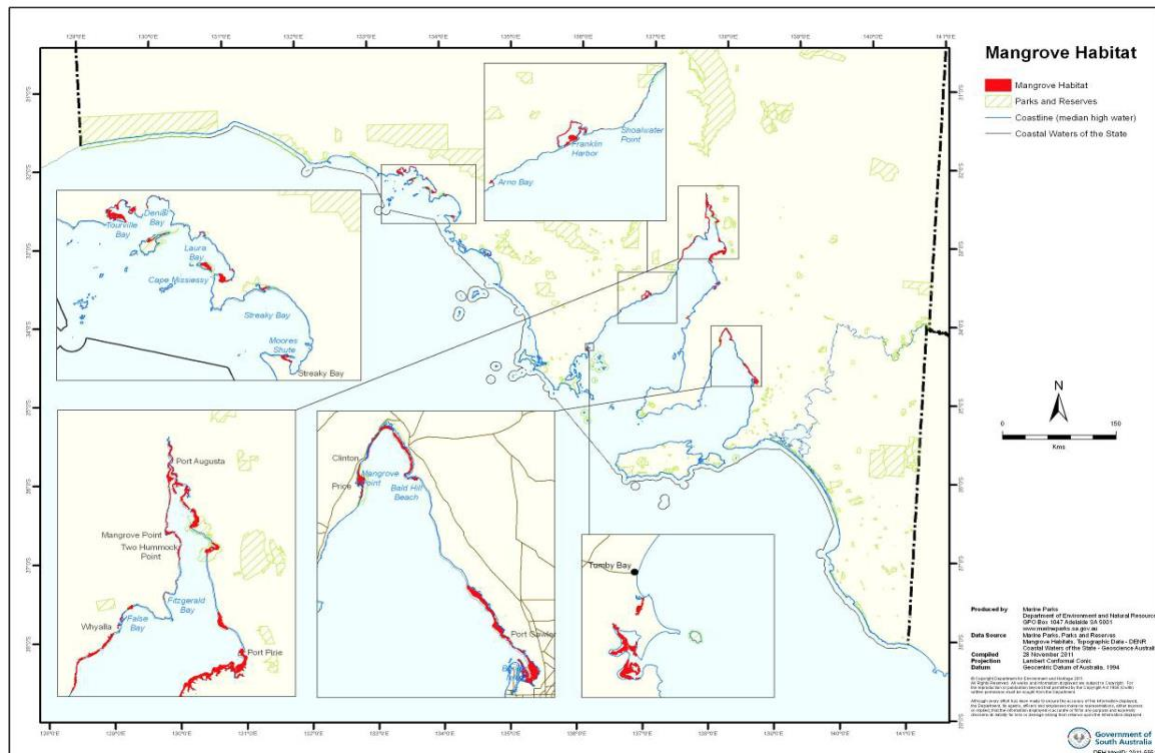
Figure 1.3 Map of mangrove distribution around South Australia (Scientific Working Group 2011)

Mangroves are adapted to live in coastal soils with their physiology allowing them to tolerate the waterlogged and saline environment (Alongi 2009; Craft 2016b). For example, mangroves control salt uptake to maintain an acceptable balance of salt and water with different salt restricting or resisting strategies (i.e. exclusion, extrusion, storage, compartmentalisation and osmoregulation) (Alongi 2009; Craft 2016b). Mangroves also develop specialised root structures (i.e. aerial roots) that grow from the main stem and above the soil, figure 1.4, facilitating oxygen and gas uptake directly from the atmosphere (Kaiser et al. 2005; Siikamäki et al. 2013). The extensive root architecture of mangroves also facilitates the trapping of suspended nutrients, peat and sediments and reduces the energy of incoming tides that might otherwise cause inland erosion (Siikamäki et al. 2013). The production of below ground structures and the accumulation of organic matter also allows mangrove environments to

maintain their elevation as sea levels rise (Craft 2016b).
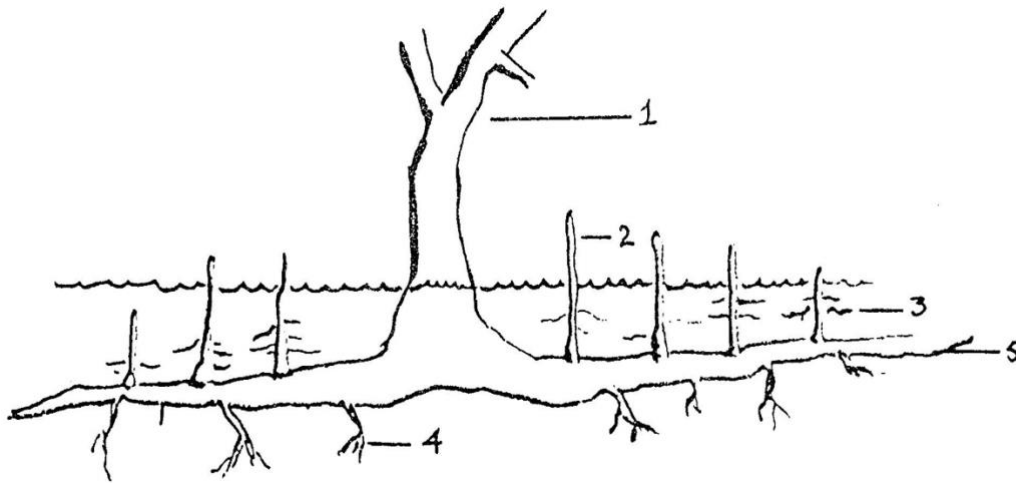
Figure 1.4 Simplified diagram of a mature *Avicennia marina* mangrove root system (1) Main trunk; (2) Pneumatophores; (3) Nutrition roots; (4) Anchor roots; (5) Cable roots (reproduced from (Crumbie 1987).

Mangroves are classified on their distribution within the environmental setting they occupy (Craft 2016b; Siikamäki et al. 2013). For examples, riverine mangroves inhabit the banks of river systems and are the most productive forests receiving high sediment and nutrient loads as a result of frequent flooding with river water (Craft 2016b). Fringe mangroves occur on the seaward edge of shorelines and are regularly inundated with sea water. Fringe mangroves are less productive than riverine mangroves but more productive than basin mangroves. Basin mangroves are found on the landward edge of both riverine and fringe mangroves occurring higher in the tidal frame (Craft 2016b). Basin mangroves are rarely inundated and have high salt contents due to high evaporation. This results in saline and anaerobic conditions and low forest productivity (Craft 2016b).

Tidal marshes

Tidal marshes are salt tolerant plants that grow within the range of mean low and high tide and are found in areas that can be inundated by seawater (Baker 2015; Caton et al. 2009; Craft 2016a; Daly 2013; Kaiser et al. 2005; Macreadie et al. 2017). The distribution of tidal marshes is global and extends from subpolar to tropical climes (Craft 2016a; Siikamäki et al. 2013). The coastal zone that supports tidal marshes is determined by the area that is subject to tidal inundation and grows and shrinks accordingly (Craft 2016a). Tidal marshes can be found in the intertidal, supra-tidal and stranded tidal zones i.e. areas that can become inundated by seawater in periods of extreme high tide or flooded during extreme storm events (Baker 2015; Caton et al. 2009; Daly 2013; Kaiser et al. 2005). Plant biomass and density is greater along the seaward/river edge of the marsh and decreases inland. The landward edge of a marsh system receives infrequent tidal inundation and is dominated by plant species adapted to lower salinity and less anaerobic conditions (Craft 2016a; Saintilan et al. 2013). The global extent of tidal marshes is estimated to be roughly 51,000 km2 and they cover approximately 178,752 ha of the South Australian coastline (Siikamäki et al. 2013).

They are categorised into three broad classes by their zonation in the landscape as tidal salt, brackish and tidal fresh marshes (Craft 2016a). Zonation of the vegetation is determined by their position in the landscape and species richness increases with elevation (Craft 2016a). Conditions of the lower elevations where tidal salt marsh and brackish marshes inhabit are more saline and anaerobic than higher in the tidal zone creating a stressful environment that only a few marsh species can tolerate (Craft 2016a). Tidal freshwater marshes, in contrast, are located in upper estuarine regions and are only subjected to freshwater inundation (Craft 2016a). Communities of high salinity tolerant plants generally occupy tidal marshes, with *Sarcocornia spp.* and *Tecticornia spp*. being the most common and abundant tidal marshes in

South Australia (Baker 2015; Daly 2013). Tidal marshes are more terrestrially adapted than other coastal vegetation; for example, although they can tolerate high saline soils and survive tidal or storm and floodwater inundation for short periods of time, prolonged periods prevent reproduction and promotes mangrove encroachment (Craft 2016a; Daly 2013)

Tidal marshes straddle the coastal margins, seaward fronted by mangroves and landward backed by saltbush. In the past, tidal marshes have been undervalued and viewed as wastelands with many tidal marsh areas drained, reclaimed and degraded by anthropogenic activity. Yet, the tidal marsh habitat acts as a buffer between the terrestrial and aquatic environment attempting to purify coastal waters, regulate salinity levels, and collect and recycle soluble nutrients back into the estuarine system (Baker, 2015). For example, soluble ammonia, silicates, phosphate and dissolved organic nitrogen are recycled through tidal marsh environments and replenished in estuary waters when tidal inundation occurs (Baker, 2015). These nutrients are transformed through microbial uptake into forms that can be easily absorbed by estuarine plants and animals. Tidal marsh environments are, therefore, important sites for the accumulation, storage and re-mineralisation of organic matter.

Vegetated coastal ecosystems and carbon

Vegetated coastal ecosystems, including mangroves and tidal marshes, extend from cold polar regions to the tropics and occur on sheltered, low-lying marine and estuarine coastlines (Chmura et al. 2003; Pendleton et al. 2012; Siikamäki et al. 2013). Their global extent is estimated to be 509,170 km$_2$ with seagrasses having the greatest global coverage followed by mangroves and then tidal marshes (Siikamäki et al. 2013). Seagrasses have a global distribution while mangroves are dominant in tropical latitudes and tidal marshes prevail in temperate regions (Alongi 2002; Feher et al. 2017; Twilley et al. 1992).

The carbon captured and stored by vegetated coastal wetlands is referred to as blue carbon (McLeod et al. 2011; Nellemann et al. 2009; Pendleton et al. 2012). Blue carbon and is stored in the living above (i.e. stems, leaves and branches) and below-ground biomass (i.e. roots), the non-living biomass (i.e. litter and deadwood) and in the soils (McLeod et al. 2011; Nellemann et al. 2009; Pendleton et al. 2012; Siikamäki et al. 2013). Carbon can be deposited into the coastal environment through various avenues but additions are primarily through the primary productivity of the coastal vegetation (Alongi 2009; Alongi 2014). Soil carbon additions are ascribed to the deposition and breakdown of autochthonous (generated *in situ*) detritus and the lateral import, deposition and retention of allochthonous (generated externally) carbon from adjacent environments (Ewers Lewis et al. 2020; Kelleway et al. 2016; McLeod et al. 2011; Middelburg et al. 1997; Saintilan et al. 2013). Blue carbon ecosystems are, therefore, carbon sinks representative of a much larger area than that of which they occupy (McLeod et al. 2011).

Current estimates of the global blue carbon stocks (including the above and below ground biomass and soils to 1 m) are 11.25 Peta-grams of carbon (Pg C; Siikamaki et al. 2013). The carbon budgets of mangrove, tidal marsh and seagrass environments 6.5 Pg C, 2 Pg C and 2.3 Pg C, respectively (Siikamäki et al. 2013). However, with estimates of 448–468 tons of organic carbon per hectare (t C ha^{-1}) in mangroves, 285–393 t C ha^{-1} in tidal marshes and 72–157 t C ha^{-1} in seagrasses, mangrove and tidal marsh environments are greater carbon sinks on a per unit area basis than seagrasses (Pendleton et al. 2012; Siikamäki et al. 2013). Sediment accumulation and carbon burial rates in blue carbon ecosystems are high as a result of frequent inputs of autochthonous and allochthonous organic matter (Howe et al. 2009; Kelleway et al. 2016; McLeod et al. 2011; Sanderman et al. 2018). Global carbon sequestration rates for mangroves average 31.1 ± 5.4 to 34.4 ± 5.9 Tg C yr^{-1} and 10.1 ± 1.1 Tg C yr^{-1} for tidal marshes with carbon burial rates of 20–949 g C m^{-2} yr^{-1} for mangroves and rang from 18–1713 g C m^{-2}

yr-1 for tidal marshes (McLeod et al. 2011; Ouyang & Lee 2014). These carbon sequestration estimates are, however, based on the global area covered by the vegetation and a significantly larger area is covered by seagrasses than mangroves and tidal marshes, the latter of which are still not well known (Siikamäki et al. 2013). Mangroves and tidal marshes, therefore, sequester significantly more carbon than seagrass habitats. In addition, despite their significantly lower land coverage the carbon sequestration rates of blue carbon habitats are comparable to the sequestration rates of terrestrial forests (i.e. Temperate 53 ± 10.4 Tg C yr-1, tropical 78.5 ± 9.8 Tg C yr-1 and Boreal forests 63 ± 28.8 Tg C yr-1), see figure 1.5 (Atwood et al. 2017; Donato et al. 2011; McLeod et al. 2011). However, blue carbon is still not considered in budgets of global climate change mitigation or carbon trading schemes (Duarte et al. 2005; Nellemann et al. 2009).
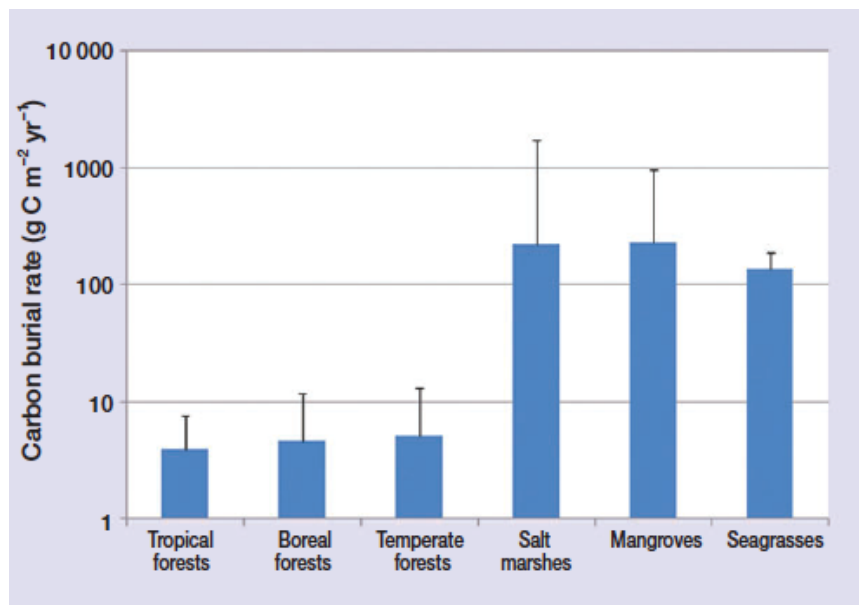
Figure 1.5 Mean long-term carbon sequestration (g C m-1 yr-1) in terrestrial forest soils and vegetated coastal ecosystem sediments. Error bars showing maximum accumulation rates (McLeod et al., 2011) © The Ecological Society of America.

The respiration of CO_2 by the mangrove and tidal marsh vegetation is exceeded by their net primary productivity and their plant biomass is a significant source of carbon additions to the

coastal zone (Adame et al. 2015; Duarte & Cebrián 1996; Duarte et al. 2013; Duarte et al. 2005; Lovelock et al. 2014b). For example, the dense above-ground canopies result from the allocation of carbon to photosynthetic tissues that increases the net primary productivity of the vegetation and generate autochthonous carbon additions to the substrate (Kelleway et al. 2017a; Owers et al. 2016a; Owers et al. 2018). In addition the allocation of carbon to below-ground tissues such as the roots and rhizomes facilitates the accretion of tidally borne (allochthonous) sediments and contributes to the below-ground soil carbon pool (Cartaxana & Catarino 1997; Duarte et al. 2013; McLeod et al. 2011; Owers et al. 2020). Therefore, supply of carbon far exceeds the vegetation's requirements and results in a surplus of organic carbon that is stored in the soil (Duarte & Cebrián 1996; Duarte et al. 2013; Duarte et al. 2005).

Soil carbon and its biogeochemistry

Soils are the most significant blue carbon reservoir accounting for an estimated 80 % of the overall carbon stocks stored in blue carbon habitats (Chmura et al. 2003; Duarte et al. 2005; McLeod et al. 2011; Nellemann et al. 2009; Siikamäki et al. 2013). Deposition of litter produced by the vegetation, in combination with organic compounds and mucus secretions from the below ground plant biomass and the laterally transported organic matter are the major sources of carbon additions to coastal soils (Hemminga et al. 1996; Saintilan et al. 2013). They are characterised by thick waterlogged mud (classified by grain size including clay [<0.002mm] and silt [0.02mm-0.06mm]) that is rich in organic matter (Holmer 2003; Kelleway et al. 2016; McLeod et al. 2011; Segar 2007). If not respired, consumed or transported offshore soil carbon is stored below ground in the coastal zone (Adame et al. 2015; Bouillon et al. 2003; Duarte & Cebrián 1996; Duarte et al. 2005). The preservation of carbon in coastal soils is a function of the delayed organic matter decomposition that occurs due to the frequent tidal inundation of coastal environments (Lovelock et al. 2014a; McLeod et al. 2011; Owers et al.

2020; Sanders et al. 2016). Preservation is also influenced by the quality and burial depth of deposited organic matter (Cartaxana & Catarino 1997; Duarte & Cebrián 1996; Kelleway et al. 2017a; Kelleway et al. 2016; Twilley et al. 1992). This is because the structural tissues of the vegetation are nutrient poor and thick making them harder to decompose (Duarte & Cebrián 1996). In addition, soil conditions become anaerobic with increasing depth and prevents the efficient decomposition of tissues (Duarte & Cebrián 1996). These factors result in the long-term storage (i.e. millennia) of carbon in blue carbon environments (Chmura et al. 2003; Duarte et al. 2005; McLeod et al. 2011).

Organic residues supplied to soil and soil surfaces through the deposition of dead plant tissues are converted into organic carbon forms that closely bond with soil minerals through physical and chemical processes (Lehmann & Kleber 2015). Organic matter cycles through soil in a balanced exchange of organic material input driven by photosynthesis and inorganic mineral output driven by microbial respiration during decomposition. Depolymerisation, microbial assimilation and mineralisation are the key processes resulting in SOM decomposition, see Figure 1.6 (Baldock & Broos 2012; Friesen et al. 2018; Macdonald & Baldock 2010). Depolymerisation involves the breakdown of polymeric compounds, too large to cross biological membranes, into smaller molecules that can be used as carbon, energy and nutrient sources for the microbial biomass (Kristensen et al. 2008; Macdonald & Baldock 2010). Microbial assimilation utilises the smaller monomers of organic and/or inorganic compounds for growth and maintenance. Mineralisation results in the conversion of carbon, nitrogen and phosphorus into CO_2 ammonium (NH_4^+) and phosphate (PO_4^{2-}) through microbial respiration (Macdonald and Baldock, 2010).
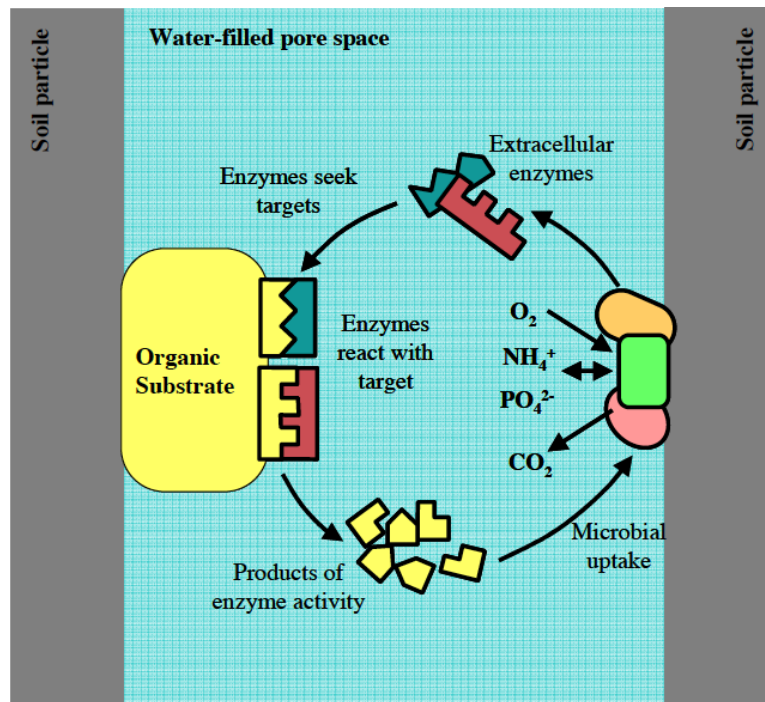
Figure 1.6 Schematic of organic substrate decomposition in soil, representing: the release of extracellular enzymes by soil micro-organisms that bind with organic substrates, followed by depolymerisation producing smaller monomers that are utilised by microorganisms for growth and metabolic activity, while excess nutrients are released in mineral form. Sourced from (Macdonald and Baldock, 2010) © 2010 CSIRO.

In agricultural systems, a portion of the plant biomass produced, and the associated nutrients derived from biological cycling are removed during harvest (Macdonald and Baldock, 2010). While in aquatic systems and in the coastal zone, autochthonous organic matter that is not transported to adjacent ecosystems through tidal flow is deposited in coastal soils and subsequently degraded or modified by microorganisms (Barreto et al. 2018; Friesen et al. 2018; Kristensen et al. 2008). In the presence of tidal flow, the transportation of allochthonous organic matter may also be deposited into the ecosystem. This creates a large potential for both autochthonous and allochthonous organic matter to accumulate in coastal soils (Holmer 2003).

The physical structure of coastal soils can also have a major impact on the abiotic processes and biotic conditions that aid in the recycling of organic matter and oxidation of sediments (Holmer 2003; Kelleway et al. 2016). In higher intertidal densely vegetated sediments macro fauna activity transport organic matter and aerate sediments (e.g. the active population of crabs in mangroves can create burrows that aid in sediment aeration while also deposit litter into the sediment) (Friesen et al. 2018; Holmer 2003; Kristensen et al. 2008). Under aerobic conditions, decomposition of organic matter is promoted; however, aerobic degradation of materials is rapid and limited to the surface of mangrove sediments, as O_2 rarely penetrates greater than 2 mm (Barreto et al. 2018; Holmer 2003). Oxygen is an efficient electron acceptor, however under anoxic conditions, due to limited O_2 supply, mineralization of organic matter requires manganese (Mn^{4+}); nitrate (NO_3^-); iron (Fe^{3+}) and sulphate (SO_4^{2-}) ions as electron acceptors for anaerobic microorganisms to oxidize depolymerised molecules formed by competitive prokaryotes (Kristensen et al. 2008). Thus, most mangrove sediments contain high levels of reduced inorganic sulphur in the form of pyrite (FeS_2) and sulphur (S) and iron mono-sulphides (FeS) (Kristensen et al. 2008). Yet, 50% of organic matter oxidation still takes place under aerobic conditions, due to mixing and reburial O_2 exposure (Holmer 2003). Aerobic respiration and anaerobic sulphate reduction are usually considered the most important respiration processes in mangrove sediments (Holmer 2003; Kristensen et al. 2008). However, the biological, physical and chemical processes occurring in coastal sediments are somewhat lacking in scientific research. For example, the cycling of soil organic matter is complex and still being understood in terrestrial ecosystems, while marine research tends to have focused more on the 'soluble' environment.

Carbon methodologies

Understanding that vegetated ecosystems, both terrestrial and marine, are important for climate

mitigation through the capture of atmospheric CO_2 and long-term sequestration of carbon is widespread. However, while plants play a major role in the capture and deposition of atmospheric CO_2 through photosynthesis, long term storage and sequestration potential is more likely achieved through the retention of carbon within soil. Soil organic matter (SOM) can be broadly described as naturally derived organic material that is found and cycled through soils both rapidly and gradually depending on the physiochemical and biological nature of the ecosystem it is in (Baldock & Broos 2012; Schmidt et al. 2011). Soil organic matter retains nutrients and pollutants in the soil, thus can aid in improving plant health and protecting water quality (Lehmann & Kleber 2015).

Measures of total organic carbon stored in soils and sediments can provide an indication of the storage potential natural and rehabilitated ecosystems can have. In order to understand how carbon storage can be promoted or what may prevent natural ecosystems continuing to function as carbon sinks it is important to know what the carbon stocks of an ecosystem are and in what (chemical) forms they are present. Analytical techniques, such as dry combustion analysis, allow for total (TC), organic (OC) and inorganic carbon (IC) and nitrogen (TN) values to be measured, calculated and used to infer stocks for comparable ecosystems and soil types (Baldock et al. 2013). However extensive, time consuming and costly analytical methods are required to gain appropriate values. Since the 1990's, advances in technology such as diffuse reflectance infrared spectroscopy (IR) and solid-state ^{13}C nuclear magnetic resonance spectroscopy (NMR) have allowed scientists to look at the chemical structure of SOM and in particular, the chemical changes occurring during decomposition (Baldock et al. 1997; Lehmann & Kleber 2015). In terrestrial soils, TC, OC, IC and TN contents and the allocation of soil OC in the particulate, humus and recalcitrant fractions can be predicted using IR in combination with multivariate partial least-squared regression analysis (PLSR) enabling more

time efficient analysis of soils and potentially sediments (Baldock et al. 2013; Janik et al. 2007; Soriano-Disla et al. 2014; Viscarra Rossel et al. 2006).

In solid state ^{13}C NMR, different chemical structures in organic materials are differentiated based on their chemical shift values, expressed in units of parts per million (ppm), values of the magnetic field of the (NMR) spectrometer (Baldock et al. 1997). An estimate of the extent of decomposition of the organic carbon in a sample can be inferred from the ratio of signal intensity associated with the alkyl (45-0 ppm) region to that of the O-alkyl region (110-45 ppm) (Baldock et al. 1997). NMR techniques have been extensively used in the analysis of agricultural soils, yet there has been little application of these techniques in coastal sediments. The application of such techniques to coastal soils, if successful, would improve soil carbon stock estimates of coastal wetlands and better inform the long-term storage potential of carbon in coastal soils.

Discussion

The increase of GHG concentrations in the atmosphere can be directly attributed to the increase of anthropogenic activity on earth. Although naturally occurring in our atmosphere, the increased concentrations of atmospheric GHGs such as CO_2, CH_4 and N_2O through the burning of fossil fuels; cement production; land use change and agriculture are the leading cause of increased temperatures on earth, see '*The carbon cycle*' (McLeod et al. 2011; Pendleton et al. 2012). The carbon cycle can be described as a balanced exchange of atmospheric CO_2 to carbon deposited into the land and oceans then re-mineralised into the atmosphere. However, anthropogenic activity has caused an increase in the output of CO_2 into the atmosphere that results in an unbalanced exchange in the carbon cycle as the output of CO_2 into the atmosphere is greater than the input back through natural processes. Carbon can be stored in different pools

that are all important contributors to the reduction of atmospheric CO_2 through long- and short-term carbon storage. However, carbon sequestration in the environment needs to increase in order to reduce the effect of anthropogenic GHG emissions and to achieve a balance, or net gain, in the carbon cycle.

Blue carbon environments have a high capacity for carbon storage in their soils as a function of the highly productive vegetation, efficient trapping and high burial of carbon-rich materials, and slow decomposition rates of the organic matter (Bouillon et al. 2003; Duarte et al. 2005; Lovelock et al. 2014a; Owers et al. 2020). However, soil stocks across blue carbon habitats are highly variable as a result of local, regional and global differences in their climatic, hydrodynamic and geomorphic features; and the socio-economic pressures on their environment (Chmura et al. 2003; Ewers Lewis et al. 2020; Feher et al. 2017; Owers et al. 2020; Rogers et al. 2019; Saintilan et al. 2013). The supply and preservation of blue carbon are, therefore, linked to the environmental setting of the blue carbon environment. However, in the blue carbon environment the long-term storage and persistence of carbon is driven by their soil characteristics (Kelleway et al. 2016; McLeod et al. 2011; Owers et al. 2020; Saintilan et al. 2013). For example, slowed decomposition due to soil saturation supports the ongoing accumulation of carbon in coastal soils as the carbon is not re-mineralised and released back to the environment (Atwood et al. 2017; Duarte et al. 2013). In addition, soils do not become carbon saturated because of the continuous sediment/organic matter supply leading to vertical accretion (Hayes et al. 2017; McLeod et al. 2011). Soils will, therefore, continue to sequester carbon over time, effectively acting as an infinite carbon sink (Chmura et al. 2003; Duarte et al. 2013; Macreadie et al. 2017; McLeod et al. 2011). But, while sediments have the potential to be carbon sinks, they can also shift to a source of emissions through climate change (precipitation/temperature changes); atmospheric composition effects (CO_2 fertilization,

nutrient deposition, pollution); and land use change (deforestation, agricultural practices). The destabilisation of soil carbon as a result of land clearing or degradation of blue carbon habitats to accommodate such activities will drive the loss of the stored carbon and the release of greenhouse gases (CO_2 and CH_4) into the atmosphere, turning the environment into a net carbon source as opposed to a sink (Pendleton et al. 2012; Twilley et al. 2017).

The accumulation and storage of carbon in blue carbon habitats is reasonably well-studied (Adame et al. 2015; Atwood et al. 2017; Bouillon et al. 2003; Chmura et al. 2003; Ewers Lewis et al. 2018; Hayes et al. 2017; Owers et al. 2016b, 2018; Saintilan et al. 2013) and the indicators of blue carbon in coastal landscapes are heavily dependent on the scale of observation (i.e. global, national, regional, local). The spatial variability of blue carbon stocks is strongly linked to differences in vegetation structure across different environmental settings, particularly as blue carbon environments extend to lower latitudes (Chmura et al. 2003; Ewers Lewis et al. 2018; Ewers Lewis et al. 2020; Hayes et al. 2017). However, there are conflicting views on whether or not above-ground biomass is reflective of soil carbon stocks in the blue carbon environment and improved understanding on the relationship between above-ground biomass and below-ground soil stocks are required (Atwood et al. 2017). Therefore, employing techniques in the blue carbon environment that could successfully predict carbon stocks across a variety of different environmental settings, and account for the variability, could be the most viable option to improve global blue carbon stock estimates necessary for the implementation of carbon offsetting schemes. In addition, better understanding of the fate of soil carbon in a changing blue carbon environment (i.e. more work on the mechanisms underpinning the preservation of carbon in blue carbon soils) would improve long-term storage estimates.

Objectives of the thesis

Coastal wetlands play an important role in climate change mitigation through their natural abilities to sequester significant amounts carbon in their environment. They are responsible for a significant contribution of global carbon stores. However, their global expanse and complex nature makes generalisations of carbon storage dynamics inherently difficult. The future implications of climate change on coastal wetlands brings to the fore the need to better understand the processes surrounding carbon storage in these environments. The following chapters of this thesis add to a growing body of literature aimed at improving our understanding of the blue carbon environment. Specifically, this thesis focuses on the chemical characterisation of organic carbon in temperate coastal wetlands.

The main objectives of the work presented in this thesis were to:

- Quantify and compare surface soil (top 10 cm) carbon and nitrogen stocks of the primary *in situ* vegetation, mangroves and tidal marshes, in temperate vegetated coastal wetlands across selected South Australian sites (*Chapter 2, part 1*).

- Explore the spatial variability of soil carbon and nitrogen stocks within mangroves and tidal marsh vegetation at nine selected South Australian sites (*Chapter 2, part 2*);

- Assess the capability of combined infrared resonance spectroscopy and partial least squared regression analyses (IR/PLSR) in predicting concentrations of carbon and nitrogen in blue carbon soils collected from around Australia (*Chapter 3*);

- Determine the allocation of SOC to particle size fractions (POC, HOC and ROC) in temperate vegetated coastal soils (*Chapter 4, part 1*);

- Predict the allocation of OC to the particle size fractions by developing fraction specific infrared and partial least squared regression (IR/PLSR) models (*Chapter*

4, part 2);

- Identify and quantify the chemical composition of the blue carbon in temperate blue carbon soils (*Chapter 5*).

I hypothesise that:

- o Mangroves will have higher surface soil carbon and nitrogen stocks than tidal marshes that are driven by the structural differences of the vegetation types (*Chapter 2, part 1*);

- o The spatial variability of the carbon and nitrogen stocks will be high between the different vegetation types and the higher variability will be within the tidal marshes as a result of irregular patterns of tidal inundation (*Chapter 2, part 2*).

- o Coastal soils will have higher allocation of OC to the POC fraction as a result of slowed SOM decomposition (*Chapter 4, part 1*).

- o O-alkyl carbon will be the most prevalent form of carbon in the coastal soils as a result of the accumulation of organic matter with slow turnover; and mangrove soils and their size fractions will be enriched with lignin and proteins as a result of their woody structure unlike tidal marshes (*Chapter 5*).

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Chapter Two

Quantifying blue carbon and nitrogen stocks in surface soils of temperate coastal wetlands.

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Abstract

Vegetated coastal wetlands play an important role in combating perturbations induced by climatic changes and anthropogenic activity by removing carbon dioxide from the atmosphere and regulating nutrient supply to adjacent environments. This is facilitated by the capture and storage of a significant amount of carbon in the vegetations above and below ground biomass and soils; their soils accounting for a majority of the ecosystem carbon stocks. The accumulation and storage of carbon and nutrients in the system are, however, driven by characteristics of the ecosystems environmental setting. In this study the carbon and nitrogen content of mangrove and tidal marsh surface soils (top 10 cm) within nine temperate coastal wetlands were quantified and compared. Overall, carbon and nitrogen contents of mangrove and tidal marsh soils did not significantly differ and averaged 18.4 Mg OC ha$_{-1}$ and 17.6 Mg OC ha$_{-1}$, respectively, for organic carbon (OC) and 1.8 Mg N ha$_{-1}$ of total nitrogen (TN) for both. However, within sites the OC stocks significantly differed with mangroves at Clinton and Port Augusta having higher OC than tidal marshes while the opposite was found at Torrens Island. These results highlight vegetation is not an exclusive driver of soil carbon and nitrogen stocks within sites but rather the environmental setting of the blue carbon habitat. In conjunction, the geomorphology, environmental conditions and potentially the organic matter supply within sites rather than above ground vegetation contribute to the spatial variability found in carbon and nitrogen stocks. Future studies quantifying blue carbon in coastal ecosystems need to consider the local scale variability for accurate quantification of the carbon and nitrogen stocks.

Key Words: Blue carbon, Mangroves, Soil carbon, Soil nitrogen, Temperate wetlands, Tidal marshes.

Introduction

Occurring at the interface between land and sea, vegetated coastal wetlands are dynamic ecosystems that play an important role in global carbon and nutrient cycles (Duarte et al. 2013). These ecosystems, including mangrove forests and tidal marshes, absorb significant amounts of carbon dioxide (CO_2) from the atmosphere and capture nutrients leached or eroded from their adjacent environments (Atwood et al. 2017; Chmura et al. 2003; Moseman-Valtierra et al. 2011). Carbon and nitrogen originate from the *in-situ* vegetation or can be deposited with sediments during tidal inundation (Bouillon et al. 2003; Hayes et al. 2017; Saintilan et al. 2013). The carbon in the system is stored in the above and below-ground plant biomass, and soils, with their soils accounting for a majority (49−99%) of the OC stored in the environment (Breithaupt et al. 2012; Donato et al. 2011; Fourqurean et al. 2014; Reddy & DeLaune 2008; Sanders et al. 2016).

The current global carbon stocks of the marine environment, referred to as "blue carbon", is estimated to be 11.25 Peta-grams of carbon (Pg C) which includes the above and below-ground biomass and soils to 1 m (Siikamäki et al. 2013). Of the global blue carbon stocks, mangrove environments account for 6.5 Pg C, tidal marshes for 2 Pg C and seagrasses for 2.3 Pg C (Duarte et al. 2013; Siikamäki et al. 2013). Similar estimates for nitrogen are not available but often as a limiting nutrient in marine environments it regulates the primary productivity of the vegetation and is an important indicator of potential blue carbon productivity (Lovelock et al. 2007; Reddy & DeLaune 2008; Sanders et al. 2014). When particulate organic matter rich in carbon and nitrogen enters a coastal wetland it can become trapped in the vegetation's extensive root system then buried in the soil where it remains long-term due to the slow turnover rate caused by the saline and anoxic soil conditions (Duarte et al. 2013; Howard et al. 2017; Kelleway et al. 2016; Mitra & Zaman 2014).

The accumulation and storage of carbon in blue carbon habitats is inherently linked to the characteristics of their environmental setting (Bouillon et al. 2008; Ewers Lewis et al. 2020; Lovelock et al. 2014; Owers et al. 2020). For example, the temperature, precipitation patterns, geomorphology and hydrodynamics of an environment influence the structure and colonisation of coastal vegetation (Ewers Lewis et al. 2020; Owers et al. 2016b). As such, variability of carbon stocks of surface soils in the blue carbon environment can primarily be explained by the current *in situ* vegetation as they contribute a significant amount of carbon to the soils (Saintilan et al. 2013). In addition, the type of above ground vegetation can be a good indicator of below-ground carbon stocks as a result of its influence on autochthonous carbon additions (primary productivity) and allochthonous carbon capture (sediment accumulation) (Ewers Lewis et al. 2020; Lamb et al. 2006).

The structure and function of the blue carbon environment are, however, susceptible to change in response to climatic perturbations and anthropogenic activity, including temperature increases/decreases, sea level rise, subsidence, erosion, nutrient supply and land use change (Brown et al. 2016; Reddy & DeLaune 2008; Wong et al. 2014). Blue carbon habitats extend from cold polar regions to the tropics and often occur on sheltered, low-lying marine and estuarine coastlines (Chmura et al. 2003; Pendleton et al. 2012; Siikamäki et al. 2013). A significant proportion of the human population also lives on or near coastal margins which increases the deposition of sediments and can contribute to the accumulation of nutrients in the blue carbon environment (Bouillon et al. 2003; Duarte et al. 2008; Rogers et al. 2019; Sanders et al. 2014). Quantification of nitrogen in the blue carbon environment can, therefore, highlight regions influenced by coastal eutrophication and can improve projections of ecosystem carbon storage potential. For example, rates of OC accumulation reported from anthropogenically

impacted regions are substantially higher than non-impacted regions (Sanders et al. 2014).

Previous studies have found patterns in soil organic carbon (OC) stocks to be highly variable across different geomorphic settings. In Chmura et al. (2003), for example, mangrove soils had significantly higher carbon stocks than tidal marsh soils when compared globally. These finding were driven, however, by a greater representation of mangroves from the tropics. This is the result of vegetation from tropical zones having greater primary productivity than sub-tropical or temperate latitudes where tidal marshes are found (Feher et al. 2017; Sanders et al. 2016). Brown et al. (2016) also found mangroves had higher soil carbon stocks than tidal marshes, however, this was driven by the tidal marsh soils having significantly lower dry bulk densities than the mangrove soils. In contrast, when compared within the same region, Howe et al. (2009) found tidal marsh soils had carbon densities 65 % greater than mangrove soils. This was the result of greater mineral sediment accumulation in mangroves due to their marine setting and the tidal inundation patterns of the wetland studied. In addition, studies that investigated carbon stocks between mangrove and tidal marsh soils across different geomorphic settings found carbon stocks to be highly variable as a result of differences in sediment supply (Ewers Lewis et al. 2018; Hayes et al. 2017).

In this study we aimed to quantify and compare surface soil (top 10 cm) carbon and nitrogen stocks of the primary *in situ* vegetation, mangroves and tidal marshes, within sites across different environmental setting across South Australia. We hypothesised that mangroves will have higher surface soil (top 10 cm) carbon and nitrogen stocks driven by the structural differences of the different vegetation types. In addition, we explored the spatial variability of soil carbon and nitrogen stocks across mangroves and tidal marsh vegetation within sites. We hypothesised the spatial variability of the carbon and nitrogen stocks would be high between

the different vegetation types, with higher variability within the tidal marsh vegetation as a result of irregular patterns of tidal inundation. Such information can provide future studies, especially those intending to assess blue carbon ecosystems for accounting purposes, a better understanding of the within site spatial variability of carbon and nitrogen stocks.

Materials and Methods

Study site and sample collection

Nine sites along South Australia's coastline spanning the eastern side of Gulf St Vincent and Spencer Gulf were sampled (Figure 2.1) during the (Austral) spring of 2016 and 2017, with permission from the Department of Environment and Water (Permit number U26525-1). Study sites included Mutton Cove, Torrens Island, Port Gawler, Port Wakefield, Clinton, Port Broughton, Port Pirie, Port Paterson and Port Augusta, characterised in Table 1 (Bourman et al. 2016). At all sites, vegetation was dominated by two tidal marsh species, the beaded samphire (*Sarcocornia quinqueflora*) and scrubby samphire (*Tecticornia arbuscular*) and the single species of mangrove present in South Australia, the grey mangrove (*Avicennia marina*). At each site, three 35 m transects spanning the tidal marsh and mangrove eco-tone were sampled. The eco-tone was determined by the presence of mangrove seedling establishment within the tidal marsh dominant vegetation. Transects were run horizontally from the first distinct mangrove equal distance into the tidal marsh/mangrove habitat. On each transect, eight 0–10 cm soil samples were collected at 5 m intervals (i.e. eight sampling positions per transect) across the tidal marsh and mangrove eco-tone such that a total of 12 samples were collected from each vegetation type. Soil samples were collected with 12 cm high $\times$ 8 cm diameter cores inserted to a depth of 10 cm. We decided to focus on the top 10 cm as it was expected to be the most representative of the short-term influence of the overlying vegetation type and the current

carbon and nitrogen stocks (Kelleway et al. 2017; Yando et al. 2016). For example, with increasing depths carbon stocks can be representative of vegetation that previously but no longer inhabits the sampling location (Owers et al. 2020).

A total of 216 (108 tidal marsh and 108 mangrove forest) soils were collected across the nine sites. Following collection, intact cores were stored at $<4_o$C for transportation back to the laboratory where they were frozen within 5 hours of collection.

Soil processing and analysis

Frozen soil samples were lyophilised (Cuddon freeze dryer, Blenheim, New Zealand), weighed, and bulk densities (BD) calculated as dry weight (g)/soil volume (cm_3), prior to being crushed and sieved ($\leq$2 mm) to remove gravel and root material. A riffle box (12×13 mm slotted box; Civilab Australia, Sydney, Australia) was used to collect a representative sub-sample (approx. 25 g) for fine grinding (Standard Ring Mill, SRM-RC-3P; Rocklabs Ltd, Auckland, New Zealand, fitted with a stainless-steel head, CARB-40-BLP). Samples were analysed for total carbon (TC) and total nitrogen (TN) contents by high temperature dry combustion (LECO TruMac CN analyser, LECO Corporation, St. Joseph, MI, USA).

The presence of inorganic carbon (IC) in samples was determined using diffuse reflectance infrared (IR) spectra collected as described in Baldock et al. (2013) using a Nicolet 6700 FTIR spectrometer (Thermo Fisher Scientific Inc., Waltham, MA, USA). Inorganic carbon has a distinct and easily observable absorption at a frequency of 2560—2480 cm_{-1} (Vohland et al. 2014). Samples containing IC were acidified repeatedly with 1 M hydrochloric acid (HCl; approximately 25 mL) until effervescence ceased, washed (three times) with deionised water, frozen and lyophilised followed by dry combustion carbon analysis (as described above) to

determine OC content. The difference between TC and OC contents was used to calculate IC content. For the soils containing no IC, OC contents were equated to the measured TC contents. Stocks of soil OC; IC and TN (Mg ha$_{-1}$) in the 0-10 cm soil layer were calculated according to Equation 1 using OC as an example.

$$\text{Equation (1):} \quad Soil\ OC\ Stock\ (Mg\ OC\ ha^{-1}) = \frac{OC \times BD \times Thickness \times (1 - P_{>2mm})}{10}$$

Where: OC = dry equivalent $\leq$2 mm soil OC content (g OC kg$_{-1}$ soil); BD = dry bulk density (Mg whole soil/m$_3$ soil); Thickness = the total layer thickness (10 cm); $P_{>2mm}$ = the proportion of total dry soil mass that was retained on the 2 mm sieve.

Statistical analysis

For all nine sites, the effects of transect and position were tested in both mangrove and tidal marsh vegetation. Differences between vegetation types, mangrove and tidal marsh were analysed in a combined analysis across all sites as well as separately within each site. A linear mixed effects model, fit by restricted maximum likelihood (REML), was used to assess the influence of vegetation type on BD, OC, IC and TN contents and carbon to nitrogen (OC:TN) ratios. Within the model, vegetation type (i.e. mangrove vs. tidal marsh) was set as the fixed effect and site, vegetation type nested within site and transect nested within vegetation type and site were set as random effects. Homoscedasticity and normality were confirmed for all test parameters. Likelihood ratio tests were applied to the full model with effect of vegetation type included against the model without the effect in question to obtain p-values. Univariate analysis was applied to the BD, OC, IC, and TN stocks data with the linear model as described above. Three sites (Torrens Island, Mutton Cove and Port Broughton) were excluded for the analysis of IC due to either its absence in the samples or its presence in only 3 samples.

Additionally, the corresponding bivariate ANOVA with random effects of site and site by vegetation was performed for OC and TN. Statistical analysis and graphic outputs were performed using GenStat 19 (VSN International 2017) and R studio for R (R Core Team 2017; RStudio Team 2016) with packages 'lme4' (Bates et al. 2015); 'ggplot2' (Wickham 2016) and 'grid extra' (Auguie 2016).

Results

The linear mixed model fit by REML showed the variance introduced by site, as the random effect, for BD (0.04 > 0.02), OC (51.5 > 17.9), IC (169.1 > 92.59), TN (0.2 > 0.1) and C:N ratio (2.7 > 1.7) was greater than the residual variance and thus could not be ignored and was analysed further. Conversely, the random effect of vegetation type nested in site and transects nested in vegetation type and site did not significantly influence the effect of vegetation on BD, OC, IC, TN and C:N ratio and could be ignored. Therefore, the subsequent results presented are for the response of each soil property to vegetation type, across and within sites, explored with bivariate and univariate ANOVA.

Organic carbon

Average 0–10 cm soil OC stocks across all sites were 18.4 Mg OC ha^{-1} for mangroves (n = 108) and 17.6 Mg OC ha^{-1} for tidal marshes (n = 108; Figure 2.2a). Overall at the broad geographical scale, soil OC stocks were not significantly different between the two vegetation types ($p > 0.05$). However, within sites, mangroves at Clinton and Port Augusta had significantly higher ($p < 0.001$ and $p < 0.05$, respectively) average soil OC stocks of 16.9 Mg OC ha^{-1} and 9.8 Mg OC ha^{-1} than tidal marshes which had respective average soil OC stocks of 12.5 Mg OC ha^{-1} and 6.5 Mg OC ha^{-1} (Figure 2.3a). Conversely at Torrens Island, tidal

marsh soil OC was significantly higher, averaging 33.7 Mg OC ha-1, in comparison to 25.5 Mg OC ha-1 for mangroves ($p < 0.001$; Figure 2.3a). At the other six sites sampled there were no significant differences in average soil OC stocks based on vegetation type (Figure 2.3a). To further explore soil OC patterns at the site level, stocks by the eight positions along transects (n = 3) were compared (Figure 2.4a). Analyses confirmed samples positioned within the mangroves (i.e. sampling positions 5–8) at Clinton had significantly higher soil OC ($p < 0.001$) stocks than samples positioned within tidal marshes (position 1–4; see also Figure 2.4a). In contrast, at Mutton Cove soil OC stocks were significantly different by position ($p < 0.05$) but were equally variable for both vegetation types (see also Figure 2.4a). At Port Augusta and the other sites, soil OC stocks were not found to significantly differ by position ($p > 0.05$; see also Figure 2.4a).

Inorganic carbon

At six of the nine sites sampled, IC accounted for more than 50% of the TC measured. Where IC was present, average 0–10 cm soil IC stocks were 31.9 Mg IC ha-1 for mangroves (n = 72) and 35.1 Mg IC ha-1 for tidal marshes (n = 75; Figure 2.2b). As for OC, IC stocks were not significantly different between the two vegetation types when all sites were combined ($p > 0.05$). Within sites, tidal marshes at Port Paterson and Port Augusta had significantly higher ($p < 0.001$ for both) soil IC stocks of 41.3 Mg IC ha-1 and 18.6 Mg IC ha-1 compared to average soil IC stocks of 24.3 Mg IC ha-1 and 9.7 Mg IC ha-1, respectively, for mangroves (Figure 2.3b). Average soil IC stocks within the other five sites containing IC did not significantly differ by vegetation type (Figure 2.3b). As with OC, IC stocks at the individual site level were compared between the eight positions along transects (n = 3) with no differences between vegetation types observed (Figure 2.4b). However, within the mangroves at Port Augusta, soil IC stocks were significantly higher at position five (15.6 Mg IC ha-1) than positions six, seven and eight

(9.3 Mg IC ha$_{-1}$, 11.6 Mg IC ha$_{-1}$ and 9.5 Mg IC ha$_{-1}$, respectively; $p < 0.05$).

Total Nitrogen

Average total nitrogen (TN) stocks in surface soils were 1.8 Mg TN ha$_{-1}$ for both mangroves (n = 108) and tidal marshes (n = 108; Figure 2c). With all sites combined, TN stocks were not significantly different between the two vegetation types ($p > 0.05$). Within sites, mangroves at Clinton had significantly higher ($p < 0.05$) average TN stock of 1.9 Mg TN ha$_{-1}$ than tidal marshes, average TN stock of 1.6 Mg TN ha$_{-1}$ (Figure 2.3c). Conversely at Torrens Island, tidal marsh TN stocks were significantly higher, averaging 2.7 Mg TN ha$_{-1}$, in comparison to 2.1 Mg TN ha$_{-1}$ for mangroves ($p < 0.05$; Figure 2.3c). Average TN stocks for the other seven sites did not significantly differ in within site based on vegetation type (Figure 2.3c). Moreover, no significant differences in TN stocks were found when the eight positions along transects (n = 3) at each individual site were compared (Figure 2.4c).

Carbon to Nitrogen ratio

Carbon to nitrogen ratios (C:N) averaged 9.9 ($\pm$ 0.2) for mangroves (n = 108) and 9.4 ($\pm$ 0.2) for tidal marshes (n = 108; Figure 2.2d). With all sites combined, C:N were not significantly different between the two vegetation types ($p > 0.05$). Within sites, tidal marshes at Torrens Island and Mutton Cove had significantly higher ($p < 0.05$ and $p < 0.001$, respectively) C:N ratios of 12.6 and 12.4 compared to mangrove (C:N ratios of 12.1 and 11.3, respectively, Figure 2.3d). In contrast, C:N ratios for the mangroves were significantly higher ($p < 0.05$ for all) at Clinton (9.0), Port Pirie (9.6) and Port Augusta (11.3) compared to tidal marshes (7.5, 8.6 and 9.0, respectively; Figure 2.3d). At Mutton Cove, the comparison of vegetation type by positions (n = 3) showed significantly lower average C:N for samples taken at position seven in the

mangroves (10.6) compared to all other positions (C:N range = 11.3 – 12.7; $p < 0.05$; see also Figure 2.4d).

Bulk density

As shown by equation (1), soil carbon and nitrogen stocks are determined by the relationship of content, bulk density and depth. Therefore, we also compared soil BD to determine whether any differences in stocks found between vegetation types were due to significant differences in soil BD. With all sites combined, soil BD did not significantly differ between the two vegetation types ($p > 0.05$). Within sites, tidal marsh soils had significantly higher ($p < 0.0001$ and $p < 0.001$) BD of 0.95 g/cm3 and 0.96 g/cm3 at Clinton and Port Paterson than soil BD of 0.48 g/cm3 and 0.81 g/cm3, respectively, for mangroves (Figure 2.5). Soil BD was also marginally significantly higher ($p = 0.05$) in tidal marshes (0.71 g/cm3) than mangroves (0.58 g/cm3) at Port Pirie. Conversely at Mutton Cove, mangrove soil BD was significantly higher, averaging 0.46 g/cm3, in comparison to 0.31 g/cm3 for tidal marsh soils ($p < 0.0001$; Figure 2.5). At the other six sites sampled there were no significant differences in soil BD based on vegetation type (Figure 2.5). To further explore patterns at the site level, BD of the soils by the eight positions along transects (n = 3) were also compared (Figure 2.6). Analyses showed samples positioned within the tidal marshes (1–4) had significantly higher soil BD at Clinton ($p < 0.0001$), Port Pirie ($p < 0.05$) and Port Paterson ($p = 0.02$) than samples positioned within mangroves (positions 5–8; see also Figure 2.6). Alternatively, for Mutton Cove, samples positioned within the mangroves (5–8) had significantly higher soil BD ($p < 0.001$) than samples positioned within the tidal marsh (1–4; see also Figure 2.6).

Discussion

Comparison of mangrove and tidal marsh OC, IC, and TN stocks (Mg ha-1), composition and BD (g/cm3) in surface soils collected from nine temperate wetlands with different environmental settings revealed no significant differences between vegetation types. However, when compared within sites, significant differences in surface soil carbon and nitrogen stocks, composition and BD were found. This suggests that at a regional scale, above ground vegetation is not having an effect on surface soil carbon and nitrogen stocks in temperate vegetated coastal wetlands. Within sites, the impact of vegetation type on surface soil carbon and nitrogen stocks is dependent on the specific site. These results are consistent with findings from previous studies comparing soil carbon stocks between mangrove and tidal marsh vegetation (Ewers Lewis et al. 2018; Hayes et al. 2017; Owers et al. 2016a; Saintilan et al. 2013). For example, previous found no significant differences between the two vegetation types overall (Ewers Lewis et al. 2018; Hayes et al. 2017); or significantly higher soil OC densities in either tidal marsh (Howe et al. 2009; Livesley & Andrusiak 2012) or mangrove vegetation (Adame et al. 2015; Chmura et al. 2003) depending on their geomorphology and location. This suggests that surface soil carbon stocks measured were influenced by location of sample collection and geomorphic attributes of the site, not by the transition of the overlaying vegetation type. This work was not intended, however, to be an assessment of blue carbon and nitrogen stocks to be compared with regional or global blue carbon stocks, given the shallow sampling depth (top 10 cm), but rather to quantify the vegetation driven contribution of carbon and nitrogen to the soil in different environmental settings. In addition, assessment of the spatial variability also revealed that each site behaved differently, and transition of vegetation type only explained these differences for some sites.

The average OC and TN stocks and soil BD measured for mangrove and tidal marsh surface

soils in this study (18.4 Mg OC ha^{-1} and 17.6 Mg OC ha^{-1}, respectively; 1.8 Mg TN ha^{-1} for

both; and 0.59 g/cm^3 for mangroves and 0.66 g/cm^3 for tidal marshes) did not significantly

differ between vegetation types. Previous research showed a similar pattern in mangroves and

tidal marshes co-occurring in southeast Australian wetlands (Ewers Lewis et al. 2018) and

Moreton Bay, Qld, with no significant difference in average soil OC stocks between the two

vegetation types reported (Hayes et al. 2017). However, when compared within sites,

mangroves at Clinton and Port Augusta had significantly higher surface soil OC and TN stocks

than tidal marshes, while the opposite was true for Torrens Island. Mangroves at Port Augusta

also had significantly higher OC stocks than the tidal marshes, but the same was not true for

TN. These results agree with previous blue carbon studies that also found significant

differences within sites between the different vegetation types (Adame et al. 2015; Chmura et

al. 2003; Ewers Lewis et al. 2018; Hayes et al. 2017; Howe et al. 2009; Livesley & Andrusiak

2012). For example, when comparing soil OC stocks across different geomorphic settings,

mangroves had higher soil OC stocks than tidal marshes in riverine settings and the opposite

was true for mixed settings (Hayes et al. 2017). Taken together, this suggests that differences

in geomorphology at Clinton, Port Augusta and Torrens Island may be important drivers of

differences in soil OC stocks between the vegetation types. And in turn, the soil TN stocks

increase with increases in OC stock due to the close interaction between the carbon and

nitrogen cycles (Adame et al. 2015). This is represented by the TN pattern following the same

pattern as surface soil OC stocks within each site.

Torrens Island, unlike Clinton and Port Augusta, is located within the Port Adelaide River-

Barker Inlet estuary with limited influence from oceanic inputs. Higher tidal marsh OC and TN

stocks for this site are consistent with findings of Donato et al. (2011), who reported lower soil

OC stocks in estuarine versus oceanic mangroves due to significant differences in the depth of

the peat layer of substrate, a key driver of soil OC stocks in coastal ecosystems. Increased allochthonous organic matter input is likely occurring in the tidal marsh vegetation of Torrens Island due to natural subsidence of the low laying LeFevre Peninsula area where Torrens Island is located. This results in frequent tidal flooding of the supratidal tidal marsh dominated region (Bourman et al. 2016) and increasing sediment deposition. This suggests that differences in local geomorphic conditions including tidal range, elevation, suspended sediment supply and differences in allochthonous versus autochthonous inputs within sites are key drivers of differences in OC and TN stocks in combination with vegetation type for some sites (Donato et al. 2011; Ewers Lewis et al. 2018; Hayes et al. 2017).

Soil BD can be used as a measure of soil compaction, generally found to increase with soil depth or when wetland soils are drained (Howe et al. 2009). Thus, the trend of higher soil BD in tidal marsh soils at Clinton, Port Paterson and Port Pirie or mangrove soils at Mutton Cove could represent greater soil compaction or higher drainage of the respective vegetation types at these sites. Clinton and Port Paterson occupy shallow, sheltered, low energy environments with carbon rich allochthonous offshore sediments deposited by frequent tidal inundation (Bourman et al. 2016). Low energy tidal environments such as these can have higher mineral fraction deposition closer to the tidal source (i.e. in the mangroves), causing soils that occur deeper in the wetlands and away from the tidal source (i.e. the tidal marsh), to have a lower mineral fraction in their soils (Howe et al. 2009). This pattern is in contrast to our findings that suggest a higher mineral fraction in the tidal marsh soils to account for the significantly higher soil BD observed for Clinton and Port Paterson. However, differences in soil BD could also just be the result of significantly higher proportions of gravel in tidal marshes soils (0.09 g > 0.04 g, $p < 0.0002$). As carbon and nitrogen stocks are a product of both the carbon or nitrogen content and BD, site-specific differences in soil BD can have significant implications for future

carbon accounting investigations if a site with significant differences in BD is present within the study.

Limited fresh water inputs, low rainfall and high evaporation in the relatively dry environment of South Australia can lead to enhanced production of biogenic carbonate material (Bourman et al. 2016; Gostin et al. 1988); this may explain the presence of IC at seven of the nine sites sampled. The high IC stocks of the South Australian blue carbon soils are unlike other regions where a majority of the soil carbon was present as organic carbon (Howe et al. 2009). High salinities, higher water temperatures, low precipitation and high evaporation in the tidal dominated upper northern Spencer Gulf coastline promote production and accumulation of carbonate materials (Bourman et al. 2016). Therefore, higher surface soil IC stocks measured in the tidal marsh vegetation of Port Augusta and Port Paterson likely arises due to amplified tidal range of the sites where the supratidal habitat of the tidal marsh is frequently flooded (Bourman et al. 2016). The amplified tidal range would also account for the significantly higher soil IC stocks of the mangroves positioned at the transitional zone, position five, at Port Augusta. Of interest, however, is the lack of IC at both Torrens Island and Mutton Cove, which may be the result of both sites being located in a sheltered embayment and is worthy of further investigation.

Carbon to nitrogen ratios can be used as an indicator for determining the quality, rate of decomposition and source of soil organic matter (SOM) (Batjes 1996; Gonneea et al. 2004; Kristensen et al. 2008; Matsui et al. 2015). The low C:N ratios of the mangrove (9.9) and tidal marsh soils (9.4) in this study suggests the rate of SOM decomposition is rapid for both vegetation types. Significant differences in the C:N ratios between the vegetation within sites however suggest more rapid decomposition of the mangrove SOM at Torrens Island and

Mutton Cove, and tidal marsh SOM at Clinton, Port Pirie and Port Augusta. Greater SOM decomposition in these ecosystems is potentially driven by less frequent tidal inundation; more frequent fresh litter inputs to the vegetation with higher C:N ratios; or inputs of higher quality OM. If more decomposed, this also suggests the SOC stocks in the mangroves at Torrens Island and Mutton cove, or tidal marshes at Clinton, Port Pirie and Port Augusta, will remain as long-term stable soil OC stores. Future studies should consider quantifying nitrogen stocks to better understand the quality of the SOM and its potential decomposition rates.

Conclusions

There is high variability in the patterns of carbon and nitrogen stocks between mangrove and tidal marsh surface soils. Sampling the ecotone between mangroves and tidal marshes highlighted the spatial variability of carbon and nitrogen stocks within sites, particularly at Clinton and Mutton Cove. These findings show vegetation type may be a significant contributor to differences in blue carbon and nitrogen stocks but characteristics of their environmental setting (i.e. geomorphology, tidal inundation) is also a key driver. Mangroves had significantly higher surface soil carbon and nitrogen stocks in some sites, while the opposite or no difference was true for others. In addition to the saline, anoxic and nutrient limited nature of blue carbon soils that already promotes storage of significant amounts of carbon for long periods of time (i.e. millennia). It is important to acknowledge the significant differences shown between sites for future carbon accounting considerations in these ecosystems, as limiting sampling intensity to one or a few location(s) may be unrepresentative of the spatial variability of carbon and nitrogen stocks in mangrove and tidal marsh ecosystems, particularly across different geomorphic settings. Overall, the differences in carbon and nitrogen stocks within the blue carbon environment appears to be dependent on site-specific

geomorphology, environment and SOM inputs.

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Table 2.1 Region and site characteristics of temperate coastal wetlands sampled. Summarised from Bourman et al. (2016).

Region	Location	Characteristics	Vegetation	Sedimentation/Deposition	Tidal Range
Gulf St Vincent	*35.13°S, 138.23°E*	Tectonically formed St Vincent basin; low energy environment; inverse estuary; limited freshwater inputs; high evaporation rates.	Peritidal sequence of subtidal seagrass meadows, intertidal sandflats, mangrove and marsh habitats; supratidal marsh.	Mostly marine derived sediments; strong influence of Pleistocene and Holocene sedimentation.	~ 3 m
Mutton Cove	*34.78°S, 138.51°E*	Reinstated tidal flow from low energy estuarine environment of the Port Adelaide River; natural subsidence.	Recent regeneration of mangrove woodlands; currently dominated by supratidal Samphire.	Carbonate quartz silts, muds and sands with organic material and cyanobacteria mats.	<2 m
Torrens island	*34.79°S, 138.53°E*	Tidal dominant; fronted by low energy estuarine environment of Barker Inlet and the Port Adelaide River; natural subsidence; subject to flooding of supratidal zone.	Subtidal and midtidal seagrasses; intertidal mangrove woodlands and marsh habitat; supratidal Samphire.	Carbonate quartz silts, muds and sands with organic material and cyanobacteria mats.	<2 m
Port Gawler	*34.65°S, 138.48°E*	Tidally dominated coast; occasional inputs from The Gawler River.	Subtidal and midtidal seagrasses; intertidal mangrove woodlands and marsh habitat;	Carbonate quartz silts, muds and sands with organic material; occasional storm driven sand and shell detritus; fine-	<2 m

		supratidal Samphire.	grained sand deposits during heavy rainfall.	
Port Wakefield	*34.18°S, 138.15°E*	Shallow, sheltered and low energy coastline; predominant tidal inlet, with occasional freshwater flows from The Wakefield River during wet years.	Continuous mangrove woodlands backed by flat tidal marsh plain and stranded Samphire.	Carbon rich allochthonous offshore deposits.	~ 3 m
Clinton	*34.22°S, 138.02°E*	Shallow, most sheltered and lowest energy part of GSV; dominated by tides and local wind waves; high gravel content; vegetation growth on top of rocky coastline.	Continuous mangrove woodlands backed by flat intertidal tidal marsh plain.	Carbon rich allochthonous offshore deposits.	~ 3 m
Spencer Gulf	*34.30°S, 136.98°E*	Tidal dominated inverse estuary; high water salinities (34-49 ppt); evaporation exceeds freshwater inputs; water temperatures 13-38°C.	Thick seagrass meadows; wide intertidal sandflats; mangrove woodlands and supratidal marsh.	Pleistocene and Holocene marine sedimentation	2.5-4.1 m
Port Broughton	*33.58°S, 137.94°E*	Complex tidal inlet; hyper saline.	Subtidal seagrass beds, intertidal sandflats with mangrove fringe; extensive	Holocene marine deposits	2.5-3 m

			intertidal and supratidal Samphire flats and bare supratidal saline flats.		
Port Pirie	*33.17°S, 138.01°E*	Low-gradient, low-energy environment; distinct zonation of vegetation and geomorphology; tidal dominated; hydrostatic uplifting coast over the past 6700 years; fall in sea level.	Subtidal seagrass beds, intertidal sandflats with mangrove fringe; extensive intertidal and supratidal Samphire flats and bare supratidal saline flats.	Holocene marine deposits	2.5-3 m
Port Paterson	*32.55°S, 137.82°E*	Shallow embayment; low energy coastal plain.	Fringing mangrove woodlands; intertidal and supratidal Samphire flats.	Accretion of sand, shells, silts and clays.	Amplified tidal range (4.1 m)
Port Augusta	*32.49°S, 137.79°E*	Narrowest part of the estuary; Shallow, sheltered, low energy, protected wave environment.	Mangrove woodlands; intertidal and supratidal Samphire flats.	High production and accumulation of biogenic materials.	Amplified tidal range (4.1 m)
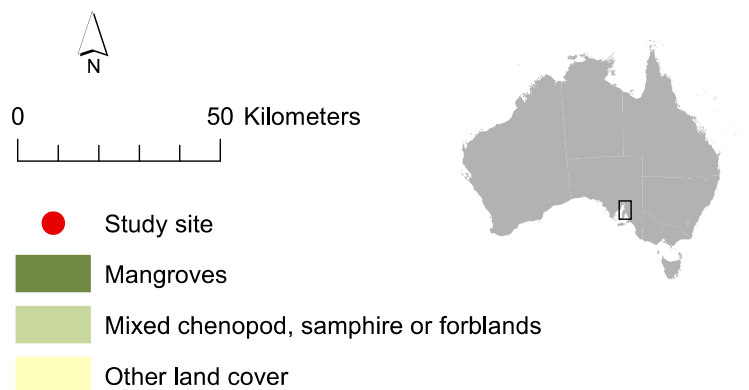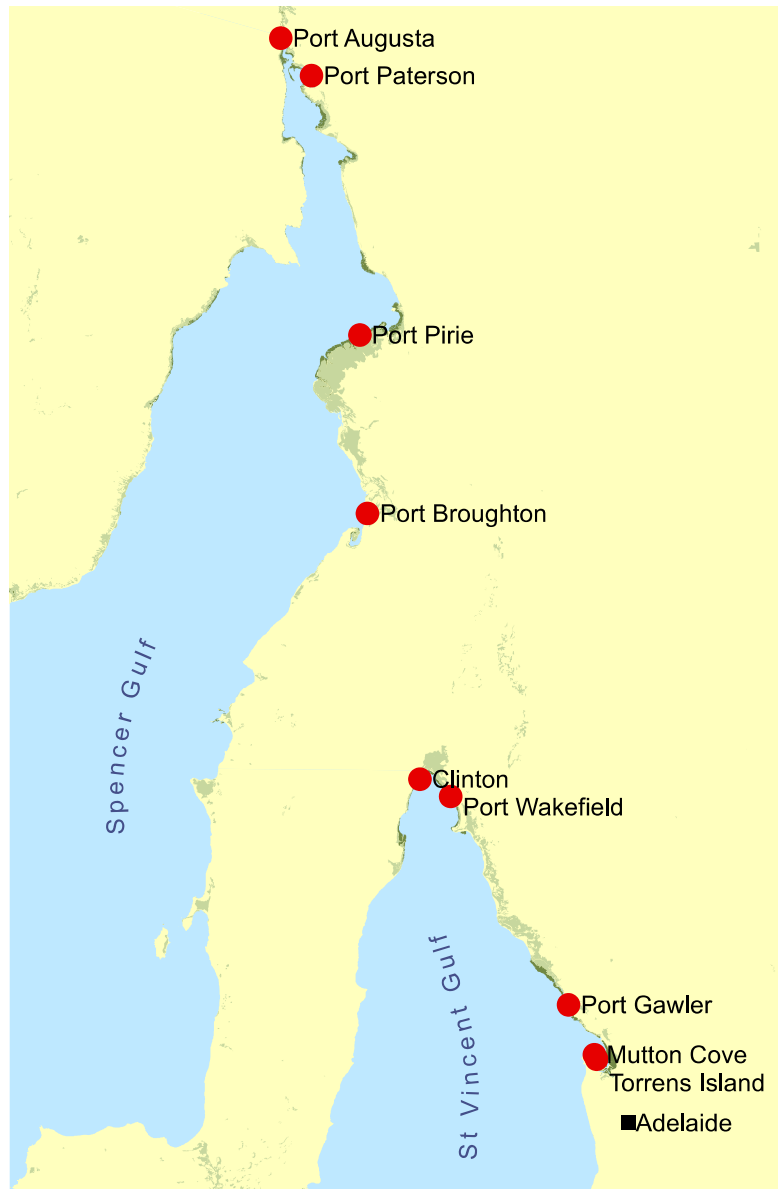
Figure 2.1 Location of the nine sampling sites where soil samples were collected for analysis from vegetated coastal ecosystems of South Australia, Australia.
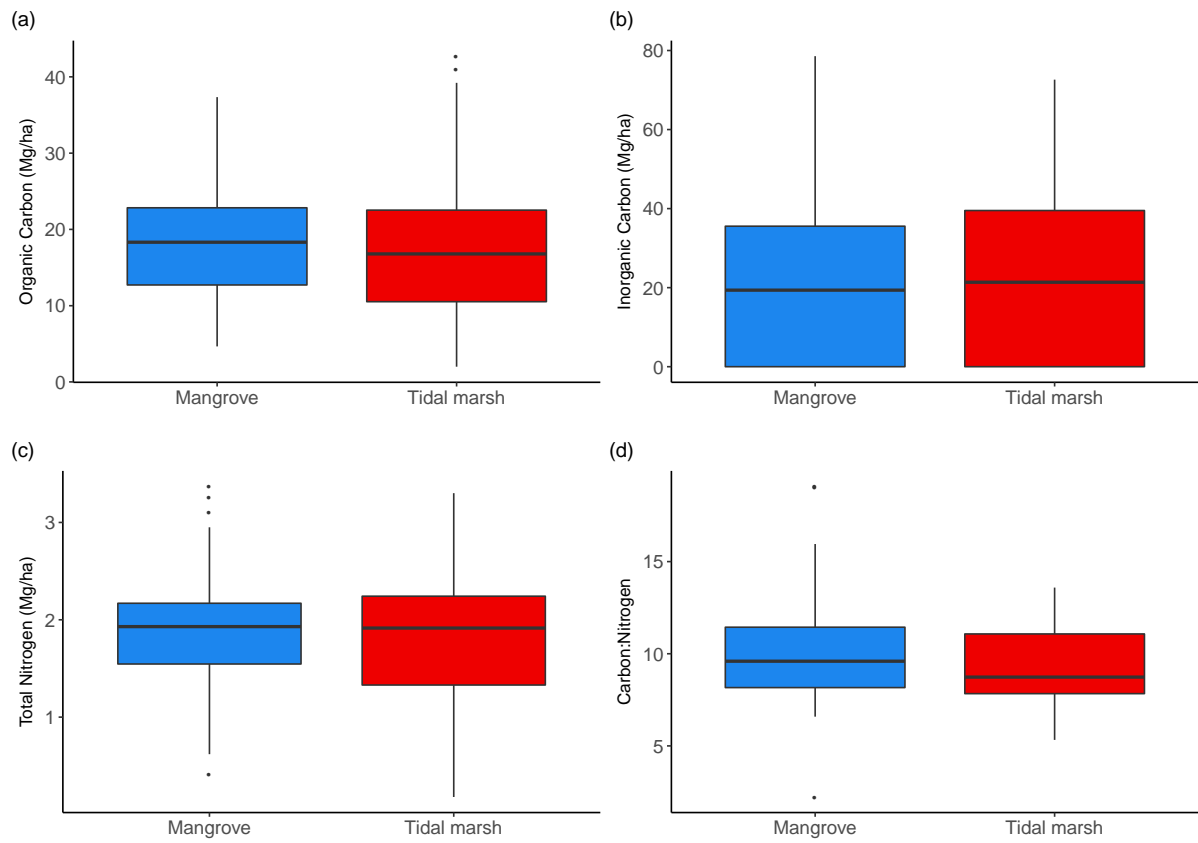
Figure 2.2 Distribution of soil (a) organic carbon stocks; (b) inorganic carbon stocks; (c) total nitrogen stocks (Mg/ha); and (d) C:N ratios contents in mangrove (n = 108) and tidal marsh (n = 108) soils from temperate vegetated coastal wetlands.
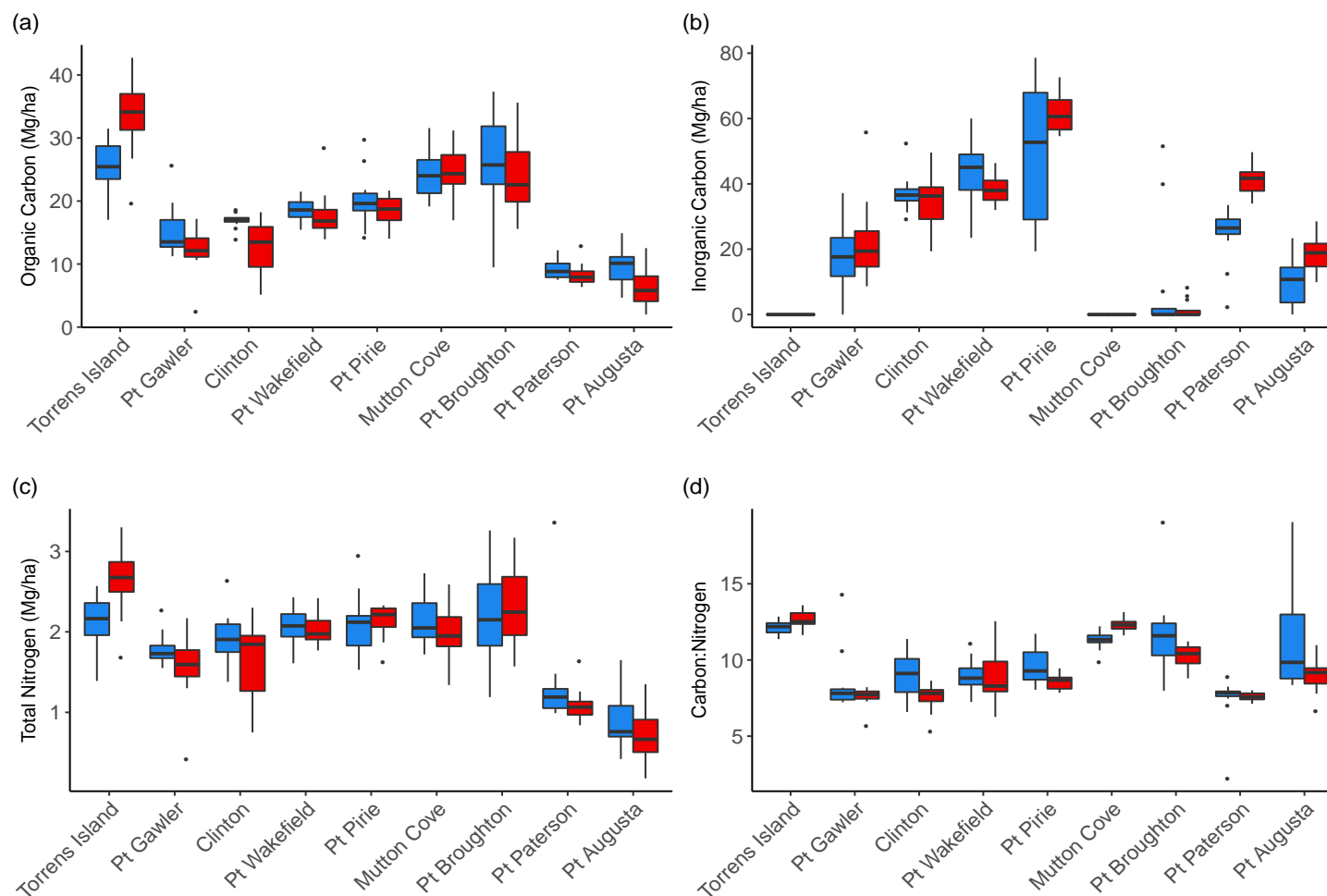
Figure 2.3 Distribution of soil (a) organic carbon stocks; (b) inorganic carbon stocks; (c) total nitrogen stocks (Mg/ha); and (d) C:N ratios in mangrove (blue; n = 12) and tidal marsh (red; n = 12) soils within each of the nine temperate wetland sites.
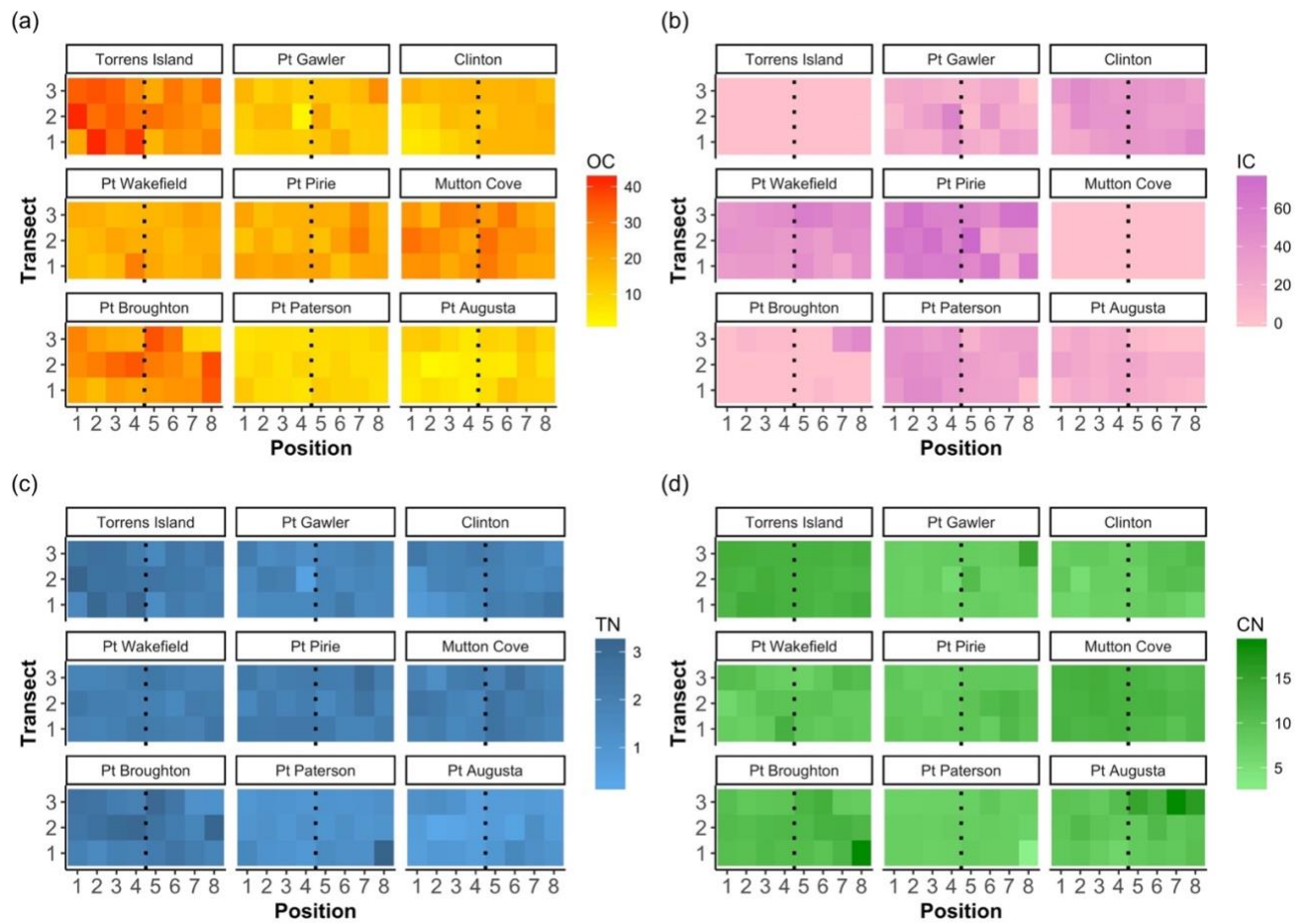
Figure 2.4 Spatial distribution of soil (a) organic carbon stocks; (b) inorganic carbon stocks; (c) total nitrogen stocks; and (d) C:N ratios by position vs. transect in tidal marsh (positions 1–4) and mangrove (positions 5–8) soils, separated by the dashed line, within each of the nine coastal wetlands sampling sites in South Australia.
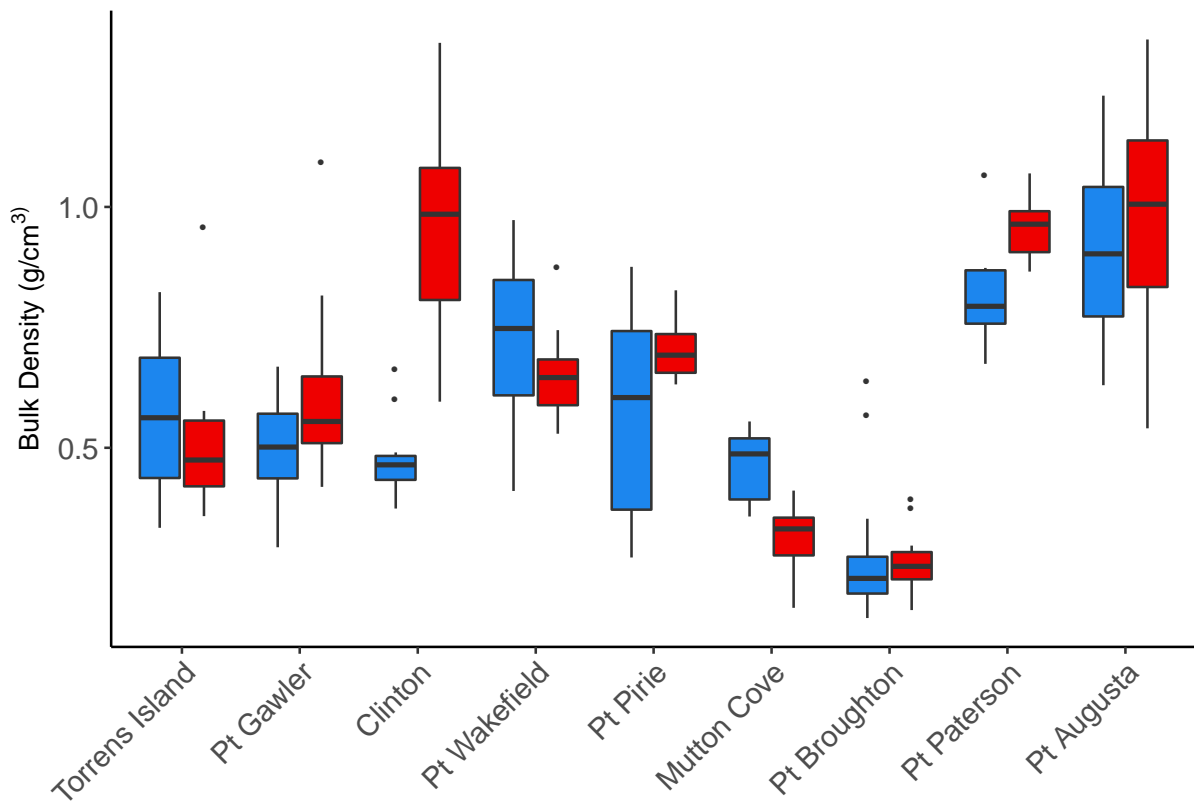
Figure 2.5 Distribution of soil bulk density (g/cm3) of the top 10cm for mangroves (n = 12 at each site; blue) and tidal marshes (n = 12 at each site; red) at each of the nine coastal wetlands sampling sites in South Australia.
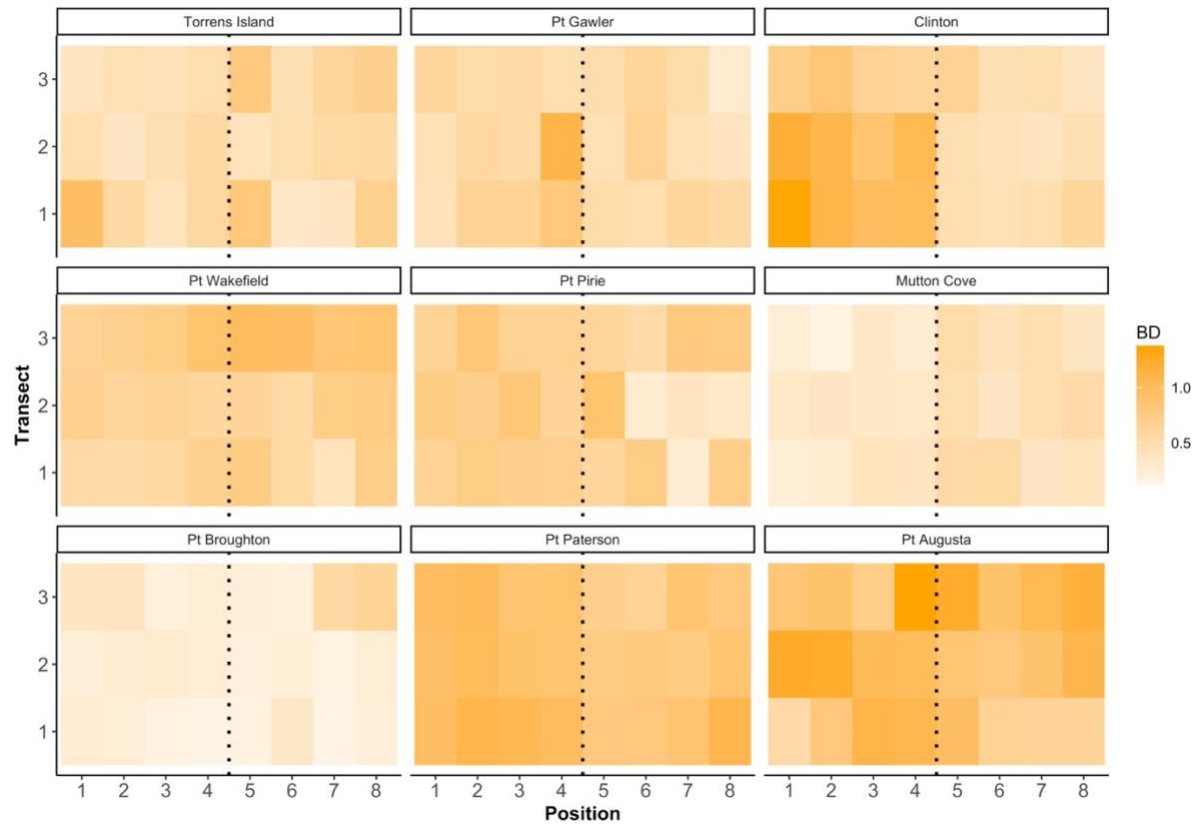
Figure 2.6 Spatial distribution of soil bulk density (g/cm3) of the top 10 cm by position vs. transects in tidal marsh (positions 1–4) and mangrove (positions 5–8) soils within each of the nine coastal wetlands sampling sites in South Australia.

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Chapter Three

Predicting carbon and nitrogen concentrations in Australian blue carbon soils using diffuse reflectance infrared spectroscopy.

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Abstract

Coastal blue carbon environments (mangroves, tidal marshes and seagrass meadows) can contain significant stocks of soil organic carbon and can accumulate additional organic carbon through the capture and retention of organic materials derived from autochthonous and allochthonous sources. Assessing soil organic carbon stocks present in these environments requires quantification of soil organic carbon concentration. Developments in the combined use of infrared spectroscopy and partial least squares regression analyses (IR/PLSR) have demonstrated an ability to provide cost effective measurement of organic carbon concentration in agricultural soils and additionally provide values for the concentrations of inorganic carbon and total nitrogen from one analysis. The objective of this study was to assess the capability of IR/PLSR analyses to provide accurate values for total (TC), organic (OC) and inorganic (IC) carbon and total nitrogen (TN) concentrations in soil samples derived from blue carbon environments. A total 1201 samples were used to derive the IR/PLSR predictive algorithms. Diffuse reflectance infrared spectra were acquired over the 6000-600 cm_{-1} spectral range and TC, OC, IC and TN analytical data were obtained using automated dry combustion analysers (LECO TruMac, C-144 or CNS-2000) with the application of acid pre-treatment to soils containing carbonate. Independent sets of 200-300 and 385-901 samples were used respectively to develop and then validate IR/PLSR predictive algorithms. Robust IR/PLSR predictive algorithms were obtained for TC, OC, IC and TN concentrations as evidenced by values derived for the coefficient of determination (R_2=0.91-0.96) and the ratio of performance to interquartile range (RPIQ = 3.7-8.5). The general applicability of the IR/PLSR predictive algorithms to Australian blue carbon soils was assesses through their application to 3830 Australian blue carbon test soils for which IR spectra had been acquired. Projection of the test soils onto the factor scores of the IR/PLSR models and plots of Hotelling's T_2 and inlier distance values showed that the models could be confidently applied to predict concentrations

of TC, OC, IC and TN. The IR/PLSR approach, once adequately calibrated, provided a rapid and cost-effective alternative approach to quantifying concentrations of the various forms of carbon and total nitrogen in Australian blue carbon soils.

Keywords: Blue carbon soils, mangrove, tidal marsh, seagrass, organic carbon, inorganic carbon, total nitrogen, partial least squares regression

Introduction

Natural organic matter, is the largest reactive reservoir of reduced carbon on Earth, with global carbon stocks estimated at 1,600 Pg C for soils, 550 Pg C for terrestrial vegetation, 1,000 Pg C for sediments and 685 Pg C (as dissolved organic matter) for oceans, compared to the global atmospheric CO_2-C reservoir (780 Pg C) (Bianchi, 2011, Houghton, 2005). Enhancing the sequestration of CO_2-C within these organic carbon reservoirs is being promoted as one mechanism to abate increasing concentrations of greenhouse gases in the atmosphere (IPCC, 2006). The potentially high stocks of soil carbon in coastal mangrove, tidal marsh and seagrass ecosystems (collectively referred to as blue carbon) and respective global annual burial rates of 34, 87 and 112 Tg C yr_{-1} (Mcleod et al., 2011) have led to an increasing interest in including blue carbon in national inventories and carbon accounting schemes.

Australia has more than 10,000 km_2 of mangroves, 15,000 km_2 of tidal marshes and 125,000 km_2 of seagrass meadows with estimated stocks of 257 Tg, 234 Tg and 1,035 Tg of organic carbon to a depth of 1 m (Serrano et al., 2019). As a result, Australia is investigating the development of an Emissions Reduction Fund methodology capable of quantifying the level of carbon abatement achieved through altered management of coastal ecosystems to enhance

organic carbon storage and/or avoid greenhouse gas (GHG) emissions (Kelleway et al., 2017). Australia is also voluntarily reporting storage of organic carbon within mangrove ecosystems as part of its National Inventory Report (Australian Government, 2019). Given the diversity of environmental, geomorphic, vegetative and edaphic conditions existing within Australian blue carbon environments and the potential range of responses to management, development of efficient and cost-effective protocols for quantifying soil carbon stock is required to meet the requirements of carbon accounting and national inventory processes.

Deriving values for soil organic carbon stocks requires measurement of organic carbon concentration, bulk density and thickness of the soil layer sampled. Quantifying the concentration of organic carbon in blue carbon soils has relied on the use of loss-on-ignition, wet chemical oxidation techniques or dry combustion total carbon analysers requiring specialised equipment. The use of diffuse reflectance infrared spectroscopy (IR), combined with multivariate partial least-squares regression analysis (PLSR), has been demonstrated to offer a more cost-effective method, relative to traditional laboratory methods, to derive estimates of the terrestrial soil organic carbon concentration (Baldock et al., 2013b). No treatment of soil other than drying and grinding is required, and the concentration of multiple elements or properties of interest may be predicted from a single spectrum, provided acceptable prediction algorithms have been generated and validated.

The objective of this study was to examine whether IR/PLSR predictive algorithms could be derived to successfully predict concentrations of total carbon (TC), organic carbon (OC), inorganic carbon (IC) and total nitrogen (TN) in blue carbon soils collected from around Australia.

Methods

Soil samples and sample preparation

The soils used in this study were collected from Australian blue carbon ecosystems including mangroves, tidal marshes, seagrasses, coral reefs, estuaries and unvegetated sites located around the Australian coastline (Figure 3.2). A range of approaches were used to collect the samples including coring with core depths ranging up to 2 m, grab sampling of soil layers from depths up to 0.3 m and collection of material accumulating on the surface of existing soils. A total of 5031 soils were included in this study and of these 1201 samples were included as calibration/validation samples in the development of IR/PLSR predictive algorithms. All 1201 calibration/validation samples were either air-dried in a fan forced oven set at 40°C to constant mass or freeze dried to constant mass. The gravimetric water content of all air-dried samples was determined by drying a subsample to 105°C to allow subsequent carbon and nitrogen concentration data to be expressed on an oven dry basis. The extent of drying achieved by freeze drying was found to be equivalent to oven drying at 105°C and no water content measurements nor corrections were applied to those samples. The calibration/validation samples were prepared for MIR and elemental C and N concentration analyses by grinding in a Retsch MM400 ball mill set to an operating frequency of 28 Hz for a duration of 180s using a 35-mL zirconium oxide-lined milling cup and a 15-mm zirconium oxide milling ball. The 3280 samples not involved in the derivation of IR/PLSR predictive algorithms were used as test samples to investigate the general applicability of the IR/PLSR algorithms derived in the study. The test samples were prepared for MIR analysis in the same manner as the calibration/validation samples, but no elemental C and N concentrations were determined.
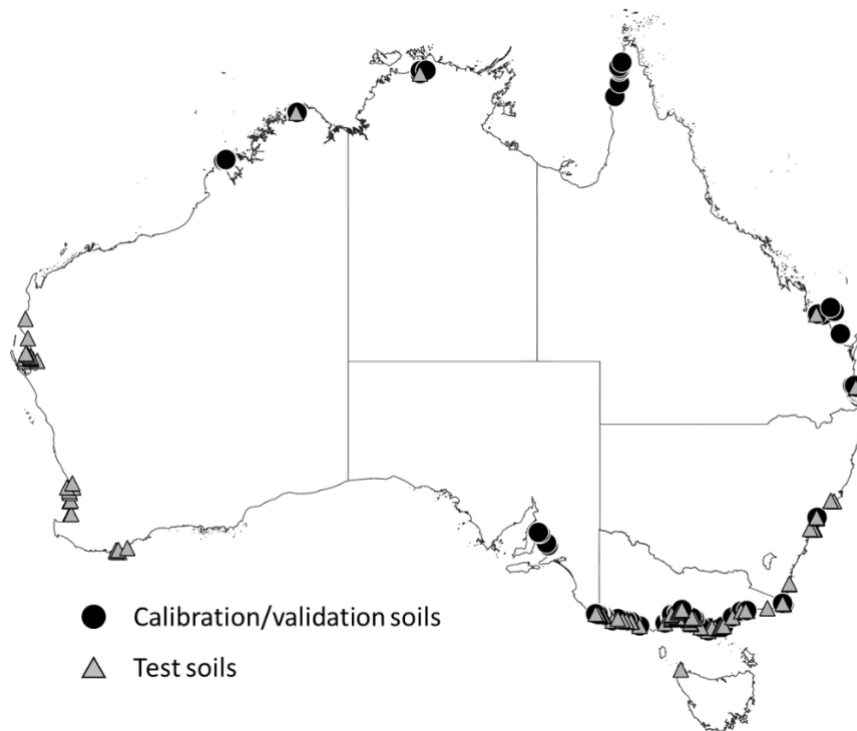
Figure 3.2 Map of Australia showing the locations where the 1201 calibration/validation soils used to build and validate the PLSR prediction models (black circles) and the 3280 test soils (grey triangles) were collected.

Elemental analysis

Concentrations of total carbon (TC), organic carbon (OC), inorganic carbon (IC) and total nitrogen (TN) were measured on 0.5-1.0 g subsamples of the dried and finely ground soil samples using dry combustion analysers (LECO TruMac, LECO C-144 or LECO CNS-2000 (LECO Corporation, St Joseph, MI, USA). All elemental concentrations were not measured on all samples. For TC, OC, IC and TN the number of samples analysed were 1201, 1159, 584 and 1198, respectively. Samples containing IC were identified as those that effervesced on addition of 1M hydrochloric acid (HCl) to an aliquot of the finely ground soil. For samples containing no IC, the concentration of OC was equated to that of TC. For soils containing IC, OC concentrations were determined after removal of all IC by pre-treatment with acid (either

1 M HCl or 6% H_2SO_3 was used). To remove the IC, 0.8 g of ground soil was placed into a nickel lined LECO C-144 ceramic analysis boat. The samples were placed on a hot plate set to 100°C and 1 ml of acid was added. Once the sample was dried an additional 1 ml aliquot of acid was added and the sample dried again. This process was repeated until no effervescence was noted on addition of a 1 ml aliquot of acid. Once completed, the dried acid pre-treated soils were analysed on the LECO C-144 analyser. The concentration of OC was calculated as the mass of carbon detected by the LECO-144 analyser expressed as a function of the initial equivalent dry mass of untreated soil placed in the nickel lined boat prior commencing the acid pre-treatments. The concentration of IC was calculated as the difference between the concentrations of TC and OC. If the difference between TC and OC concentrations was <1.0 mg C/g soil, the IC concentration was below detection limits and the soil was considered to contain no inorganic carbon.

Infrared analyses

Infrared spectra were acquired for all 5031 soils included in this study. Approximately 100 mg of the finely ground soil was placed into 9 mm diameter stainless steel auto sampler cups and the surfaces levelled in preparation for diffuse reflectance IR analysis. The samples were loaded onto a 60 sample Pike AutoDiff-Automated diffuse reflectance sampler (Pike Technologies, Madison, WI, USA). Diffuse reflectance IR spectra were acquired using a Nicolet 6700 FTIR spectrometer (Thermo Fisher Scientific Inc., MA, USA) equipped with a KBr beam-splitter and a DTGS detector and. Spectra were acquired over 8000 – 400 cm^{-1} with a resolution of 8 cm^{-1}. The background signal intensity was quantified by collecting 240 scans on a silicon carbide disk prior to analysing each set of 60 soil samples and subtracted from the signal obtained for each soil sample. Two standard soils were included with each set of samples (one at the start and one at the end of each run) and used to confirm adequate performance of

the spectrometer. A total of 60 scans were acquired and averaged to produce a reflectance spectrum for each individual sample. The Omnic software Version 8.0 (Thermo Fisher Scientific, Waltham, MA, USA) was used to convert the acquired reflectance spectra into absorbance spectra (log transform of the inverse of reflectance).

Chemometric analyses

All IR spectral transformations and chemometric analyses were conducted using the Unscrambler 10.5 software (CAMO Software AS, Oslo, Norway). The acquired IR absorbance spectra were smoothed using a Savitzky-Golay transformation with a second order polynomial and 5 points to the left and right of the point being smoothed. A baseline offset transformation applied after smoothing and all spectra were mean centred prior to conducting chemometric analyses.

A principal component analysis (PCA) using full cross validation was applied to the 1201 IR spectra associated with the calibration/validation samples for which TC concentration data existed. A subset of 300 of the 1201 samples that best accounted for variations in signal intensity within the acquired IR spectra was identified by applying a Kennard-Stone algorithm to the scores derived from the PCA analysis. The identified 300 samples were designated as the calibration set of samples and the remaining 901 samples were designated as an independent set of samples to validate the partial least squares regression (PLSR) analysis completed for TC. For OC, IC and TN separate PCA analyses were performed using only the spectra associated with samples having analytical data. Separate calibration and validation set of samples were defined by applying the Kennard Stone algorithm to the scores derived in the PCA analyses. For OC, IC and TN the calibration sets contained 300, 200 and 300 samples and the independent validation sets contained 859, 384 and 898 samples, respectively.

Predictive PLSR models were derived for square root transformed concentrations of TC, OC, IC and TN from the IR spectra. Application of a square root transformation to all analytical data was required to produce linear PLSR models with a homogenous distribution of residuals. Due to the potential overlapping of signals derived from organic carbon and carbonate in the 2900–2700 cm$_{-1}$ IR spectral region, additional PLSR models were derived to predict the square root transformed OC concentrations in soils containing inorganic carbon and those containing no inorganic carbon. For these PLSR models, a PCA analysis combined with a Kennard-Stone selection (as described previously) was used to identify respective calibration and validation sets of 200 and 384 samples for soils with carbonate and 200 and 375 soils without carbonate. A range of statistical parameters commonly applied to chemometric analysis of soils were used to evaluate the quality of the PLSR predictions including the coefficient of determination (R_2) for the relationship between measured and predicted values, the root mean square error for prediction (RMSEP), the standard error of prediction (SEP), the ratio of performance to deviation (RPD) and ratio of performance to interquartile range (RPIQ) (Baldock et al., 2013b; Bellon-Maurel et al., 2010; Bellon-Maurel and McBratney, 2011).

The IR/PLSR models produced were applied to all collected soils left out of the calibration/validation process (i.e. the set of 3830 test soils) to derive predicted values for the square root transformed TC, OC, IC and TN concentrations. The suitability of the IR/PLSR models to predict these concentrations was assessed in two ways based on plots and statistics provided by the Unscrambler 10.5 software. Firstly, the spectra acquired from the test soils were projected onto the factor scores plot derived for the calibration soils used to develop the PLSR models. This projection allowed detection of test soils with spectral characteristics that deviated from the spectral components of the calibration soils used to derive the PLSR models.

Secondly, the Hotelling's T_2 statistics (multivariate distance to the centre of the calibration dataset) were plotted against the Inlier statistics (minimum Mahalanobis distance to the calibration soils) for all of the test soils. Where the Hotelling's T_2 or Inlier statistics were greater than the limits set with $\alpha=0.05$, the confidence associated with the predicted values declined.

Results and Discussion

Elemental concentrations of the calibration/validation soils

The concentrations of TC, OC, IC and TN within the 1201 calibration/validation soils ranged from 0.1–413.7 mg TC g_{-1} soil, 0.1–413.7 mg OC g_{-1} soil, 0.1–119 mg IC g_{-1} soil and <0.01–22.6 mg TN g_{-1} soil respectively. All distributions were positively skewed towards larger concentrations with skewness values of 2.5, 4.2, 1.4, and 3.4 respectively for concentrations of TC, OC, IC and TN. The frequency distributions of square root transformed concentrations of TC, OC, IC and TN (denoted as sqrtTC, sqrtOC, sqrtIC and sqrtTN, respectively) for the calibration/validation samples used to build the PLSR predictive algorithms are provided in Figure 3.3. Even after application of the square root transformation the frequency distributions of all variables retained a positive skew. The distribution of sqrtIC values displayed a negative kurtosis, while those of the sqrtTC, sqrtOC and sqrtTN values all had a positive kurtosis.

The correlation between the square root transformed elemental concentrations is provided at the base of Figure 3.3. All correlation coefficients were significant ($p<0.01$) with the strongest correlation exiting between sqrtOC and sqrtTN with 88% of the variation in sqrtTN explained by that of sqrtOC. Such a strong correlation indicated that the majority of the nitrogen present in the samples existed in an organic form with the with an average OC:TN ratio of 13.9. Strong correlation coefficients (0.76 – 0.88) were also obtained for sqrtTC with sqrtOC, sqrtIC and sqrtTN. However, the correlations between IC and OC or TN were weak ($r = 0.14$ and $r = 0.25$, respectively) suggesting little relationship between the amount of carbonate and organic materials in the soils.
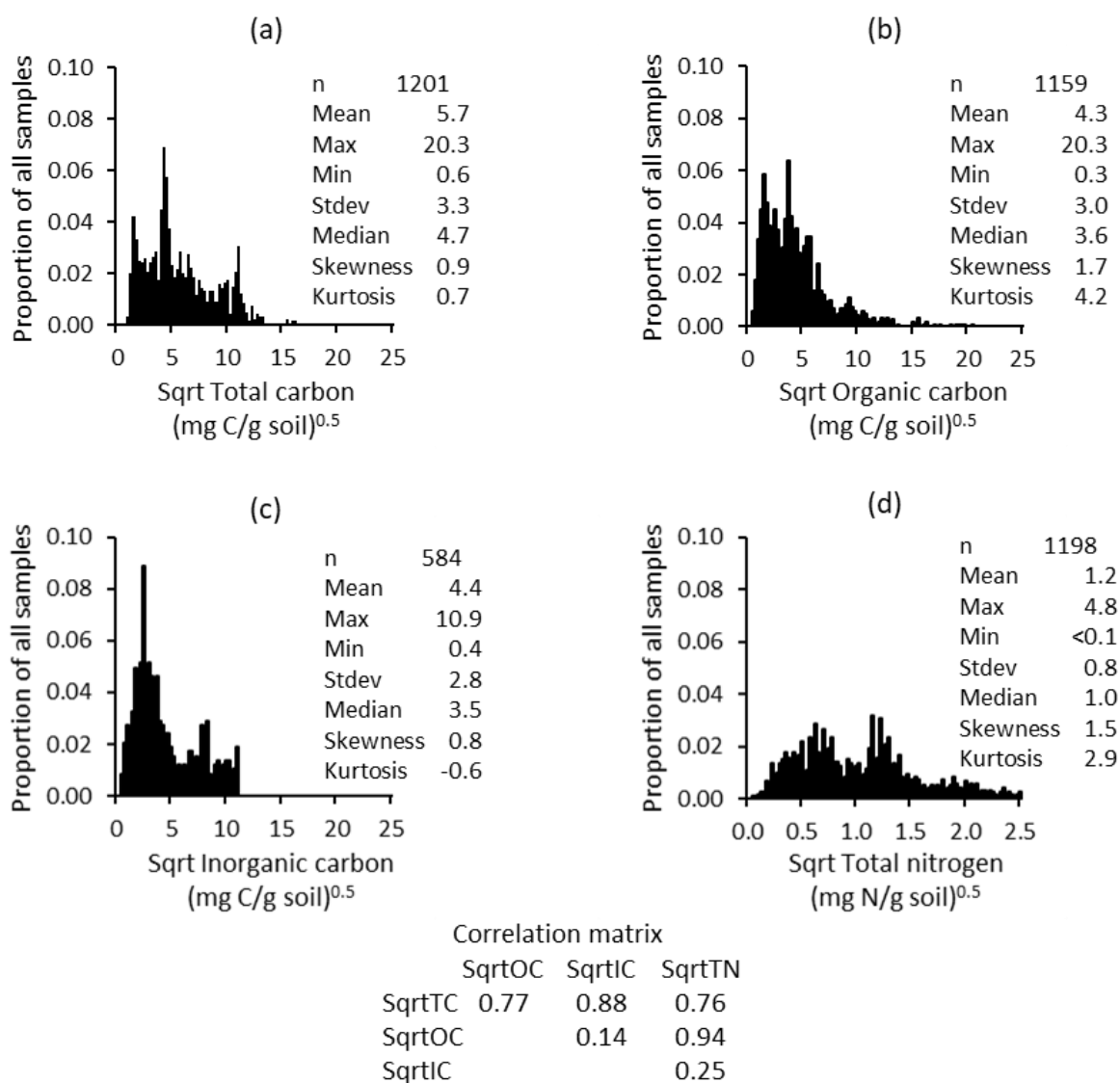
Figure 3.3 Distribution of square root transformed measured concentrations of (a) TC, (b) OC, (c) IC and (d) TN for the calibration/validation soils as well as the correlation matrix between the transformed concentrations.

Infrared spectra

The acquired IR spectra acquired for the 1201 calibration/validation soils displayed a wide range in signal intensities over the $6000 - 600$ cm$_{-1}$ spectral region (Figure 3.3). At wavenumbers >4000 cm$_{-1}$, eleven soils showed significant signal intensity; while, all other

spectra displayed low signal intensity over this spectral region. The low signal intensity within the 2600 – 2200 cm$_{-1}$ wavenumber range for the eleven samples and the application of a baseline offset transformation to the acquired spectra accounted for the elevated signal intensity at wavenumbers >4000 cm$_{-1}$. The approximate equidistant spacing of the minimum and maximum signal intensities from the median and that associated with the 75$_{th}$ and 25$_{th}$ percentiles from the median over the 4000-600 cm$_{-1}$ wavenumber range suggested a near normal distribution at each wavenumber. Average values for skewness and kurtosis over this region were 0.33 and 0.29, respectively, supporting a near normal distribution of signal intensity at each wavenumber across the calibration/validation soils.

Important spectral features that varied across the calibration/validation soils included signals representative of clay minerals, a broad OH stretch, organic carbon and carbonate. The sharp signals over the 3700 – 3600 are indicative of the presence of 1:1 and 2:1 clay minerals (e.g. kaolinite and montmorillonite, respectively) (Madejová, 2003, Parikh et al., 2014). The broad O-H stretching signal (3600 – 3200 cm$_{-1}$) may originate from a range of sources including water and mineral or organic components. The two absorptions near 2920 and 2850 cm$_{-1}$ result from C-H stretching originating from organic components and have been found to be diagnostic in the prediction of organic carbon concentrations (Baldock et al., 2013a, Janik et al., 2007). Additional absorptions can be attributed to soil organic matter (see Parikh et al., (2014)); however, the presence overlapping adsorptions derived from a range of soil minerals makes it difficult to reliably attribute these solely to soil organic matter. The strong absorption observed over the 2660-2450 cm$_{-1}$ wavenumber region originates from carbonate and is associated with absorptions near 1800, 1450, 880 and 700 cm$_{-1}$ (Comstock et al., 2019). The carbonate absorptions between 1800 and 700 cm$_{-1}$ can be difficult to conclusively attribute to carbonate due to overlap with other soil components. The presence of quartz is associated with

three absorptions near 1990, 1860 and 1800 cm^{-1} and a sharp absorption near 1160 cm^{-1} (Le Guillou et al., 2015, Sila et al., 2016).
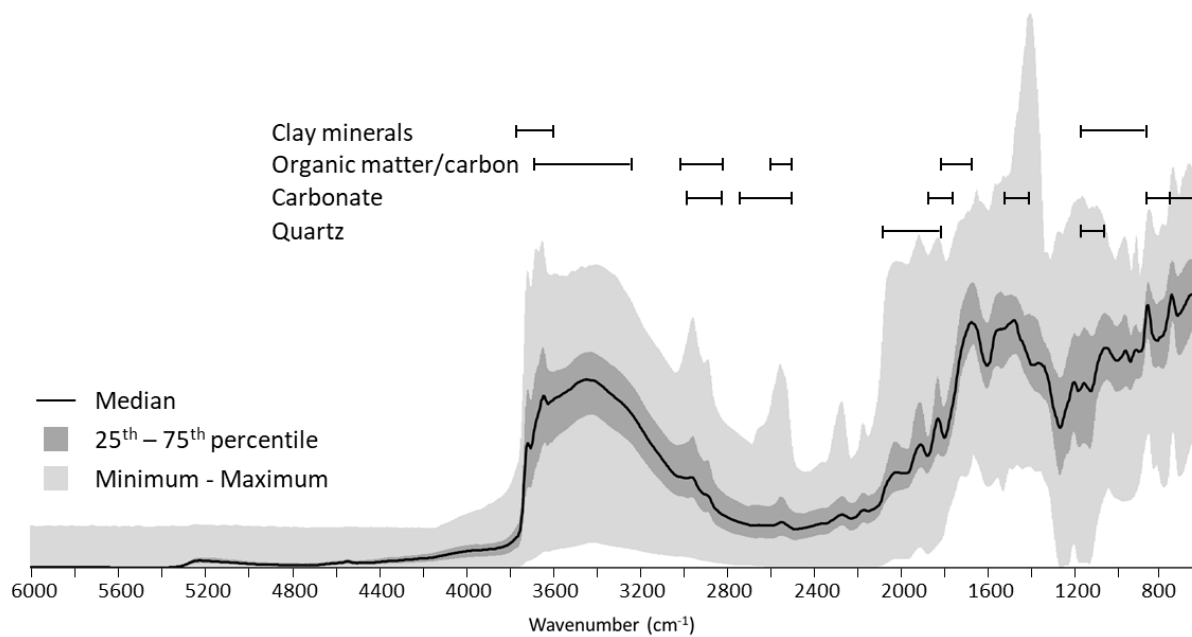
Figure 3.4 IR spectra acquired for the 1201 calibration and validation soils showing the spectral intensities associated with the maximum, 75th percentile, median, 25th percentile and minimum spectral intensities observed at each wavenumber as well as some infrared regions typically associated with particular soil components.

Principal components analysis.

A PCA was applied to the IR spectra of the 1201 calibration/validation soils for the purpose of 1) identifying outlier samples, 2) defining what spectral features accounted for variations in spectral intensities across the soils and 3) selecting a representative set of calibration soils that could be used in the subsequent PLSR analyses to derive predictive algorithms for TC, OC, IC and TN concentrations. A total of six principal components contributed significantly to explaining 97% of the variations in spectral intensity across the samples with the first three components accounting for 86.5%. The scores plots for PC1 versus PC2 or PC3 (Figure 3.5a

and b, respectively) did not contain any obvious outliers. Thus, all 1201 samples were retained in in all subsequent chemometric analyses. The absence of significant contributions of signal intensity attributable to organic or inorganic carbon within the loadings spectra for PC1 and PC2 (Figure 3.5c) suggested that differences in the composition of soil minerals (excluding carbonate) contributed most to the variations in the acquired IR spectra. Important contributions of signals from organic ($2900 - 2800$ cm$_{-1}$) and inorganic ($2660 - 2450$ cm$_{-1}$) carbon became evident in the loadings spectra associated with PC3 through to PC6. The 300 soils selected by the Kennard-Stone algorithm to be included in the subsequent PLSR analysis of TC concentration (shown as black circles within Figure 3.5a and b) accounted for the variations in spectral signal intensity of all 1201 samples. Similar results (not shown) were obtained for the selection of 300, 200 and 300 soils for used as calibration soils in the PLSR analyses of OC, IC and TN, respectively.
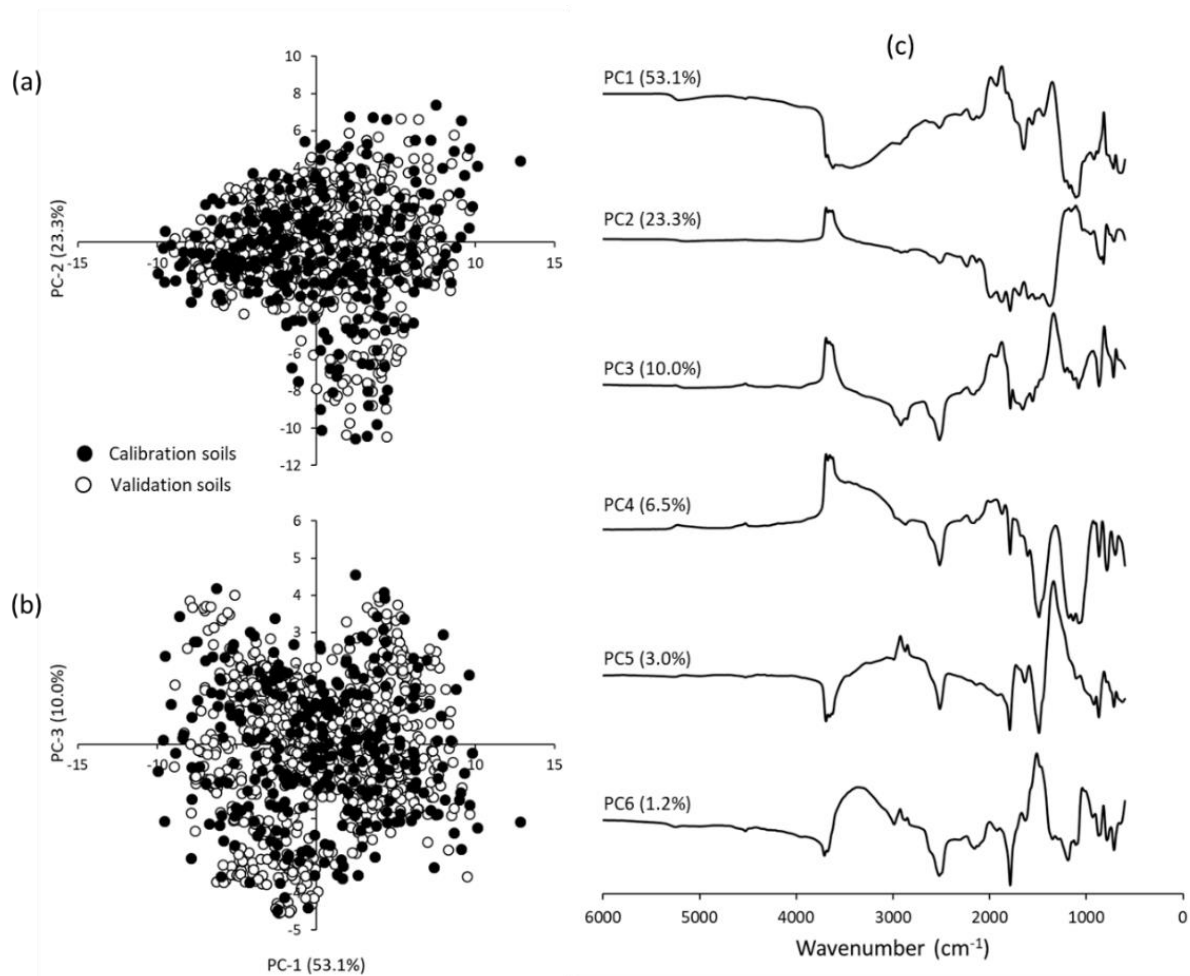
Figure 3.5 Results of the principal components analysis of the IR spectra acquired for the 1201 calibration/validation soils showing (a) PC1 versus PC2 scores (b) PC1 versus PC3 scores and (c) the loading spectra associated the six significant principal components. Closed and open circles within the scores plots (a and b) identify the soils selected to the calibration (n = 300) and validation (n = 901) sets for derivation of the TC prediction algorithms.

Partial least squares regression analyses

The relationship between IR/PLSR modelled and the measured values of sqrtTC, sqrtOC, sqrtIC and sqrtTN are provided in Figure 3.6 for both the calibration samples used to build the IR/PLSR models and the independent test sets of samples used for validation. Based on the results obtained for the independent validation samples, strong predictive IR/PLSR models were constructed ($R_2 \geq 0.91$ and RPIQ ≥ 3.7). The β-coefficient spectra associated with each IR/PLSR prediction algorithm (Figure 3.7) provide an indication of the contributions (negative or positive) that different spectral regions make to the predicted values. It is important to note that it is the product of the β-coefficient and the spectral intensity for a given wavenumber that defines the contribution to the predicted value. As a result, a small β-coefficient value can still impact the final predicted value significantly if the spectral intensity associated with it is high. An examination of the β-coefficient spectrum for sqrtTC showed positive contributions to the predicted values by signals potentially originating from clay minerals (3700 – 3600 cm$_{-1}$) and organic matter C-H (3000 – 2800 cm$_{-1}$) and amide (1700 – 1600 cm$_{-1}$) with reduced contributions from carbonate derived signals (2600 – 2400 and 1800 cm$_{-1}$). The sqrtOC β-coefficient spectrum was similar to that derived for sqrtTC; however, the carbonate derived signals become negative and more pronounced, consistent with a need to remove carbonated based signal intensity when predicting sqrtOC rather than sqrtTC concentrations. The majority of the features present in the β-coefficient spectrum for the sqrtIC predictive algorithm are opposite to those present in the sqrtOC algorithm. The sqrtTN algorithm is similar to the sqrtOC spectrum consistent with the high correlation between sqrtOC and sqrtTN measured concentrations.
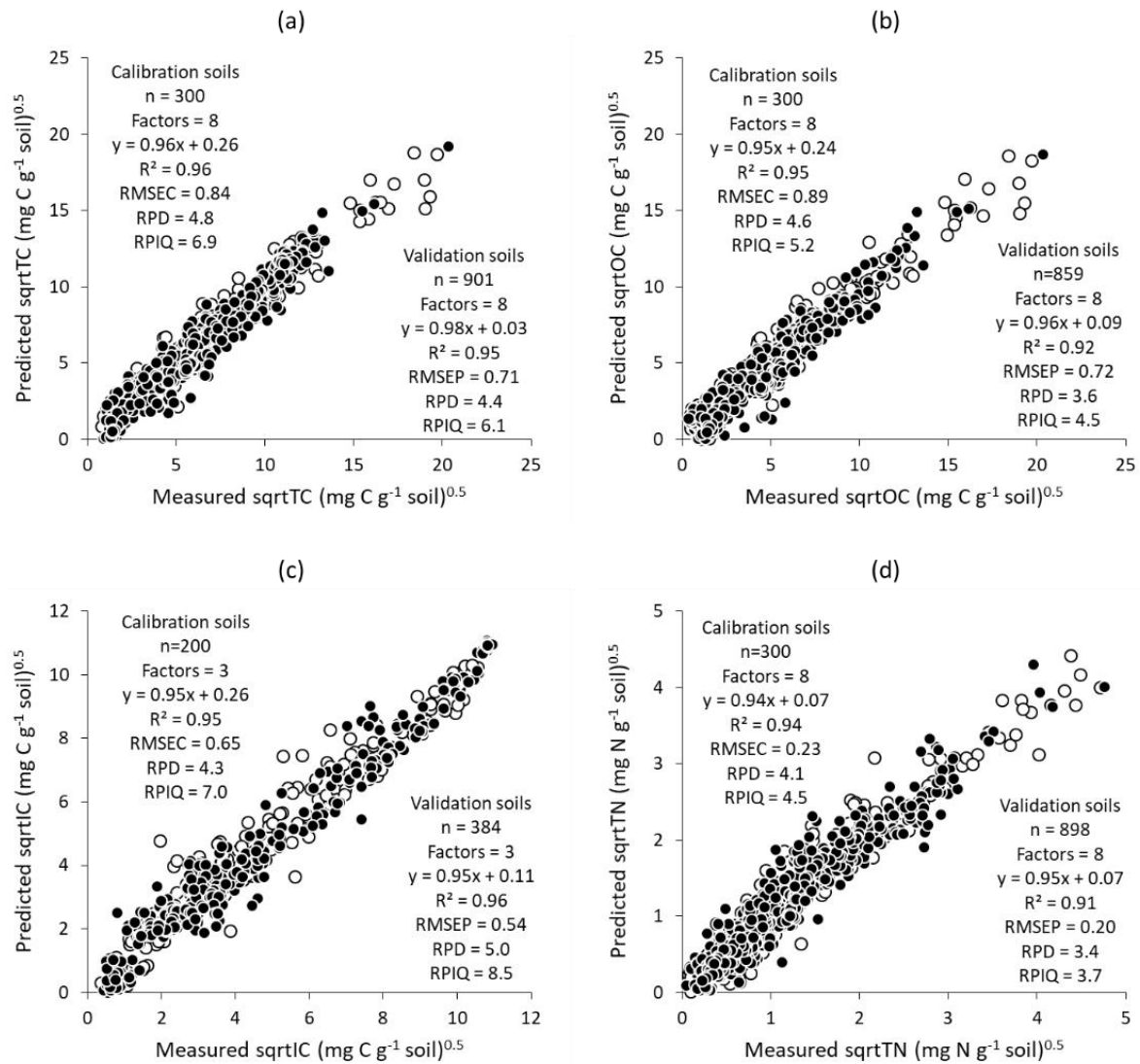
Figure 3.6 Relationship between measured and PLSR predicted values for the calibration (white circles) and validation (black circles) soils, number of significant PLSR factors, coefficient of determination (R_2), residual mean square error of calibration (RMSEC) or prediction (RMSEP), ratio of performance to deviation (RPD) and ratio of performance to interquartile range (RPIQ) for (a) sqrtTC, (b) sqrtOC, (c) sqrtIC, and (d) sqrtTN.
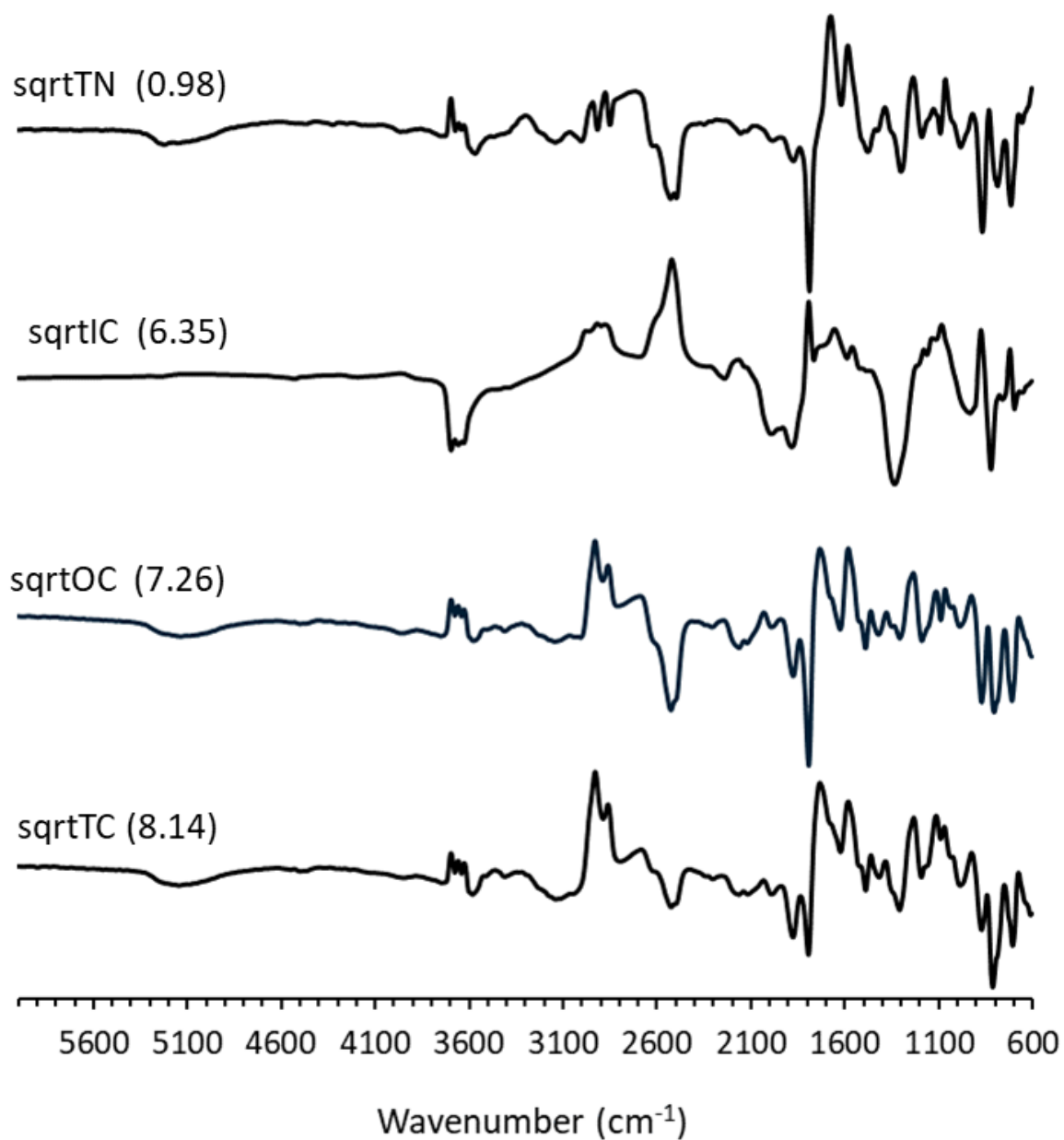
Figure 3.7 β-coefficient spectra of the PLSR predictive algorithms derived for sqrtTC, sqrtOC, sqrtIC and sqrtTN. Values in parentheses provide the intercept of the predictive algorithms. The scale of the β-coefficients for each predictive algorithm vary.

Application of the PLSR predictive algorithms

The suitability of the IR/PLSR predictive algorithms to Australian blue carbon soils was assessed using the 3830 test soils not included in the generation or validation of the IR/PLSR predictive models. The first assessment involved determining whether the variation in the spectral features contributing to the prediction of sqrtTC, sqrtOC, sqrtIC and sqrtTN within the calibration soils encompassed the variation in those same features within the test soils. To make this assessment, the IR spectra obtained for the test soils were projected through the loadings of the respective IR/PLSR models onto the IR/PLSR model factor scores (Figure 3.8). For the IR/PLSR models derived from the calibration soils to be applicable to the test soils, all test soils should fall within the limits of the factor scores defined for the calibration soils. With few exceptions (<5 test soils), the factor scores generated for the test soils fell within those of the calibration soils for the sqrtTC, sqrtOC and sqrtTN IR/PLSR predictive algorithms indicating that these algorithms were applicable to the test soils.

For the sqrtIC PLSR algorithm, although the majority of test soils fell within the limits of the factor scores obtained for the calibration soils; a significant number of test soils fell outside the factor score limits. This difference arose because only calibration soils that tested positive for the presence of IC were included in the derivation of the sqrtIC IR/PLSR predictive algorithm; whereas, the test soils were not tested for the presence of IC and all 3830 samples were projected onto the sqrtIC factor scores. As a result, more uncertainty would exist in the application of the sqrtIC IR/PLSR predictive model to the 3830 test soils. Application of an acid fizz test to define which of the 3830 test soils contained IC and projection of only IC containing soils onto the sqrtIC PLSR factor scores would likely reduce the number soils that fell outside the factor score limits of the calibration soils.
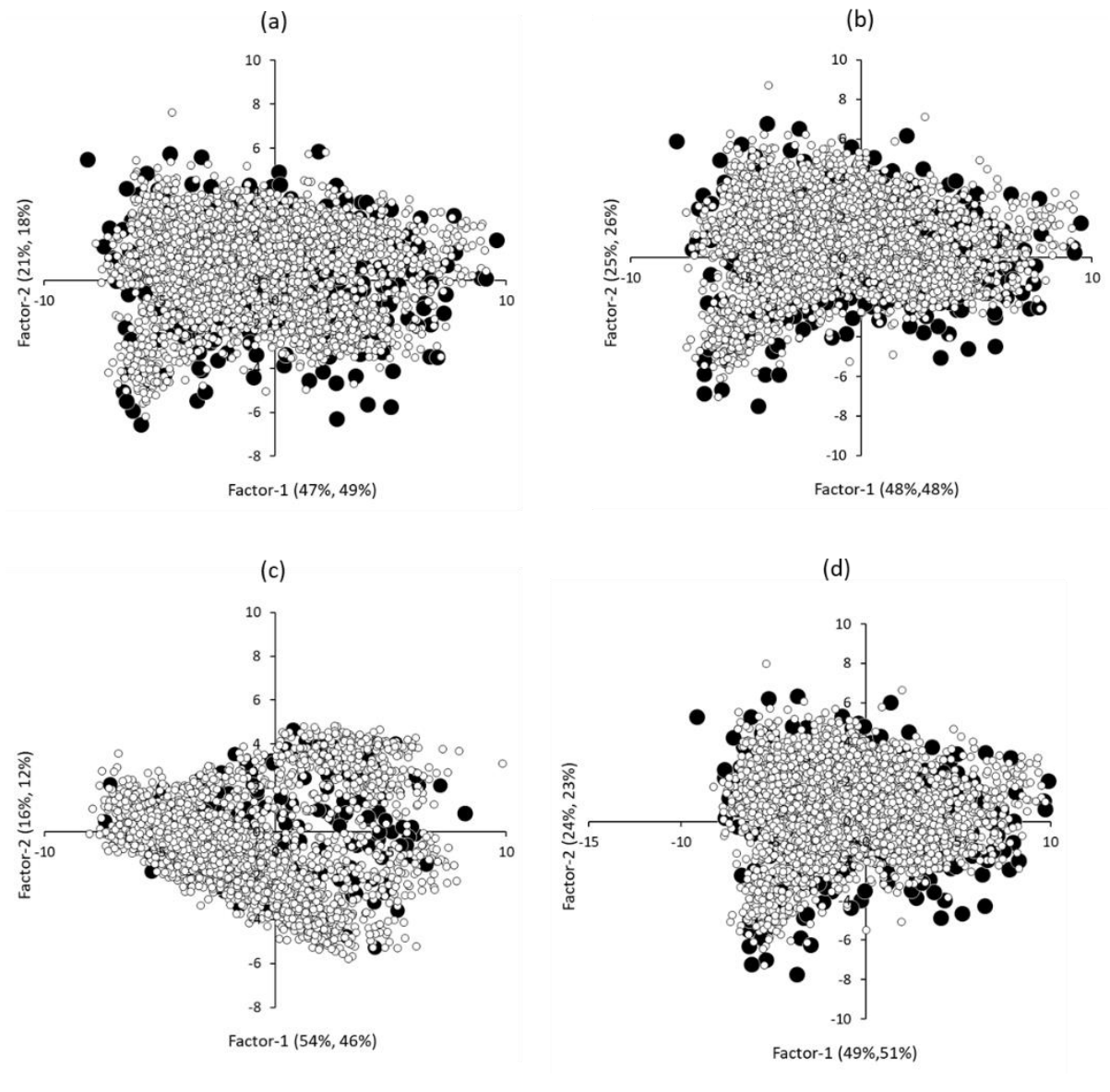
Figure 3.8 Projection of the IR spectral features contributing to the prediction of (a) sqrtTC, (b) sqrtOC, (c) sqrtIC, and (d) sqrtTN for the 3830 test soils (smaller white filled circles) onto the factor scores plot for the calibration soils (larger black filled circles) used to develop the PLSR models.

The suitability of the IR/PLSR predictive algorithms to the test soils was also assessed by examining the relationship between the Hotelling's T_2 and the inlier distance statistics obtained after applying the IR/PLSR algorithms to the test soils (Figure 3.9). As the magnitude of the

Hotelling's T2 statistic (distance from the centre of the data set) or the Inlier distance (minimum Mahalanobis distance from a calibration sample) increases, the confidence in the accuracy of the predicted value declines. Test soils that plot in the bottom left quadrant of each of the graphs presented in Figure 3.9 are both close enough to the centre of the dataset and close to a calibration sample. The proportions of the 3830 test soils that appeared in the lower left quadrant were 0.97, 0.97, 0.94 and 0.97 for sqrtTC, sqrtOC, sqrtIC and sqrtTN indicating that the IR/PLSR models were able to predict the values well. Of the samples that did not fall in the lower left quadrant, the predicted values derived for those that appear in the upper right quadrant have the lowest confidence. Soils plotting in the upper right quadrant should be analysed and added to the calibration dataset to extend the range of the calibration soils so that it becomes more representative of Australian blue carbon soils.
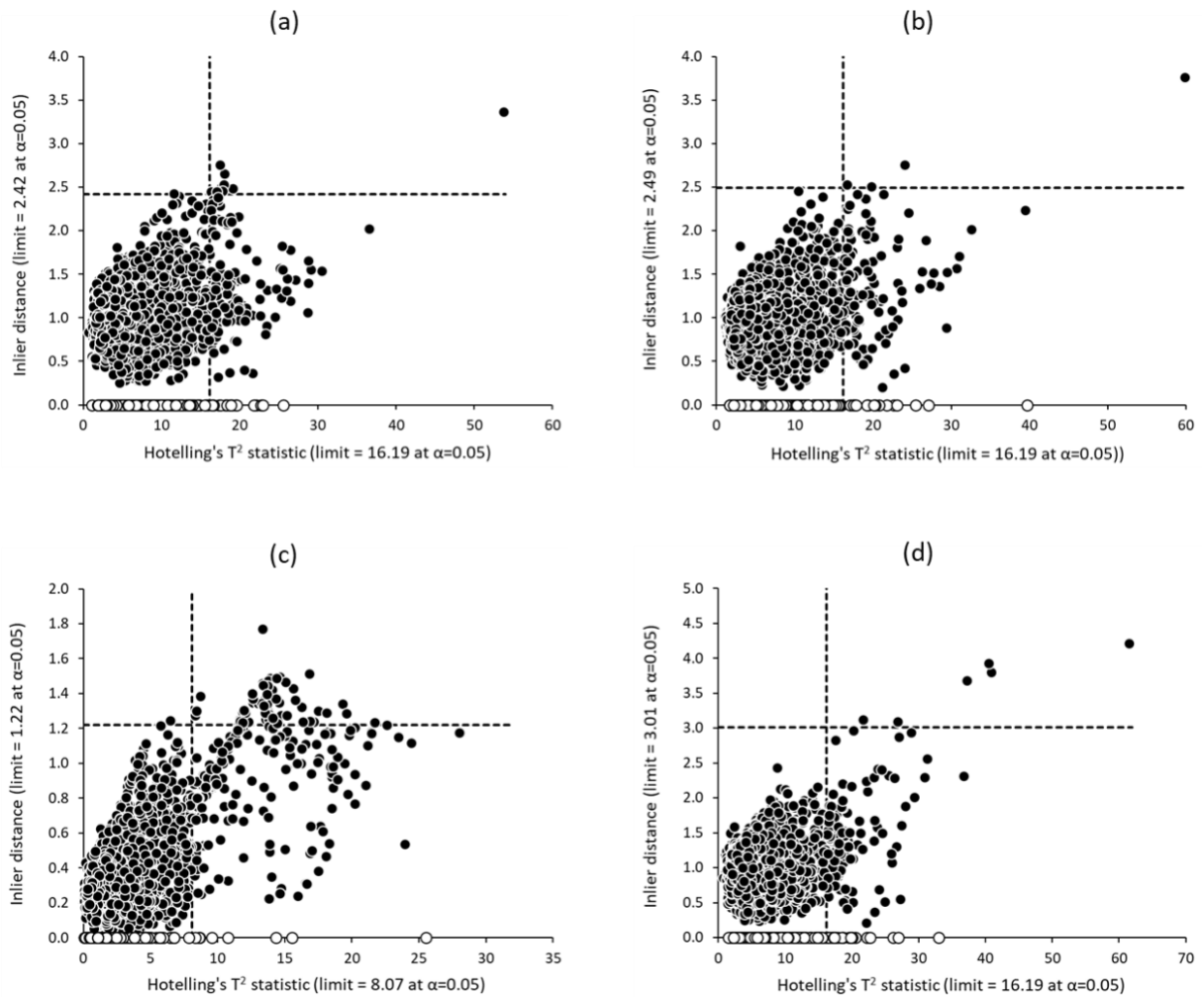
Figure 3.9 Plot of Hotelling's T2 statistics versus inlier distances for the calibration soils (white filled circles and the 3830 test soils (black filled circles) with the dashed lines show the limits of these statistics at α=0.05.

Conclusions

IR/PLSR predictive algorithms were successfully derived for sqrtTC, sqrtOC, and sqrtTN using 300 calibration soils selected to represent the 1201 calibration/validation soils that had both IR spectra and measured analytical data. On application of these IR/PLSR predictive algorithms to 3830 test soils, 97% of the test soils were well predicted. For sqrtIC, a strong IR/PLSR predictive algorithm was obtained, but on application to the test soils 94% of the soils

could be considered well predicted. A significant number of the test soils (55 soils, equivalent to 1.4% of all test soils) could be considered to be predicted poorly and should be sent for analysis to improve the representativeness of Australian blue carbon soils.

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Statement of Authorship

Title of Paper	Determining the allocation of soil organic carbon to below ground carbon storage pools in temperate coastal wetlands.
Publication Status	☐ Published ☐ Accepted for Publication ☐ Submitted for Publication ☑ Unpublished and Unsubmitted work written in manuscript style
Publication Details	Not applicable.

Principal Author

Name of Principal Author (Candidate)	Christina Helen Asanopoulos
Contribution to the Paper	• Experimental design; • Field work; Processing and analyses of samples; Data analyses; • preparation of manuscript
Overall percentage (%)	85%
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.
Signature	Date 13|12|2019

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

   i.   the candidate's stated contribution to the publication is accurate (as detailed above);

   ii.   permission is granted for the candidate in include the publication in the thesis; and

   iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

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Chapter Four

Determining the allocation of soil organic carbon to below ground carbon storage pools in temperate coastal wetlands.

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Abstract

Vegetated coastal wetlands (mangrove forests, tidal marshes and sea grass meadows) and the carbon (blue carbon) they store make a significant contribution to global carbon budgets. The quantification of blue carbon stocks is of increasing importance with the development of carbon off-setting initiatives. These initiatives require reliable and effective estimates of carbon budgets in blue carbon environments. Infrared and partial least squared regression (IR/PLSR) analyses allow for the rapid quantification of soil properties with minimal preparation required and high productivity. The objective of this study was to determine the allocation of organic carbon (OC) to size fractions in blue carbon soils and examine the suitability of using an infrared and partial least squared regression (IR/PLSR) approach to predict the allocation of carbon in temperate coastal wetlands. A selection of blue carbon soils collected across nine sites in the South Australian region were separated into particle size fractions of particulate organic carbon (POC), humus organic carbon (HOC) and recalcitrant organic carbon (ROC). Analytically derived data for the POC, HOC and ROC fractions contents of these blue carbon soils were then used to developed three predictive models. The allocation of carbon across the different particle size fractions did not significantly differ but the highest proportion of OC in both the mangrove (58 %) and tidal marsh (53 %) soils were found in the HOC fractions. In addition, the IR/PLSR models for each of the fractions were found have strong collinear relationships with the OC IR/PLSR model. This suggests models are not fraction specific and OC data can be used to predict the allocation of carbon to the different size fractions. IR/PLSR models can successfully be used to predict allocation of carbon to different pools in the blue carbon environment but a larger sample size is required to validate the correlation between OC and the size fractions.

Key words: Blue carbon, wetlands, FTIR-DRIFT spectroscopy, partial least squared

regression, predictions, particulate organic carbon (POC), humus organic carbon (HOC), recalcitrant organic carbon (ROC).

Introduction

A significantly large amount of carbon (blue carbon) is stored in the soils of mangrove forest and tidal marsh environments (Chmura et al. 2003; Duarte et al. 2013). The accumulation of soil carbon in these environments is facilitated by the high burial rates of organic matter and its retention under soil conditions that slow its decomposition (Barreto et al. 2018; Donato et al. 2011; McLeod et al. 2011). In the blue carbon environment, soil organic matter (SOM) is derived from the autochthonous litter and wood deposits generated through the primary productivity of the vegetation and allochthonous inputs of tidally transported marine or riverine material (Friesen et al. 2018; Saintilan et al. 2013)

Macrofauna facilitate the decomposition of SOM in the blue carbon environment through the consumption and burial of leaf litter (Friesen et al. 2018; Kristensen et al. 2008). The consumption and burial by crabs breaks down litter tissues and simplifies the structure of the SOM freeing up cellulose and hemicellulose structures for microbial decomposition (Friesen et al. 2018). In addition frequent tidal inundation induces leaching of dissolved organic matter that enhances decomposition of litter in the blue carbon environment (Friesen et al. 2018). Beyond the breakdown of large pieces of litter microbes facilitate further breakdown of complex organic materials into simple compounds (Friesen et al. 2018). Soil organic matter is a heterogeneous mixture of organic residues, ranging from large and labile to small and recalcitrant structures (Baldock & Broos 2012; Christensen 1992). Once part of the soil matrix the decomposition of large labile organic material is transformed to smaller molecules that

eventually become recalcitrant to further microbial decay (Macdonald & Baldock 2010). With greater extent of decomposition reduction in the particle size of the organic matter will occur (Baldock et al. 2013b; Macdonald & Baldock 2010). The rate of SOM decomposition is controlled by a variety of factors including environmental conditions, soil type, soil properties, the chemical composition of the SOM and its accessibility and the functional potential of the soil biota (Baldock & Broos 2012; Friesen et al. 2018).

Jenkinson (1990) modelled the behaviour of SOM in agricultural soils during decomposition and found carbon is allocated to pools with different rates of turnover. This model is called the Rothamsted model (RothC) and it has become one of the most referenced among such models (Schmidt et al. 2011; Sollins et al. 1996). The RothC model simulates the behaviour of SOM during decomposition within conceptual carbon pools that are identified by their different rates of decomposition and microbial accessibility. RothC can therefore be used to calculate the rate of carbon loss or gain from a system. However, Skjemstad et al. (2004) later found the theoretical carbon pools proposed by Jenkinson (1990) could be replaced with measured pools, that improved the model outputs. The measured pools, derived by Skjemstad et al. (2004) were later modified in Baldock et al. (2013b) with the addition of a third carbon pool which is almost entirely recalcitrant to further decomposition, the recalcitrant organic carbon (ROC) fraction. These advances were made possible through the application of analytical techniques such as solid state ^{13}C NMR (Preston 1996).

These organic carbon fractions represent pools of biological significance, in terms of their accessibility to microbial decomposition, that can be used as indicators for long-term carbon storage potential in soils (Baldock et al. 2013b). The allocation of organic carbon to these pools can therefore improve our understanding of microbial accessibility to organic carbon in natural

ecosystems, including vegetated coastal wetlands. However, the allocation of soil organic carbon (SOC) to these size fractions has never been quantified and the physical fractionation approach taken by Baldock et al. (2013b) has not been attempted on blue carbon soils.

The quantification of carbon stocks in vegetated coastal habitats is of increasing importance with the development of carbon off-setting initiatives. Programs like the 'Reducing Emissions from Deforestation and forest Degradation Plus' (REDD+) and the Australian 'Emissions Reduction Fund' (ERF) encourage the conservation and restoration of blue carbon habitats (Ahmed & Glaser 2016; Serrano et al. 2019). These programs are low cost options for climate change mitigation that will potentially result in the reduction of anthropogenic emissions and provide payments for ecosystem services provided by blue carbon habitats (Ahmed & Glaser 2016). The adoption and implementation of strategies such as REDD+ or the ERF, however, require reliable estimates of carbon budgets across blue carbon environments (Lovelock et al. 2014; Macreadie et al. 2019; Owers et al. 2020; Siikamäki et al. 2013). Including details pertaining to the allocation of carbon to particle size fractions in blue carbon soils can provide a level of sophistication to estimated long-term carbon budgets. The key objective of this study was to determine the allocation of OC to particle size fractions (POC, HOC and ROC) in temperate vegetated coastal soils. We hypothesised that coastal soils will have higher allocation of OC to the POC fraction as a result of slowed SOM decomposition.

In addition, infrared and partial least squared regression (IR/PLSR) techniques allow for the rapid quantification of soil properties with minimal preparation required and facilitates the analysis of numerous samples in short time scales (Baldock et al. 2013a; Soriano-Disla et al. 2014; Viscarra Rossel et al. 2006). Therefore, given the inherent difficulty in sampling coastal soils and the time consuming and costly analytical procedures needed to make these

quantifications, the secondary objective was to predict the allocation of OC to the particle size fractions by developing fraction specific infrared and partial least squared regression (IR/PLSR) models.

Methods

Study sites

This study was carried out across the eastern coastlines of northern Gulf St. Vincent and Spencer Gulf in South Australia. Both gulfs are characterised as inverse estuaries with low freshwater inputs and high evaporation rates (Bourman et al. 2016). Their low energy environment support an estimated 16 420 ha of the grey mangrove, *Avicennia marina,* and 19 760 ha of intertidal, supratidal and stranded samphire tidal marshes, *Sarcocornia spp.* and *Tecticornia spp.* (Baker 2015; Foster et al. 2019). The Gulf St. Vincent sites included Mutton Cove (34.78°S, 138.51°E); Torrens Island (34.79°S, 138.53°E); Port Gawler (34.65°S, 138.48°E); Port Wakefield (34.18°S, 138.15°E) and Clinton (34.22°S, 138.02°E). The Spencer Gulf sites included Port Broughton (33.58°S, 137.94°E); Port Pirie (33.58°S, 138.01°E); Port Paterson (32.55°S, 137.83°E) and Port Augusta (32.50°S, 137.79°E). Further summary of the study sites can be found in chapter 2. During the collection of soil samples from the Gulf St Vincent coast (austral spring of 2016) the total rainfall and temperature ranged from 100–200mm and 6–24 degrees Celsius (°C), respectively (Bureau of Meteorology, 2019). While, the average rainfall and temperature ranged from 25–100mm and 9–27°C for the Spencer Gulf coast during the soil sample collection period (austral spring, 2017) (Bureau of Meteorology, 2019).

Soil sample collection and preparation

A sampling plot (35m × 20m) was established at each site that spanned the transitional zone of the mangrove (*Avicennia marina*) and intertidal marsh (*Sarcocornia quinqueflora*) vegetation, as mangrove and tidal marsh hereinafter. The transitional zone was determined to begin at the edge of the mangrove forest where fully established trees were observed. In total, 216 soil samples were collected from across the Gulf St Vincent and Spencer gulf sites with twenty-four (12 × mangrove and 12 × tidal marsh) surface soil samples (0 – 10cm) collected from each site. Three transects, spaced 10m apart, were run across the mangrove and tidal marsh ecotone and eight (four × mangrove and four × tidal marsh) surface soil samples were collected at 5 m intervals along each transect. The soil samples were collected using a PVC corer with an internal diameter of 80 mm by manual percussion and rotation. Intact soil cores were bagged and stored at 4°C immediately after collection and frozen within 5 hours of collection. Soil samples were later lyophilised (Cuddon freeze dryer, Blenheim, New Zealand) prior to being crushed and sieved to ≤2 mm. A representative sub-sample was separated from the bulk ≤2 mm sample by being passed through a 12 × 13 mm slotted riffle box (Civilab Australia, Sydney, Australia) to be used for subsequent analysis.

Particle size fractionation of the <2 mm soils

Fifty-four (27 mangrove and 27 tidal marsh) samples were selected for particle size fractionation. Selected samples included three mangrove and three tidal marsh samples from each site. Samples were fractionated using an automated wet sieving system (Vibratory Sieve Shaker Analysette 3 PRO; FRITSCH GmbH, Idar-Oberstein, Germany) fitted with sprinklers in the Perspex lid and supplied deionised water via a peristaltic pump (Masterflex L/S Model 7553-79 with a Masterflex L/S Modular Controller; Cole-Parmer, Vernon Hills, IL, USA),

following the procedure described by Baldock et al. (2013b). In short, a 10g aliquot from the representative sub-sample of each sample selected for fractionation was dispersed by shaking overnight in 40 mL of 5 gL_{-1} sodium hexametaphosphate solution. Dispersed soil was transferred and passed through a 50 μm sieve fitted onto the automated wet sieving system which vibrated at an amplitude of 2.5 mm in 20 second intervals for three minutes. In the event that after three minutes the water flowing from the system was not clear or undispersed aggregates were observed on the sieve, the system was reset and run again. The portion of each sample that remained on the sieve, the coarse fraction (>50 μm), was then transferred into pre-weighed 250 mL LPDE bottles. The portion that passed through the sieve, the fine fraction (<50 μm), was transferred into pre-weighed 500 mL LDPE bottles. All bottles were frozen and later lyophilised until completely dry before being weighed and removed from the bottles. The coarse and fine fractions were then homogenised and ground into a fine powder. Coarse fractions were ground for 1 minute on a Standard Ring Mill (SRM-RC-3P; Rocklabs Ltd, Auckland, New Zealand) fitted with a stainless-steel head (CARB-40-BLP) and fine-fractions were hand ground by mortar and pestle.

To obtain the necessary mass of OC within the size fractions for further analysis, i.e. [13]C Nuclear Magnetic Resonance spectroscopy (NMR), the fractionation process as described above was repeated with additional steps for both coarse and fine fractions, as follows. Several 10g aliquots were again dispersed overnight, the number of aliquots required was determined by their carbon contents measured through dry combustion as discussed below. The numerous aliquots were combined on to the sieve and shaken until water flowing from the system was clear. The organics of the accumulated coarse fractions were then separated from the >50 μm minerals (i.e. quartz) by a stream of water and manual oscillation into a separate container. The accumulated organics of the coarse fractions and the fine fractions were again collected, frozen

and lyophilised until completely dry. The accumulated fine fractions were then treated with 2% hydrofluoric acid (HF). Hydrofluoric acid was added to the fine fractions, 3 g weighed into 50 mL centrifuge tubes (as many as required) and extracted with nine 50 mL aliquots of 2% HF and shaken 5 times for 1 hour; 3 times for 16 hours; and 1 time for 64 hours. Before each new addition of 2% HF, samples were centrifuged (2,000 rpm for 7 mins) and the supernatant discarded. The fine fractions residue was then washed repeatedly with deionised water after the final 64-hour extraction, and lyophilised. The organics of the coarse fraction and the treated fine fraction residues were again finely ground as described above. Separation of the organics in the coarse fraction and HF treatment of the fine fraction was required to concentrate the OC and remove paramagnetic materials, for the latter, to improve solid state ^{13}C NMR spectral resolution (Baldock et al. 2013b; Skjemstad et al. 1994).

Measuring carbon contents of the coarse and fine size fractions

Prior to carbon analysis and HF treatment, all samples were tested for the presence of inorganic carbon (IC) by adding a few drops of 1M hydrochloric acid (HCl). Any samples that were identified with IC present, when addition of HCl showed effervescence, were first acidified with 1M HCl until effervescence ceased (~25 – 40mL, as required), washed repeatedly with deionised water, and lyophilised. The organic carbon (OC) contents for the coarse and fine fractions was then determined by dry combustion on a LECO TruMac CN analyser (LECO corporation, St Joseph, MI, USA) as follows. A set of 6 – 8 laboratory standard soils were run at the beginning and end of each run. Aliquots of 0.5 – 0.8 g of the finely ground coarse and fine fractions were run in sets of ten, with every tenth sample run in duplicate and followed by a known standard soil.

Allocation of organic carbon to the particulate, humus and recalcitrant fractions

Solid state ^{13}C NMR (200 Avance spectrometer equipped with a 4.7 T, wide-bore superconducting magnet running at a resonance frequency of 50.33 MHz; Bruker Corporation, Billerica, MA, USA) was used for determining the proportion of OC allocated to recalcitrant OC (ROC) of the accumulated coarse and fine fractions. The ROC fraction was determined by the proportion of OC, in the coarse and fine fraction, that was allocated to poly-aryl and aryl C. A detailed description of the ^{13}C NMR methods and the procedure followed for acquisition of 108 NMR spectra for the coarse (n = 54) and fine (n = 54) fractions are explained by Baldock et al. (2013b). All ^{13}C NMR spectra acquired were processed with the Bruker TopSpin 3.5 software.

Following determination of OC allocation in the coarse and fine fractions to ROC, the contents of the particulate OC (POC) and humus OC (HOC) were calculated as per equations 1–3 reproduced from Baldock et al. (2013b):

$$POC = (2000\text{—}50\mu m\ OC)(1 - fROC_{2000})(MF_{2000}) \tag{1}$$

$$HOC = (\leq 50\mu m\ OC)(1\text{-}fROC_{50})(MF_{50}) \tag{2}$$

$$
\begin{aligned}
ROC &= ROC_{2000} + ROC_{50} \\
&= (2000\text{—}50\mu m\ OC)(fROC_{2000})(MF_{2000}) \\
&\quad + (\leq 50\mu m\ OC)(fROC_{50})(MF_{50})
\end{aligned}
\tag{3}
$$

In equation (1) the POC fraction is calculated using the measured OC content (g OC kg^{-1}) of the coarse fraction (2000–50 μm OC); the amount (g ROC/g >50 μm) of the coarse fraction OC determined to be poly-aryl and aryl C by ^{13}C NMR (fROC[2000]); and the total soil mass (g >50 μm fraction/g ≤2 mm soil) of the coarse fraction (MF[2000]). In equation (2) the HOC fraction

is calculated using the measured OC content (g OC kg$_{-1}$) of the fine fraction ($\leq$50 μm OC); the amount (g ROC/g $\leq$50 μm) of the fine fraction OC determined to be poly-aryl and aryl C by $_{13}$C NMR (fROC$_{50}$); and the total soil mass (g $\leq$50 μm fraction/g $\leq$2 mm soil) of the fine fraction (MF$_{50}$). The ROC fraction is then is the total amount of poly-aryl and aryl allocated OC in the coarse and fine fractions as per Equation (3).

Infrared and partial least-squares regression analysis

An aliquot of each $\leq$2 mm soil sample (n = 216) was homogenised and finely ground using a Standard Ring Mill (SRM-RC-3P; Rocklabs Ltd, Auckland, New Zealand) fitted with a stainless-steel head (CARB-40-BLP). Baldock et al. (2013a) showed fine grinding improved predictions of OC, POC and ROC contents derived from infrared spectra and partial least-squares regression (IR/PLSR) algorithms. Diffuse reflectance infrared fourier transform spectra (IR) was then collected as described in Baldock et al. (2013a) for each sample on a Nicolet 6700 FTIR spectrometer (Thermo Fisher Scientific Inc., Waltham, MA, USA; fitted with a KBr beam splitter; a DTGS detector; and an AutoDiff-Automated diffuse reflectance accessory from Pike Technologies, Madison, WI, USA) without variation. Acquired spectra was converted from reflectance to absorbance spectra using the Omnic software (Version 8.0; Thermo Fisher Scientific Inc.)

The IR spectra of the $\leq$2 mm samples that were selected for fractionation (n = 54) and the OC contents calculated for the POC, HOC and ROC fractions were used to develop predictive, cross validated partial least-squares regression (PLSR) models. Overview of the PLSR method is described in Viscarra Rossel et al. (2006). Each PLSR model was generated using a full leave-one-out cross-validation method available in the Unscrambler X software (Version 10.5; CAMO Analytics AS, Oslo, Norway). The frequency range of the IR spectra used to derive the

PLSR models was $6000 - 600 cm^{-1}$. Shortening of the spectra, from the $8000 - 400 cm^{-1}$ collected, removed unnecessary noise from the predictive algorithms but also reduced the probability of important signal loss. A baseline-correction (baseline-offset transformation and mean centred) was applied to the shortened spectra and a square root transformation was applied to calculated POC, HOC and ROC contents. Square root transformation of the OC contents was used to improve model linearity and homogeneity of the residuals (Baldock et al. 2013a; Janik et al. 2007).

Statistics

A linear mixed effect model (LMMs), fit by maximum likelihood (ML), was used to compare the allocation of OC to the POC, HOC and ROC fractions between mangroves and tidal marshes. Vegetation type (i.e. mangrove vs. tidal marsh) and size fractions (i.e. coarse vs. fine) were defined as fixed effects for the LMMs and site as a random effect. The hierarchical structure of the experimental design required fractions to be grouped within vegetation that were in turn grouped within sites. The significance of vegetation type was tested by a Type II Wald chi-squares test. Graphical outputs and statistical analysis were completed in Microsoft Excel for Mac (version 16.3; Microsoft, 2019) and R studio for R (R Core Team 2017) with packages ggplot2 (Wickham 2016); gridExtra (Auguie 2016); lme4 (Bates et al. 2015); car (Fox & Weisberg 2011) and MASS (Venables & Ripley 2002). Performance of the IR/PLSR models were assessed by the correlation coefficient of (r_2) between the measured and predicted values; the root mean square error for the calibration (RMSEC) and cross-validation (RMSECV) samples. Further assessment of the model reliability in the prediction of unknown samples based on ratio of the performance to the inter-quartile distance index (RPIQ) and the proportion of total variance, or the adjusted R-square ($_{adj}R_2$) are also presented.

Results and Discussion

Soil organic carbon allocation

The average organic carbon (OC) content of mangrove soils was 9.46 ($\pm$8.72) mg POC_{-1} g, 23.66 ($\pm$21.48) mg HOC_{-1} g and 7.39 ($\pm$7.85) mg ROC_{-1} g for the POC, HOC and ROC fractions, respectively. The range of the OC of the mangrove POC, HOC and ROC fractions was 1.12–35.46 mg POC_{-1} g, 2.84–93.58 mg HOC_{-1} g and 1.07–32.15 mg ROC_{-1} g, respectively (Figure 3.1). For the tidal marsh soils, the average OC content was 11.98 ($\pm$ 13.59) mg POC_{-1} g, 21.80 ($\pm$ 13.43) mg HOC_{-1} g and 7.68 ($\pm$ 7.22) mg ROC_{-1} g for the POC, HOC and ROC fractions, respectively. There were no significant differences in the allocation of OC to the POC, HOC and ROC fractions between the mangrove and tidal marsh soils ($p > 0.05$ for all). The range of the OC of the tidal marsh POC, HOC and ROC fractions was 0.65–49.29, 3.39–52.48 and 0.72–23.66 mg OC_{-1} g, respectively (Figure 4.1). The POC, HOC and ROC distributions were all positively skewed (< 3) irrespective of vegetation type, with high kurtosis (1.76, 4.39 and 3.59) in the mangrove dataset and low kurtosis (0.70, -0.92 and -0.71) in the tidal marsh dataset. Within the two datasets, one tidal marsh and eight mangrove samples were greater than 1.5 times the upper interquartile range (IQR) of the distribution (Figure 4.1). Overall, a majority (53% and 58%) of the OC was allocated to the HOC fraction followed by POC (29% and 23%) and ROC (19% and 18%) fractions in the tidal marsh and mangrove soils. Similar variations in OC content and allocation to the size fractions was reported by Baldock et al. (2013b). However, they found a greater proportion of OC in agricultural soils was allocated to the ROC fraction (26%) than the POC fraction (19%).

The relationship between the measured total organic carbon (TOC) contents of the $\leq$2 mm soils and their respective measured POC, HOC and ROC fractions are displayed in Figure 4.2a – f. There was significant correlation between the calculated POC/ROC fractions and the measured

TOC values ($r_2 = 0.83$; $p < 0.05$ for both), however, scatter increased in conjunction with an increase in the measured TOC and calculated POC/ROC fraction contents (Figure 4.2a and c). The correlation between the calculated HOC and measured TOC values had a stronger relationship ($r_2 = 0.93$) with TOC than the POC or the ROC fractions had with TOC (Figure 4.2b). The relationship between the calculated HOC fraction contents and the measured TOC contents were also significantly correlated ($p < 0.05$). Applying a square root transformation to the TOC, POC, HOC and ROC fractions contents increased the magnitude of the slope for all correlations (Figure 4.2d – f). Transformation also improved the correlation coefficient of the measured sqrtPOC to measured sqrtTOC ($r_2 = 0.83$ vs. 0.90; Figure 4.2a and d). The correlation between the fractions and TOC, particularly the HOC fraction, reflects covariance between the calculated OC contents of the fractions and the TOC content of the ≤2 mm soils.

IR/PLSR Models

Predictive IR/PLSR models derived from the square root transformed POC, HOC and ROC fraction contents performed better than models derived from non-transformed data (Table 4.1). Transformation improved spread of the data which untransformed was skewed towards low values, which is common for soil sample sets (Bellon-Maurel et al. 2010). Non-transformed models resulted in negative predictions when OC contents were < 1.6, < 3.5 and < 1.5 mg/g for the POC, HOC and ROC fractions, respectively (Figure 4.3a – c). Square root transformation of the measured values also removed curvature (Figure 4.3a – c) in the models and improved homogeneity of the model residuals (difference between measured and predicted). Square root transformation of measured TOC contents in Janik et al. (2007) and total (TC), OC and inorganic carbon (IC) and nitrogen (TN); and POC, HOC and ROC fraction contents in Baldock et al. (2013a) had the same affect for the soil properties predicted by IR/PLSR models. Therefore, only the predictive IR/PLSR models derived from the square root

transformed POC, HOC and ROC contents of the 54 mangrove and tidal marsh soils chosen for fractionation are further discussed.

The optimal number of factors suggested by the calibration model for predicting sqrtPOC were three, and four factors for predicting both sqrtHOC and sqrtROC contents. However, models using all the suggested factors potentially overfitted the calibration data, although the optimum number of suggested resulted in the smallest RMSE. Model selection based on the lowest possible RMSE relates to the predictive power of the PLSR model (Haaland & Thomas 1988; Viscarra Rossel et al. 2006). But this can also lead to overfitting as RMSE is infinite and subjective to the number of samples within the calibration. Thus the optimum model becomes the one that requires the fewest number of factors and where RMSE is not significantly greater than the RMSE of the optimum (Haaland & Thomas 1988). The number of factors chosen for the predictive IR/PLSR models used in this study were therefore one less than the optimal number suggested (Table 4.1).

There was a strong relationship between the analytically measured and IR/PLSR predicted values for all three models (Figure 4.3). Overall, the correlation between the predicted and measured values for the calibration sets had r_2 values >87 % and validation sets had r_2 values >82 % (Table 4.1). The sqrtROC model performed best, the calibration set having an r_2 of 0.92 and the smallest RMSEC of 0.35 mg $ROC_{(0.5)-1}$g and the validation set had an r_2 of 0.89 and an RMSECV of 0.41 mg $ROC_{(0.5)-1}$g (Figure 4.3f). The sqrtPOC and sqrtHOC models also performed well with an r_2 of 0.87 for both the calibration sets and RMSEC values 0.56 mg $POC_{(0.5)-1}$g and 0.60 mg $HOC_{(0.5)-1}$g, respectively (Figure 4.3d and e). The validation sets of the sqrtPOC and sqrtHOC models also performed well (r_2 of 0.83, 0.82, respectively) but had higher dispersion (RMSECV of 0.63 mg $POC_{(0.5)-1}$g and 0.69 mg $HOC_{(0.5)-1}$g respectively;

Figure 4.3d and e). Furthermore, the RPIQ and $_{adj}R_2$ values of the sqrtPOC ($_{adj}R^2_{cv} = 0.83$; RPIQ$_{cv}$ = 2.77), sqrtHOC ($_{adj}R^2_{cv} = 0.82$; RPIQ$_{cv}$ = 2.37) and sqrtROC ($_{adj}R^2_{cv} = 0.88$; RPIQ$_{cv}$ = 3.03) suggested good predictive power of the models, again the most accurate model being the sqrtROC. The RPIQ of each model was used in favour of using the ratio of performance to deviation (RPD) to assess the reliability of predicting additional samples. This was due to the skewed distribution of the data set which is better represented in the RPIQ index (Bellon-Maurel et al. 2010). The RPIQ index provides a more robust index for determining the accuracy and quality of a predictive IR/PLSR model (Bellon-Maurel et al. 2010).

Beta (β) coefficients and β coefficient correlations

Visual inspection of the beta (β) coefficients derived for the sqrtPOC, sqrtHOC and sqrtROC predictive IR/PLSR models appeared almost identical (Figure 4.4b – d). Beta coefficients allow for qualitative characterisation of the spectra and plotting the coefficients as a function of their wavenumber allows for identification of spectral features contributing to the predictions (Baldock et al. 2013a; Haaland & Thomas 1988). Within the derived β coefficients the positive and negative peaks correspond with chemical components that resonate at specific wavelengths (Viscarra Rossel et al. 2006). The β coefficients, for all the models, had positive peaks at 2927 cm$_{-1}$, 2846 cm$_{-1}$, 1677 cm$_{-1}$ and 1577 cm$_{-1}$ associated with resonance of organic matter including alkyl C ($_{-}$CH$_2$) and protein amides (OC-NH), respectively. Another contributing factor appeared to resonate from aromatic structures, present for all models, at 1230 cm$_{-1}$. In conjunction, the negative peaks at 3725 – 3571 cm$_{-1}$, 2520 cm$_{-1}$ and 1793 cm$_{-1}$ associated with kaolinite and carbonates interfered with the model's ability to predict (Figure 4.4a–c). The β coefficients of the POC, HOC and ROC models also appeared identical to the sqrtOC derived IR/PLSR β coefficients (Figure 4.4a). The magnitude (β$_0$) of the sqrtOC coefficient was,

however, greater ($\beta_0 = 3.30$) than the sqrtHOC ($\beta_0 = 2.47$), sqrtROC ($\beta_0 = 1.87$) and sqrtPOC ($\beta_0 = 1.53$).

Therefore, the relationship between the β coefficients derived for the sqrtPOC, sqrtHOC and sqrtROC to the sqrtOC were assessed to investigate their collinearity. In particular, given the strong correlation between the calculated sqrtPOC, sqrtHOC and sqrtROC fractions and measured TOC content as discussed above (Figure 4.2). Plotting the sqrtPOC, sqrtHOC and sqrtROC β coefficients as a function of the sqrtOC β coefficients model showed all three models were positively correlated (Figure 4.5). The weakest correlation was between the sqrtOC and the sqrtPOC β coefficients due to slight deviation from the line of best fit, however, they still had a significant positive correlation ($r_2 = 0.94$; $p < 0.000$). For the sqrtHOC and SqrtROC models, the relationship between β coefficients with the sqrtOC model was significantly correlated ($r_2 = 0.98$ for both; $p = <0.000$). The strong correlation between the β coefficients confirmed the predictions derived from all four models used the same IR spectral features. Similar findings of collinearity between the sqrtOC, sqrtTN ($r_2 = 0.88$) and sqrtHOC ($r_2 = 0.97$) were observed in Baldock et al. (2013a), however in contrast, their sqrtPOC and sqrtROC models appeared to be fraction specific.

Predictions of sqrtPOC, sqrtHOC and sqrtROC contents

Based on the confidence in the reliability of the IR/PLSR predictive algorithm performance the sqrtPOC, sqrtHOC and sqrtROC contents for the remaining 162 coastal wetland soils were predicted. A projection of the predictions over the known samples and the associated test statistics are presented in Figure 3.6a–f. Aside from one predicted POC sample (Figure 4.6d) and one predicted ROC sample (Figure 4.6f) falling outside of the inlier distance limit (Lim:

1.53; 1.46, respectively), the unknown samples fit well within the range of the modelled data for all predicted fractions (Figure 4.6a – c). The range of the predicted sqrtPOC fractions was -0.5 – 7.14 ($\pm$ 0.19 – 1.52) mg $POC_{(0.5)-1}$ g with an average of 2.93 ($\pm$ 0.59) mg $POC_{(0.5)-1}$ g. The range of the predicted sqrtHOC fractions was 0.78 – 9.16 ($\pm$ 0.22–1.84) mg HOC_{-1} g with an average of 4.53 ($\pm$ 0.67) mg $HOC_{(0.5)-1}$ g and was 0.02 – 5.90 ($\pm$ 0.13–1.14) mg $ROC_{(0.5)-1}$ g with an average of 2.51 ($\pm$ 0.40) mg $ROC_{(0.5)-1}$ g for the predicted sqrtROC fractions. As the prediction of the sqrtPOC, sqrtHOC and sqrtROC contents is strongly related to sqrtOC content, wide spread use of these models, provided samples fit within the OC range of the present dataset, would be acceptable (Soriano-Disla et al. 2014). However, predictions of soil properties through the application of IR/PLSR can become less accurate as the geographic range of samples increases (Chang et al. 2001). Therefore, it is recommended that at least 25% of a new sample population be analysed as described above and added to the model to improve the robustness of the IR/PLSR models (Janik et al. 2007; Soriano-Disla et al. 2014).

Conclusion

The primary objective of this study was to determine the allocation of OC to particle size fractions (POC, HOC and ROC) in vegetated temperate coastal wetland soils. We found the allocation of OC to the size fractions varied slightly with vegetation type, but overall, the HOC fraction accounted for 53 – 58 % of the OC, the POC fraction accounted for 23 – 29 % and 18 – 19 % is allocated to the ROC fraction. The higher allocation of OC to the HOC fraction is in contrast to our hypothesis and suggests OC is being degraded and should persist in the environment. The determination of OC allocation to the different size fractions in a blue carbon soils could be used to allocate blue carbon into pools with different levels of susceptibility to loss or degradation. However, further details on the chemical composition of blue carbon soils

and their respective size fractions would be required to quantifying the relationship between particle size fractions and reduced susceptibility.

In addition, the application of the combined IR spectroscopy and PLSR analysis approach appears well suited for predicting the allocation of OC to size specific fractions in vegetated coastal wetlands. Overall our study found the IR/PLSR models were not fraction specific for the samples included in this study. Therefore, the OC content of the bulk ≤2 mm soil can be used to determine the allocation of OC to the POC, HOC and ROC size fractions. We recommend that future blue carbon projects also include IR analysis and laboratory quantification of OC allocation across size fractions for at least 25% of the sample population. This data will enable a spectral database to be developed that covers the depth profile and a diverse range of sites and climates the blue carbon environment spans. Such information will improve calibration models and allow for cost effective and more reliable predictions of future blue carbon stocks through the application of predictive IR/PLSR algorithms.

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Table 4.1 Summary statistics of the IR/PLSR models derived for the non-transformed and square root transformed particulate (POC), humic (HOC) and recalcitrant (ROC) organic carbon contents.

Variable	Set	model factors	Slope	intercept	r^2	R^2	$RMSE_{c,cv}$	Bias	$SE_{C,CV}$	RPIQ
POC	Calibration	2	0.788	2.270	0.788	0.788	5.238	0.000		1.823
	Validation		0.739	2.878	0.733		5.881	0.081		1.624
SqrtPOC	Calibration	2	0.869	0.379	0.869	0.869	0.559	0.000		3.115
	Validation		0.825	0.518	0.834		0.629	-0.012		2.767
HOC	Calibration	3	0.752	5.647	0.752	0.752	8.857	0.000		2.000
	Validation		0.692	7.111	0.679		10.076	0.098		1.758
SqrtHOC	Calibration	3	0.868	0.593	0.868	0.868	0.598	0.000		2.250
	Validation		0.851	0.658	0.823		0.693	0.008		2.371
ROC	Calibration	3	0.827	1.302	0.827	0.827	3.106	0.000		1.833
	Validation		0.785	1.685	0.789		3.429	0.063		1.660
SqrtROC	Calibration	3	0.917	0.205	0.917	0.917	0.348	0.000		3.535
	Validation		0.920	0.192	0.888		0.405	-0.005		3.033
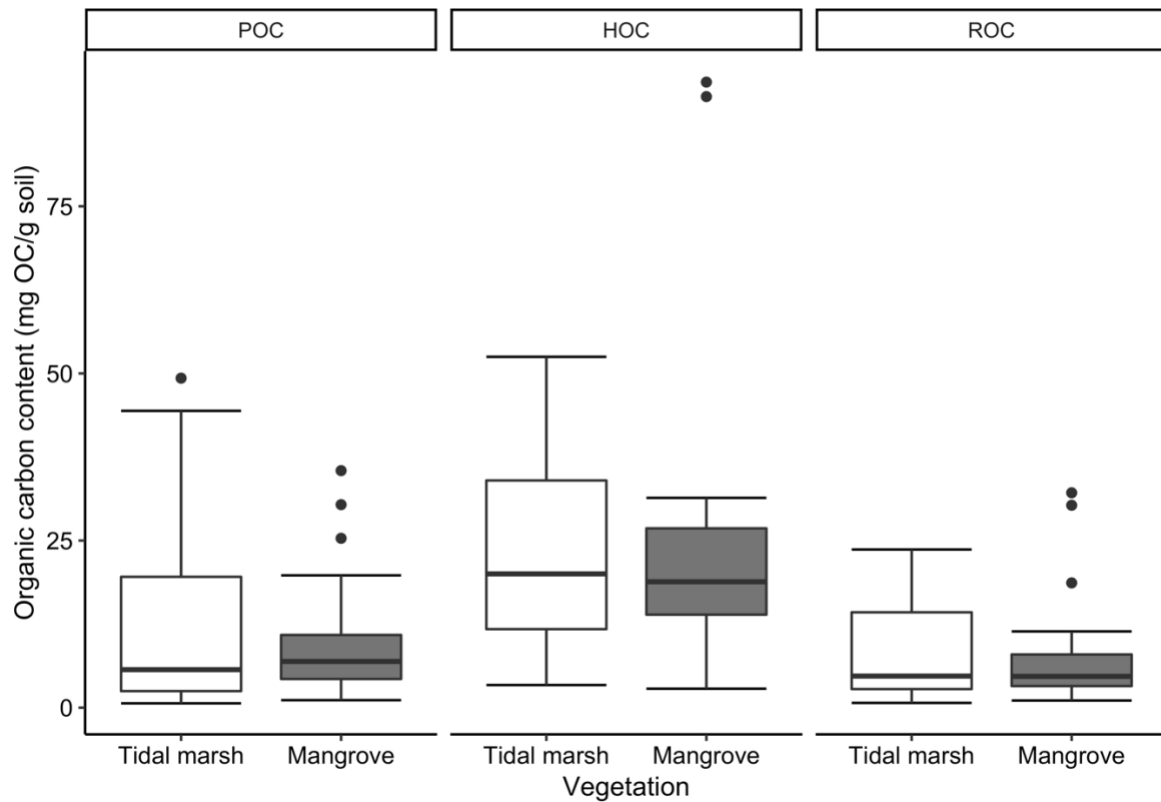
Figure 4.1 Box and whisker plots for the allocation of organic carbon (mg/g) to the particulate (POC), humus (HOC) and recalcitrant (ROC) soil storage pools in mangrove (n = 27) and tidal marsh (n = 27) soils of temperate coastal wetlands. The horizontal line within the box indicates the median, boundaries of the box indicate the 25th and 75th percentile, and the whiskers indicate the highest and lowest values 1.5* greater than the inner quartile range (IQR) of the results. Values greater than 1.5* the IQR are plotted as black dots.
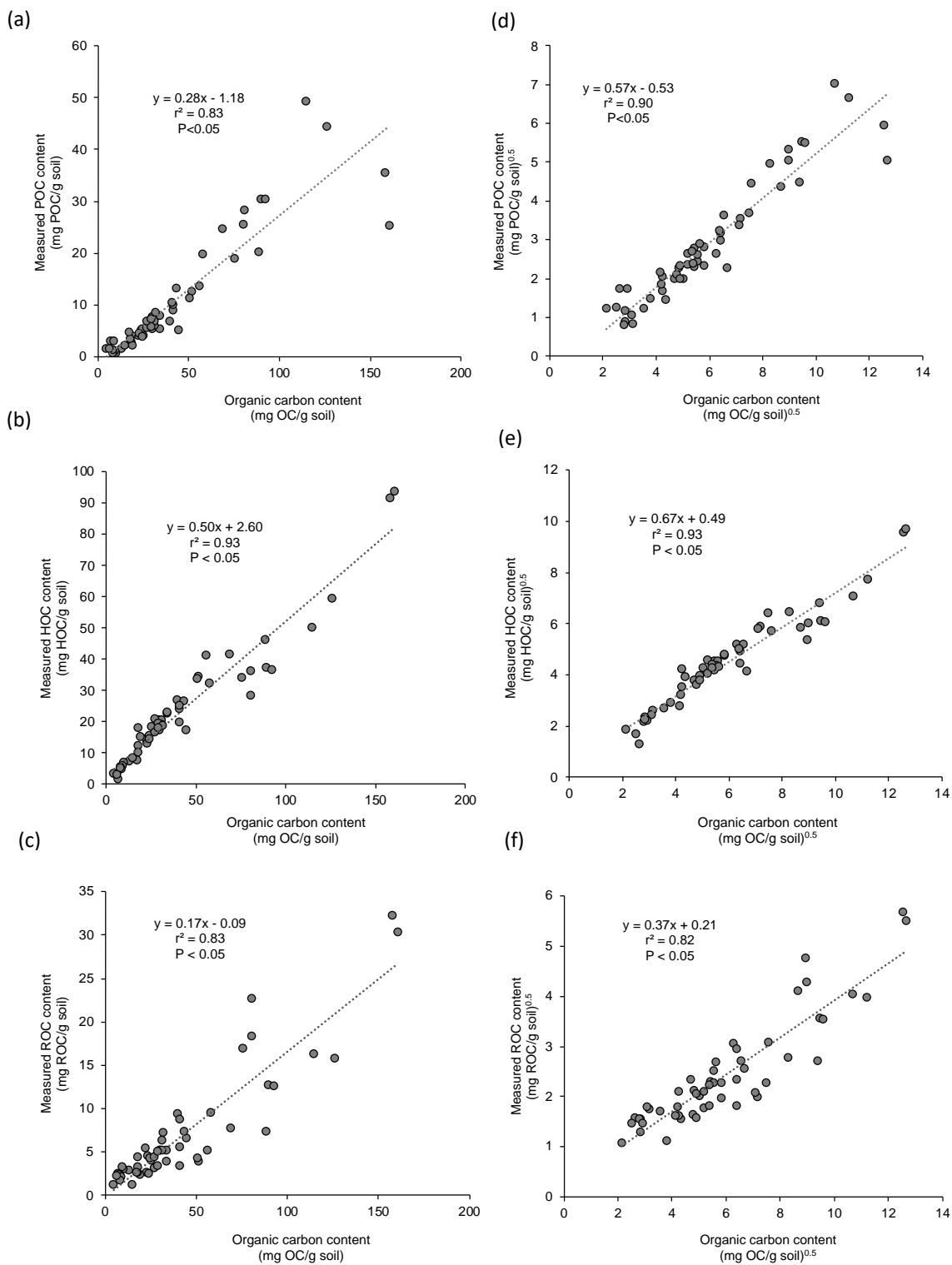
Figure 4.2 Relationship between the measured (a) particulate (POC), (b) humus (HOC) and (c) recalcitrant (ROC) organic carbon contents and the total organic carbon (TOC) contents; and square root transformed (d) sqrtPOC, (e) sqrtHOC, and (f) sqrtROC contents and the sqrtTOC for 54 mangrove (n = 27) and tidal marsh (n = 27) soils in temperate coastal wetlands.
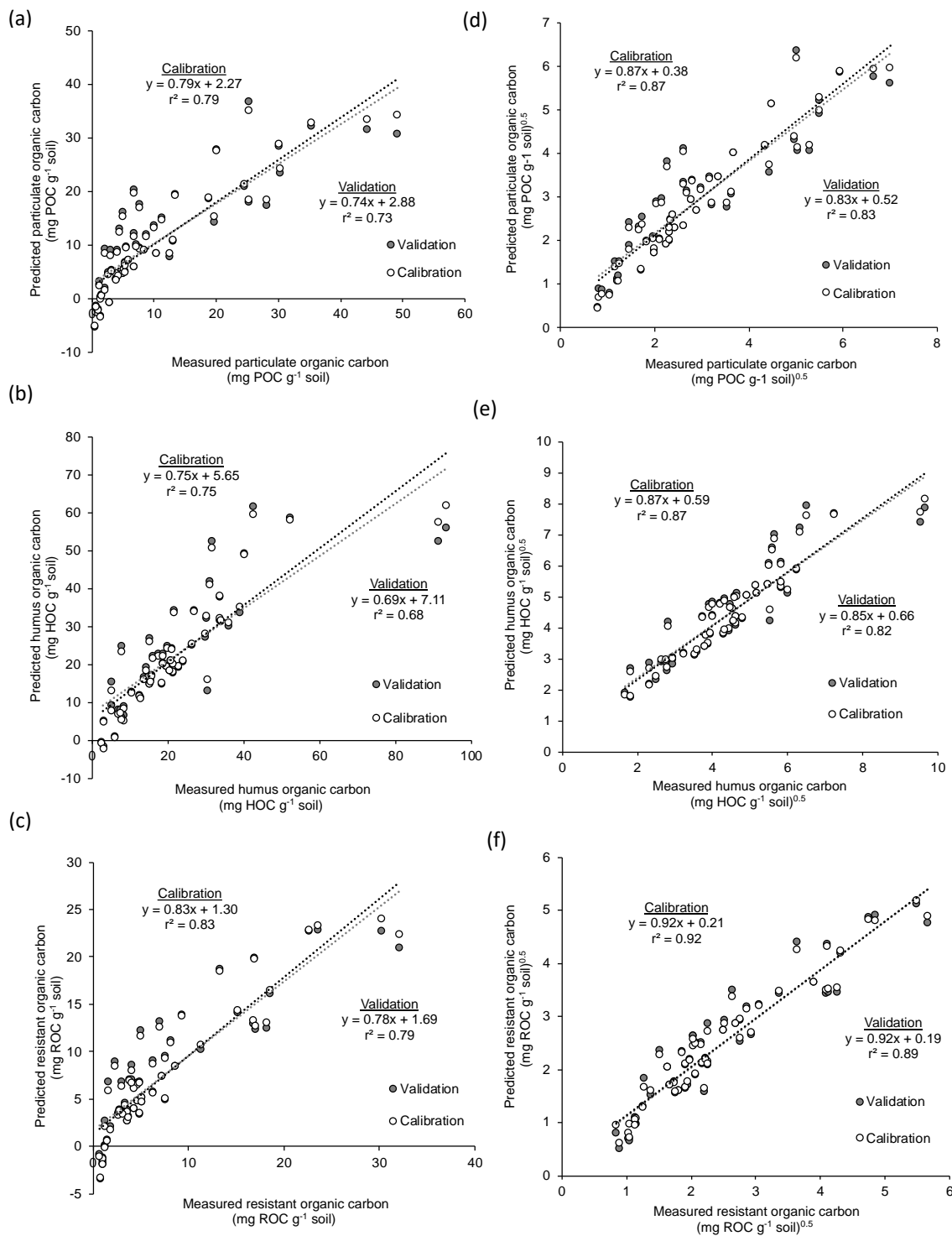
Figure 4.3 Relationship between the measured and IR predicted (a) particulate (POC), (b) humus (HOC) and (c) recalcitrant (ROC) organic carbon contents; and the square root transformed (d) sqrtPOC, (e) sqrtHOC and (f) sqrtROC contents and the sqrtTOC for 54 mangrove (n = 27) and tidal marsh (n = 27) soils in temperate coastal wetlands.
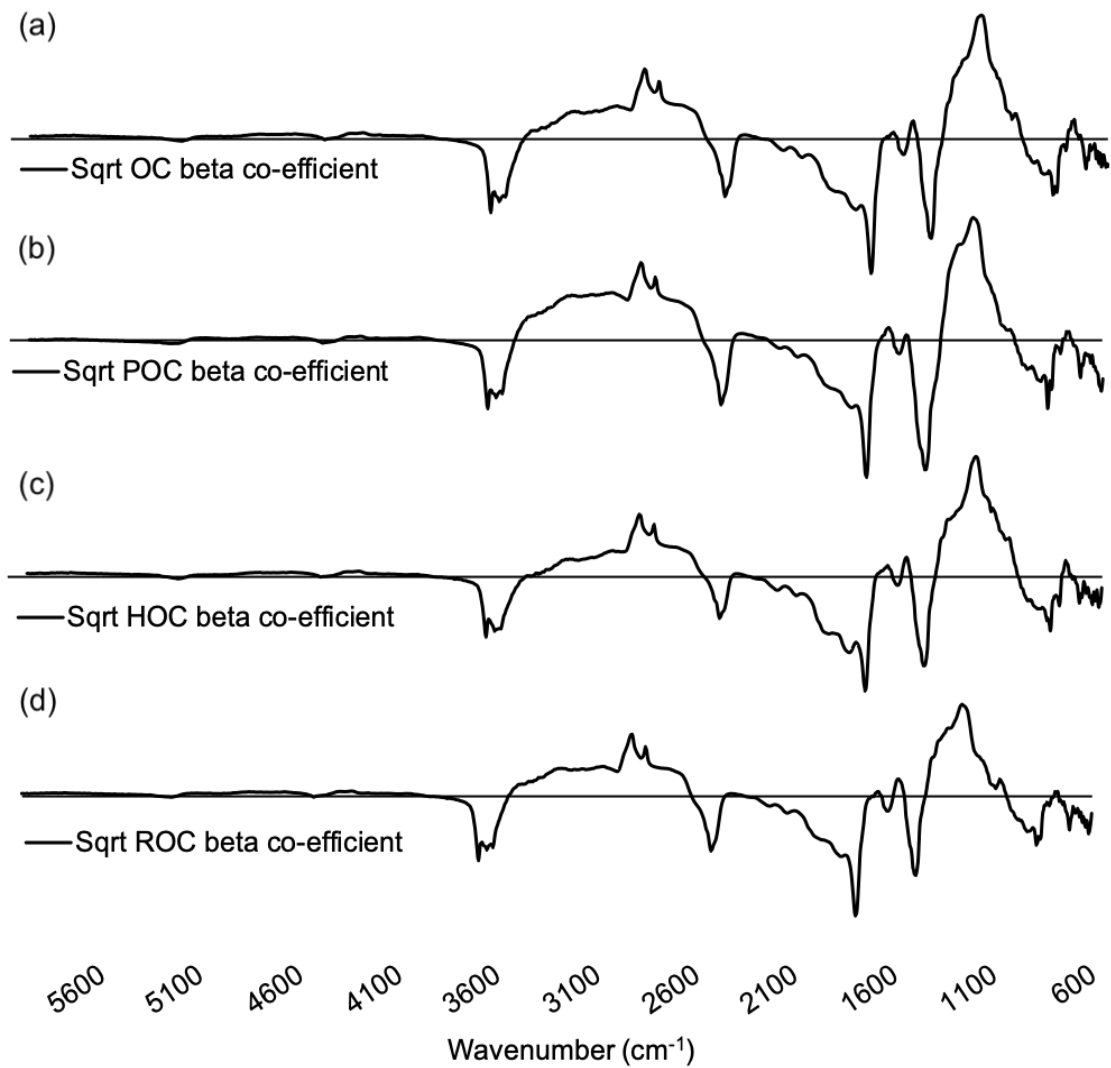
Figure 4.4 Beta (β) coefficients derived for the square root (a) organic carbon (OC); (b) particulate (POC); (c) humus (HOC); and (d) recalcitrant (ROC) organic carbon content IR/PLSR models for 54 selected mangrove (n=27) and tidal marsh (n=27) soils in temperate coastal wetlands.

(a)

y = 0.56x + 0.00
r² = 0.94
P < 0.05

Sqrt particulate organic carbon beta Coefficents

Sqrt organic carbon beta coefficients

(b)

y = 0.67x + 0.00
r² = 0.98
P < 0.05

Sqrt humus organic carbon beta Coefficents

Sqrt organic carbon beta coefficients

(c)

y = 0.48x - 0.00
r² = 0.98
P < 0.05

Sqrt resistant organic carbon beta Coefficents

Sqrt organic carbon beta coefficients
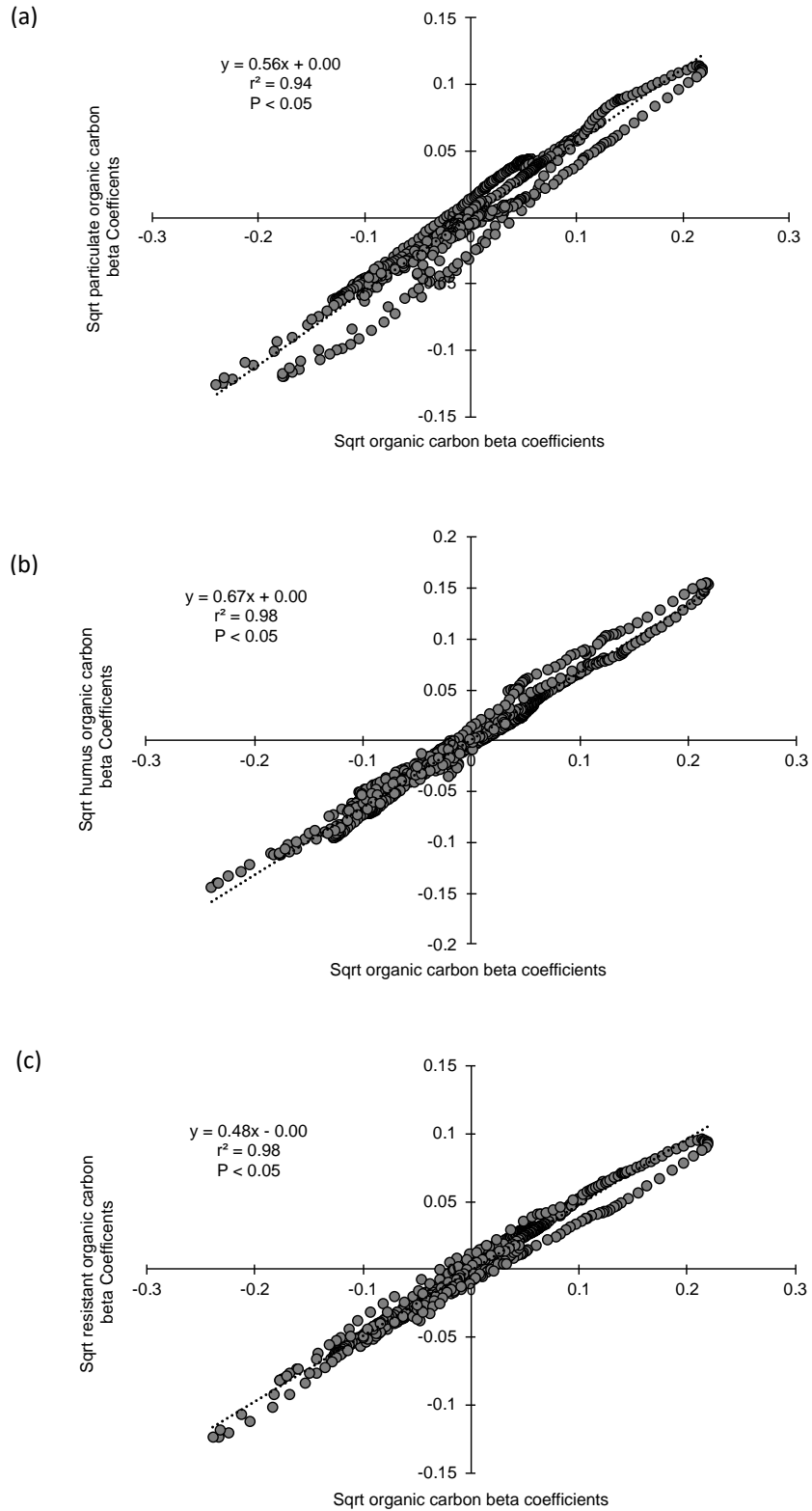
Figure 4.5 Correlation of the square root organic carbon beta co-efficient with the (a) particulate (POC), (b) humus (HOC) and (c) recalcitrant (ROC) organic carbon beta co-efficient of the 54 temperate mangrove (n = 27) and tidal marsh (n = 27) wetland soil IR/PLSR

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models.

Figure 4.6 Projection of the IR/PLSR predicted (a) particulate (POC); (b) humus (HOC); and (c) recalcitrant (ROC) organic carbon contents and the corresponding test statistics for the predicted (d) POC; (e) HOC; and (f) ROC of 162 mangrove (n = 81) and tidal marsh (n = 81) soils in temperate coastal wetlands.
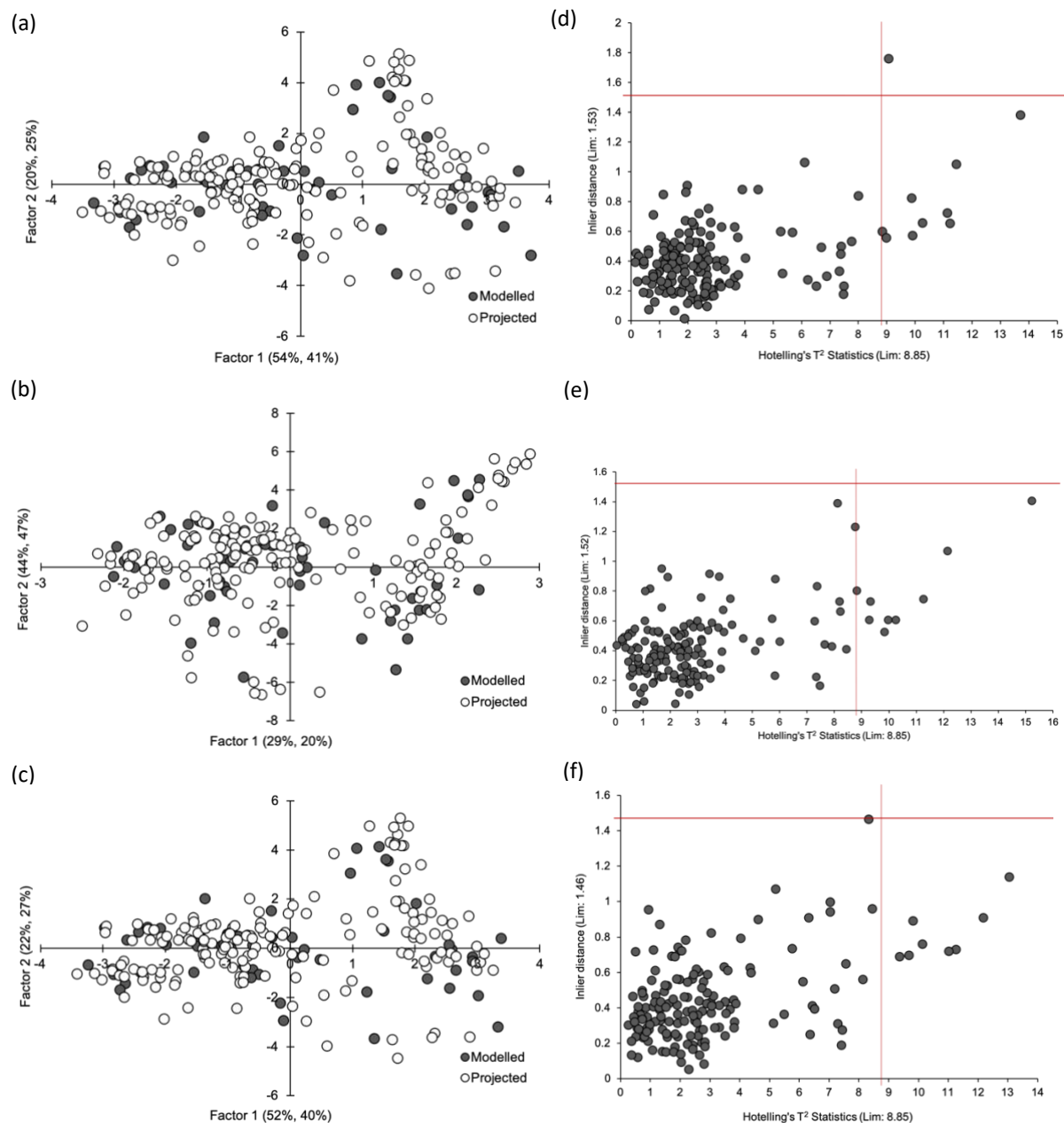
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Statement of Authorship

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Contribution to the Paper	• Experimental design; • Field work; Processing and analyses of samples; Data analyses; • Preparation of manuscript
Overall percentage (%)	85%
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.
Signature	Date 13/12/2019

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i.   the candidate's stated contribution to the publication is accurate (as detailed above);

ii.   permission is granted for the candidate in include the publication in the thesis; and

iii.   the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

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Chapter Five

Chemical structure of soil organic carbon in temperate vegetated coastal wetlands.

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Abstract

In mangrove and tidal marsh environments, turnover of soil organic carbon (SOC) is hindered by anoxic soil conditions that promote long-term storage of carbon. The long-term storage of SOC is a function of the environmental conditions of the ecosystem, the physical properties of the soil and the chemical composition of the SOC. Characterising the chemical composition of the SOC can therefore improve understanding of the potential fate of carbon in a changing environment. In this study we quantified and characterised the chemical composition of the SOC in selected mangrove and tidal marsh soils collected across nine temperate coastal wetlands. Solid state 13C nuclear magnetic resonance (NMR) spectroscopy of mangrove and tidal marsh soils and separation of soils into coarse (>50 μm) and fine (≤50 μm) sized fractions was used for characterisation of chemical composition. O-alkyl carbon was the predominant type of carbon present in both mangrove and tidal marsh soils (29% and 31% respectively). The proportion of alkyl carbon, however, was the key differentiator of the SOC between the mangrove and tidal marsh soils. Particle size fractions revealed fine fractions to be relatively similar in chemical composition irrespective of the vegetation type. Coarse fractions derived from mangrove soils contained a greater proportion of alkyl carbon while those derived from tidal marsh soils contained a greater proportion of O-alkyl/di-O-alkyl carbon. The higher alkyl content of mangrove soils indicates a greater decomposition of SOC in these soils than the tidal marsh soils. Overall, however, the dominance of labile carbon forms (O-alkyl) in the surface soils, irrespective of vegetation types, is suggestive of SOC stocks that are vulnerable to rapid decomposition should soils become aerobic.

Keywords: Blue Carbon; Soil Organic Matter (SOM); Soil carbon; Chemical composition; Particle size fractions; Solid-state 13C NMR; Spectroscopy.

Introduction

Vegetated coastal wetlands cover less than 2 % of the earth's surface but account for 47 % of the carbon stored in ocean sediments and 80 % of that carbon is stored in their below ground soils (Duarte et al. 2013; McLeod et al. 2011; Siikamäki et al. 2013). Over the past 50 years, however, 25% to 50% of the area they occupy has been lost as a result of anthropogenic activity and climate change (Duarte et al. 2013; McLeod et al. 2011; Murray et al. 2011; Pendleton et al. 2012). In light of the threats from urbanisation, degradation or removal and climatic threats to these ecosystems, quantification of soil organic carbon (SOC) stocks and rates of change in coastal wetlands has received increased research attention (Adame et al. 2015; Breithaupt et al. 2012; Chmura et al. 2003; Macreadie et al. 2017; Sanderman et al. 2018). Research in this area is important in supporting the conservation and restoration of coastal environments.

Coastal wetlands are dynamic ecosystems that encompass components of both a terrestrial and marine habitat (Hedges & Oades 1997). They are dominated by salt tolerant vascular plants (i.e. mangroves and tidal marshes) that contribute significant amounts of autochthonous organic matter to underlying soils. These wetlands are also regularly inundated by tides that can provide deposits of carbon rich allochthonous organic matter. Thus, blue carbon stocks (i.e. the carbon stored in coastal wetlands) are expected to reflect organic inputs from both terrestrial and marine environments. As a result of the frequent tidal inundation, anoxic conditions predominate in coastal soils, preventing rapid degradation of the organic matter through microbial transformation (Kristensen 2000; Reddy & DeLaune 2008).

The flow and transformation of carbon and other elements between the atmosphere, ocean, land, and lithosphere are controlled by biotic and abiotic processes (Ciais et al. 2013). Soil organic matter is a heterogeneous mixture of organic residues at different stages of

decomposition (Baldock & Broos 2012; Baldock et al. 1997; Christensen 1992). The quality and composition (i.e. protein, polysaccharides, lignin etc.) of organic matter affects nutrient cycling, ecosystem net primary productivity, and SOC storage (Grandy & Neff 2008; Hedges & Oades 1997; Kristensen 2000). The rate of SOC decomposition is influenced by the environmental conditions (e.g. moisture, temperature, light and oxygen availability) of the ecosystem and its soil environment (Kristensen 2000). In conjunction, the turnover of SOC is driven by its chemical composition (e.g. functional groups and solubility) and physical properties (e.g. surface area, particle size, mineral binding potential and porosity) (Baldock et al. 2004; Bianchi et al. 2018; Christensen 1992; Kleber & Johnson 2010).

Within the soil matrix, components of SOC will decompose and accumulate at different rates based on the lability of their molecular structure and microbial accessibility (Baldock et al. 2004; Kögel-Knabner & Rumpel 2018). With a greater extent of SOC decomposition and humification, the molecular structure is transformed from labile to more recalcitrant forms. The transformation results in the accumulation of carbon that is more recalcitrant to further decomposition (Kleber & Johnson 2010). The chemical structure of SOC and its inherent susceptibility to alteration are important for defining its turnover. Therefore, the quantification of SOC in coastal wetlands combined with information surrounding its chemical structure can provide information on its origin and resistance to decomposition, as has been demonstrated in terrestrial settings (Kögel-Knabner & Rumpel 2018),

In addition to chemical changes, as the extent of decomposition increases, particle size of SOC is reduced (Baldock et al. 2013b). The physical separation of soils allows for the quantification and characterisation of carbon associated with the different particle sizes (Baldock et al. 2013b). In agricultural soils, the SOC pool structure provides important information on carbon

turnover rates (Baldock et al. 2013b; Skjemstad et al. 2004). Different turnover rates, resulting from differing composition, highlights the structural and functional differences associated with different carbon pools (Christensen 1992; Golchin et al. 1994). A decrease in particle size also coincides with a decrease in C:N ratio, reflecting the loss of labile carbon relative to nutrient content, and representing a less energy rich source for the microbial community (Christensen 1992). The dynamics of the distribution and differences in chemical composition, as revealed by particle size fraction, may then be used to improve estimates for the stability of blue carbon stocks.

This study focused on the quantification and characterisation of the chemical composition of SOC in temperate coastal wetlands. The objective was to determine the chemical composition of the blue carbon in temperate wetlands. Specifically, we quantified and characterised the chemical composition of surface soils and their size fractions beneath the dominant vegetation, mangroves and tidal marshes, in temperate coastal wetlands. We hypothesise that O-alkyl carbon will be the most prevalent form of carbon in the coastal soils as a result of the accumulation of organic matter with slow turnover and mangrove soils and their size fractions will be enriched with lignin and proteins as a result of their woody structure unlike tidal marshes.

Methods

Sample collection

Soil samples were collected from nine temperate coastal wetlands located on the eastern borders of Gulf St Vincent and Spencer Gulf, South Australia. More details on the study sites and sampling effort can be found in *Chapter 2*. Briefly, at each site, three 35 m parallel

transects, each separated by 10 m, were established. Each transect spanned the transitional zone of mangrove (*Avicennia marina*) and tidal marsh (*Sarcocornia quinqueflora*) vegetation. Eight soil samples, spaced 5 m apart from each other, were collected on each transect using a soil corer with an internal diameter of 80 mm. In this way, twenty-four 0–10 cm surface soil cores in total were collected from each site within the two dominant vegetation types, i.e. 12 × mangrove and 12 × tidal marsh. The top 10 cm is the most representative of the current overlying vegetation type and (Kelleway et al. 2017; Owers et al. 2020; Yando et al. 2016). 2Immediately after collection intact soil cores were stored at 4°C for transportation back to the laboratory where they were frozen within 5 hours of collection.

Sample preparation, carbon content analyses

All frozen soil samples were lyophilised (Cuddon freeze dryer, Blenheim, New Zealand), crushed and sieved to ≤2 mm. A representative sub-sample to be used for subsequent analyses was split (approx. 100g) from each ≤2 mm bulk soil using a riffle box (12 × 13 mm slotted box; Civilab Australia, Sydney, Australia), minimising any bias associated with the sub-sample separation. Following separation, an aliquot of the sub-sample was ground to a fine powder using a ring mill (SRM-RC-3P; Rocklabs Ltd, Auckland, New Zealand) fitted with a stainless-steel head (CARB-40-BLP).

Diffuse reflectance infrared spectra (IR) were collected for the finely ground sub-samples using a Nicolet 6700 FTIR spectrometer (Thermo Fisher Scientific Inc., Waltham, MA, USA) following the procedure described by Baldock et al. (2013a). All samples found to have a calcium carbonate ($CaCO_3$) peak (2,560 cm^{-1} – 2,480 cm^{-1}) in the IR spectra were acidified with 1 M hydrochloric acid, washed repeatedly with deionised water, and lyophilised. Following carbonate removal, where required, carbon contents were determined on a 0.8 g

subsample by high temperature dry combustion (LECO TruMac CN analyser, LECO Corporation, St. Joseph, MI, USA) as described in Baldock et al. (2013b).

Analyses for chemical composition of SOM

Fifty-four (27 mangrove and 27 tidal marsh) representative samples were selected from the original 216 collected. The representative samples included three mangrove and three tidal marsh samples from each sampling site. The selected mangrove samples were all located at position eight on the transect because it was the mangrove sample furthest into the forest. The selected tidal marsh samples were all located at position two on the transect because it was where the tidal marsh vegetation was most uniform. An unground subsample of each of the selected soils was also fractionated to allow for characterisation and quantification of carbon within the different particle sizes, i.e. coarse (>50 μm) and fine (≤50 μm) fractions. Soils were fractionated following the automated particle size fractionation method as described in Baldock et al. (2013b) and the OC contents of the respective coarse and fine fractions were measured as described above for the ≤2 mm soils.

The chemical composition of the organic carbon found in each of the selected soils and their respective fractions was determined by solid-state 13C NMR as described in Baldock et al. (2013b). The spectra of biomolecules, with structures representative of those derived from plant residues or created by decomposer organisms (e.g. lignin, cellulose, chitin and gliadin) and algae were also acquired. Solid-state 13C NMR cross-polarisation (CP) spectra were acquired using a 200 MHz Avance spectrometer (Bruker Corporation, Billerica, MA, USA) equipped with a 4.7 T, wide-bore superconducting magnet running at a 13C resonance frequency of 50.33 MHz. Bruker TopSpin 3.5 software was used for processing all 13C NMR spectra acquired. The fine fractions and ≤2 mm soil samples that had less than 5% OC content

were treated with 2% hydrofluoric acid (HF) to concentrate the carbon and improve spectral resolution (Skjemstad et al. 1994). To complete the HF digestions, finely ground ≤2 mm soil subsamples and fine fractions (two 50 mL centrifuge tubes × 3 g) were treated with nine 50 mL aliquots of 2% HF and shaken for 5 × 1 hour; 3 × 16 hours; and 1 × 64 hours. Between each extraction and addition of 2% HF, samples were centrifuged (2,000 rpm for 7 mins) and the supernatant discarded. After the final 64-hour extraction, the soil residue was washed repeatedly (three times) with deionised water and lyophilised.

To provide estimates of broad carbon types, the spectra were integrated using the chemical shift limits defined by Baldock et al. (2013b): 45 – 0 ppm (alkyl C), 65 – 45 ppm (methoxyl and N-alkyl C), 90 – 65 ppm (O-alkyl C), 110 – 90 ppm (di-O-alkyl C), 145 – 110 ppm (aryl and unsaturated C), 165 – 145 ppm (O-aryl C), 190 – 165ppm (carbonyl and amide C), and 215 – 190 ppm (ketone C). Glycine was used as an external standard for NMR observable OC (Baldock et al. 2013b; Baldock & Smernik 2002). Due to poor total 13C NMR signal intensity resulting from low organic carbon contents even after HF pre-treatment, two ≤2 mm tidal marsh and two mangrove samples were excluded from the data set. The proportion of O-alkyl and alkyl carbon was also used to calculate the alkyl to O-alkyl ratio (A:OA) as an index for extent of decomposition (Baldock et al. 1997).

Statistical Analysis

Relative contributions of 13C NMR signal intensity to spectral regions as defined above were determined. The proportion allocated to each region were arcsine transformed prior to a comparison of each carbon type between mangroves and tidal marshes with a linear mixed effect model (LMMs), fit by maximum likelihood (ML). Within the LMMs, vegetation type (i.e. mangrove vs tidal marsh) and size fractions (i.e. coarse vs fine) were set as the fixed effect

with site as a random effect. Due to the hierarchical structure of the experimental design, fractions were grouped within vegetation, which in turn, were grouped within sites. A Type II Wald chi-squares test was subsequently applied to each of the LMMs to test the significance of the fixed effect, i.e. vegetation type. Homoscedasticity and normality were also confirmed for all test parameters. Principle components analyses (PCA) were applied to the normalised NMR spectral data of ≤2 mm soils and size fractions to further investigate dissimilarities in spectral chemistry between the soils. The greatest variation in the samples are identified by the PCA and explained through its principle components as linear combinations of the original variables (Ringér 2008). The first PC identifies the largest variation while the subsequent PCs explain further, uncorrelated, variations. Sub-spectra are generated from the significant components that can be used to interpret the importance of each variable (Nordén & Albano 1989). Unscrambler 10.2 software (CAMO Software AS, Oslo, Norway) was used for all principle component analysis and generation of corresponding loading plots. All other statistical analysis were performed in R studio for R (R Core Team 2017) with packages lme4 (Bates et al. 2015); car (Fox & Weisberg 2011) and MASS (Venables & Ripley 2002). Graphs were generated in excel and R studio for R (R Core Team 2017) with packages ggplot2 (Wickham 2016) and gridExtra (Auguie 2016).

Results

Elemental carbon and nitrogen contents, C:N ratio, and carbon composition in the ≤2 mm soils and size fractions were similar for both vegetation types (Table 5.1). The most abundant type of SOC in both the tidal marsh and mangrove ≤2 mm soils was O-alkyl which accounted for 31% and 28% respectively of their organic carbon (Table 5.1). However, the proportions of carbonyl ($p < 0.05$), di-O-alkyl and O-alkyl ($p < 0.001$ for both) carbon was significantly higher

in the tidal marsh soils compared to the mangrove soils (Table 5.1). Conversely, mangrove soils had a significantly higher proportion of alkyl carbon compared to the tidal marsh soils ($p < 0.001$; Table 5.1). The remaining types of SOC (i.e., ketone, O-aryl, aryl and N-alkyl carbon) did not significantly differ in the soils under each vegetation type ($p > 0.05$ for all; Table 5.1). Despite the similar abundance of O-alkyl carbon, ≤ 2 mm mangrove soils had a significantly higher ($p < 0.001$) A:OA ratio (0.8 ± 0.1) than the tidal marsh soils (0.6 ± 0.1; Table 5.1).

In both the size fractions, O-alkyl carbon was the most abundant SOC type in both tidal marsh and mangrove soils, accounting for $25 - 35\%$ of the total carbon (Table 5.1). The proportions of ketone, carbonyl, di-O-alkyl and O-alkyl carbon were significantly higher for the coarse fraction of the tidal marsh soils than the coarse fraction of the mangrove soils ($p < 0.001$ for all; Table 5.1). For the fine fractions, the proportions of carbonyl, di-O-alkyl and O-alkyl carbon was also significantly higher in tidal marsh soils compared to mangrove soils ($p < 0.05$; $p < 0.001$; and $p < 0.001$, respectively; Table 5.1). Conversely, the proportions of N-alkyl, and alkyl carbon were significantly higher for both the coarse ($p < 0.001$ for both) and fine fractions ($p < 0.05$ for both) of the mangrove soils (Table 5.1). The proportions of the remaining carbon types (i.e. O-aryl and aryl carbon) did not significantly differ ($p > 0.05$) between vegetation type for both fractions (Table 5.1). The A:OA ratio of the coarse (0.6 ± 0.2) and fine (1.0 ± 0.1) mangrove size fractions was also significantly higher ($p < 0.001$ and $p < 0.01$, respectively) than for the corresponding coarse (0.4 ± 0.1) and fine (0.8 ± 0.2) tidal marsh fractions (Table 5.1).

Solid-state 13C NMR spectra of the biomolecule's lignin, cellulose, chitin and gliadin and algae are shown in Figure 5.1, while the average 13C NMR spectra of the ≤ 2 mm mangrove and tidal marsh soils and their respective size fractions are displayed in Figure 5.2. Coinciding with the

integrated regions, the average spectra for all the samples had major signals at around 172 ppm, 150 ppm, 130 ppm, 105 ppm, 72 ppm, 55 ppm and 30 ppm (Figure 5.3a – f). The PCA of the NMR spectra of the ≤2 mm soils converged for an optimum number of six principle components (PCs). The PCA model accounted for 97% of the variation in spectral intensity, with 85% explained by the first three PCs (Figure 5.3 a and b). The first PC described 46% of the variation within the sample population while PC2 and PC3 explained a further 27% and 12%, respectively. Scores plots showed segregation for a majority of the sample population and clustering based on vegetation type in PC1 revealing differences in their chemistry (Figure 5.3a). The PC1 loading spectra features an inverted peak at 33 ppm attributed to alkyl carbon (Figure 5.1c). Examination of the scores plot and loading spectrum for PC1 together show a greater contribution of alkyl peak for mangrove than tidal marsh soils. In contrast, similarity in the spectral chemistry of the sample population is displayed by the overlap across the PC2 axis (Figure 5.3a). Attributes of the spectral data contributing to the spread of the population in PC2 were associated with resonances at 171 ppm, 105 ppm, 72 ppm and again 33 ppm representative of proteins and polysaccharides (Figure 5.3d). In contrast to PC1, the PC2 scores indicated that variation in the NMR spectral signals associated with the various biomolecules occurred across the entire sample population and were not associated with vegetation type (Figure 5.1 and 5.3d). The PC3 loadings spectrum (Figure 5.3e) contained signals at 152 ppm, 130 ppm, 117 ppm, 105 ppm 72 pm, 56 pm and 33 ppm consistent with lignin (Figure 5.1a). The similar range of PC3 scores for soils from both vegetation types (Figure 5.3b) suggested that changes in lignin were not reflective of vegetation type.

The PCA of the NMR spectra of the coarse fractions converged after six PCs. The PCA model accounted for 95% of variation in the spectral intensity, with 84% explained by the first two PCs. Examination of the scores plot shows 68% of the divergence between samples in PC1

was driven by differences in the chemical composition between the sample population (Figure 5.4a). The loading spectrum of PC1 (Figure 5.4b) revealed samples with positive scores, the majority being tidal marsh, had greater spectral resonance at 105 ppm and 73 ppm attributed to di-O-alkyl and O-alkyl carbon (Figure 5.4b). The inverted peak at 33 ppm showed that soils with negative PC1 scores contained a greater proportion of alkyl carbon and tended to be associated with the mangrove vegetation (Figure 5.4b). A further 16% of the spectral variation was described by PC2 (Figure 5.4a). The PC2 loading spectrum contains features representative of the presence of proteins and cellulose within the sample population with little indication of a separation on the basis of vegetation type (Figure 5.1b, c and d and 5.3c).

The PCA of the fine fraction NMR spectra converged after six PCs. The model accounted for 95% of variation in the spectral intensity, with 80% explained by the first two PCs. The scores plot showed that PC1 and PC2 described 56% and 24% of the spectral variability (Figure 5.5a). The overlap of the sample population across the PC1 and PC2 axes highlighted the similarity of the spectral chemistry within the sample population (Figure 5.5a). The main attributes of the spectra contributing to the spread of the population across PC1 were positive resonances at 76 ppm and 72 ppm (indicative of carbohydrates) and negative resonances at 172 ppm, 56 ppm and 33 ppm as well as 106 ppm and 72 ppm (indicative of proteins and carbohydrates, respectively). The positive resonances were representative of O-alkyl and carbonyl carbon while the negative resonances suggest the presence of alkyl and N-alkyl carbon within the sample population (Figure 5.5b). The loading spectrum of PC2 features the spectral signals of alkyl carbon, carbohydrates, cellulose and proteins (Figure 5.1b, c and d and 5.5c).

Discussion

Variations in chemical composition

Understanding the carbon composition of SOC stocks is important for improving our understanding of the long-term stability of blue carbon stocks and their vulnerability to loss under changing environmental conditions. Integration of the ^{13}C NMR spectra showed the highest relative proportion of carbon was allocated to the O-alkyl region for both soil types and their size fractions. However, mangrove soils and their size fractions also contained a significant proportion of alkyl carbon. O-alkyl carbon is present in many biomolecules but is particularly prevalent in carbohydrates and is generally found in greater amounts in less decomposed organic material (Preston et al. 1989). Alkyl carbon is also present in many biomolecules but is particularly prevalent in lipids, which contain long chain polyethylene structures; alkyl carbon generally represents a greater proportion of total carbon in soils where SOC is more decomposed (Baldock et al. 1997; Baldock & Preston 1995; Kögel-Knabner 1997). Baldock et al. (1997) described a consistent correlation between decreasing O-alkyl carbon with increasing alkyl carbon during decomposition of most types of plant biomass other than wood. Such findings gave rise to use of the A:OA ratio, derived from the ^{13}C NMR data, in terrestrial soils as an indicator for extent of SOM decomposition (Baldock et al. 1997).

The A:OA ratios of the ≤2 mm soils, suggest SOC in mangrove environments was more decomposed than the tidal marsh SOC. However, this was not supported by their C:N ratios as they were much closer and suggested both soil types to be at a similar extent of decomposition. The A:OA ratio increased with decreasing particle size in both environments, as did C:N ratios, both of which are consistent with an increased degree of decomposition for the smaller fraction. Similar findings were reported by Baldock et al. (1992) where C:N ratios of the coarse fractions were greater than the fine fractions, while correspondence between decreasing C:N ratios and

increasing A:OA ratios associated with greater extent of decomposition in fine fractions was also observed in agricultural soils by Leifeld and Kögel-Knabner (2005).

In further support of lower levels of microbial processing, the coarse fraction contained a larger proportion of O-alkyl carbon than the corresponding whole soil, irrespective of vegetation type. Lower levels of microbial processing in coarse fractions has often been identified in studies that apply either particle size or density fractionation schemes (Baldock et al. 1997; Baldock et al. 1992; Baldock et al. 2013b; Golchin et al. 1994). Thus, the high carbon contents, higher C:N ratios, lower A:OA ratios and abundance of O-alkyl carbon all support the notion that coarse fraction SOC is less decomposed. These findings suggest a majority of the coarse fraction carbon appears to have originated from the vascular plant community (Bouillon et al. 2003; Kristensen et al. 2008; Middelburg et al. 1997). For example, Bouillon et al. (2003) and Middelburg et al. (1997) showed when high carbon stocks coincided with high C:N ratios, the isotopic signature of the carbon ($\delta^{13}C$) values were similar to the $\delta^{13}C$ of the *in situ* vegetation. Carbohydrates and other polysaccharides that resonate in the O-alkyl region are, in general, more labile carbon forms that are rapidly depleted from soils (Arndt et al. 2013; Friesen et al. 2018). However, the anaerobic conditions and slower turnover rates of coastal sediments may inhibit the microbial degradation of labile carbon forms and encourage its accumulation (Barreto et al. 2018; Friesen et al. 2018).

Benefits of applying chemometric analyses

The assessment of ^{13}C NMR spectra through integration of the chemical regions allows for the extent of SOC decomposition to be assessed by parameters such as the A:OA ratio (Baldock et al. 1997; Nordén & Albano 1989). However, unlike multivariate approaches, such as PCA, quantification of SOC composition through integration is constrained by the chemical shift

regions that are defined for integration. The advantage of a multivariate approach is that the information pertaining to the variations in spectral features across the sample population can be directly observed (Nordén & Albano 1989; Ringér 2008; Wold et al. 1987). The sub-spectrum generated, presented as loading spectrum in this study, of each PC produced are consistent with the composition of known biomolecules. Such information cannot be interpreted from use of the integrated regions.

Combined interpretation of the ≤2 mm soils and coarse fractions PCAs and their loading spectra identified that the largest variation in the samples was associated with vegetation type. Consistent with the integral region data for both ≤2 mm and coarse fractions, mangrove associated samples in PC1 diverged from tidal marsh samples on the basis of alkyl carbon. In agreement with the integrated regions, PC2 also identified samples that had high O-alkyl carbon irrespective of vegetation type and its loading spectrum had attributes consistent with cellulose. These results are consistent with previous ^{13}C NMR assessments of coastal soil where a significant proportion of the carbon in surface soils was allocated to carbohydrates (Kelleway et al. 2017). For the fine fractions, the PCA was again consistent with the integrated regions and represented their homogeneous nature and greater extent of decomposition. These findings are attributed to the alkyl and carbonyl peaks produced by the loading spectra indicative of fatty acids, waxes and resin and carboxylic, amide or esters, respectively (Baldock & Preston 1995). The preservation of cellulose is highlighted by the loading spectrum of PC2 for the fine fractions is the preservation of cellulose and is consistent with the accumulation of labile carbon. In contrast for the ≤2 mm soils, PC3 and its loading spectrum highlighted the presence of lignin. These biomolecules contain molecular components that resonate within several of the differentiated integral regions (Baldock & Preston 1995). The observation of lignin and cellulose preservation in coastal soils could not have been achieved without the

application of PCA. Combining the conventional approach of integrating the spectra and chemometrics allowed for comprehensive assessment of the chemical structure of SOC in temperate coastal soils.

Quantifying extent of decomposition and potential vulnerability to change

The C:N ratio often used as an indicator for extent of decomposition was not as sensitive to determining decomposition as the A:OA ratio was, in particular for the ≤2 mm samples. Although determining extent of decomposition using the A:OA ratio in the marine environment requires further investigation, our preliminary findings indicate it to be appropriate for temperate coastal wetlands. This is due in part to the terrestrial nature and dominance of organic matter inputs derived from the vascular plant community. More information on the composition of the decomposer community and chemical nature of the organic inputs across the two vegetation types would however further validate its use. In addition, physical separation of the temperate coastal wetland soils provided more detail than the ≤2 mm soils on the chemical structure of the SOC. Fractions revealed (1) carbohydrates were predominant in the coarse fractions; (2) mangrove associated coarse fractions were more decomposed than tidal marsh coarse fractions; while (3) fine fractions were homogenous and more decomposed than the coarse fractions. Therefore, allocation of carbon content to different carbon pools within the soil matrix through size fractionation can improve estimates of the long-term stability of SOC in temperate coastal wetlands.

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Table 5.1 Carbon and nitrogen stocks (Mg/ha), C:N ratio, and carbon composition of <2 mm soil, coarse (>50 μm) and fine (<50 μm) sized fractions across mangrove and tidal marsh soils. Data are averages of 54 samples, ± standard deviation. *denotes significant higher proportion across vegetation type.

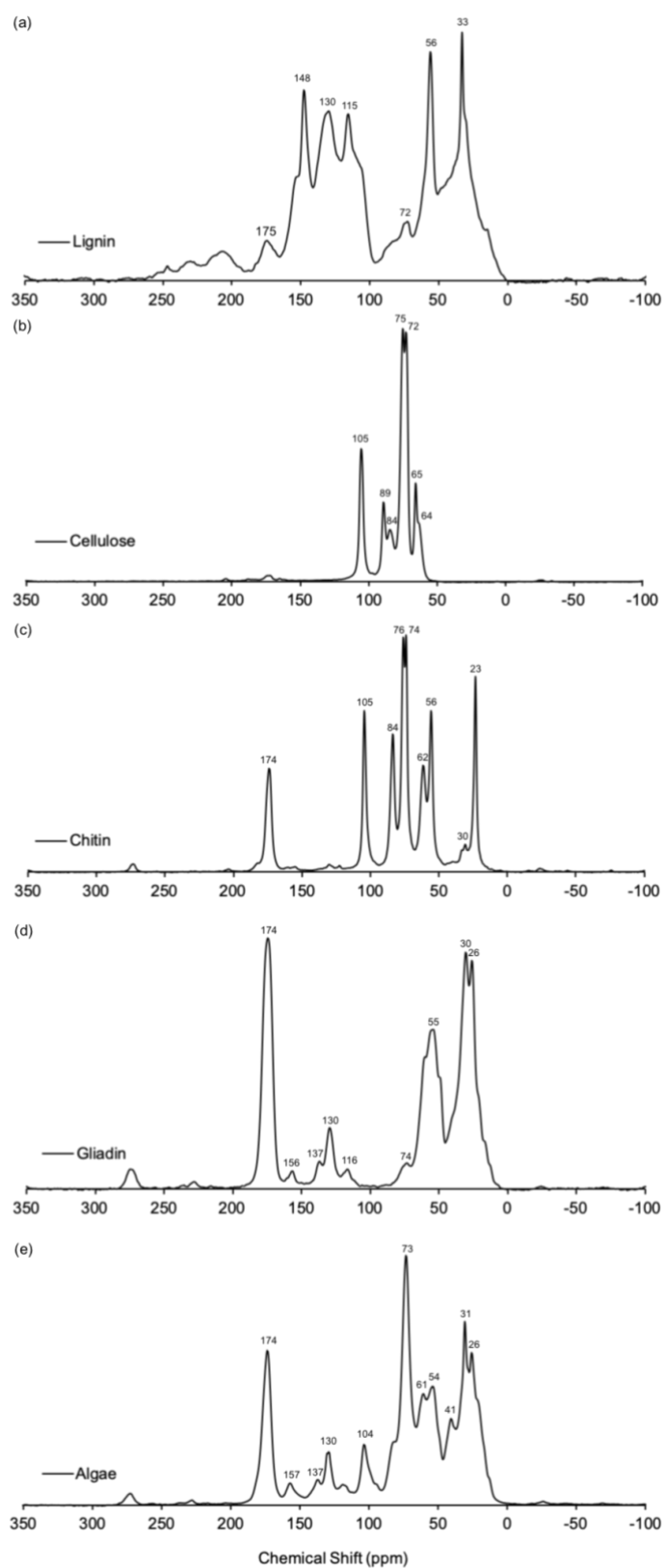|	Average (±*SD*)					
	Mangrove			Tidal Marsh		
Variable	<2mm	Coarse	Fine	<2mm	Coarse	Fine
C (Mg/ha)	18.0 (7.3)	34 (27.6)	18 (8.9)	17 (9.2)	34 (31.2)	17 (7.1)
N (Mg/ha)	1.8 (0.5)	1.9 (1.5)	1.9 (0.8)	1.8 (0.7)	2.1 (1.8)	1.8 (0.6)
C:N	10 (2.4)	19 (4.8)	9 (1.0)	9 (2.0)	17 (4.2)	9 (1.0)
Proportional (%) carbon composition (as determined by NMR)						
Ketone	1.4 (0.4)	1.2 (0.3)	1.7 (0.5)	1.40 (0.5)	1.6 (0.5)*	1.8 (0.6)
Carbonyl	11.1 (1.7)	8.0 (1.4)	13.6 (1.1)	11.8 (1.3)*	9.8 (2.2)*	14.2 (1.3)*
O-aryl	5.7 (0.7)	6.5 (0.8)	5.5 (0.8)	5.4 (0.9)	6.7 (1.2)	5.5 (0.9)
Aryl	15.1 (1.6)	17.3 (1.4)	14.3 (1.9)	14.8 (2.0)	18.0 (2.2)	14.2 (2.1)
di-O-alkyl	6.4 (0.4)	7.3 (0.6)	5.7 (0.4)	7.0 (0.5)*	8.3 (0.8)*	6.1 (0.6)*
O-alkyl	28.6 (1.7)	31.8 (3.1)	25.4 (2.1)	31.1 (2.0)*	34.8 (3.4)*	27.2 (2.6)*
N-alkyl	8.8 (0.7)	7.9 (0.5)*	9.1 (0.8)*	8.6 (1.0)	7.3 (0.8)	8.5 (1.1)
Alkyl	22.9 (2.3)*	19.9 (3.5)*	24.6 (2.5)*	19.8 (3.0)	13.6 (3.9)	22.5 (3.1)
A:OA	0.8 (0.1)*	0.6 (0.2)*	1.0 (0.1)*	0.6 (0.1)	0.4 (0.1)	0.8 (0.2)

Figure 5.1 Solid state 13C NMR spectra of common biomolecules including (a) lignin; (b) cellulose; (c) chitin; (e) gliadin; and (f) algae.
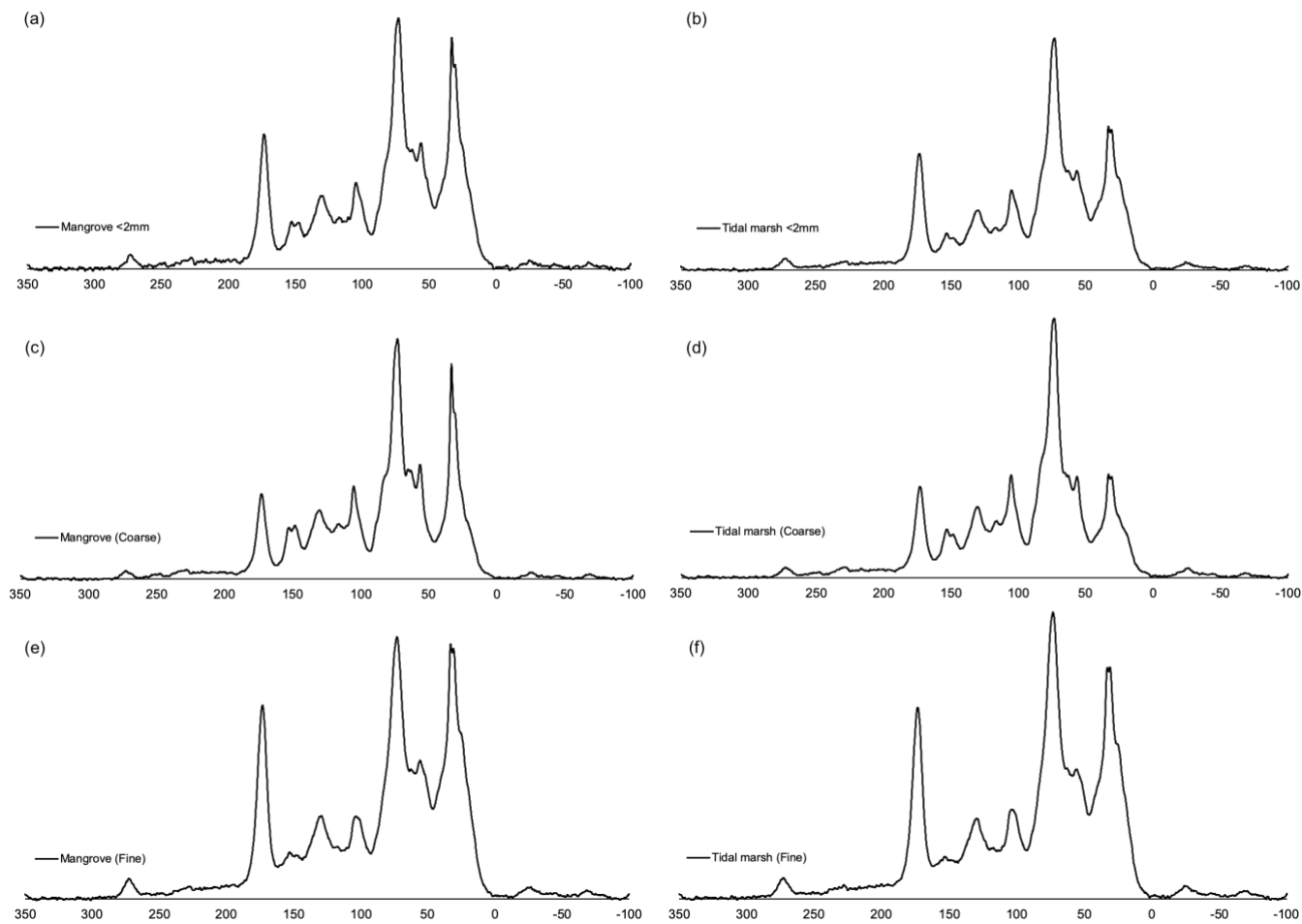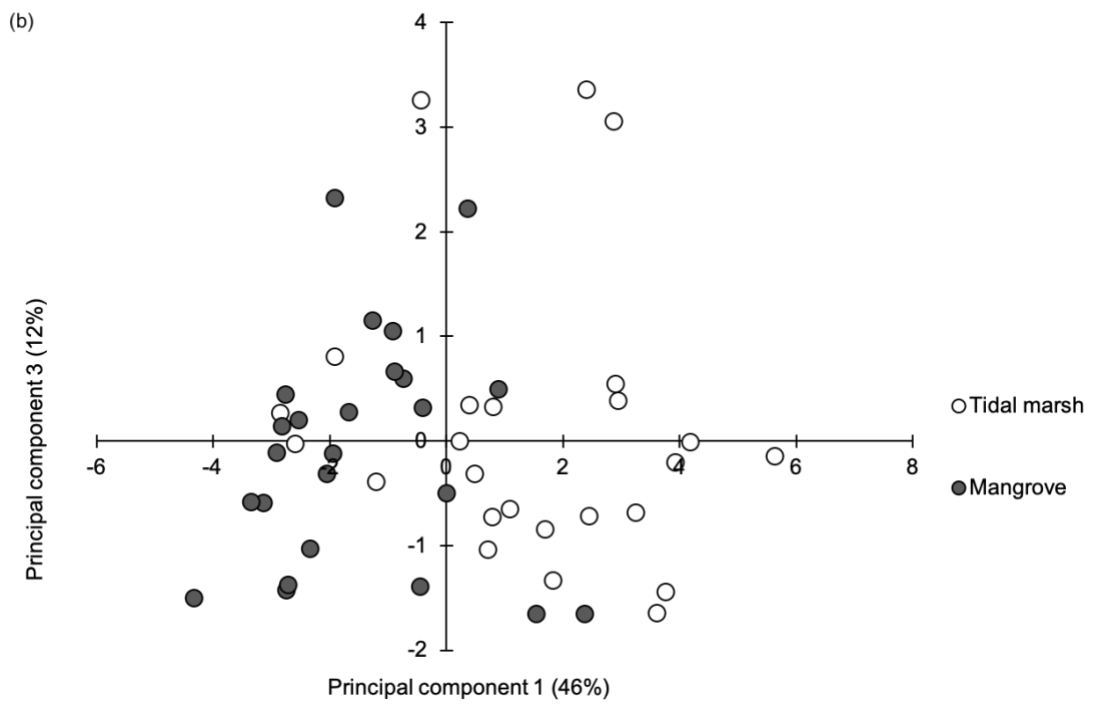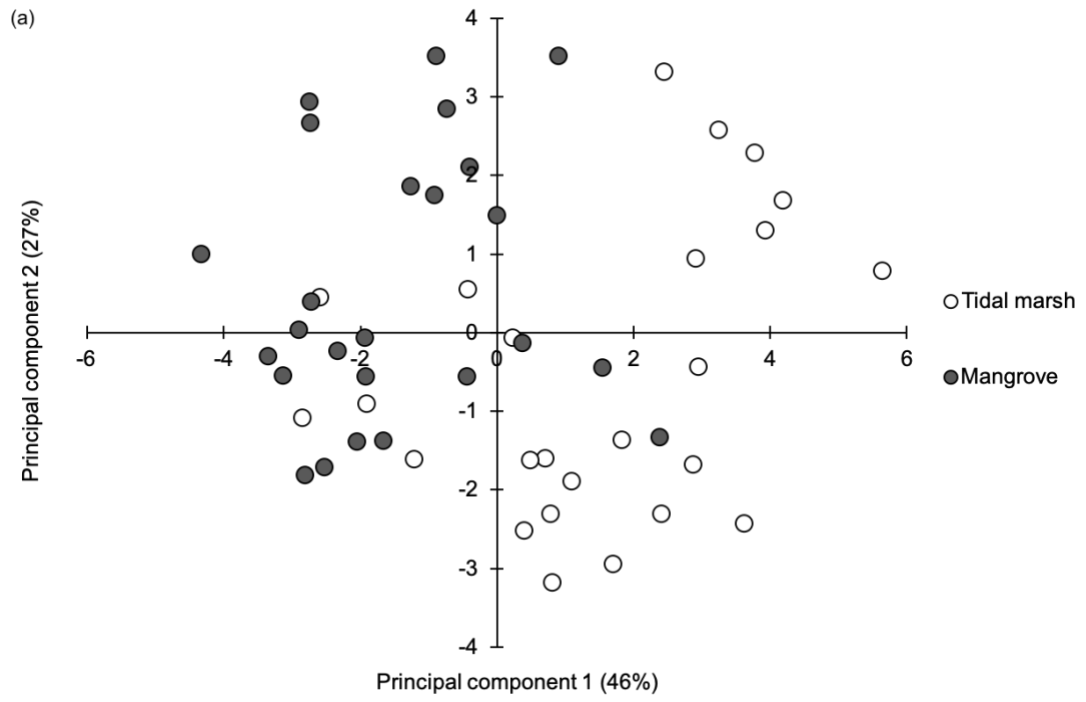
Figure 5.2 Average solid state 13C NMR spectrum for <2 mm (a) mangrove and (b) tidal marsh soils; coarse (c) mangrove and (d) tidal marsh fractions; and fine (e) mangrove and (f) tidal marsh fractions.
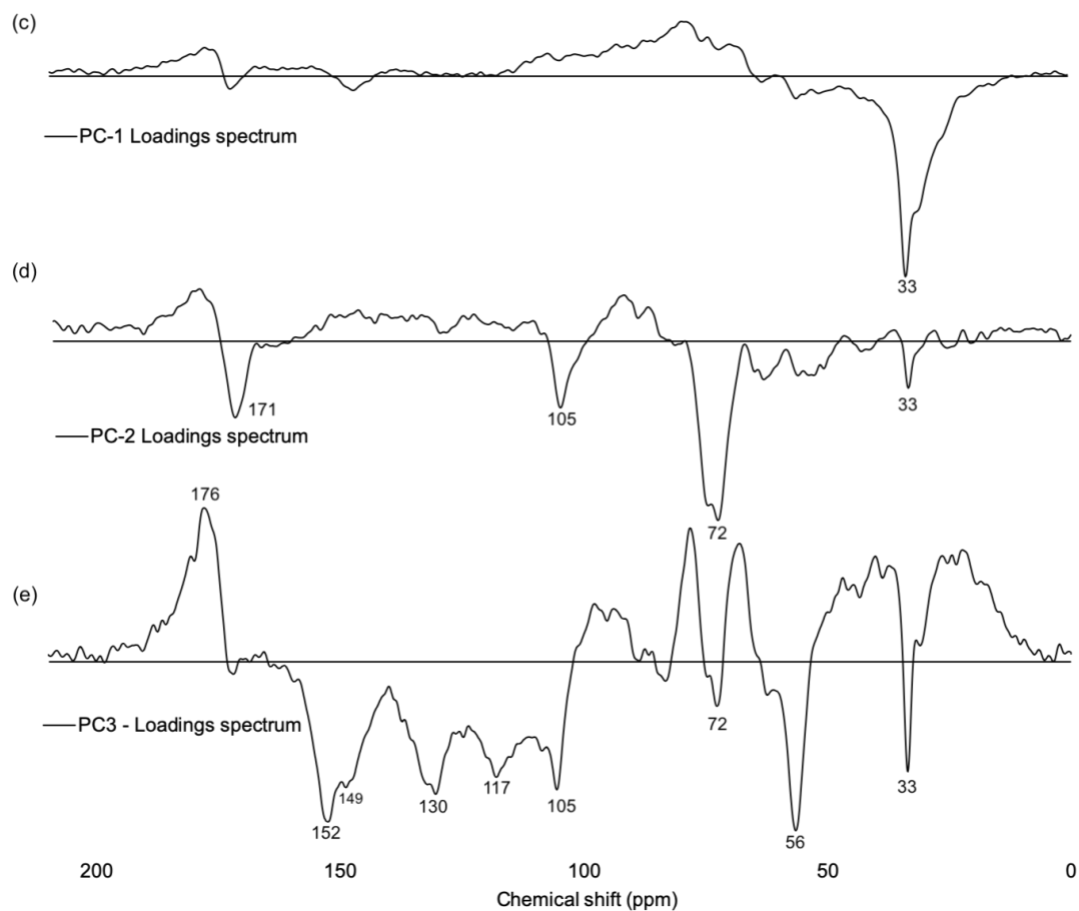
(a)

Principal component 2 (27%)

Principal component 1 (46%)

○ Tidal marsh
● Mangrove

(b)

Principal component 3 (12%)

Principal component 1 (46%)

○ Tidal marsh
● Mangrove

Figure 5.3 Principle component analysis score plots for (a) PC1 versus PC2; and (b) PC1 versus PC3, grouped by vegetation types (tidal marsh = white; mangrove = grey) and the corresponding loading spectra for (c) PC1; (d) PC2; and (e) PC3 derived from the NMR spectra acquired for the 0 – 10 cm layer of 50 (25 mangrove and 25 tidal marsh) soils.

Figure 5.4 Principle component analysis score plot for (a) PC1 versus PC2 grouped by vegetation type (tidal marshes = white, n = 27; and mangroves = grey; n = 27) and the corresponding loading spectra for (b) PC1 and (c) PC2 derived from the NMR spectra acquired for the coarse (>53 μm; n = 54) particle size fractionated mangrove and tidal marsh soils.
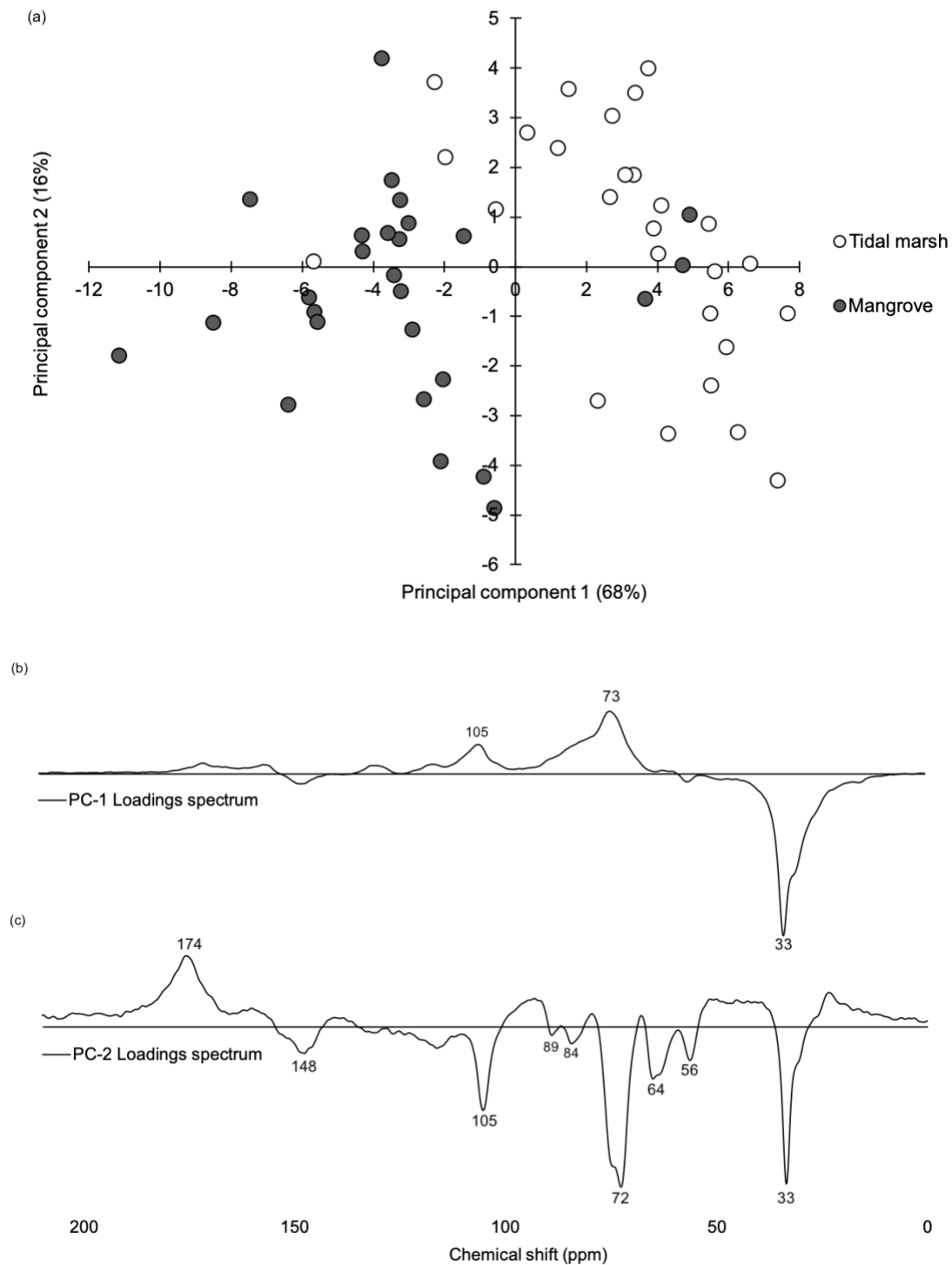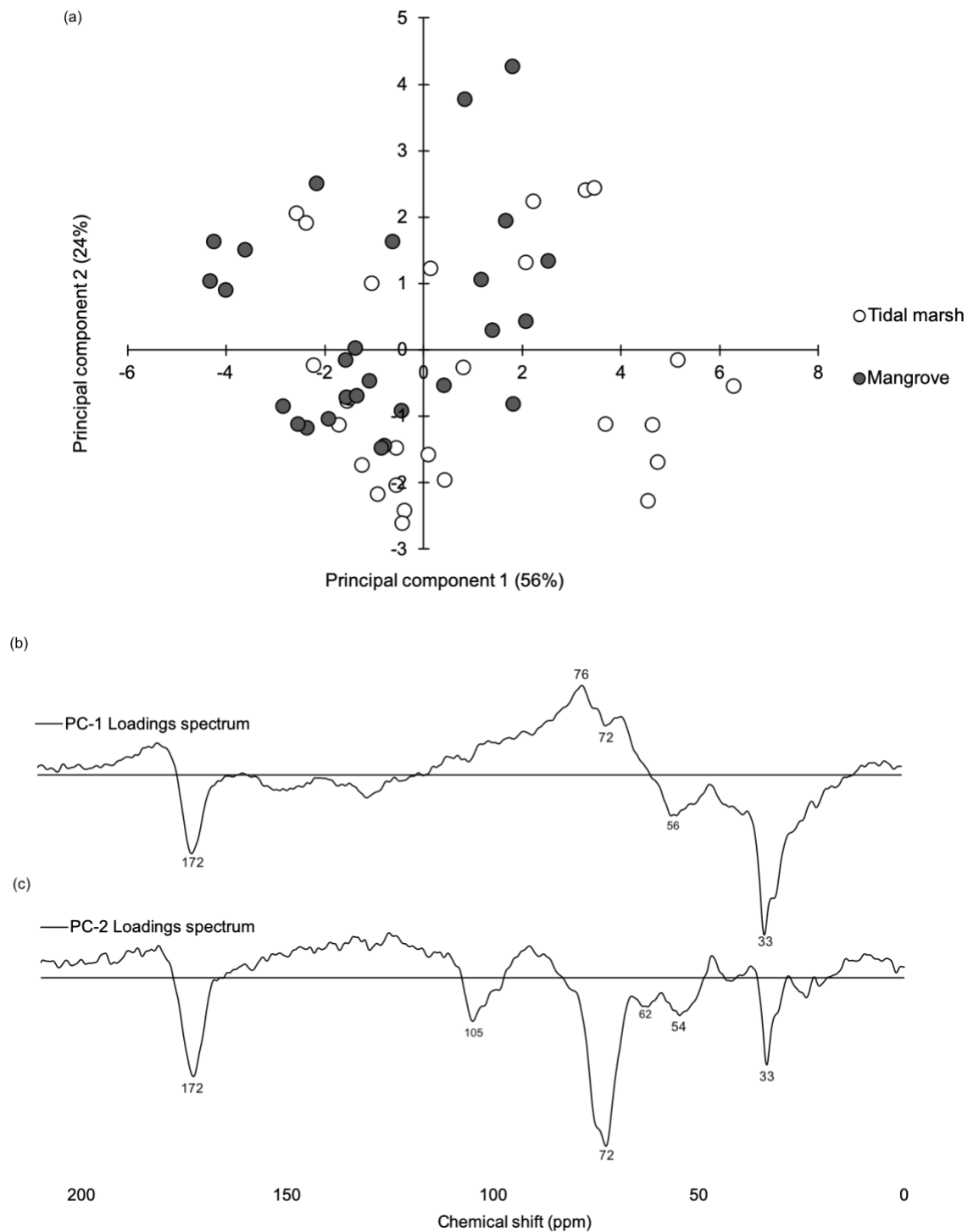
Figure 5.5 Principle component analysis score plot for (a) PC1 versus PC2 grouped by vegetation type (tidal marshes = white, n = 27; and mangroves = grey; n = 27) and the corresponding loading spectra for (b) PC1 and (c) PC2 derived from the NMR spectra acquired for the fine (<53 μm; n = 54) particle size fractionated mangrove and tidal marsh soils.

Chapter Six

General discussion, conclusion and future directions.

The overarching aim of the work presented in this thesis was to advance understanding of soil organic carbon (SOC), specifically its chemistry, in temperate blue carbon environments. Chapter one gives a broad overview and brief introduction to blue carbon and concludes with the objectives and hypotheses of the research (Figure 6.1). Mangroves and tidal marshes are the focus of this research as they are the two primary blue carbon environments of carbon sequestration. In addition, carbon sequestration rates in mangrove and tidal marsh environments are comparable to the carbon sequestration rates of the most productive temperate forests, i.e. tropical, boreal and peat, despite covering significantly less land (McLeod et al. 2011; Siikamäki et al. 2013).
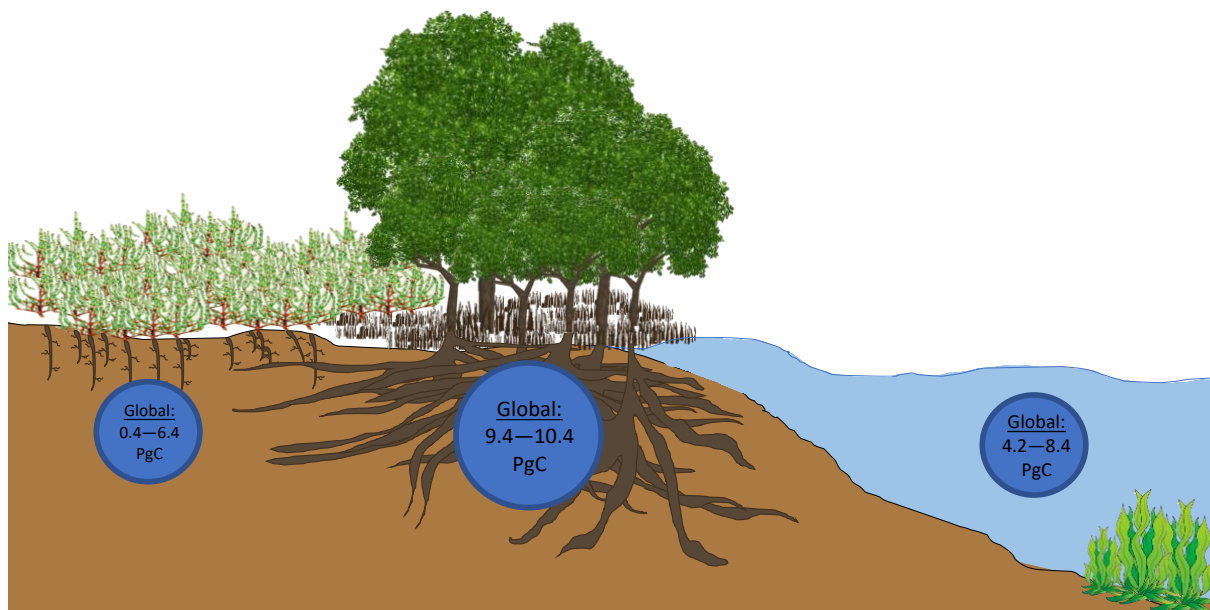

Figure 6.1 Information surrounding carbon stocks in vegetated coastal wetlands *Chapter One*.

The first research chapter, chapter two, quantifies and compares surface soil (top 10 cm) carbon and nitrogen stocks, and its spatial variability, in mangrove and tidal marsh soils of nine temperate blue carbon sites across South Australia. It was hypothesised that mangroves would have higher soil carbon and nitrogen stocks driven by higher primary productivity rates. In addition, the structural differences (i.e. the mangroves extensive root systems and dense canopies) combined with the frequent tidal inundation at lower elevations where mangroves occur would result in higher surface soil carbon stocks than the tidal marshes. In contrast, the variability of surface soil carbon stocks would be greatest in tidal marshes due to less frequent tidal inundation and more frequent oxygen exposure. The second research chapter, chapter three, assessed the application of IR/PLSR algorithms to the prediction of total carbon (TC), organic carbon (OC), inorganic carbon (IC) and total nitrogen (TN) concentrations in a diverse set of blue carbon soils. The objective of the chapter was to examine how successful the IR/PLSR approach would be in predicting the concentrations of the various carbon forms and TN in blue carbon soils. In chapter four, the allocation of OC to particulate, humic and recalcitrant organic carbon (POC, HOC and ROC, respectively) particle size fractions in temperate vegetated coastal soils was quantified. It was expected that the POC fraction associated organic matter, that has a particle size of >50 μm, would be representative of less decomposed material and therefore a less stable long-term carbon store in the environment. Then the same predictive IR/PLSR algorithm approach used in chapter three was applied to the prediction of the allocation of OC to each of the three particle size fractions. The final research chapter, chapter five, characterised the chemical composition of the SOC in temperate coastal wetland soils, and their size fractions. It was hypothesised that accumulation of organic matter with slow turnover rates would result in the prevalence of O-alkyl carbon in coastal soils and the woody structure of mangrove tissues would enrich their soils with lignin and proteins.

Taken together, this work provided a comprehensive assessment of SOC in temperate blue carbon ecosystems.

In this study, soil carbon and nitrogen stocks were found to be driven by the geomorphic setting and inherent environmental conditions of each site (*Chapter Two*). These findings are in agreement with previous studies from other regions (Adame et al. 2015; Chmura et al. 2003; Ewers Lewis et al. 2018; Hayes et al. 2017; Howe et al. 2009; Livesley & Andrusiak 2012) that also concluded carbon stocks to be driven by differences in suspended sediment supply, hydrology; distance of sampling location from the water's edge; and in some cases, vegetation type. Figure 6.2 highlights the contribution of chapter two to the blue carbon field. The majority of the SOC in the samples collected was found in the humic fraction, which suggests that the carbon in these sediments is likely to remain stored over the long-term (*Chapter Four*). However, the longevity of blue carbon is often reported in the literature as a factor of the slow decomposition rates, caused by the saline and anoxic nature of the soils and large contribution of below ground biomass (Duarte et al. 2013; Kelleway et al. 2017b). This work highlights it is also a factor of its allocation to the humic-organic carbon (HOC) carbon pool, which has slower decomposition rates driven by its chemical composition. These finding are novel to the blue carbon literature. The contribution of chapter four to the blue carbon field are highlighted in Figure 6.3. Mangrove associated SOC was also found to have a chemical composition consistent with greater decomposition compared to that of SOC in the tidal marsh soils (*Chapter Five*). This does not support the hypothesis that the SOC in mangrove soils would be less decomposed than tidal marshes due to the prevailing saturated soil conditions. Decomposition was inferred from the A:OA ratio of the soils, rather than the C:N ratio which is often used to make such inferences (*Chapter Five*). This is an important advance in knowledge as this approach is more reliable (Baldock et al. 1997). Analysis of soil fractions in

this study also enabled more detailed information on the chemical composition of the soils to be collected and suggest that preservation of labile carbon (i.e. O-alkyl) was occurring in fine fractions (*Chapter Five*). Moreover, a distinct lignin signature was also found to be a key feature of the fine fractions and supported notion that the vascular plant community predominates the carbon inputs of the environment (*Chapter Five*). Figure 6.4 highlights the contribution of chapter five to the blue carbon field.
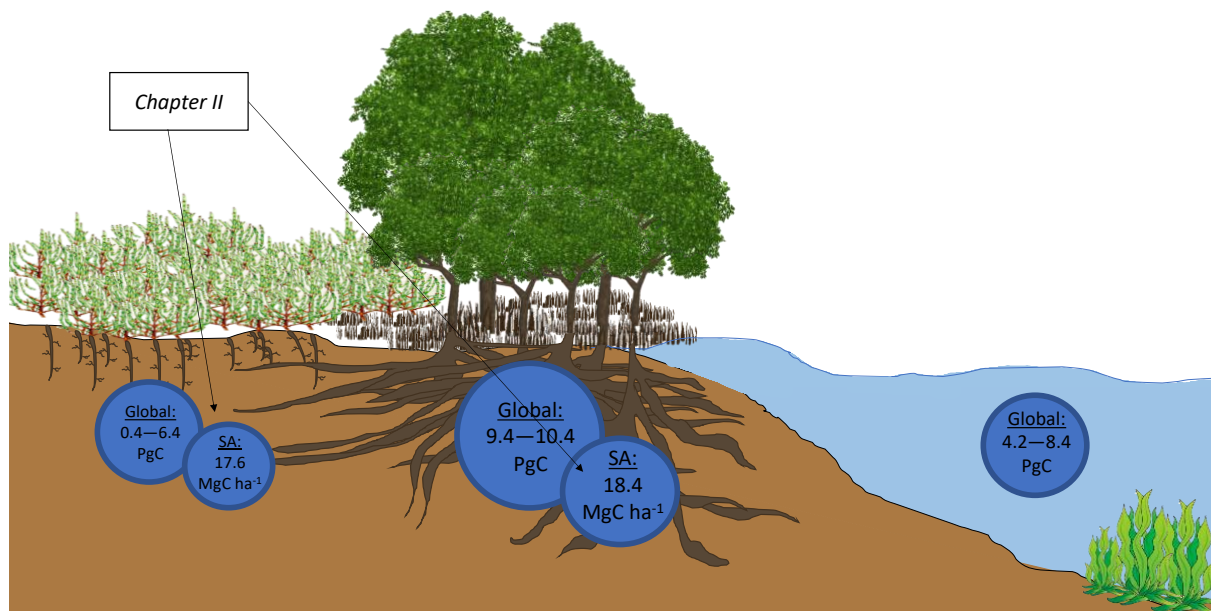

Figure 6.2 Contribution of this thesis, *Chapter Two*, to the scientific literature on mangrove and tidal marsh soil organic carbon stocks in temperate coastal wetland surface soils (0–10 cm), South Australia.

Figure 6.3 Contribution of this thesis, *Chapter Four,* to scientific literature on the allocation of soil organic carbon stocks to the particulate-, humic- and recalcitrant- organic carbon fractions of mangrove and tidal marsh soils in temperate coastal wetland surface soils (0–10cm), South Australia.

Figure 6.4 Contribution of thesis, *Chapter Five*, to scientific literature on the chemical composition of the mangrove and tidal marsh soil organic carbon stored in temperate coastal
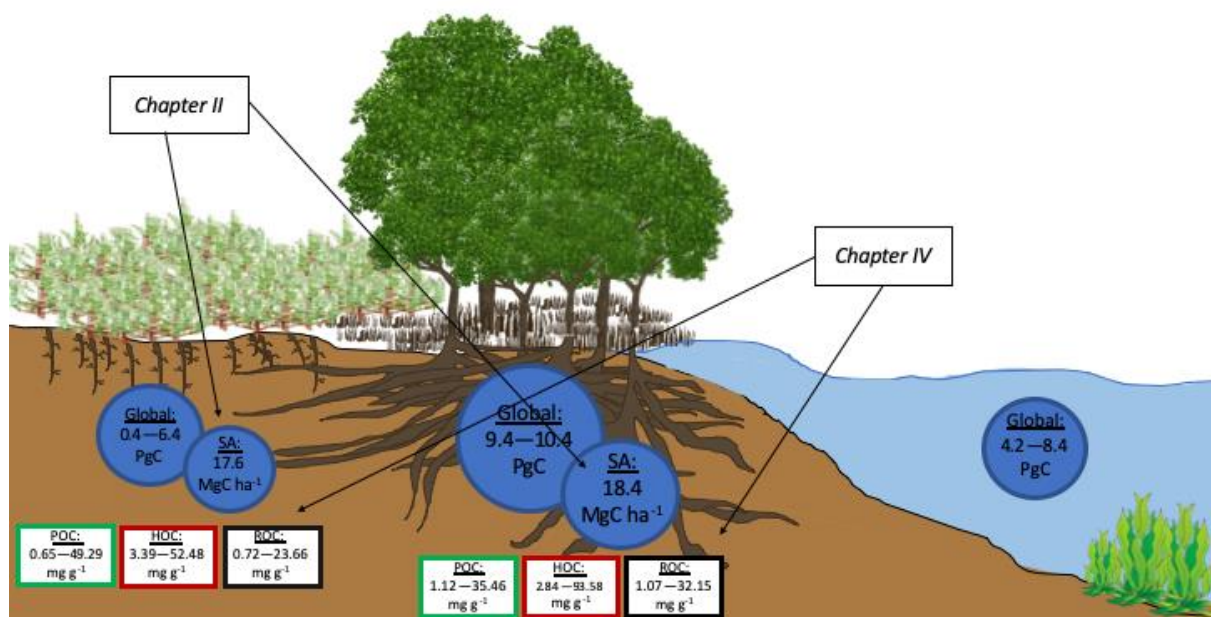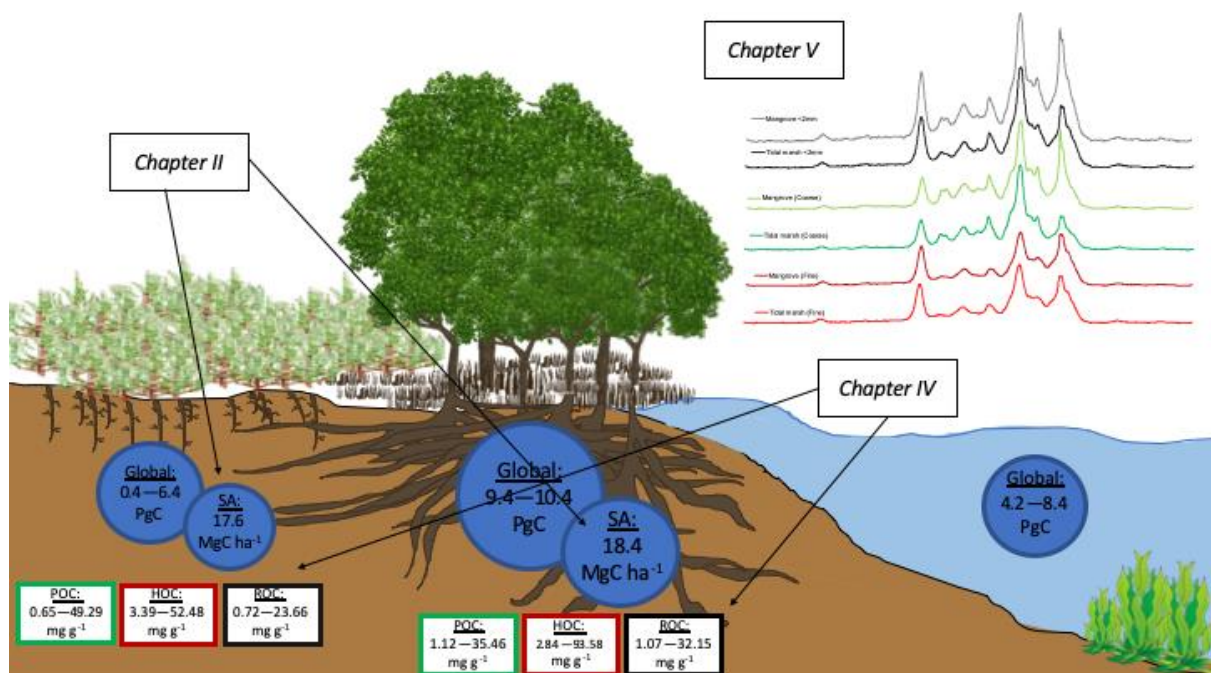
wetland surface soils (0–10cm), South Australia.

The IR/PLSR models have until now only applied to predicting soil properties in terrestrial soils (Baldock et al. 2013; Soriano-Disla et al. 2014; Viscarra Rossel et al. 2006). This researched showed reliable prediction of SOC and nitrogen concentrations was possible in temperate coastal soils and their size fractions (*Chapter Three and Four*). This finding is important, as it provides a less labour intensive and cost-effective approach for making predictions of soil organic carbon stocks in temperate coastal wetlands. In addition, through IR/PLSR I found the allocation of carbon to the sqrtPOC, sqrtHOC and sqrtROC fractions can be determined through quantification of SOC contents as the models were found not to be fraction specific (*Chapter Four*). Taken together, this means that the allocation of OC to the size fractions can be predicted using the SOC contents, rather than needing to complete the (time consuming) fractionation process on future samples.

The results of *Chapter Two*, in particular, highlight the importance of increasing sample collection when quantifying stocks in vegetated coastal wetlands. As the SOC stocks are greatly influenced by the location of the sampling plot and the number of cores taken within a site. My findings surrounding the spatial variability of stocks highlighted the need for far more intensive within-site sampling for studies of blue carbon than is the norm. My work suggests that current estimates (based on very small sample, often one–three core(s) per site) fail to adequately capture the variability in soil carbon. This is perhaps not surprising given the heterogeneous nature of carbon inputs in these systems (detritus deposits and below-ground biomass inputs). Furthermore, my discovery that tidal marshes are just as important contributors to soil carbon storage (on a per unit area basis) as mangroves in temperate coastal wetlands highlight the need to prioritise their conservation, at least within South Australia.

Moreover, the development of IR/PLSR models provides a reliable and cost-effective method for quantifying carbon stocks in vegetated coastal wetlands, inherently difficult to sample, which can be used to improve the future stock estimates of blue carbon environments. In addition, long-term storage estimates in vegetated coastal wetlands can be improved by characterising the chemical composition of their stored carbon which in-turn informs their potential vulnerability to loss, particularly with a changing climate.

The focus of this study was on surface sediments (0–10cm layer); as such, the estimates of carbon stocks presented in this thesis (*Chapter Two*) do not adhere to the blue carbon sampling methodology for quantification of soil carbon stocks in coastal wetlands (0–60 cm) (Howard et al. 2014). This approach was taken so that sampling and analytical effort could be directed at questions of spatial variability and chemical composition of SOC derived from the current *in-situ* vegetation. However, this also limited my ability to assess SOC decomposition through the depth profile (see Future Research below). Taking deeper cores would allow for changes in the characteristics of carbon to be observed through the depth profile and this would improve future estimates of the persistence of blue carbon. It should be noted, however, that carbon content generally decreases with soil depth (>30cm) and increase in soil bulk density (Adame et al. 2013; Ewers Lewis et al. 2018; Hayes et al. 2017; Kauffman & Bhomia 2017; Saintilan et al. 2013; Sanderman et al. 2018; Sanders et al. 2016).

Conclusions

The preceding chapters of this thesis have aimed to advance the knowledge of soil organic carbon, also known as blue carbon, in temperate coastal wetlands. Blue carbon environments are some of the most productive habitats in the world and are globally significant carbon sinks (Chmura et al. 2003; McLeod et al. 2011). Their current carbon stocks are estimated to be 11.25

Pg C with mangroves and tidal marsh soils accounting for more than 80 % of the stored carbon (Duarte et al. 2005; Siikamäki et al. 2013). Current blue carbon research is heavily focused on quantifying stocks and its variability in vegetated coastal margins and has highlighted the influence of climate, ecology, geomorphology, hydrodynamics and anthropogenic activity on carbon stocks, see chapter one (Ewers Lewis et al. 2018; Ewers Lewis et al. 2020; Feher et al. 2017; Hayes et al. 2017; Kelleway et al. 2017a; Owers et al. 2016, 2020; Rogers et al. 2019; Saintilan et al. 2013; Sanders et al. 2016). This thesis has not attempted to add to estimates of carbon stocks from the temperate blue carbon region but rather improve the understanding of the characteristics of the carbon within these unique environments using temperate wetlands as a case study.

Overall, the summation of this work has highlighted that carbon stocks of temperate mangrove and tidal marsh soils are highly variable and are driven by the characteristics of their environments (chapter two), but there is also distinct differences in the molecular structure of their carbon additions (chapter five). The application of IR/PLSR predictive models, however, can overcome the variability of carbon and nitrogen stocks in the blue carbon environment. Their application can provide rapid, cost effective and reliable carbon and nitrogen concentration estimates that can be used to improve future blue carbon stocks (chapter three and four). Temperate wetlands, similar to those that occur in South Australia, are often co-inhabited by mangrove and tidal marshes unlike tropical wetlands and provided a unique opportunity to compare carbon stores across the two vegetation types influenced by the same environmental conditions. The limitations of the presented work prevents quantification of soil carbon stocks for accounting purposes and did not assess the changes in carbon chemistry through the depth profile. But herein lies a framework that can be applied to deeper soil cores across vegetated coastal wetlands that will add a significant contribution, as this work has, to

the blue carbon field.

Future directions

In undertaking this study, a number of potential avenues for future research became apparent, as follows:

- It is recommended that an analytical approach, as outlined herein, be applied to future soil carbon projects in the blue carbon environment, sampling to a depth of at least 1m or as required by prevailing carbon accounting methods.

- The sampling intensity of all future blue carbon studies, within a given site, should also be increased to allow for improved quantification surrounding the spatial variability of blue carbon stocks.

- A spectral database for blue carbon soils should be established to improve the robustness of the IR/PLSR models presented in *Chapter's Three and Four*. The development of region and carbon content specific models is also encouraged, as this will result in better performance and reflect the nature of the sample population being predicted which will result in more precise prediction of carbon stocks.

- Further investigation of the chemical composition of blue carbon soils and their component fractions; and the allochthonous and autochthonous carbon sources, should be undertaken utilizing analytical techniques such as solid state ^{13}C NMR. Similar techniques should also be explored to investigate the composition of other nutrients such as phosphorus and nitrogen should also be explored.

- The biological significance of component fractions and the quantification of carbon pools in blue carbon environments needs to be further explored.

- An assessment of the mechanisms surrounding the stabilisation and destabilisation of SOC, in the blue carbon environment is needed to improve our understanding

of carbon storage in vegetated coastal wetlands.

- Understanding of the microbial population in blue carbon environment such as their functioning under anoxic conditions and their response to increased nutrient loads would greatly improve our understanding of carbon cycling in the blue carbon environment.

- The chemical composition of blue carbon soils could be compared to terrestrial soils to quantify the allochthonous and autochthonous carbon sources in blue carbon soils.

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