BIOCHEMICAL COMPOSITION OF COASTAL WETLAND BLUE CARBON ALONG THE TEXAS COAST

A Thesis

by

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ABSTRACT

This study represents a detailed analysis of the biochemical composition of organic carbon (OC) preserved in coastal wetland systems. Coastal wetlands are sensitive to anthropogenic disturbances with habitats undergoing development into both urban and farm areas. Additionally, these ecosystems are sensitive to climate change and have been documented to shift with accelerated sea-level rise and changing temperature regimes. The estimated magnitude of organic carbon (OC) stocks contained in the first meter of US coastal wetland soils represents ~10% of the entire OC stock in US soils (4 vs. 52 Pg, respectively). Because this stock extends to several meters below the surface for many coastal wetlands, it becomes paramount to understand the fate of OC under ecosystem shifts, varying natural environmental constraints, and changing land utilization. Here we analyzed stable isotopic data ($\delta^{13}C$), total hydrolysable neutral sugars, amino acids, phenols, and cutin acids at two study sites located on the Texas coastline to investigate the nature of OM stored in two wetland ecosystem types along the Texas coastline, *Avicennia germinans* (black mangrove) and *Spartina alterniflora* (cordgrass). Northward expansion of the mangrove ecotone has occurred over the past 100 years with the decreased occurrence of freezing events during winter periods. Due to the recognized efficiency of C burial in these environments we opted to take a closer look at the effects of this shift on sedimentary C dynamics. Results showed sharp declines of neutral sugar yields with depth occurred parallel to increases in lignin degradation ratios indicating substantial decomposition of both the polysaccharide and lignin components of litter

detritus. Neutral sugar compositions allowed reconstruction of past dominant vegetation by utilizing unique hemicellulosic carbohydrate compositions. While overall magnitude of OC in these sediments was almost equal, the chemical composition of buried OC was notably different regardless of supposed lability or recalcitrance of the different biochemical classes. Overall, biomarker data suggest litter chemistry is the primary control of C preservation in these wetland ecosystems.

DEDICATION

This document is dedicated to the family, friends, and advisors who have stuck with me throughout this process; I would not be at this stage in my education and life without your constant support and love.

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CONTRIBUTORS AND FUNDING SOURCES

Contributors

This work was supervised by a thesis committee consisting of Dr. Karl Kaiser of the Marine Sciences Department and Oceanography Department, Dr. Rainer Amon of the Marine Sciences Department and Oceanography Department, and Dr. Peter Santschi of the Marine Sciences Department and Oceanography Department.

All work conducted for this thesis was completed by the student independently.

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NOMENCLATURE

OC	Organic Carbon
OM	Organic Matter
SOM	Sedimentary Organic Matter
SOC	Sedimentary Organic Carbon
DLAA	D and L Amino Acids
THAA	Total Hydrolysable Amino Acids
THNS	Total Hydrolysable Neutral Sugars
NPP	Net Primary Production
SLR	Sea Level Rise

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CHAPTER I

INTRODUCTION

The global carbon cycle facilitates the transportation, transformation, and longevity of organic matter (OM) in the various Earth reservoirs. Each global reservoir represents a unique and individual subsection of the planetary system, possessing its own respective controls and dynamic conditions, which govern the movement of OM. Sediments, and the OM contained within is one of the largest planetary reservoirs with concentrations of OM far exceeding that of the atmospheric or terrestrial plant pools; 3500-4800 Pg C, 829 Pg C, and 420-620 Pg C, respectively (Schlesinger and Andrews, 2000; Lehmann and Kleber, 2015). Due to its considerable magnitude understanding the complexities that govern the flux and storage of OM in terrestrial sediments becomes of great importance. Indeed, much work has been done to further the comprehension of the scientific community in this field, and the beginning portion of this study serves as a synopsis of what is known, unknown, and remains to be contested.

Taking a closer look at subdivisions within the field of sedimentary organic matter (SOM) one classification of ecosystems has garnered considerable interest of late for its recognized efficiency in the long-term storage of carbon (C); coastal wetland habitats and the C contained within its boundaries are referred to as blue carbon. These coastal barriers and their C content are a result of the relationship between highly productive vegetation and a unique set of physicochemical conditions that facilitate the persistence of SOM in these ecosystems (Chmura et al., 2003; Duarte et al., 2004; McLeod et al., 2011;

Hopkinson et al., 2012; Bianchi et al., 2013). Unfortunately, as a result of their coastal position tidal saline wetlands are highly sensitive to anthropogenic land-use and precautions need to be taken to ensure their protection. Additionally, shifting temperature and precipitation regimes in a world with a changing climate is reflected in the distribution and extent of dominant plant vegetation for these ecosystems (Ross et al., 2000; Zhang et al., 2008; Feher et al., 2017; Kohl et al., 2017; Moomaw et al., 2018). This study takes a closer look at SOM dynamics, under a biochemical lens, for two dominant plant species along the Gulf of Mexico that have experienced shifting land-coverage as a result of climatic and anthropogenic forcing; *Avicennia germinans* (black mangrove) and *Spartina alterniflora* (cordgrass). While united by many qualities there are key differences between the individual plant ecotones that have the potential to result in considerable differences in sequestration affecting both C pool size and physicochemical makeup.

1.1 Sedimentary Organic Matter

The OM reservoir contained within the Earth's sediments represents a vast stock of C estimated at approximately 2500 Pg C (Dungait et al., 2012). Findings by the 2007 Intergovernmental Panel on Climate Change stated C stored within sediments was three times the magnitude of the C pool in the atmosphere or living plant biota (IPCC, 2007; Schmidt et al., 2011). This heterogenous mélange can extend deep below ground and exhibits diversity in both chemical composition and longevity with different components persisting in sediments for tens, to tens of thousands of years (Schlesinger and Andrews,

1999; Trumbore and Czimczik, 2008; Dungait et al., 2012). SOM will hereafter be used to describe both OC and OM contained within the sediment matrix. The storage and exchange of SOM represents an ever-shifting balance between primary production, respiration, and stabilization. Additionally, this equilibrium is sensitive to external pressures such as anthropogenic activity and climate change, therefore comprehensive understanding of cause and effect relationships with respect to SOM is of the utmost importance (Deyn et al., 2008). Sediments are both a source and sink of atmospheric CO_2, remaining in relative equilibrium however, environmental conditions and changing land use can result in the development of these environs into either a source or sink of CO_2 highlighting the need for understanding dynamics governing the balance (Marschner et al., 2008). There is the potential for sediments to be strategically utilized for mitigation of anthropogenically driven atmospheric CO_2 increases; developing these reservoirs to maximize drawdown and of this prodigious greenhouse gas.

The formation of SOM results from the interaction of the biotic and atmospheric systems in the form of primary production where carbon dioxide (CO_2) and OM are utilized by plant life to create plant biomass and oxygen (O_2) (Keyn et al., 2008; Trumbore and Czimczik, 2008; Dungait et al., 2012). This newly produced C-rich material in turn acts as a nutrient source for biological, microbial and faunal, organisms as well as fuel for chemical, and physical processes; ultimately plant-derived material makes its way to the

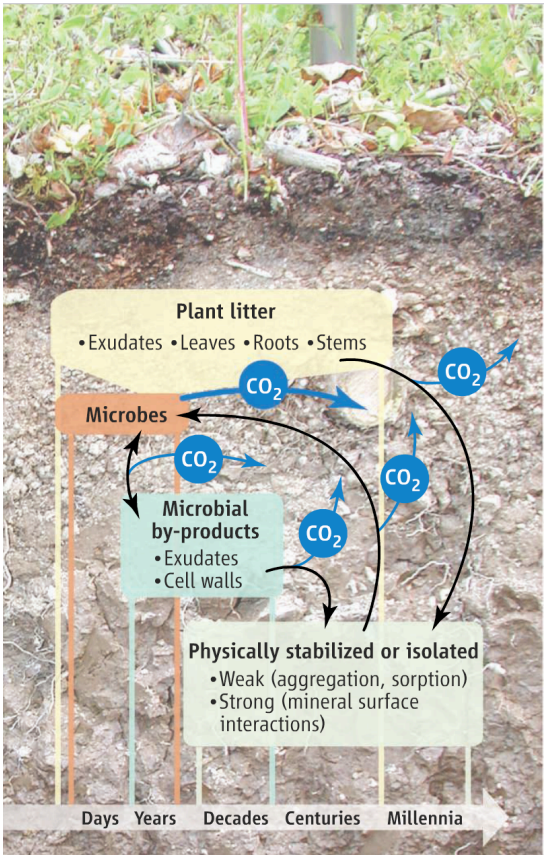

Figure 1. Taken from Trumbore and Czimczik 2008 this figure shows the transformation processes and steps involved in the formation of SOM and respiration of CO_2 as well as the resultant SOM pool age based on transformation type.

sedimentary interface where over time burial, respiration and transformation into SOM occurs. Freshly produced litter material is considered as reactive with respect to OM however, as it's interactions with external influences increase it is widely understood that stabilization occurs (Raich and Schlesinger, 1992; Sollins et al., 1996). Alternatively, litter material that is utilized and respired instead of stored takes the form of CO_2 which is then released back into the atmosphere highlighting the role of SOM as both a source and sink of atmospheric CO_2 (Fig. 1). It should be noted that while the bulk of SOM is dominated by plant-derived material (both fresh and decayed), it also includes microbially- and faunally-produced detritus and exudates as well as nutrients (Marschner et al., 2008; Dungait et al., 2012). The dynamic combination of elements that result in the formation of SOM are also crucially important with respect to sediment fertility which once again highlights the need for understanding.

One crucial question regarding the longevity of SOM remains to this day; while first posed by Hedges et al. (2000) the scientific community strives to understand why, despite its thermodynamic reactivity, SOM persists in sediments for millennia (Table 1).

Table 1. Taken from Dungait et al. (2012) shows the different pools contained within the SOM class according to mean residence times (MRTs).

Litter	Metabolic	DPM	0.1–0.5	10–25	Simple sugars Amino acids Starch
	Structural		2–4	100–200	Polysaccharides
SOM	Active	BIO DPM	1–2	15–30	Living biomass POM Polysaccharides
	Slow	RPM	15–100	10–25	Lignified tissues Waxes Polyphenols
	Passive	HUM IOM	500–5000	7–10	Humic substances Clay: OM complexes Biochar

DPM, decomposable plant material; BIO, microbial biomass; RPM, resistant plant material; HUM, humified organic matter; IOM, inert organic matter; POM, particulate organic matter; OM, organic matter.

Traditional kinetic theory accredits molecular structure with the governance of SOM longevity, more complex biochemicals being "recalcitrant" and therefore less susceptible to respiration (Raich and Schlesinger, 1992; Sollins et al., 1996; Hedges et al., 2000; Schmidt et al., 2011; Dungait et al., 2012). Understanding the complexities behind turnover and storage of OM in sediments which accounts for approximately 2/3 of the terrestrial C pool is of paramount importance in an era of anthropogenically exacerbated climate change (Marschner et al., 2008). Comprehension will lead to the development of environmental strategies which have the potential to maximize the utilization of SOM storage for climate change mitigation.

1.2 Controls of Storage and Respiration of SOM

Investigation into the processes controlling formation SOM, it's long term storage and turnover, highlight recalcitrance not as a molecular quality but an ecosystem one (Fig. 2) (Schmidt et al., 2011; Dungait et al., 2012). Investigation of the chemical nature of SOM shows the dominance of two groups of OM the first of which is characterizable using analytical techniques and the second uncharacterizable, a heterogeneous mixture of compounds which we are currently unable to classify on a molecular level with modern techniques (Hedges et al., 2000; Tremblay and Benner, 2005). Difficulties in pinning down the cause and effect relationships governing SOM turnover and storage arise due to the diversity of sediment composition and environmental factors across the globe. While

there is still considerable debate over the magnitude of the different physicochemical forcing's which cause the formation of these two groups, the following will serve to highlight the different mechanisms involved in OM storage and turnover.

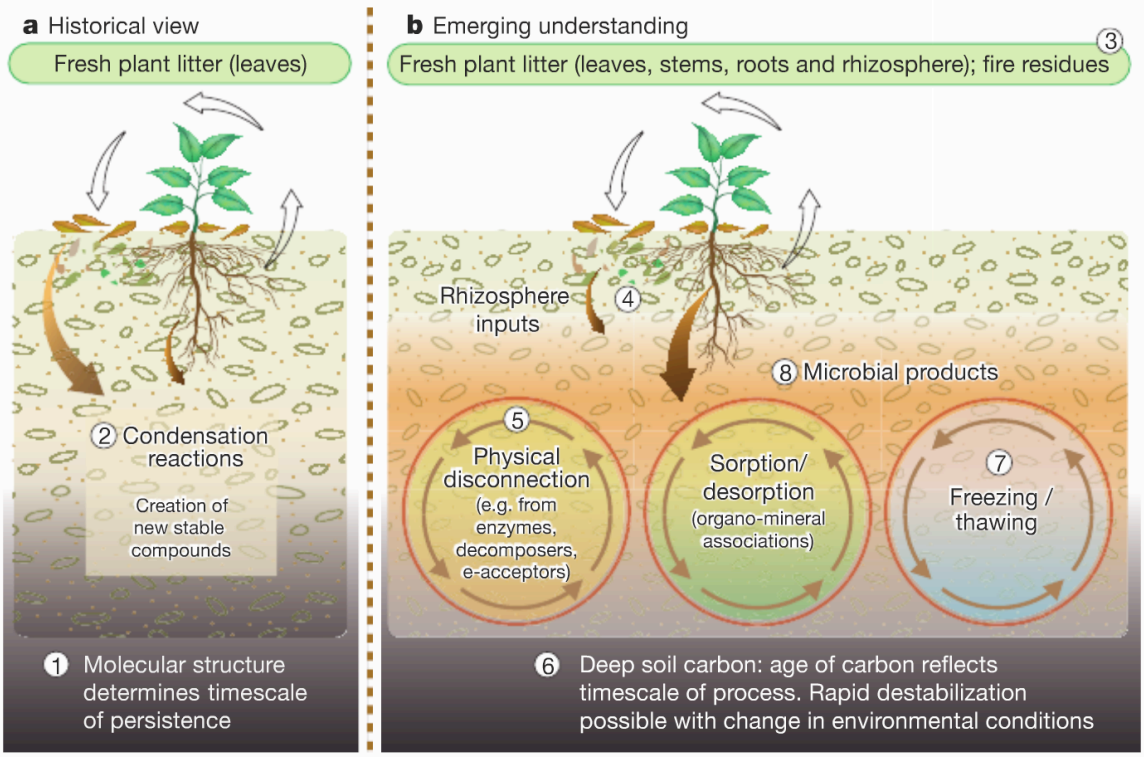

Figure 2. Taken from Schmidt et al. (2011) this figure highlights the change in perception amongst the scientific community for the formation and turnover of OM in sediments. (a) The traditional view holds that plant C input comprises the bulk of SOM in the upper sediment horizon. This material is processed based molecular availability resulting in the formation of more stable compounds. Recent research has finessed the understanding of SOM cycling (b) and has proven that the molecular structure plays a secondary role. Instead cycling results from a combination of environmental and physicochemical effects.

Molecular Structure

The molecular composition of fresh plant litter has long been lauded as a main control for the longevity, or lack thereof, for sediment C. Selective preservation of organic

molecules based on their ease of digestion by both biological and physical diagenetic processes has been erroneously credited as the key to long-lived SOM formation (Lutzow et al., 2006; Schmidt et al., 2011; Lehmannn and Kleber, 2015). This hypothesis follows the understanding that burial and exposure of plant litter to decomposers follows a diagenetic pathway with the utilization of desirable, or labile, compounds first and only thereafter will more recalcitrant materials be exploited, this reasoning follows traditional kinetic theory (Dungait et al., 2012). Along this decomposition continuum, molecular components of OM become highly fragmented and smaller in size compared to the initially large litter-composing macromolecules such as lignin phenols and carbohydrates. According to Lehmann and Kleber (2015) the processing and fragmentation of organic molecules results in an increase of both polar and ionizable groups within the OM matrix which not only increases microbial availability but mineral complexation as well. While enhanced microbial processing would result in further transformation of OM and loss of characterizable material, mineral complexation acts to preserve the chemical identity of the OM.

The molecular composition of plant litter is highly variable depending on plant species, but the essential building blocks consist mainly of polysaccharides (50-60%) and lignin (15-20%), in addition to proteins, polyphenols, chlorophyll, cutins and suberins, as well as lipids and waxes (10-20%). Each of these individual molecules possess inherent qualities (size, polarity, ether bridges, quaternary C-atoms, three-fold substituted N-linkages, phenyl- and heterocyclic- N groups, as well as long chain hydrocarbons) that will either aid or hinder the oxidation process upon burial (Lutzow et al, 2006). Some

biopolymers, tannins for example, also have the effect of enzyme retardation, which act to inhibit enzymatic activity thereby prolonging preservation upon burial (Dungait et al., 2012). Structure of these plant-derived biomolecules plays a key role with recalcitrance increasing with increasing aromaticity; alternatively, the presence of hydrolytic bonds will increase lability. In possession of aromatic structures, lignin, is considered to be far more resistant to decomposition compared to the polysaccharide component of plant litter and the effect of these structural differences can be observed in the initial stages of decomposition (Lutzow et al., 2006). Closer analyses in numerous studies show varied degradability within the group of lignin monomers some of which are rapidly oxidized while other exist in their unadulterated form for extended periods. Incubation studies of turnover time show the vanillyl lignin phenols to decompose slowest, followed by syringyl and then cinnamyl units (Marschner et al., 2008; Bianchi et al., 2013). A study by Marschner et al. (2008) divided lignin into two subgroups, the first, comprising the majority of lignin (95%), of which degraded rapidly upon burial (<1 year residence time); the remaining fraction persisted considerably longer with a mean residence time of ~20 years. Considering the vast majority of lignin was observed to undergo oxidation in a short time span Marschner et al. (2008) concluded there to be no inherent recalcitrance of the molecule itself and its observed stability and longevity in soil to be a result of mineral complexation.

The conundrum presented by selective preservation based on molecular structure presents itself in the persistence of structurally labile compounds which retain their chemical identity in highly oxidized and long-lived sediments. Additionally, the rapid

decomposition of recalcitrant molecules has been observed in numerous research studies. These inconsistencies prompted further investigation of SOM stabilization and shown selective preservation to take a secondary role in preservation.

Sediment Fauna – Macro and micro-biota

Oxidation of OM buried in sediments can follow a number of pathways but is largely governed by the biotic utilization of plant litter at all depths in the sediment profile. This processing creates a continuum of progressively smaller fractions of the original plant matter. Coincidentally the organisms responsible for this process also follow a continuum of progressively smaller mass. In the upper levels of the sediment-atmosphere-plant interface a host of both vertebrate and invertebrate organisms utilize and digest plant litter (Sollins et al., 1996; Middleton and McKee, 2001; Kristensen et al, 2008). There is however an exception to this trend which promotes preservation of OM as larger sediment animals (worms, isopods, beetles, etc.) aggregate OM in the form of fecal pellets which often possess binding agents secreted by the organism. These binding agents act as a physical and chemical barrier, resistant to further decomposition by smaller fauna and also limits extracellular synthesis reactions (Sollins et al., 1996). Biotically driven aggregation also takes place on a smaller, microbially-driven stage via several mechanisms. It should be noted however that while sediment animals do indeed form fecal pellets, in some instances these aggregates can in turn be utilized by other sediment animals thereby negating the preserving effects of the initial processing. A study by Sollins et al. (1996) concluded that aggregation promotes the preservation of OM in sediments, limiting the

accessibility of organic compounds to oxidation. In addition, formation of aggregates often depletes the conglomeration of oxygen due to greatly reduced pore space, this in turn will also act to promote preservation. The full extent of this faunally-driven preservation is difficult to quantify and plays only a small role in the preservation of SOM.

Large sediment fauna play another role in the physical breakdown of plant litter in the form of communution, the breaking down of material into progressively smaller pieces. This acts to increase the available surface area of senescent litter to decomposers however plays only a minor role in SOM storage/turnover dynamics. Additionally, while this process often promotes degradation via increasing microbial availability, it has been shown by Scheu and Wolters (1991) to hinder degradation when litter material was over-fractionated (Sollins et al., 1996).

Sediment macrophytes are also credited as ecosystem engineers, with organisms such as earthworms, and ants physically modifying the sediment structure and transporting OM to different levels in the sediment profile. This is turn can also promote the oxygenation of the sediment column. To summarize, the transportation of OM to less accessible areas can help to stimulate sequestration however, oxidation can also be promoted depending on where the OM is being transported and how the sediment profile is oxygenated (Deyn et al., 2008).

Following the continuum of progressively smaller particle size, sediment organisms also become smaller and smaller. Microbial activity is credited by many to have the largest effect on SOM storage and turnover (Boshker et al., 1999; Schmidt et al., 2011; Dungait et al., 2012; Lehmann and Kleber, 2015). Activity of the microbial biomass in

sediments plays a two-part role in SOM turnover, firstly in the decomposition and breakdown of plant biopolymers, and secondly in the formation of microbial products that in turn become important and characterizable fractions of SOM in the sediment matrix, lasting for variable lengths of time (Schmidt et al., 2011). Upon burial of plant material processing of OM begins with accessibility and sediment characteristics (eg, pH, temperature, enzyme availability) having considerable effect on the availability of OM for microbial digestion by both heterotrophs and autotrophs (Kögel-Knabner, 2001; Deyn et al., 2008; Dungait et al., 2012). At this initial meeting of microbial decomposer with plant material, molecular structure will play a larger role in the order of decomposition; the most readily and easily available biomolecules undergoing rapid utilization followed by less accessible and less accessible biomolecules. As digestion of plant material continues however, microbial organisms will turn their attention to less desirable molecules in order to maintain metabolism. This processing results in the initial partitioning of SOM into purportedly recalcitrant and labile pools, however as time proceeds its effect is diminished (Sollins et al., 1996).

As microbial organisms process decaying OM of plant origin they in turn transform said material into microbial biomass which takes a number of forms e.g. carbohydrates, D-amino acids, lipids, muramic acid (Kögel-Knabner, 2001; Tremblay and Benner, 2005; Darien et al., 2007; Kaiser and Benner, 2008). These secondary compounds have their own chemical identity, and in turn requirements for decomposition that result in either storage or turnover via other processes. Additionally, use of analytical tools in the characterization of SOM is able to track the activity of microbial communities,

biogenic sources and diagenetic alterations of OM via the production of these bacterial biomarkers (Kaiser and Benner, 2008). This has proven to be a useful tool in furthering the understanding of the scientific community with respect to sediment cycling controls and dynamics. Each local environment represents a unique combination of factors that have developed to facilitate a healthy ecosystem. Dominant plant species controls the molecular nature of OM input into the sediment matrix. Micro and macro-fauna are then locally specialized to efficiently digest the material that they are provided in the given environment where they exist. This leads to further specialization and the development of the heterogeneity that is observed when comparing ecosystems of varying plant and physicochemical traits (Tamura and Tharayil, 2014). Deyn et al. (2008) accredits sediment sequestration potential to be determined by intrinsic factors (e.g. minerology, topography, hydrography, texture) in combination with biota and climate. The pairing of plant type with microbial community has also been observed to have a priming effect as plants stimulate sediment micro-organisms via the release of labile root exudates. These "primed" microbes and fungi have the largest effect in nutrient poor sediments as uptake of root exudates by microorganisms facilitate the production of lignin oxidizing enzymes (Deyn et al., 2008; Mueller et al., 2016). Understanding these feedback relationships between plant type and microbial community are tantamount to furthering the comprehension of the scientific community with respect to SOM dynamics.

Physicochemical Environment

Physicochemical environment will have a large effect on the nature of microbial community at each level in the sediment profile. The chemical nature and characteristics of the sediments themselves and the physical environment in which they exist plays a key role in the governance of sequestration and turnover.

Each environmental position will possess its own unique physical characteristics (e.g. temperature, sunlight regime, precipitation, hydrology, pH) that will affect the relationship between flora and fauna both above and below ground. Temperature regimes will stimulate or inhibit net primary production for the given plant species topside and also act to promote/inhibit uptake of plant litter by sediment biota (Valiela et al., 1985; Mcleod et al., 2011; Kirwan et al., 2014). Increases in temperature have been shown by Kirwan et al. (2014) to stimulate microbial communities and in turn degradation and turnover of plant litter material. These results have been echoed in numerous other research studies however it should be noted that there is a threshold for temperature increases having a positive effect on microbial activity; too high a temperature increase will inhibit activity. Additionally, the magnitude of the effect of temperature shifts on sequestration is a topic of contention and more research is necessary to determine its extent (Dungait et al., 2011; Kohl et al., 2017).

Another physical sediment quality that ties environment to sequestration potential is oxygen and nutrient concentrations. It is possible to breakdown bacteria into two main groups based on their necessity for molecular oxygen; anaerobic and aerobic (Benner et al., 1984; Philben et al., 2014). In general terms the turnover of OM in oxygenated

sediments occurs an order of magnitude faster than in anoxic ones, highlighting the importance of this physical quality on SOM sequestration. In sediments the dominant plant species will have considerable control over sediment oxygenation as it is linked to NPP, sediment trapping by roots, and root characteristics. In the absence of oxygen there is still decomposition and remineralization occurring via alternative terminal electron acceptors utilized by anaerobes (Kristensen et al., 1995; Hartnett et al., 1998; Arndt et al., 2013; Mueller et al., 2016). An external environmental factor that will also influence oxygen concentration in sediments is the depth of the water table. This once again highlights the co-mingling of characteristics that work in concert to govern overall storage and turnover of OM in sediments (Dungait et al., 2011). Additionally, the presence of reactive oxygen species (ROS) in the presence of sunlight and transition metals such as iron (Fe), has been shown to play a role in the molecular modification of terrigenous OM as it enters the aquatic realm (Waggoner et al., 2017). These reactions tend to have the greatest effect on lignin phenols and have been observed by Waggoner et al. (2017) to considerably alter the molecular structure with ·OH being the most prevalent ROS form. Previously, loss of lignin has been accredited to remineralization, however in light of these observations it has been proposed that lignin is not completely removed from the system it is instead modified via these ROS reactions. The cumulative effect of these reactions will lead to the inability to determine the newly modified molecules as being of terrestrial origin.

Sunlight and iron play an additional role at the terrestrial to aquatic interface. As terrigenous dissolved OM enters the aquatic system photochemical processing occurs and alters DOM both compositionally and structurally. Exposure to sunlight in the presence of

iron (both Fe(II) and Fe(III)) has been shown by Chen et al. (2014) to produce aliphatic and aromatic compounds which closely resemble black carbon, an aromatic-heavy group of compounds derived from combustion of terrigenous material and known for its resistance to degradation (Schmidt et al., 2011; Chen et al., 2014; Waggoner et al., 2015). These newly produced molecules have the tendency to precipitate out and in turn end up in sediments.

Hydrography of a given region will have a secondary effect as well which is most evident at the sediment surface. Precipitation, tidal inundation and flooding introduces water to soluble components of plant litter (e.g. Tannins). Dissolution of these soluble components has the net effect of transporting OM away from the local environment resulting in a net loss of OM from the system (Middleton and McKee, 2001).

Physicochemical interactions between sediment minerals and plant-produced compounds act to shield biopolymers from enzymatic and microbial attack (Sollins et al., 1996; Schmidt et al., 2011; Dungait et al., 2012; Clemente and Simpson, 2013; Tamura and Tharayil, 2014). Many clays and minerals act as aggregate surfaces upon which plant biopolymers are adsorbed. Clay minerals, amorphous iron and amorphous aluminum colloids possess large, charged surfaces which are prime locations for the sorption of SOM. In this way plant biopolymers maintain their chemical identity yet are protected from biotic attack and therefore able to persist in sediments for millennia (Sollins et al., 1996; Schmidt et al., 2011; Dungait et al., 2012).

1.3 Blue Carbon

Coastal wetland systems have recently received considerable attention for their ability to sequester and store organic carbon over decadal to centennial scales (Fig. 3). In the past decade there has been a marked increase in the study of the newly deemed blue carbon; C captured and sequestered in coastal wetlands, now recognized as a sink in the global C cycle (Mcleod et al., 2011; Bianchi et al., 2013). Worldwide, wetland ecosystems are estimated to be responsible for about 42.6 ± 4 Tg C yr^{-1} (global area of $203 \cdot 10^3$ km^2) though focus of these inventories is centered on the role of freshwater wetlands; bog and peatland ecosystems (Table 2; Chmura et al., 2003). Coastal wetlands have the potential to play a crucial role in the global C budget due to their efficiency in C burial; while emitting negligible amounts of Methane (CH_4) and Nitrous Oxide (N_2O) (Chmura et al., 2003; Bianchi et al., 2013). A study done by Chmura et al. (2003) estimates that coastal wetlands in the United States sequester 5 Tg C yr^{-1} (Chmura et al., 2003). This annual buildup of C in sediments leads to the development of massive sediment C reservoirs; a paper by Thorhaug et al. (2019) estimates the magnitude of stocks in the coastal wetlands of the Gulf of Mexico to be 480.48 Tg C. When combined with their terrestrial wetland counterpart (peatlands) they sequester an approximately equal volume of C as forests, globally (Moomaw et al., 2018).

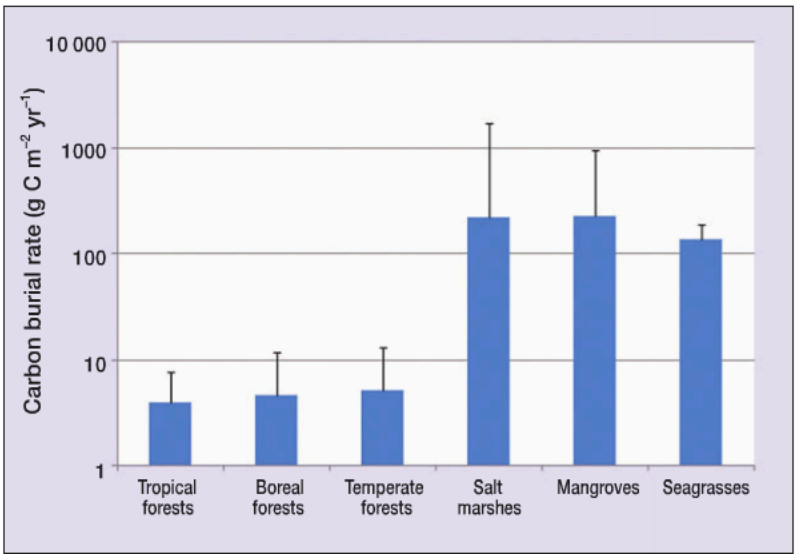

Figure 3. Taken from McLeod et al. (2011) shows a comparison in long-term rates of C sequestration for terrestrial and coastal wetland sediments. Error bars are included to indicate the maximum rates of accumulation.

The observed effectiveness of tidal saline wetlands highlights their importance for removal of carbon dioxide (CO_2) from the atmosphere (Fig. 3). As of 2009 atmospheric CO_2 levels had increased 38% above the preindustrial value of 280 ppm; caused in large part by burning of fossil fuels and changes in land use (Mcleod et al., 2011). These ecosystems have been shown to represent an excellent potential for mitigation of increasing atmospheric CO_2, however their geographic range makes them vulnerable to changing land use patterns and accelerated sea-level rise (SLR).

Coastal wetlands differ from terrestrial wetlands in their capability to store a much larger volume of C (Chmura et al. 2003; Bianchi et al. 2013). Plants capture CO_2, transform it through biosynthesis, and ultimately facilitate its storage in the form of OC in sediments. In terrestrial wetlands it is possible for sediments to become saturated in C which restricts the size of the C sink (Fig. 3). Coastal wetlands however, accumulate sediment vertically

in equilibrium with sea level rise and are therefore capable of increasing in both rate of sequestration and size of the C sink (Mcleod et al. 2011). Global averages of sequestration rates in tidal saline ecosystems estimate annual rate of burial to be 210 ± 20 g CO_2 m^{-2} with no significant difference between mangrove and salt marsh, however there are considerable differences between plant types with respect to soil C density; 5000 ± 400 and 430 ± 30 Tg C m^{-2} (upper 0.5 m of sediment) for mangrove and salt marsh, respectively (Table 2; Chmura et al., 2003). One potential explanation for storage capacity in mangrove systems is the increased primary productivity due to increased temperatures along the subtropical latitudes. However, microbial activity also increases with temperature and as such only accounts for <25% of the variability (Chmura et al., 2003; Kohl et al., 2018). Another explanation lies in the higher concentrations of lignin associated with woody plants (Bianchi et al., 2013); prompting a further look into the chemical components of the senescent litter material buried in these tidal saline sediments and how they affect overall C dynamics.

Table 2. Taken from McLeod et al. (2011) this table details C burial rates, global area, and global C buried for each coastal wetland ecosystem type. Division of wetlands is based on dominant plant species.

Ecosystem	Carbon burial rate ($g\ C\ m^{-2}\ yr^{-1}$) mean ± SE	Global area (km^2)	Global carbon burial* ($Tg\ C\ yr^{-1}$) mean ± SE	Sources: Global area	Sources: Carbon burial
Salt marshes	218 ± 24 (range = 18–1713) n = 96 sites	22 000**–400 000	4.8 ± 0.5 87.2 ± 9.6	Chmura *et al.* (2003); Duarte *et al.* (2005a)	Chmura *et al.* (2003); Duarte *et al.* (2005a)
Mangroves	226 ± 39 (range = 20–949) n = 34 sites	137 760–152 361	31.1 ± 5.4 34.4 ± 5.9	Giri *et al.* (2010); Spalding *et al.* (2010)	Chmura *et al.* (2003); Bird *et al.* (2004); Lovelock *et al.* (2010); Sanders *et al.* (2010)
Seagrasses	138 ± 38 (range = 45–190) n = 123 sites	177 000–600 000	48–112	Charpy-Roubaud and Sournia (1990); Green and Short (2003); Duarte *et al.* (2005b)	Duarte *et al.* (2005a); Duarte *et al.* (2010); Kennedy *et al.* (2010); Duarte (unpublished data)

Notes: *We calculated global carbon burial values using the mean carbon burial rate and the minimum and maximum global area values for salt marshes and mangroves. Global carbon burial values for seagrasses are from Kennedy *et al.* (2010). **No global inventory of salt marshes has been published, so Chmura *et al.* (2003) estimated 22 000 km^2 of salt marshes based on inventories for Canada, Europe, the US, and South Africa. SE = standard error.

This study hopes to highlight climate-driven ecosystem change and its resultant effects with respect to C storage in tidal saline wetlands. By understanding the ecological impacts of ecosystem change we are able to determine the magnitude of importance in these C storing powerhouses that are coastal wetlands.

CHAPTER II

CONTROLS OF OM PRESERVATION IN COASTAL WETLAND SOILS

2.1 Introduction

While occupying only a small fraction of the planet's surface, wetlands and their respective sediments, have been shown to contain a disproportionately large portion of the Earth's C; estimated from 3 to 68% (Buringh et al., 1984; Schlesinger et al., 1991; Whiting and Chanton, 2001; Chmura et al., 2003). Wetland sediments and their storage efficiency is the result of a culmination of conditions favorable to preservation. A high-water table and flooded sediments restrict the diffusion of oxygen and turnover, which in turn helps to preserve wetland sediment C, to aLK much greater extent than aerated sediments. Other environmental conditions primed to facilitate C storage include lower temperatures (in high latitude wetlands), rapid sediment accumulation, and elevated primary productivity; all of which help to make wetland sediments dynamic sequestration reservoirs. While wetlands are indeed highly productive areas that are responsible for a considerable amount of CO_2 drawdown from the atmosphere, numerous biological and physicochemical feedbacks also make wetlands one of the largest contributors of methane (CH_4) to the atmosphere, in some cases mitigating the effect of CO_2 uptake (Chappellaz et al., 1993; Whiting and Chanton, 2001; Chmura et al. 2003). A ratio developed by Whiting and Chanton (2001) uses the relationship of CH_4 and CO_2 for comparison of wetland C

sequestration efficiency provides a straightforward approach to understanding the capabilities of the different wetland types.

Coastal wetlands have proven themselves as a standout in wetland varieties as important global sinks for carbon with the added benefit of emitting negligible amounts of methane and nitrous oxide (Chmura et al., 2003; Bridgam et al.; 2006 Bianchi et al., 2013). Global estimates show these ecosystems bury approximately 31-34 Tg C yr^{-1} (Chmura et al. 2003). Coastal wetlands differ from their terrestrial counterparts in their capability to continuously sequester large volumes of organic carbon (OC) as they accrete sediment vertically in equilibrium with sea level rise (SLR; Chmura et al., 2003; Mudd et al, 2009; McLeod et al., 2011; Bianchi et al., 2013; Mueller et al., 2016). At the same time, their coastal location makes them vulnerable to both changing land use patterns and accelerated SLR, with global ramifications (Bridgam et al., 2006; Kirwan et al., 2014).

The coastal wetlands of Texas are a collection of diverse ecosystems each with their own ecological structure, vegetation, and carbon sequestration potential. Two ecosystems are of particular interest along the Texas coastline, which has been experiencing a shifting ecotone over the last forty years from *S. alterniflora* dominant cordgrass marshes to *A. germinans*, black mangrove, dominated marshes. Mangrove and cordgrass ecosystems, both of which are categorized as coastal wetlands, are great representations of all the characteristics that define these C-sequestering habitats. While united by many qualities there are key differences between the individual plant ecotones that have the potential to result in considerable differences in sequestration affecting both C pool size and physicochemical makeup. The extent of this shift is currently only

estimated and disagreement as to its magnitude is an issue (Perry and Mendlessohn, 2009; Comeax et al., 2012; Bianchi et al., 2013; Henry and Twilley, 2013; Doughty et al., 2016; Yando et al., 2016; Thorhaug et al., 2019). A study by Chmura et al. (2003) measured the average soil C density of mangrove swamps and cordgrass and showed mangrove soils to be considerably higher with respect to soil C content; 0.055 ± 0.004 gcm^{-3} and 0.039 ± 0.003 gcm^{-3}, respectively. Additionally, rate of sedimentation (0.53 ± 0.07 $cmyr^{-1}$ and 0.35 ± 0.06 $cmyr^{-1}$, mangrove and cordgrass, this study) temperature and precipitation regimes will also all be localized and individual to the given environment leading to the potential for further variation in sequestration potential.

This ecotone encroachment of C-rich woody mangroves (C_3) into previously herbaceous (C_4) salt marsh ecosystems are recognized to be a result of increasing global mean temperature Perry and Mendlessohn, 2009; Comeax et al., 2012; Bianchi et al., 2013; Henry and Twilley, 2013; Doughty et al., 2016; Yando et al., 2016; Thorhaug et al., 2019). Typically, mangroves are predominantly distributed in subtropical regions with a general range of 25°N and 25°S, although sometimes extend up to 38° N due to their intolerance of cold weather. As the severity and frequency of coastal winter freezes is declining, mangroves have been observed to expand northward (Comeaux et al, 2012); Cavanaugh et al., 2014). Shifts in dominant plant types have been associated with shifts in carbon sequestration, controlled by productivity, litter biochemistry, or environmental factors such as rate of soil accretion, redox, temperature, or litter input to the sediments. (Middleton and McKee, 2001; Chmura et al 2003; Parton et al., 1987; Ouyang et al., 2017;

Philben et al., 2014). Considerable debate amongst scientists in the field exists; controversy over the extent of the observed shift's combined effects remains a present issue as investigation continues into C dynamics of these coastal systems.

The approach applied in this study utilizes a suite of plant-derived biochemicals to help further the body of knowledge being complied to understand the effect of mangrove encroachment into previously cordgrass dominant ecosystems. Most of the carbon, nitrogen and phosphorus in primary biological products reside in carbohydrates, amino acids, lipids and nucleic acids (Hedges et al., 2000; Benner 2002). In plant tissues, additional biochemicals such as phenolic- (lignin,) and lipid-type (cutin acids and suberins) macromolecules provide structural support for the plant cell or form a physical barrier for protection. During decomposition, these primary biological products are either stabilized or rapidly converted to organic debris showing incredibly high chemical and structural diversity.

The prevailing theories for accumulation and stabilization of organic matter in natural environments have been sorption to mineral surfaces, photochemical reactions with iron and other transition metals, ROS alteration of lignin; abiotic condensation and transformation reactions, selective preservation of intrinsically resistant biomolecules, or availability of molecular oxygen (Keil el. 1994, Hartnett et al. 1998, Hedges et al. 2001, Mayer et al. 2002, Hebting et al. 2006; Chen et al., 2014; Waggoner et al., 2015; Waggoner et al., 2017). Few of the fundamental mechanisms and reactions are well understood. Organic matter preservation need not a priori depend on recalcitrant organic compounds or structures. A recent model for sediment organic matter decomposition frames

recalcitrance as an ecosystem property (Schmidt et al., 2011). The assumption of this model is that a variety of abiotic and biotic factors, e.g. compound chemistry, microbial community, nutrient availability, water availability etc., control the stability of organic matter and together they modulate decomposition and preservation.

Herein, we report a comprehensive analysis of major classes of biochemicals (carbohydrates, phenols, amino acids, and cutin acids) to investigate organic carbon burial in wetland ecosystems dominated by cordgrass and mangroves. The results indicate selective preservation of litter and microbial macromolecules that were different in mangrove and cordgrass dominated wetlands. Potential implications for climate-related ecosystem changes and controls on carbon sequestration are discussed.

2.2 Methods

Site selection and sampling

Sampling sites were selected based on surface vegetation, to represent the plant species of interest based upon observed ecotone shifts along the Texas coast. A mangrove-dominated (*A. germinans)* wetland was sampled near Port O'Connor, Texas (PO) (28˚25' 28.75"N, 96˚24'57.37"W), and a cordgrass-dominated (*S. alterniflora)* was sampled at Sunset Cove (SC), West Galveston, Texas (29˚9'1.99"N, 95˚2'12.00"W). Both cores (3cm diameter, 46 cm depth) were collected at each site perpendicular to the shoreline representing vegetation at mid-elevation. Belowground samples were sectioned in 1cm increments for the first 10cm and 2 cm increments for the remainder (10-46 cm). Senescent

plant matter was collected and separated into above and belowground components. Aboveground *A. germinans* plant matter was further partitioned into leaf and twig material. All samples were freeze-dried and ground prior to analysis using a ball mill.

Radiochronological dating and molecular analysis

Age and sedimentation rate were determined using the radiotracers lead 210 (^{210}Pb) and cesium 137 (^{137}Cs) to remove discrepancies that may be presented due to differences in elevation. ^{210}Pb activity was determined in sediment samples using a high-purity Germanium (HPGe) well detector. Data was verified with literature published by Comeaux et al. (2012), and only ^{210}Pb was used in the linear sedimentation rate calculation. Estimated ^{210}Pb linear sedimentation rates were applied to the excavated sediment cores when the natural log of the unsupported activity to depth had a linear correlation greater than 0.50 ($R^2 > 0.50$). The linear sedimentation rate is calculated by the following:

$$S\frac{dA}{dz} + \lambda A = 0$$

Flux of OC was calculated for both cores by dividing the OC mass accumulation by the age of the sediment determined via the linear sedimentation rate following:

$$OC\ Flux = \frac{OC\ mass\ accumulation\ (mg \cdot cm^{-2})}{Year}$$

Carbonates were removed via wet acidification with hydrochloric acid (HCL) and then dried using a steady flow of nitrogen gas. Organic carbon (OC) and total nitrogen

(TN) concentrations were determined for each sample by high-temperature combustion using a Costech elemental analyzer.

Total neutral carbohydrates and glucose cellulose analysis

Carbohydrates (fucose (Fuc), rhamnose (Rha), arabinose (Ara), galactose (Gal), glucose (Glu), mannose (Man), and xylose (Xyl)) were analyzed using two different hydrolysis techniques, one to determine the total amount of carbohydrates and another to only release carbohydrates bound in hemicelluloses. By difference, Glc bound in cellulose was calculated. Samples for total carbohydrates were weighed into 2 mL glass ampoules and drops of 1 M HCl were added to remove any inorganic form of carbon. Samples were dried with a stream of ultrapure nitrogen and then pretreated with 200 µL of 12 M sulfuric acid for 2 hours at room temperature. After pretreatment, 1.8 mL of deionized water was added. Ampules were sealed and heated for 3 hours at 100˚C in a water bath.

Samples for hemicellulosic carbohydrate analysis were treated with 1 M HCl to remove inorganic carbon, dried, and then hydrolyzed in 2 mL of 1.2 M sulfuric acid for 5 hours at 100˚C in a water bath. Sulfuric acid in both hydrolyses was removed using a Biorad A11 A8 resin (Kaiser and Benner, 2000). Salts and matrix components were removed by passing samples through a mixed bed resin (Biorad AG50 X8 H^+-form, AG2 X8 HCO_3^- - form) before chromatography. Monomeric carbohydrates were separated on a Dionex PA1 column with guard at 1 mL min-1 at 15 C and quantified by pulsed amperiometric detection.

Lignin phenol and cutin acid analysis

Lignin oxidation products were determined using the cupric oxide (CuO) oxidation developed by Hedges and Ertel (1982) with modifications (Louchouarn et al., 2011). The multiple-step method included an alkaline oxidation, clean-up followed by gas chromatography-mass spectrometry (GC/MS) analysis. Sample mass was adjusted for each vessel to contain 3-6 mg of OC. In cases where this amount was not achievable, under ~500mg sediment was used.

Lignin phenol analysis was performed with a Varian Ion Trap 3800/4000 GC/MS system fitted with a fused silica column (VF 5MS, 30 m x 0.25 mm i.d., 0.25 μm film thickness; Varian Inc.) for separate ion and quantifications of trimethylsilyl (TMS) derivatives of CuO oxidation by-products. Settings for the GC/MS analysis followed those developed by Louchouarn et al. (2010). Values for lignin phenols were corrected for CuO efficiency following Sarkanen and Ludwig (1971)[47] with 30% and 90% efficiency for vanillyl and syringyl phenols, respectively.

Cutin acid analysis utilized the spectra collected during the CuO lignin phenol method to identify the C_{14}-C_{18} hydroxylated fatty acids[48]. Quantification of the individual reaction products was achieved using the mass ion of 217 that was observed in all cutin acids but 9-Octadecene-1,18-dioic acid. For 9-Octadecene-1,18-dioic acid the mass ion of 441 was use. Calibration curves for mass ion 217 and 441 were developed from five commercially available cutin acid standards (list all five) and an average response factor was used for used for quantification of all cutin acid compounds. The following cutin acids were identified in litter and sediment cores: hexadecane1,16-dioic acid (C16DA), 9,10,18-

Trihydroxy-octadecanoic acid (9,10,ω-C18), octadecane-1,18-dioic acid (C18DA), 9-octadecene-1,18-dioic acid (C18DA:1), 8(9,10),16-dihydroxyhexadecane-1-16-dioic acid (x,ω-C16), x,18-octadecanoic acid (x,ω-C18), 7(8)hydroxyhexadecane-1,16-dioic acid (x-C16DA)

Amino acid analysis

Enantiomeric amino acids were analyzed according to Kaiser and Benner (2005) with modifications. Briefly, sediment and senescent plant material samples were weighed into 2 mL glass ampoules, and inorganic carbon was removed by addition of 1M HCl. After drying, 1 mL of 6 M HCl was added to the ampoule, mixed and then sealed followed by a 20 hr hydrolysis at 110°C in a Hewlett Packard 5980 GC oven. Samples were removed from the oven and transferred into 2 mL autosampler vials then dried under a steady stream of nitrogen (UHP) gas and low heat. Samples were resuspended in 400 uL of a 0.01 M Borate buffer (pH 10.2), closed with Teflon-lined silicone septa screw caps, vortexed briefly, and then centrifuged for 2 min. Two 150 uL sample aliquots were transferred into separate 2 mL autosampler vials with 400 uL flat bottomed inserts and frozen until analysis.

Amino acid enantiomers were separated after derivatization with o-phthaldialdehyde (OPA) and N-isobutryryl-L-cysteine (IBLC) on an Agilent Poroshell 120 EC-C-18 (4.6x100 mm) with guard at 20℃ and 1.5 ml min^{-1}. Mobile phase A was 40 nM monopotassium phosphate (KH_2PO_4) adjusted to 6.12, and mobile phase B was methanol/acetonitrile (92/8 v/v). The gradient program was 100% mobile phase A for

26.67 minutes, then 61% A at 26.67 mins, 46% A at 38.4 mins, 40% A at 42.67 mins, and returning to 100% A from 45.33 mins until completion of the individual sample run at 46.4 mins.

Analysis was performed on an Agilent 1260 Infinity Quaternary LC system with autosampler and fluorescence detector, controlled using OpenLAB software. Excitation was at 350 nm while emission of OPA derivatives was at 450 nm. Concentrations were corrected for racemization due to stereochemical inversion occurring during the hydrolysis process[49]. It should be noted that D- and L- isomers of aspartic and asparagine, and D- and L- isomers of glutamic acid and glutamine are combined due to conversion during acid hydrolysis and denoted as D- and L- Asx and D- and L- Glx, respectively[50].

2.3 Results

Biochemical composition of dominant wetland vegetation

Table 3. Biochemical composition of black mangrove (*A. germinans*) and cordgrass (*S. alterniflora*) litter.

Litter	Total	Hemicellulose	Cellulose Glucose	Phenol	Cutin Acids	AA	Fuc	Rha	Ara	Gal	Glu	Man	Xly
	(%OC)						(mol%)						
A. germinans													
Root	44.9	21.3	23.7	16.9	5.2	0.8	0	3	12	23	43	2	17
Leaves	27.0	15.8	11.2	6.4	12.2	3.1	0	10	22	6	48	2	13
Twigs	45.1	22.5	22.5	15.5	8.9	NM[a]	0	4	13	19	43	2	19
S. alterniflora													
Shoot	53.6	24.7	28.8	18.0	0.8	1.7	0	0	7	2	58	1	32
Root	32.3	18.0	14.3	NM[a]	NA[b]	3.6	0	1	14	5	47	1	33

[a.] NM, not measured

[b.] NA, not detected

Neutral carbohydrates contributed the largest fraction of organic carbon (OC) in both plant types with yields ranging from 32.3-53.6 and 27.0-45.1 %OC for cordgrass and mangrove, respectively (Table 3). Carbon normalized yields of OC was calculated via:

$$Yield\ (\%OC) = \frac{OC\ in\ biochemical}{Bulk\ OC}\ x\ 100$$

Yields of phenols represented by the sum of vanillyl, syringyl, and cinnamyl phenols and corrected for oxidation efficiency (see methods) were 18.0 %OC in cordgrass

shoots and ranged from 6.4-16.9 %OC for mangroves. Cutin acids composed a minor fraction of OC in cordgrass tissues (0.8 %OC) but were important in mangrove (5.2-12.2 %OC). Amino acids (AA) made the smallest contribution to OC in both plant types (0-3.6 %OC). Phenols were not measured for the root mass of cordgrass.

Glucose dominated carbohydrate composition in both plant types (46-52 mol%, Table 3, Figure 7). Hemicellulosic carbohydrates in *A. germinans* litter were enriched in Rha and Gal relative to *S. alterniflora*. Alternatively, Xyl was enriched in *S. alterniflora* senescent litter relative to *A. germinans.* The unique compositions of neutral sugars in mangroves and cordgrass allowed for the differentiation of dominant vegetation in sediment cores. A vegetation index (VI) was calculated using concentrations of Rha, Gal, and Xyl according to:

$$VI = \frac{Rha + Xyl}{Xyl}$$

Different components of *A. germinans* (roots, twigs, and leaves) showed index values of 1.7-2.8, In contrast, *S. alterniflora* (root and shoot) showed index values of 0.2-0.5 (Tables 5 and 6) demonstrating the separation both plant types based on their carbohydrate composition.

Vegetation, biochemical composition, and macromolecular structure in wetlands sediment cores

Organic carbon content ranged from 0.1-2.0 wt% OC in the mangrove-dominated core and from 0.1-4.4 wt% OC in the cordgrass-dominated core (Tables 5 and 6). Highest OC content was observed in the upper ten centimeters followed by sharp declines with

increasing depth in both cores (Fig. 4). Atomic C/N ratios ranged from 10-25 in surface sediments with a gradual decrease down-core. In the cordgrass dominant core, organic nitrogen (ON) was below the detection limit below 24 cm. Organic carbon burial rates were similar in both cores and decreased with increasing depth and marsh age (Fig.4).

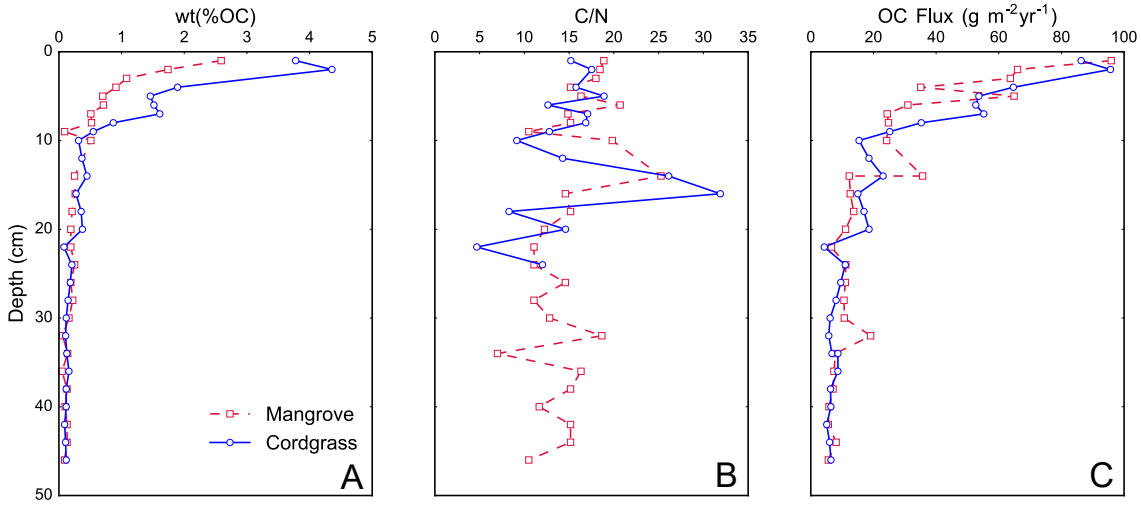

Figure 4. Weight %OC (A), atomic C/N ratio (B), and the OC flux in gm^{-1} yr^{-1} (C).

Stable carbon isotopes ranged from -26.6 to -22.4 ‰ in the mangrove-dominated core, and from -19.2 to -15.8 ‰ in the spartina-dominate core (Fig. 5). Both cores exhibited VI values similar to the dominant plant-type with a gradual increase correlated with declining yields of plant-derived biochemicals (Fig 5, Fig, 6). The gradual change of index values with depth was linked to preferential loss of Xyl (Fig 5).

Carbon-normalized concentration of major plant-derived biochemicals (THNS, phenols, cutin acids, DLAA) showed a general decrease with depth in both cores with few exceptions (Fig. 6). Depth profiles for amino acids showed an increase in concentrations mid-core for cordgrass-dominated sediments whereas mangrove-dominated sediments

showed highest concentrations in surface and at depth. Low concentrations of cutin acids were only measured in the surface sediments of both cores.

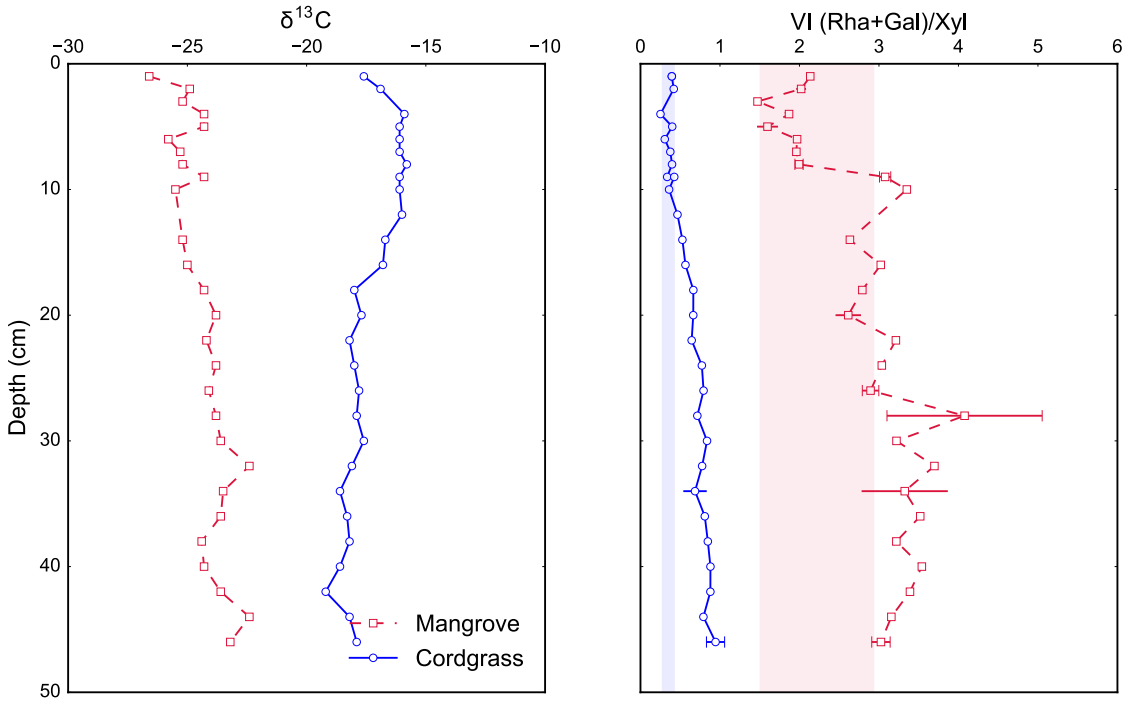

Figure 5. $\delta^{13}C$ isotopes (A) and the vegetation index (VI, B) in sediment cores. Shaded areas show VI ranges observed in senescent litter.

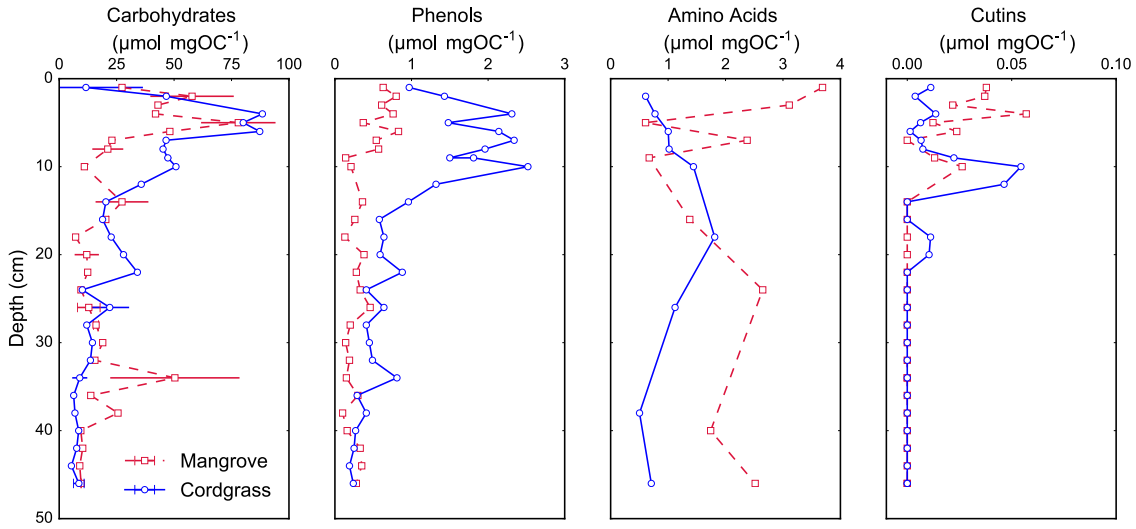

Figure 6. Carbon-normalized concentrations (μmol mg OC^{-1}) of biochemicals including carbohydrates (A), phenols (B), amino acids (C), and cutins (D) in the cordgrass and mangrove cores. Error bars highlight the relative heterogeneity in the sediment samples themselves.

There was a shift in the chemical composition between plant litter and surface sediments (Fig 7, Table 4). More OC could be assigned to carbohydrates, phenols, and amino acids in surface sediments than in plant litter but less to cutin acids. In surface sediments of the mangrove-dominated core 40-70% of OC was comprised of carbohydrates, phenols, amino acids, and cutin acids (Fig. 6). In comparison, 60-100% of OC in surface sediments of the cordgrass-dominated core was characterized by the four classes of biochemicals.

The OC comprised by analyzed biochemicals declined considerably with depth in both cores, but the decrease was less pronounced in the mangrove core. Carbohydrates and phenols were the major identifiable biochemicals for both cores at the surface. The mangrove core also showed important contributions of amino acids and cutin acids (11

%OC and 5 %OC, respectively). Downcore, all biochemicals experienced losses, with the exception of amino acids in the mangrove-dominated core. Comparison of composition from plant litter to the bottom of the cores shows preservation of different biochemicals.

Table 4. Yield (%OC) of the analyzed biochemicals for plant litter, and upper, and lower portions of the cores.

Source vegetation	Carbohydrates	Phenols	Amino Acids	Cutin Acids	Uncharacterized
	Yield (%OC)				
A. germinans					
Litter	30	12	2	9	47
Top 10cm	31.3 ± 18.6	13.0 ± 5.7	11.3 ± 7.6	5.2 ± 3.5	37.2 ± 14.6
Bottom 10cm	12.4 ± 9.1	5.0 ± 2.1	8.9 ± 4.9	ND	78.4 ± 4.5
S. alterniflora					
Litter	42	18	3	1	37
Top 10cm	40.6 ± 12.9	37.7 ± 9.9	5.4 ± 1.8	1.6 ± 1.4	22.2 ± 18.0
Bottom 10cm	6.0 ± 2.1	8.3 ± 3.9	3.1 ± 0.8	0	84.4 ± 0.6

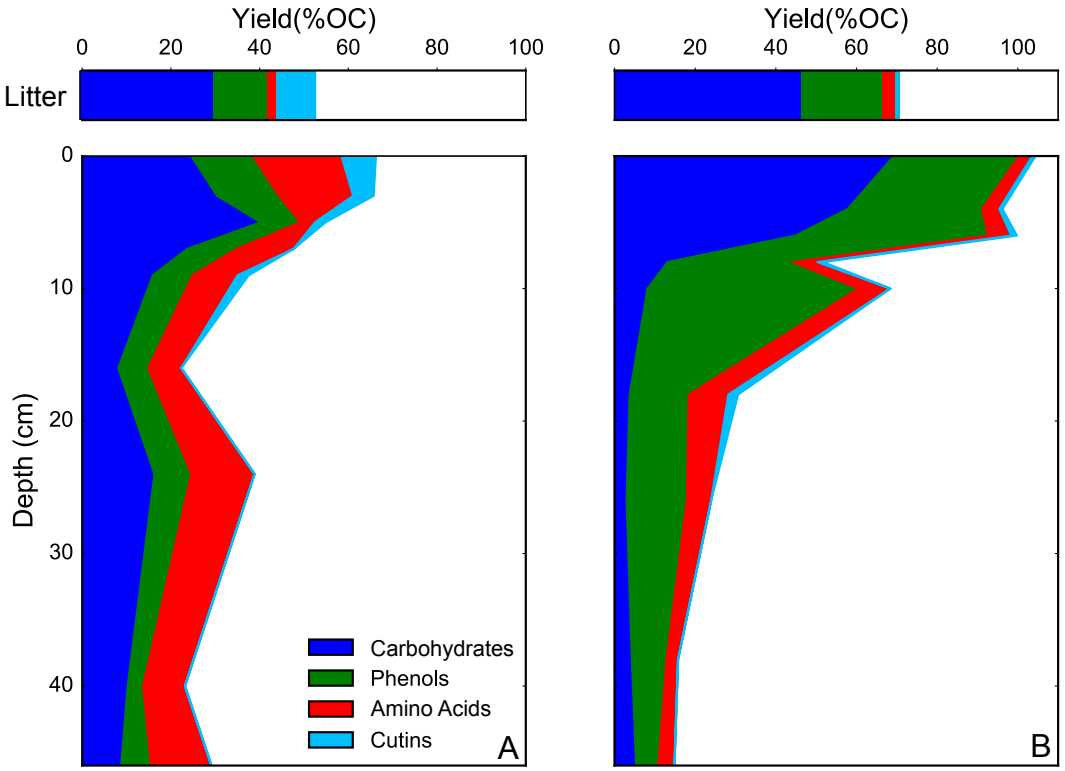

Figure 7. C-normalized yields (%OC) of major classes of biochemicals in plant litter and sediment cores dominated by black mangrove (A) and cordgrass (B).

Glucose (Glc) is the main building block of cellulose but also occurs in hemicelluloses. Cellulose-bound Glc was determined using different hydrolysis techniques (see methods). In surface sediments cellulose-bound Glc accounted for 31-59% of total neutral carbohydrates in both cores, however values in the mangrove core were slightly higher (47-59% vs. 31-43%, (Fig. 8). Cellulose-bound Glc declined rapidly, and below the first 10 cm for both cores' cellulose-bound Glc contributed <25% to total carbohydrates.

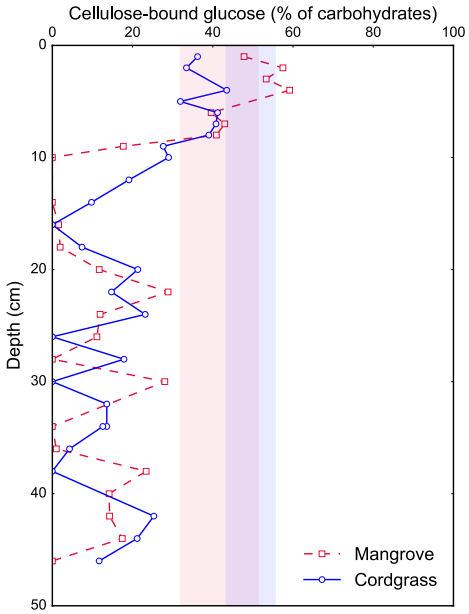

Figure 8. Contributions of cellulose-bound glucose to total carbohydrates. Shaded bars show ranges observed in plant litter (blue=cordgrass, red=mangrove).

Indicators of organic matter decomposition in sediments

A carbohydrate-based decomposition indicator was developed by dividing carbohydrate yields in surface litter by yields in sediment core samples (Fig. 9). Sugar degradation indices in sediment organic matter offset from 1 indicate either preferential loss (higher) or selective preservation (lower) of carbohydrates. The Sugar Degradation Index was < 1.5 in the upper layers of the cores (Fig. 9), indicating sediment organic matter had similar neutral carbohydrate yields compared to fresh vegetation. Below 10 cm, the index increased to >2 with a maximum value of 40 in the cordgrass core. Sugar degradation indices were much higher in the cordgrass dominated core than in the mangrove-dominated core showing more extensive removal of carbohydrates in the former.

Acid/aldehyde ratios of vanillyl (V) and syringyl (S) phenols ([Ad/Al]S,V) were used to evaluate the extent of decomposition of vascular plant-derived lignin in sediment cores (Fig. 9). A general increase of [Ad/Al]S,V ratios was observed for both dominant plant types as burial time increased. The mangrove-dominated core displayed [Ad/Al]V ratios of 0.19 – 3.33 and [Ad/Al]S of 0.09 – 1.18, whereas the cordgrass-dominated core showed [Ad/Al]V ratios of 0.14 – 0.54 and [Ad/Al]S of 0.10 – 0.32. Both cores also showed positive peaks of [Ad/Al]S,V ratios suggesting variable extent of decomposition of the lignin component. Variability observed for [Ad/Al]S,V ratios did not coincide with changes in the carbohydrate degradation index. The DL ratio shows similar trends between the two cores with values increasing in the upper 10 cm of the core ranging from 0.04-0.13. The values decline as they approach mid-core, then begin to gradually increase.

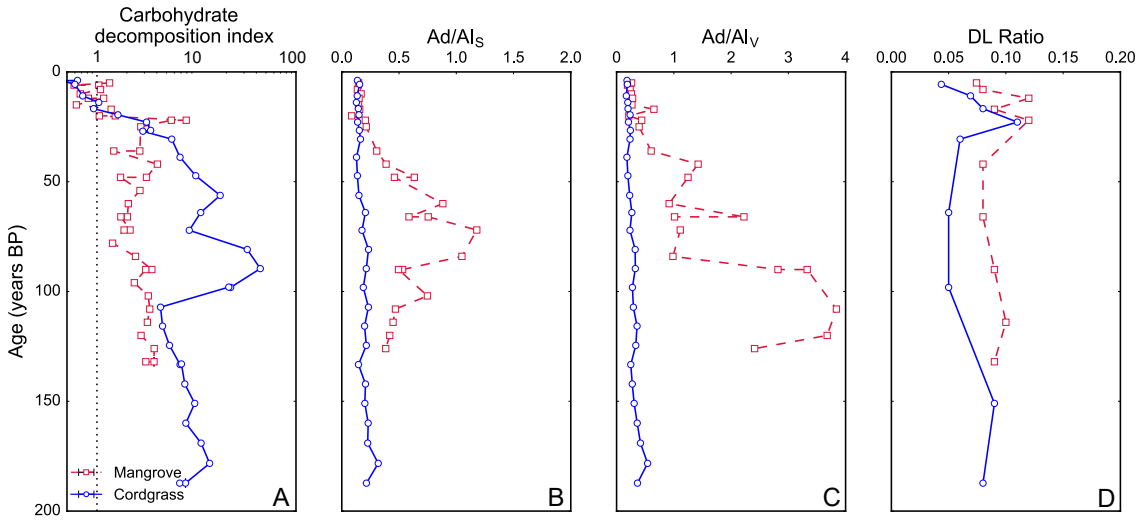

Figure 9. Profiles of carbohydrate decomposition indices (A), aldehyde ratios (Ad/Al) of syringyl and vanillyl phenols (B and C, respectively), and ratios of D- to L-amino acid enantiomers (D).

Analysis of compositional changes in OM between plant classes shows the preservation of different biochemicals in soil reservoirs. Overall quantity of OC stored in soils between these study sites shows little variability despite differences in both rates of primary production and soil accretion. The quality of OC and storage affinity for particular molecular components however, is different; resulting in preservation of distinct suites of OM based on plant type.

2.4 Discussion

Environmental History of Vegetation

Coastal wetlands along the Texas coast have been experiencing black mangrove (*A. germinans*) expansion into cordgrass salt marshes (*S. alterniflora*) over the last 20 years (Chmura et al., 2003; Bianchi et al., 2013; Comeaux et al., 2012; Ross et al., 2000; Stevens et al., 2006). This shift from C_4 to C_3 plants is related to a decline in the frequency and intensity of freezes during coastal winters to which black mangroves are relatively intolerant (Chmura et al., 2003; Bianchi et al., 2013; Comeaux et al., 2012). Mangrove expansion leads to changes in ecosystem properties with different responses to sea level rise and to a potential change of overall storage and sequestration of OC (Comeaux et al., 2012; Bianchi et al., 2013). Here we investigate the chemical composition of OC buried in mangrove and cordgrass marsh-type wetlands to characterize mechanisms controlling OC burial and potential effects of shifting plant communities.

Stable carbon isotopes and hemicellulosic carbohydrate compositions indicated the investigated cores did not experience a slow transition in the dominant plant and ecosystem type over the last 40 years as observed elsewhere along the gulf coast. The molar ratio of (Rha + Gal):Xyl separated vegetation types well as mangroves contained relatively high proportions of Rha and Gal, and cordgrass had higher relative contributions of Xyl. Both, stable carbon isotopes and the developed vegetation index responded to biogeochemical processing and the change in molecular composition (Benner et al. 1987). The relative contributions of Rha and Gal are generally higher in soil organic matter than

fresh plant material and increase during the decomposition of plant litter as a result of incorporation of microbial detritus (Opsahl and Benner, 1999; Hedges et al., 1994; Derrien et al, 2007; Dungait et al, 2011). In contrast, Xyl is more reactive than other carbohydrates resulting in decreasing relative contributions of Xyl with decomposition (Opsahl and Benner 1999). Careful selection of hemicelluloses for use in the index needs to be taken in order to successfully distinguish plant origin in all manner of vegetation (Jia et al., 2008; Philben et al., 2014; Comont et al., 2006). A study by Philben et al. (2014) utilized a similar approach and their results were successful in the characterization of dominant plant vegetation in a core spanning 2000 years. This method of plant classification in sediments has the potential to be a useful tool for vegetal identification for both newly buried and paleo-vegetation playing on the different suites of hemicelluloses synthesized by individual plant species during growth. A difference between this study and those done previously is the use of a single ratio exploiting the differences in hemicellulose dominance between plant species, instead of a ratio for each plant type. Additionally, while there are a number of other techniques that have been used in identification of plant composition of sediments (macrofossils, alkanes, alkanones, etc.) this approach allows investigation even after considerable humification and decomposition (Philben et al., 2014).

Biochemical Composition of OM

The combined analysis of the major biochemicals characterized >50 %OC in senescent litter material. Among analyzed biochemicals, carbohydrates accounted for the

largest portion of characterizable OC in senescent material for both plant types. It should be noted that methylated and in particular acidic carbohydrates are also important groups of carbohydrates in plant tissues, but they were not analyzed in this study.

A rapid compositional change occurred during the initial burial of litter material. Yields of characterizable biochemicals shifted from plant litter to surface sediments. In both cores a larger fraction of the sediments were characterized at the molecular level upon burial, which can be attributed to the loss of any soluble components of the OM upon tidal inundation (Hernes et al. 2001). With loss of leached and water-soluble fractions of OC analysis was able to characterize 40-60% OC and 85-100% OC in surface sediment for mangrove and cordgrass, respectively. A considerably smaller fraction of OC was characterizable in surface sediments within the mangrove core alluding to alternative sources of OC not analyzed in this study. It should be noted that the location of the mangrove core received little to no alluvial input and the majority of organic matter is produced locally. Benthic marsh algal mats have been observed in mangrove ecosystems and $\delta^{13}C$ values for the two species overlap complicating identification (Bianchi et al., 2013), however high yields of amino acids provide evidence of their presence (Tyler and McGlathery, 2003).

Carbon-normalized yields of identified biochemicals decline with depth giving rise to a larger uncharacterized fraction of OC. Cutin acids are confirmed to be highly sensitive to diagenetic change, similar to observations by Opsahl and Benner (1995) who found cutin acids to be a highly labile fraction of plant OC. The remaining three classes of biochemicals (carbohydrates, phenols, and amino acids) underwent shifts in relative

contribution to OC downcore all of which exhibit losses for both cores. While most biochemicals are observed to decrease in their ratio of OM, in the mangrove-dominant core amino acids are observed to increase in relative contribution.

Examination of the five components (carbohydrates, phenols, amino acids, cutin acids, and uncharacterized) contributing to total yield (%OC) show shifts in preservation for different biochemical components from surface downcore. Comparison of the bottom 10 cm of each core shows a larger portion of characterizable material in the mangrove-dominated core (~30% OC vs. <20% OC), with respect to the four analyzed biochemicals. The carbohydrate components in each respective core show great variance in preservation from one plant type to another, in the mangrove-dominant core preservation vastly exceeds that of the cordgrass core. The continued presence of carbohydrates in soils agrees with recent research disproving their previously recognized labile nature. A study by Derrien et al. (2007) concluded that the original plant substrate had a much lesser degree of effect on preservation with respect to carbohydrates than the overall sediment characteristics. It is likely that the variance observed in this study reflects sediment quality, further tying the partnership of both biological and physicochemical reactions in the preservation of OM in these coastal wetlands (Barreto et al., 2016; Kristensen et al., 2008; Schmidt et al., 2011; Alongi, 2014; Lehmann and Kleber, 2015). The phenolic component of the mangrove core preserved to a much larger extent than in the cordgrass core, where phenols show significant loss from surface to depth. Numerous studies have suggested that higher concentrations of the more labile cinnamyl and syringyl will result in selective

loss, a result of molecular bond characteristics, with ester linkages observed to be more susceptible to cleavage than other bonds occurring in vanillyl lignin phenols. Results taken during this study and corroborated with outside studies show cordgrass to have a larger fraction of these lignin phenols (not shown) this could be a contributing factor for the variability between the phenolic contributions to yield (%OC) in the bottom of these cores (Benner et al. 1991; Bianchi et al. 2001; Opsahl and Benner 1995). Additionally, it has been demonstrated by Saraswati et al. (2016) that the enzymatic latch mechanism, previously applied to northern peatlands, has a large effect with respect to the preservation of phenols in sediments. Under anaerobic conditions like those observed in mangrove sediments the activity of phenol oxidase, a phenol-reducing enzyme, is impeded resulting in storage. Mangrove sediments have been observed to accumulate sediments on a much shorter timescale than cordgrass sediments (0.39 (+/-) 0.06 and 0.23 (+/-) 0.04 g cm^{-2} yr^{-1}, respectively, this study). This results in mangrove sediments rapidly reaching an anaerobic state and triggering the enzymatic latch mechanism, promoting preservation of lignin phenols in mangrove-dominant sediments (Saraswati et al. 2016; Chmura et al 2003; Unger et al 2016).

Contribution of amino acids to yield (%OC) in each core is also preserved to a greater extent in the mangrove-dominant sediments. While a fraction of these amino acids are D-enantiomers, produced microbially in the cell wall of bacteria, it has been suggested by Philben et al. (2013) that the intrinsic chemical structure of the amino acids themselves, accompanied by molecular associations and cross-linkages between peptides promotes recalcitrance. These diphenyl ether linkages from protein to protein occur to a much

greater degree in mangrove cells walls when compared to cordgrass tissues and are expected to significantly contribute to the general recalcitrance of amino acids of mangrove biomass (Philben et al., 2013).

Controls of Preservation in Coastal Wetland Sediments

Upon analysis of yield (%OC) for each core it is possible to break down total yield into two groups, the first of which is chemically characterizable and consists of the four biochemicals analyzed in this study, while the second and considerably larger component is uncharacterizable OC. There are a number of physiochemical controls resulting in the preferential preservation of certain biomarkers depending on plant type, however in addition there are a number of biotic and abiotic factors that give rise to these two mentioned assemblages. As mentioned previously, the rate of sedimentation can have a large effect on the degree of preservation of characterizable OC in sediments (Unger et al 2016; Lutzow et al. 2006; Hemminga et al 1988; Hyun et al 2007). This is confirmed in this study with the faster accreting mangrove sediments possessing a considerably larger fraction of characterizable material accompanied by a faster sedimentation rate (see above). Additionally, it has also been suggested the pore space may also enhance preservation of biomolecules. As mentioned by Lutzow et al. (2016) faster accreting sediments tend to have lower pore space limiting the availability of plant OM to microbes and other larger decomposers as well as water content, which is a necessary element for some organisms including bacteria. With respect to the characteristics of sediments and their ability to enhance preservation, mineral content has been shown to shield plant OM

while concurrently maintaining the biochemical identity (Unger et al., 2016; Chmura et al 2003; Lutzow et al 2006; Lehmann et al 2007; Barreto et al., 2016). This process can occur via two pathways, one being the physical protection of characterizable plant OM via sorption onto mineral surfaces. The other is chemical, involving the complexation of plant OM with clay and silt sediments particles (Lehmann et al 2007; Dodla et al 2012). It has been suggested by some that temperature will have an effect on the decomposability of characterizable OM in sediments however further research has shown the effect of temperature on decomposition to be minor (Benner et al., 1991). The physical environment in which these sediments exist not only has an effect on the abiotic controls of decomposition but also has an effect on the biotic component. Tidal activity, temperature, geomorphology, nutrient delivery, etc. will all also have a direct effect on the health of the ecosystem thereby promoting or inhibiting primary production.

Increased primary productivity will in turn accelerate the transfer of plant biomass to sediments, thereby increasing the characterizable fraction of OM. In coastal wetlands as new litter material is incorporated into sediments it is exposed to a number of biotic factors that act as decomposers, increasing the fraction of uncharacterizable soil OM. In oxygenated sediments macrophytes and bioturbators have been recognized to increase the flux of litter into sediments. Additionally, these organisms are known for oxygenating sediments during burrowing, which could potentially increase decomposition along aerobic pathways. Grazing by macrophytes takes a minor role however when considering the role of bacteria in decomposition. Microbial communities are responsible for the vast majority of uncharacterizable OM produced in coastal wetland soils while also being

important component of OM themselves (Schmidt et al., 2011; Lutzow et al. 2006). While the vast majority of microbially processed OM is uncharacterizable, D-amino acids can be characterized and are produced in bacterial membranes, tracking the activity of microbial communities with respect to OM (Kaiser and Benner, 2005; McCarthy et al., 1998; Salton, 1994).

CHAPTER III

CONCLUSIONS

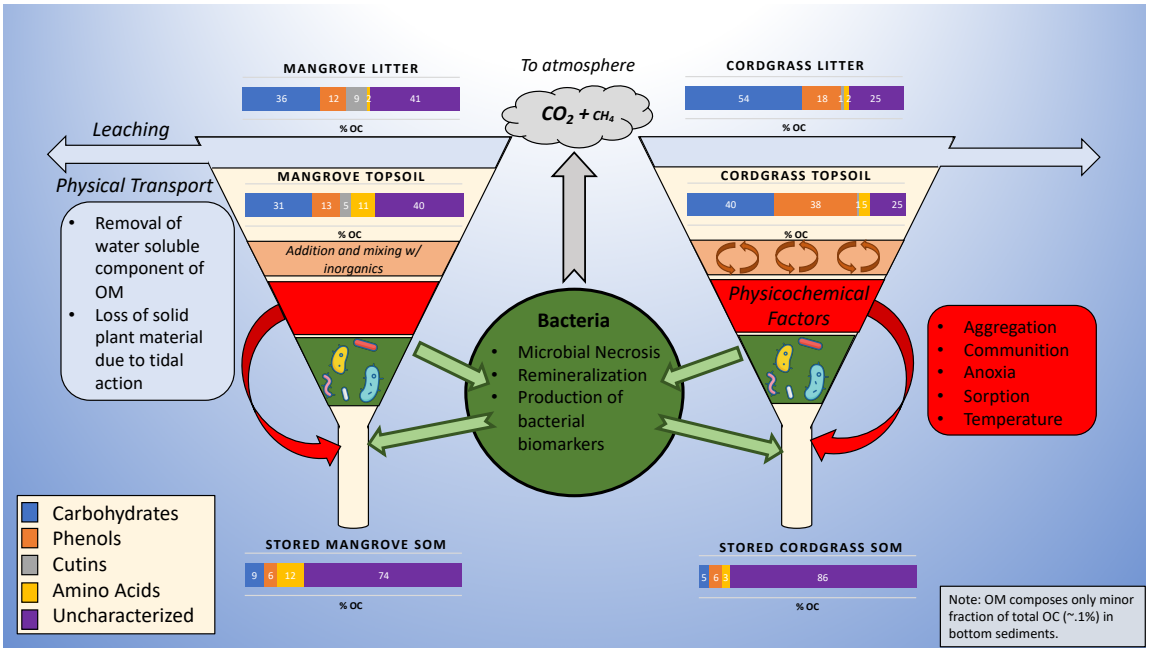

Figure 10. Depiction of factors and processes that control and influence the formation of SOM in these coastal wetland sediments. Composition of stored SOM is a reflection of the input plant material and factors that are uniquely developed over time in an individual environment.

Analysis of compositional changes in OM between plant classes showed the preservation of different biochemicals in sediment reservoirs. Overall quantity of OC stored in sediments between these study sites showed little variability despite differences in both rates of primary production and soil accretion. Wt %OC in sediments at the bottom of each core accounted for only ~1% highlighting the contribution of inorganics in the soil matrix. The quality of OC and storage affinity for particular molecular components however, was different; resulting in preservation of distinct suites of OM based on plant

type (Fig. 10). Previous research into the drivers of SOM storage and composition focused on either recalcitrance or ecosystem properties as controls. We however propose that these drivers act in concert to produce qualitatively different SOM depending on plant type. This study was unique in its approach to both quantify and characterize all the major biochemicals in the soil profiles, which provided insight into the combined effects of biological and physicochemical reactions. While chemical structure did play a role with respect to reactivity and ultimate storage, interaction with ecosystem-dependent biotic and abiotic factors will result in the compositional variability observed in these coastal wetland soils. Ultimately, as transition occurs from one dominant plant species to the next it remains unclear what the initial, or far reaching, effects on carbon storage will be. It is possible that as the mangrove ecotone crosses the threshold to previously cordgrass dominant ecosystems, specialized mechanisms involved in sedimentary cordgrass decomposition would be hampered with respect to decomposition of the C_3 plant tissue. However, as time passes sediment biota would catch up, and decomposition patterns typical of mangrove ecosystems would take hold. Further research is required to fully answer these hypotheses, and there is need for the analysis of a location where recorded transition has occurred.

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APPENDIX A

SUPPLEMENTARY DATA

Table 5. Port O'Connor - composition of analyzed biochemicals.

Source					THNS	Phenols	Cutins	DLAA
	Depth	Year BP	wt% OC	VI	(umol/mg C)			
A. germinans	0-1	5	1.97	1.34	35.97	0.63	0.04	3.69
	1-2	6	1.23	0.59	81.79	0.80	0.04	NM
	2-3	8	1.06	1.09	43.73	0.61	0.02	3.11
	3-4	10	0.55	0.68	69.05	0.76	0.06	NM
	4-5	12	0.96	0.82	57.01	0.37	0.01	0.61
	5-6	15	0.44	0.62	77.12	0.83	0.02	NM
	6-7	17	0.34	1.39	34.32	0.54	ND	2.38
	7-8	20	0.35	1.53	31.27	0.57	ND	NM
	8-9	22	0.33	7.89	29.48	0.58	0.05	2.75
	9-10	25	0.32	2.76	17.59	0.21	0.03	NM
	12-14	36	0.15	2.70	18.07	0.36	ND	NM
	14-16	42	0.15	4.05	12.01	0.26	ND	1.38
	16-18	48	0.16	3.15	15.39	0.13	ND	NM
	18-20	48	0.13	1.73	28.05	0.38	ND	NM
	20-22	60	0.08	2.07	23.45	0.28	ND	NM
	22-24	66	0.13	2.02	24.00	0.33	ND	2.65
	24-26	72	0.14	1.74	27.91	0.46	ND	NM
	26-28	78	0.12	1.87	25.64	0.20	ND	NM
	28-30	84	0.13	2.44	19.58	0.14	ND	NM
	30-32	90	0.22	3.56	13.50	0.19	ND	0.70
	32-34	96	0.10	3.09	15.52	0.15	ND	NM
	34-36	102	0.09	3.28	14.61	0.30	ND	NM
	36-38	108	0.09	3.39	14.11	0.10	ND	NM
	38-40	114	0.07	3.21	14.99	0.16	ND	1.74
	40-42	120	0.07	2.77	17.34	0.33	ND	NM
	42-44	126	0.10	3.76	12.83	0.35	ND	NM
	44-46	132	0.07	3.75	12.84	0.28	ND	2.52
Deviation (%)	-	-	-	1.75	7.81	0.01	0.01	0.31

*Vegetation Index (VI)

Table 6. Sunset Cove - Composition of analyzed biochemicals**.**

Source					THNS	Phenols	Cutins	DLAA
	Depth	Year BP	wt% OC	VI	(umol/mg C)			
S. alterniflora	0-1	4	3.78	0.39	50.56	0.97	0.01	0.61
	1-2	6	4.36	0.42	46.62	1.43	ND	0.77
	3-4	11	1.89	0.25	88.44	2.31	0.01	NM
	4-5	14	1.46	0.40	80.09	1.48	0.01	NM
	5-6	17	1.52	0.31	87.29	2.14	ND	1.00
	6-7	20	1.61	0.38	46.51	2.34	0.01	NM
	7-8	23	0.87	0.40	45.19	1.96	0.01	1.02
	8-9	27	0.55	0.34	47.29	1.50	0.02	NM
	9-10	31	0.31	0.36	50.82	1.81	0.05	1.44
	10-12	39	0.37	0.47	35.72	2.52	0.05	NM
	12-14	47	0.45	0.53	20.31	1.32	ND	NM
	14-16	56	0.27	0.57	18.88	0.96	ND	NM
	16-18	64	0.36	0.67	22.64	0.58	0.01	1.81
	18-20	72	0.38	0.66	27.99	0.64	0.01	NM
	20-22	81	0.08	0.64	34.04	0.59	ND	NM
	22-24	90	0.21	0.77	10.07	0.88	ND	NM
	24-26	98	0.18	0.79	22.00	0.41	ND	1.12
	26-28	107	0.15	0.72	12.00	0.64	ND	NM
	28-30	116	0.12	0.84	14.47	0.41	ND	NM
	30-32	125	0.11	0.78	13.59	0.45	ND	NM
	32-34	133	0.13	0.69	8.95	0.49	ND	NM
	34-36	142	0.16	0.81	6.31	0.81	ND	NM
	36-38	151	0.12	0.85	6.86	0.29	ND	0.50
	38-40	160	0.12	0.88	8.48	0.41	ND	NM
	40-42	169	0.09	0.88	7.62	0.27	ND	NM
	42-44	178	0.11	0.79	5.32	0.25	ND	NM
	44-46	187	0.12	0.94	8.56	0.19	ND	0.71
Deviation (%)	-		-	0.06	8.06	0.02	0.00	0.10