DEGRADATION STATE AND SEQUESTRATION POTENTIAL OF CARBON IN COASTAL WETLANDS OF TEXAS: MANGROVE VS. SALTMARSH ECOSYSTEMS

An Undergraduate Research Scholars Thesis

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ABSTRACT

Degradation State and Sequestration Potential of Carbon in Coastal Wetlands of Texas: Mangrove vs. Saltmarsh Ecosystems. (May 2015)

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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 use. In this project we used total hydrolysable carbohydrates, and lignin phenols at two study sites located on the Texas coastline, to characterize composition and degradation state of sedimentary organic matter (OM) to elucidate mechanisms controlling carbon burial in mangrove (*A.germinans*) and salt marsh (*S. alterniflora*) dominated wetlands. Carbohydrates are used as specific decomposition indicators of the polysaccharide component of wetland plants, acid/aldehyde ratios of vanillyl (V) and syringyl (S) phenols ($[Ad/Al]_{S,V}$) help track the decomposition of lignin. The contribution of carbohydrates and lignin phenols to the total OC pool in litter and surface sediments correspond to 20-60% and 10-40%, respectively. Sharp declines of yields with depth occur parallel to increasing $[Ad/Al]_{S,V}$ ratios indicating decomposition of both the polysaccharide and lignin components of litter detritus. Although results show surface sediment and plant litter OC to be

higher in *S. alterniflora*, rapid disappearance with depth is observed relative to *A. germinans* OC. Litter biochemistry is determined to be a greater control of OC burial then total detrital input to sediment.

CHAPTER I

INTRODUCTION

Coastal wetland systems have recently received considerable attention for their ability to sequester and store organic carbon over decadal to centennial scales. In the past decade there has been a marked increase in the study of blue carbon (BIC); carbon 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 (Chmura et al., 2003). Coastal wetlands have the potential to play a crucial role in the global Carbon 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). The observed effectiveness of tidal saline wetlands highlights their importance for removal of carbon dioxide (CO_2) from the atmosphere. As of 2009 atmospheric CO_2 levels had increased to 38% about 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 demonstrate 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.

Wetlands along the Texas coastline are a collection of diverse ecosystems each with their own ecological structure , vegetation, and potential C sequestration. This study will focus on comparing the sequestration capabilities of *Avicennia germinans* (black mangrove) and *Spartina alterniflora* (smooth cordgrass) dominated ecosystems. These two ecosystems are of particular interest as the Texas coastline has been experiencing some shifts over the last decades from dominant *S. alterniflora* salt marshes to *A. germinans* ecosystems (Bianchi et al., 2013). This ecotone shift of C-rich woody mangroves (C_3) into previously herbaceous (C_4) salt marsh ecosystems is accredited to increase in global mean temperature (Comeaux et al., 2011; Bianchi et al., 2013). Typically, mangroves are distributed predominantly in subtropical regions between a general range of 25°N and 25°S, although sometimes extending 38° N; due to their intolerance of cold weather. As the severity and frequency of coastal winter freezes declines, mangroves are observed to expand Northward (Chmura et al. 2003; Comeaux et al. 2011). Due to the recently recognized importance of coastal saline ecosystems for C sequestration, this shift is expected to in turn to affect both rates of C burial and pool size.

In this study it was anticipated that shifts in dominant plant types would result in concomitant shifts in C sequestration, controlled by either litter biochemistry, or environmental factors such as rate of soil accretion, litter input to the sediments, ambient temperature, etc.. 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 soils to become saturated in C which restricts the size of the C sink. Coastal wetlands however, accumulate soil 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 (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). 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.

To better constrain the reasons for the tenfold difference observed in sequestration for these two wetland ecosystems, this study employed a geochemical approach; using biomarkers to analyze the mechanisms controlling C sequestration in coastal wetlands. The approach relies on distinguishing the extent of OM decomposition in salt marsh sediments vs. mangrove wetlands as a means to identify the controlling factors for plant OM decomposition and ultimate storage. In order to achieve, this both carbohydrates and free lignin phenols were used to characterize differences between the two plant classes in both litter and sediments. By determining the component neutral sugars in senescent material it is also possible to distinguish plant source inputs throughout the sediments and therefore track any shifts in vegetation that may have occurred (Cowie et al., 1992; Borch, 1997). Neutral sugars, carbohydrates, have been observed

to be some of the most reactive components of the OC pool and generally concentrations decrease quite rapidly with depth relative to lignin (Borch, 1997). By analyzing the lignin and carbohydrate components of each plant type and their extent of decomposition with depth it will be possible to verify if major differences do exist in C sequestration between the two ecosystems, and if so what is the role of litter composition (biochemistry) on influencing carbon sequestration potential.

CHAPTER II

METHODS

2.1 Sample Collection

Two sample sites were selected based upon wetland type (i.e., saltmarsh vs. mangrove dominated wetlands). The first of the two sites was *A. germinans* dominant and was located at Port O'Connor, Texas (PO) (28˚25' 28.75"N, 96˚24'57.37"W). The second site was *S. alterniflora* dominant and located at Sunset Cove (SC), West Galveston, Texas (29˚9'1.99"N, 95˚2'12.00"W) (Fig 1.). Three sediment cores (3cm diameter, 46 cm depth) were collected at each site perpendicular to the shoreline to represent vegetation at low elevation with respect to the permanent water line (i.e., low-elevation, mid-elevation, and high elevation) only the mid-elevation sediment cores were used for this study. Belowground samples were sectioned in 1cm increments for the first 10cm and 2cm increments for the remainder (30-46 cm). Belowground plant matter (root mass) was not removed from the sediment core before sectioning. Live plant matter was also collected to establish input material end members. Plant material was cleaned and separated into above and below ground plant matter. The above ground *A. germinans* plant matter was also partitioned into leaf and twig material. All samples were then freeze dried and

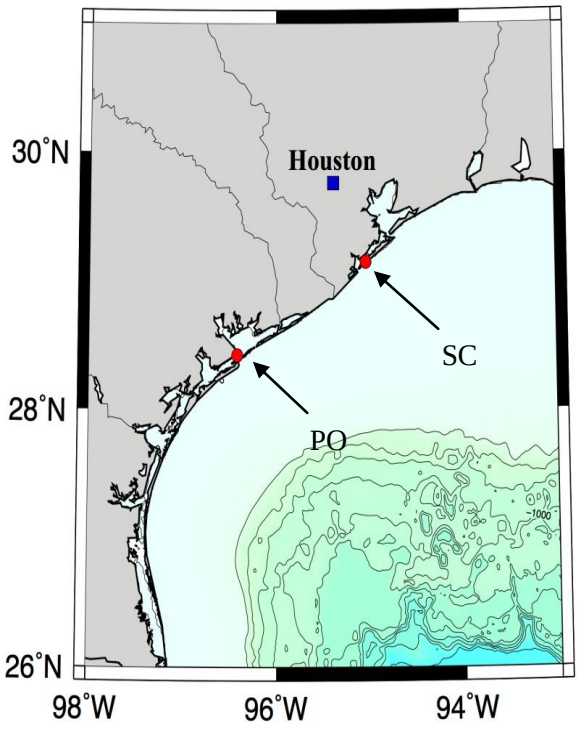

Fig 1. Location of the Sunset Cove (SC) and Port O'Connor (PO) sample sites along the Texas coastline.

ground prior to analysis using a ball mill. Carbonates were removed prior to elemental analysis using wet acidification with hydrochloric acid (HCL) and then dried. Organic carbon (OC) and total nitrogen (TN) concentrations were determined for each sample using a Costech elemental analyzer.

2.2 Carbohydrates Method

Carbohydrates were analyzed according to Skoog and Benner (1997) with some modifications (Kaiser and Benner 2000). Briefly this method involved three steps starting with a hydrolysis, followed by neutralization, and purification/desaltation via solid phase extraction. The treated samples were then analyzed with high pressure liquid chromatography (HPLC). Carbohydrate representatives consist of seven monosaccharide neutral sugars (fucose, rhamnose, arabinose, galactose, glucose, mannose, xylose).

Samples were weighed into 2 mL glass ampoules with the mass varying depending on the percent weight organic carbon (%OC) in the sample (%OC>0.5 = 2.0 mg, %OC≤0.5 = 10.0 mg, %OC≤0.2 = 50.0 mg). Prior to hydrolysis, all samples were first treated with 6 M HCl, mixed, and then dried using a steady flow of nitrogen gas. This was done to ensure the removal of any carbonates present in the sediments. The hydrolysis for particulate material was a 5 hr process that first immersed the sample in 200 µL of 12 M sulfuric acid for 2 hr. This was followed by a dilution to 1.2 M via the addition of 1800 µL of reverse osmosis (RO) water; the ampoules were then sealed and samples hydrolyzed for 3 hr at 100˚C in a water bath.

Neutralization of the samples was performed after allowing the ampoules to first cool to room temperature. It was done by running the samples through about 2 mL of a self-absorbed

retardation resin (AG11 A8, Biorad 50-100 mesh) packed in Biorad polypropylene columns. (Kaiser and Benner, 2000). Prior to the resin treatment samples were rinsed with 40 mL deionized water (20x the resin volume) to remove salts (4 mL/min), leaving about 2 mL of water on top of the resin. Samples were spiked with 50 µL of a prepared deoxyribose surrogate standard immediately prior to neutralization. About 2 mL of sample was added to the column just as the water level reached the top of the resin (2 mL/min). Once the sample was added to the column collection began, and as the column drained an addition 4 mL of deionized water was added to the column to ensure complete elution of the samples. Samples were frozen and stored overnight.

Samples were thawed then purified and desalted using a mixture of cation and anion exchange resins in a 1:1 ratio (AG50 X8, H^+ -form, 100-200 mesh:AG2 X8, HCO_3^- -form, 20-50 mesh). The columns used for this step were Alltech SPE plastic columns with Teflon frits, mounted on a vacuum manifold with attached pump (Kaiser and Benner 2000). Columns were packed with about 2 mL of mixed bed resins, rinsed and drained, followed by the addition of 2 mL of sample; at this point sample and resin were stirred to remove all carbon dioxide from the column. Samples were then collected by vacuum into glass shell vials. Prior to running through HPLC all samples were sparged with helium for 0-2 min to remove any oxygen contained within the solution (Note: sparging times for samples varied throughout the project, a water blank was run at the beginning of each sample group to determine necessary time). Samples were then analyzed for neutral sugars using anion exchange chromatography with a 20 mM NaOH mobile phase on a PA 1 column in a Dionex 500 system with pulsed amperiometric detection (PAD). Calibration was done using a set of internal and external standards. Seven neutral sugars were used: Fucose,

Rhamnose, Arabinose, Galactose, Glucose, Mannose, Xylose (Fuc, Rha, Ara, Gal, Glu, Man, Xyl). Three external standards were used to develop a calibration curve and contained all seven of the neutral sugars with variable concentrations as well as 50 µL of the surrogate standard deoxyribose. The detector setting for the system were the same as those used by Skoog and Benner (1997).

2.3 Lignin Method

Lignin oxidation products (LOPs) were determined using the cupric oxide (CuO) oxidation developed by Hedges and Ertel (1982) with modifications (Louchouarn et al., 2010). The method uses multiple steps including the initial preparation, oxidation, and extraction; followed by analysis via GC/MS. The reaction vessels used for this method were stainless steel reaction mini-vessels (3 mL: Prime Focus Inc.) that were each prepared with 150±4 mg ferrous ammonium sulfate ($Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$), 330±4 mg CuO, and a stainless steel ball bearing to ensure mixing. The amount of sample added to each vessel was adjusted to contain 3-6 mg of OC. In cases where this C amount was not achievable under ~500mg sediment was used. About 2.5 mL, of Argon (Ar) sparged (1 hr and 45 min) 2 N Sodium Hydroxide (NaOH) was added to the vessel (carefully to avoid the addition of any air bubbles to the solution). The headspace of the vessels was then purged with Ar (45 min) in a customized purging block (Prime Focus Inc.); at the end of the purge the caps are tightened. Vessels were removed from the block and checked for leaks as well as free movement of the ball bearing.

The oxidation of the samples was done in a gas chromatograph oven (Hewlett-Packard 5890) with a revolving carousel to stir samples during heating. The oxidation program lasts 3 hr and

reaches a maximum temperature of 154˚C, maintained for 2.5 hr, after heating ramp at a rate of 4.2˚C/min for 30 min.

Upon removing the vessels from the oven the extraction was performed. After opening the reaction vessels each sample was spiked with 50 µL of the surrogate standard, d-7 Cinnamic Acid (250 ng/µL) and then mixed. The vessels were then centrifuged for 5 min (setting 7-8) and the NaOH was decanted into clean, long centrifuge tubes. The sample was then rinsed (3x) with 1-2.5 mL 1 N NaOH and then transferred to a centrifuge tube. After the final rinse, about 2.5 mL of 6 N HCl was added to the centrifuge tube (until pH is 1 or less). A 3 mL volume of ethyl acetate was then added to each sample which was then vortexed for 1 min. Samples were centrifuged (6 at a time) for 5 min for phase separation. The top layer ethyl acetate was then transferred to a new vial. The ethyl acetate addition and subsequent extraction was repeated twice. All samples were then treated with sodium sulfate (Na_2SO_4) to remove any water followed by a transfer of the solution into conical centrivap vials. Samples were dried completely at 45˚C in a centrivap prior to resuspension and extraction in two 200 µL additions of pyridine.

All samples were diluted and derivatized before running on the Gas chromatography-mass spectrometry (GC/MS). A 10x dilution (sample:pyridine) was prepared and 50 µL of the new diluted sample was combined with 50 µL internal standard (IS) (d-7 Ad, 2 ng/µL) and 50 µL N,O-bis(trimethylsilyl)trifluoroacetamide (BTSFA) in a new sample vial. Standard vials were also prepared with 50 µL calibration standard (LOP std 1.0 ng/µL), 50 µL IS, and 50 µL BTSFA. Samples were gently mixed and then vortexed for 3-5 sec prior to being placed in a preheated 20-wells block heater (75˚C) to derivitize for 30 min.

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 were analogous to Louchouarn et al. (2010).

CHAPTER III

RESULTS

3.1 Elemental Composition and Organic Carbon Fluxes

Measurement of total OC (TOC) showed a range of concentrations from 0.07-2.00 wt %OC in the Port O'Connor core and 0.11-4.36 wt %OC in the Sunset Cove core (Fig. 3). The highest wt %OC were observed in the upper ten centimeters (cm) followed by sharp declines with depth in both cores. Higher wt %OC were observed in the Sunset Cove core in surface sediments, but wt% OC at depth were similar in both cores.

The next dataset looked at the change in ratio of atomic C to Nitrogen (C/N) with depth (Fig. 4). Values were calculated by converting the %OC and %N to atomic units and then taking the ratio between the two. Both cores showed an overall decrease of C/N ratios with depth. N concentrations >24 cm were below the detection limit in Sunset Cove core. C/N ratio in Port O'Connor ranged from 7-25, while Sunset Cove ranged from 5-32. There are some large fluctuations in values, however, and more investigation is needed to determine cause.

Initially, the concentration of OC characterized in lignin and carbohydrates is much higher in Sunset Cove compared to Port O'Connor resulting in a higher flux of OC into surface sediments (Fig. 5). As depth increases with age however, the Sunset Cove sediments displays sharp decline relative to Port O'Connor. While the flux at the bottom of the core is similar, Sunset Cove is shown to have higher preservation at these two sites. The observed range of OC flux is 4-72 mg/m^2yr and 5-104 mg/m^2yr for Port O'Connor and Sunset Cove, respectively.

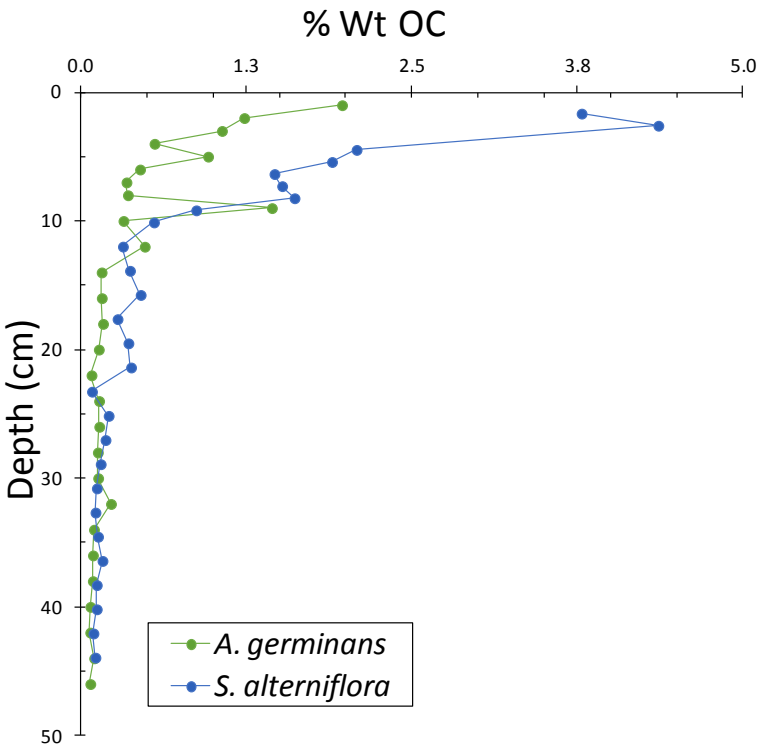

Fig 3. TOC with depth for *A. germinans* (Port O'Connor) and *S. alterniflora* (Sunset Cove).

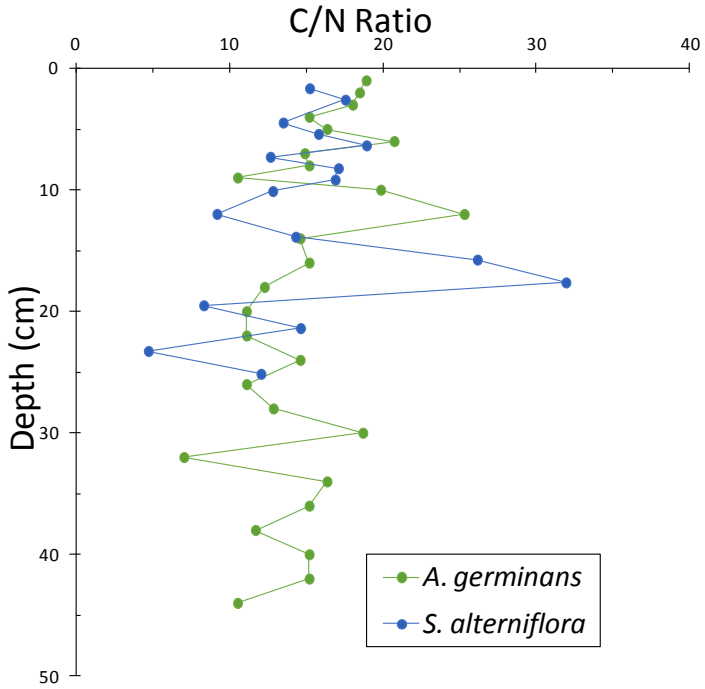

Fig 4. Atomic C/N ratio for *A. germinans* (Port O'Connor) and *S. alterniflora* (Sunset Cove).

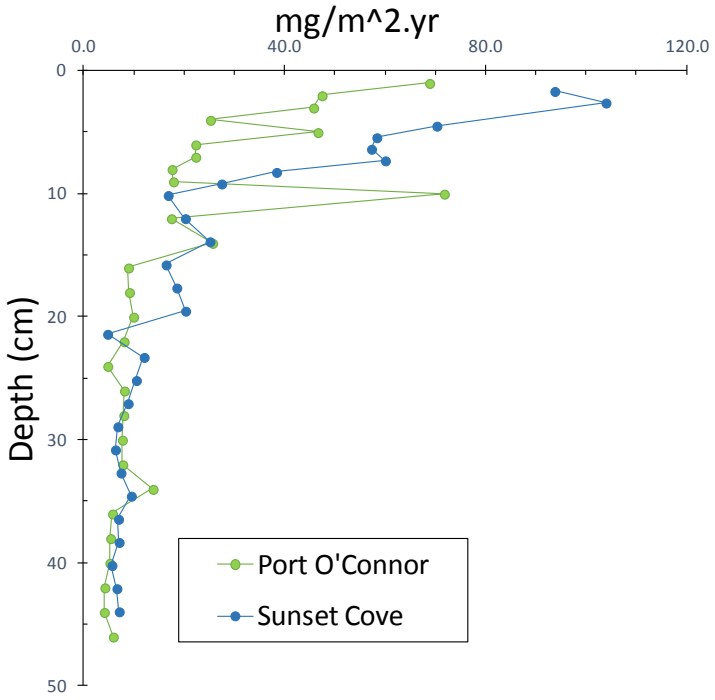

Fig 5. Total flux of OC for Port O'Connor (*A. germinans)* vs. Sunset Cove (*S. alterniflora*).

3.2 Chemical Composition

The chemical composition of dominant surface vegetation was analyzed to determine the occurrence and yields of carbohydrates and phenols (Tables 1, 2, and 3). The survey included senescent leaves, woody material and root biomass from *A. germinans*, above and below-ground tissue samples from *S. alterniflora* and Badis grass (*B. Copano*) tissue samples. Glu dominated carbohydrate compositions in all plant tissues examined. High Xyl contributions were found in Spartina and Badis Grass tissue. Composition of the remaining neutral sugars varied between the different tissues and plant types. Both plant types, *A. germinans* and *S. alterniflora*, are enriched in glucose; 45% and 58% total neutral sugars respectively. These values are averaged between tissue types of each respective plant type. *A. germinans*, however, is enriched in arabinose, galactose, and rhamnose (17%, 14%, and 7%, respectively) relative to *S. alterniflora* which itself

is enriched in xylose (32%). The observed variation in neutral sugar composition between plant types was used to develop a vegetation index (Fig. 11).

The primary tissue found in litter material is leaves in *A. germinans* and above ground shoot material in *S. alterniflora*. For this reason, calculations done using senescent litter data exclude values for *A. germinans* twigs and *S. alterniflora* below ground material. The yields of neutral sugars in *A. germinans* averaged 33.0±15.6 %OC, while *S. alterniflora* yields averaged 42.0±13.4 %OC (Table 2). Phenol yields measured averaged 12±7.4 %OC and 17.4 %OC for *A. germinans* and *S. alterniflora*, respectively (Table 3).

Table 1. Compositional background of analyzed surface vegetation.

	wt% OC	wt% N	C/N
A.germinans Root	40.58	0.56	85
A.germinans Twigs	42.11	1.55	32
A.germinans Leaves	43.02	0.84	60
S. alterniflora Shoot	40.91	1.07	45
S. alterniflora Root	27.30	NM	NM
B.copano Shoot	40.74	0.90	53

*****Note: *A. germinans* twig material was not included in calculations involving senescent values; leaf and below ground tissues were averaged. Not measured (NM)

Table 2. Neutral sugar composition in analyzed surface vegetation.

	Fuc (mol%)	Rha (mol%)	Ara (mol%)	Gal (mol%)	Glu (mol%)	Man (mol%)	Xyl (mol%)
A.germinans Root	0	3	12	23	43	2	17
A.germinans Twigs	0	4	13	19	43	2	19
A.germinans Leaves	0	10	22	5	48	2	13
S. alterniflora Shoot	0	0	7	2	58	1	32
S. alterniflora Root	0	1	14	5	47	1	33
B.copano Shoot	0	2	16	6	39	3	34

Table 3. Lignin phenol composition in analyzed surface vegetation.

	Vanillyl (mol%)	Syringyl (mol%)	Phenyl (mol%)	Cinnamyl (mol%)
A.germinans Root	8	12	75	5
A.germinans Twigs	5	13	33	50
A.germinans Leaves	6	7	83	5
S. alterniflora Shoot	3	20	32	44
S. alterniflora Root	NM	NM	NM	NM
B.copano Shoot	2	22	70	6

The major neutral sugars measured in both sediment cores were Fuc, Rha, Ara, Gal, Glu, Man, Xyl (Fig. 6). Compositional trends showed that both dominant plant types were enriched in glucose, with an average of 45% and 39% total carbohydrates for Port O'Connor and Sunset

Cove, respectively. The ratios of other sugars however showed variation between cores; Sunset Cove, (*S. alterniflora*) was enriched in xylose relative to Port O'Connor (*A. germinans*), 28% vs. 9% total sugars. Alternatively, Port O'Connor was enriched in Ara, Gal, and Rha relative to Sunset Cove; 19% vs. 13% Ara, 12% vs. 9% Gal, and 7% vs. 3% Rha.

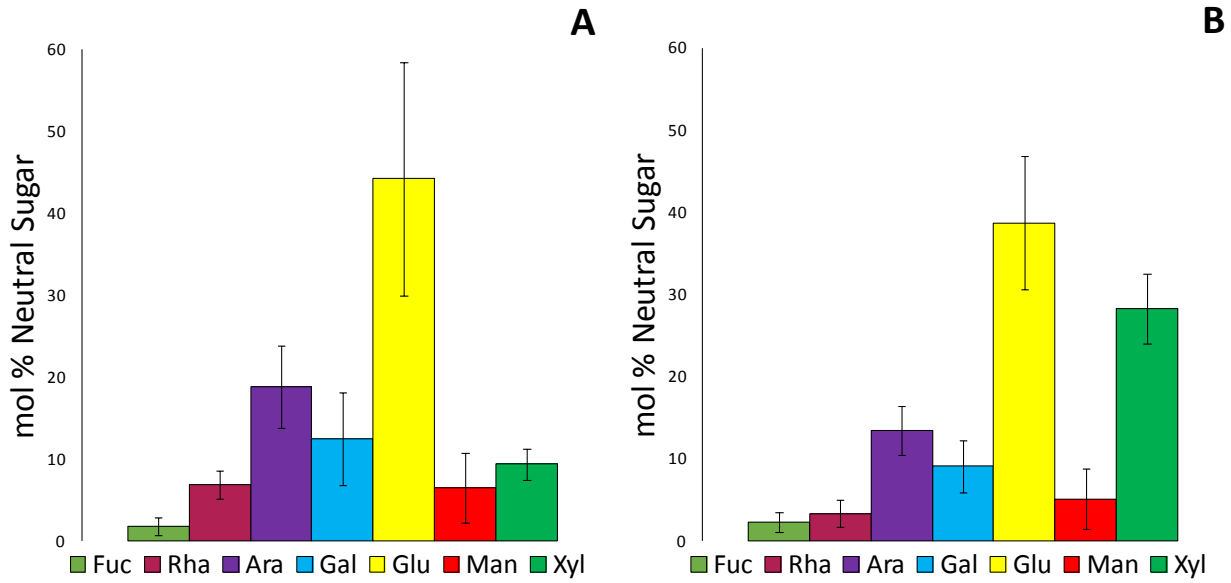

Fig 6. Mol percentages (mol%) of neutral sugars in the Port O'Connor core (*A. germinans*) (A) and Sunset Cove (*S. alterniflora*) (B).

The molar % total lignin phenols was calculated according to the method described in Louchouarn et al. (1997) to determine compositional differences between dominant plant types with respect to phenols; which are considered here as a sum of the 11 CuO oxidation products (Fig. 7) (Louchouarn et al., 1997). The included phenols are: *p*-hydroxybenzoic acid (Pd), *p*-hydroxybenzaldehyde (Pl), *p*-hydroxyacetophanone (Pn), Vanillic acid (Vd), Vanillin (Vl), Acetovanillone (Vn), Syringic acid (Sd), Syringealdehyde (Sl), Acetosyringone (Sn), *p*-coumaric acid (Cd), and Ferulic Acid (Fd) (Hedges et al., 1988). Relative abundances of phenols were calculated by combining the % total lignin for each class of phenol. Phenyl (P) = Pd+Pl+Pn, Vanillyl (V) = Vd+Vl+Vn, Syringyl (S) = Sd+Sl+Sn, and Cinnamyl (C) = p-Cd+Fd. Both cores

demonstrated a high relative abundance of S phenols with 38% and 36% total lignin for Port O'Connor and Sunset Cove, respectively. Both cores showed similar proportion of V phenols at 23% and 22% for Port O'Connor and Sunset Cove, respectively. The Port O'Connor core was enriched in P phenols relative to Sunset Cove, 20% vs. 12% P. In contrast, Sunset Cove was observed to be enriched in C phenols; 30% vs. 20% C, for Port O'Connor vs. Sunset Cove, respectively.

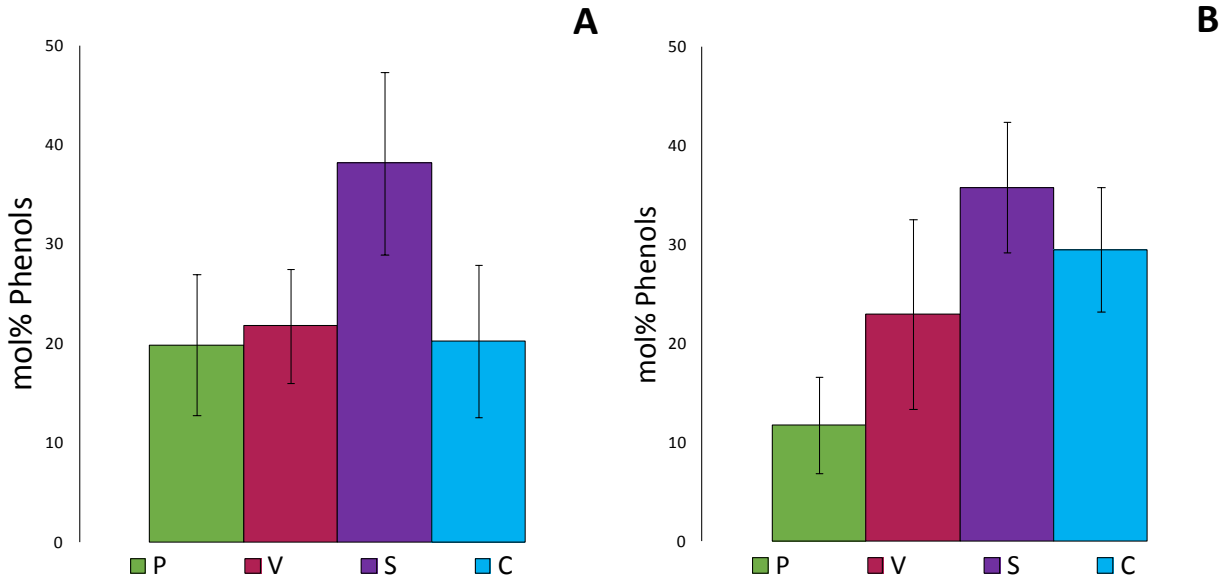

Fig 7. Mol % of phenols in the Port O'Connor core (A. *germinans*) (A) and Sunset Cove (*S. alterniflora*) (B).

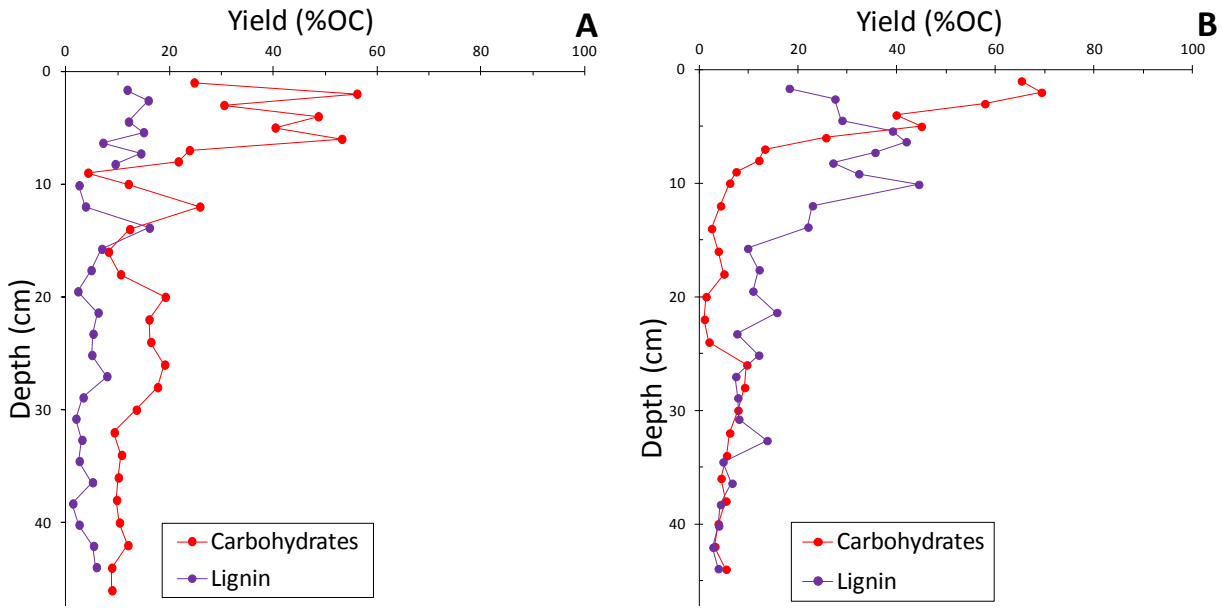

Fig 8. Compositional breakdown of both lignin and carbohydrate components of OC in the Port O'Connor (A) and Sunset Cove (B) cores.

The contribution of neutral sugar and lignin phenol C to the total C pool is presented in Fig. 8. Percentage values showed that carbohydrates comprised 4-56 % of sedimentary OC (SOC) in both cores, whereas lignin comprised 1-16 % of the OC pool. The proportion of carbohydrates and phenols were generally highest in the surface and sharply decreased with depth in both sediment cores.

The C normalized yields (%OC) of phenols and carbohydrates were summed to characterize OC composition in sediments (Fig. 9A). In the surface sediments close to 100% of all OC are classified using only free lignin phenols and carbohydrate neutral sugars demonstrating that the sedimentary OM is predominantly derived from plant OM. Sharp declines are observed after the first ten cm with only a small fraction of input OC characterized at depth, the Port O'Connor core displays a range of 71.8 – 6.7 yield %OC while Sunset Cove shows 96.7 – 5.7 yield %OC.

A ratio of carbohydrates to lignin yield %OC was plotted (Fig. 9B). The values are similar between cores in surface sediments however, a sharp decline is observed in the *S. alterniflora* dominant Sunset Cove core. Alternatively, the Port O'Connor, *A. germinans* dominant core, displays continuous oscillation in values throughout the core with a range of 7.6 – 1.5. Sunset Cove (*S. alterniflora*) shows an observe d range of 3.6 – 0.1

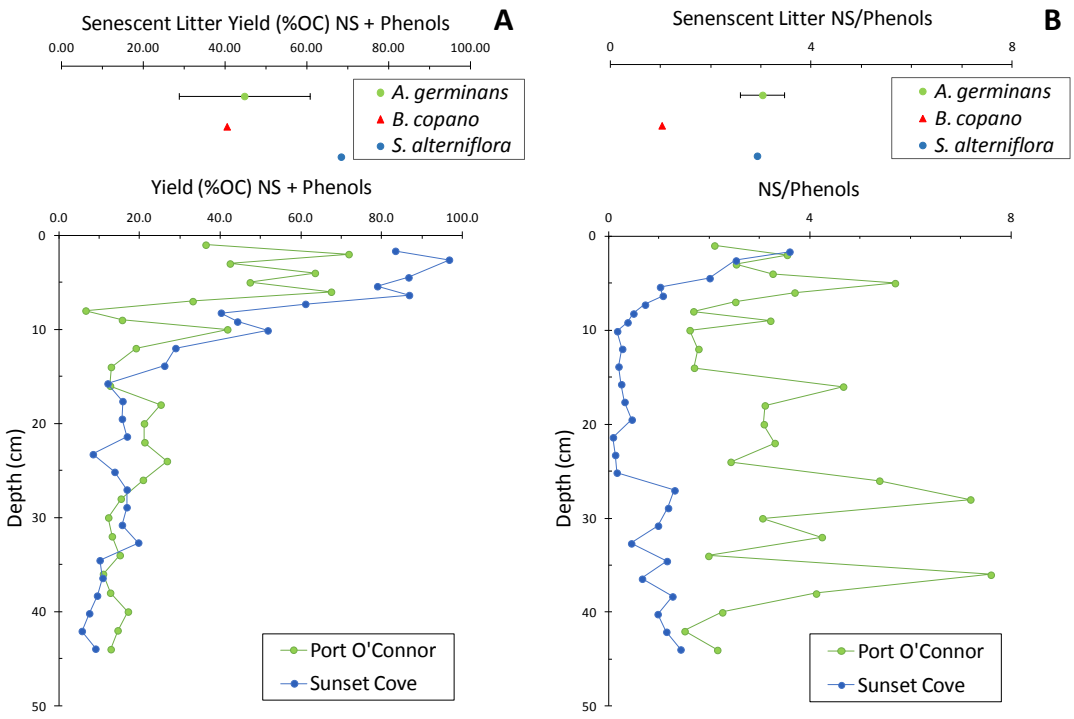

Fig 9. Sum of the two yield %OC components, lignin and carbohydrates, includes senescent litter material; in Port O'Connor (*A. germinans)* vs. Sunset Cove (*S. alterniflora*) (A). Comparison of component yields between Port O'Connor (*A. germinans)* vs. Sunset Cove (*S. alterniflora*) (B).

3.3 Vegetation Analysis

A vegetation index was calculated based on carbohydrate compositions in senescent plant materials (Figures 10 and 11). Based on this index it is possible to fingerprint source input with the potential to show any change in dominant vegetation occurring within the core.

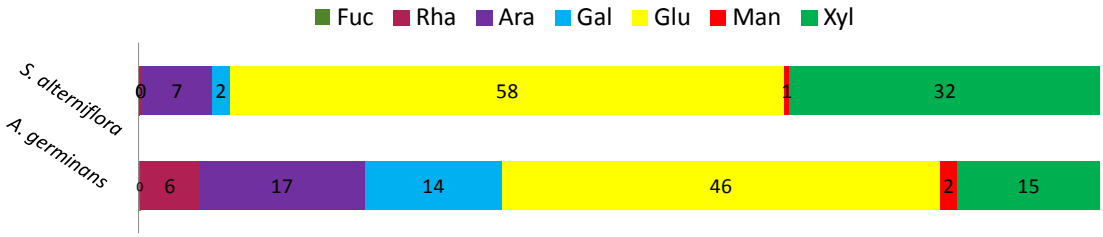

Fig 10. Mol percentages (mol%) of neutral sugars for *A. germinans and S. alterniflora* senescent litter material

A ratio was developed to compare the sum of rhamnose and galactose to the sum of arabinose and xylose in order to identify the source input type.

$$Vegetation\ Index = \frac{Rhamnose + Galactose(\frac{nmol}{mg}Sample)}{Arabinose + Xylose\ (\frac{nmol}{mg}Sample)}$$

The vegetation index ranged from a minimum of 0.14 to a maximum of 0.92 in both sediment cores (Fig. 11). The index was generally higher in the *A. germinans* dominated Port O'Connor core than in the *S. alterniflora* dominated Sunset Cove core. Both cores showed a consistent shift to more positive indices with depth, but did not indicate a shift in the dominant vegetation.

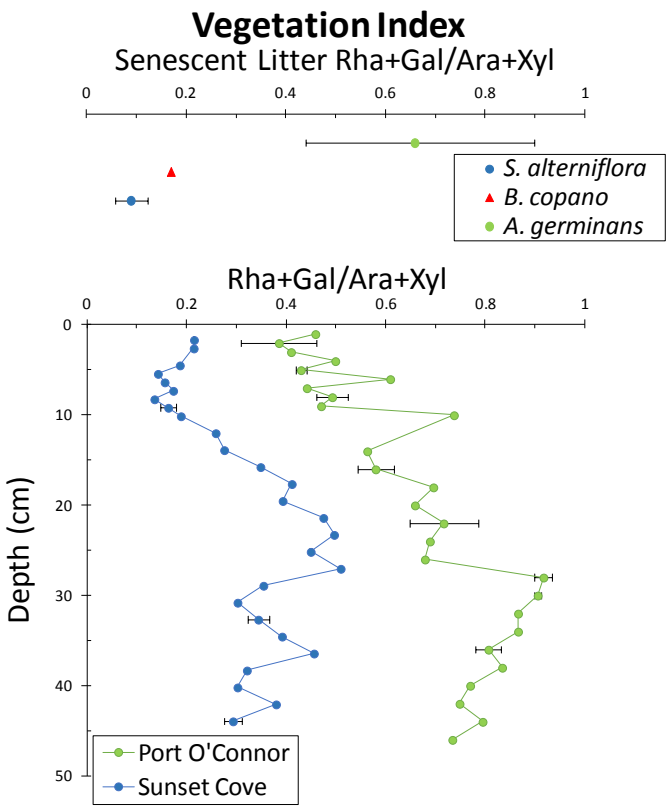

Fig 11. Vegetation index to fingerprint input plant source for each both the Port O'Connor (*A. germinans)* vs. Sunset Cove (*S. alterniflora*), including senescent material.

3.4 Decomposition Indicators

Acid/aldehyde ratios of vanillyl (V) and syringyl (S) phenols ($[Ad/Al]_{V,S}$) follow the decomposition of vascular plant-derived lignin with depth (Fig. 12). Increasing $[Ad/Al]_{V,S}$ ratios were observed for both dominant plant types as burial time increased. Port O'Connor displayed a range of 0.19 – 3.33 $[Ad/Al]_V$ and 0.09 – 1.18 $[Ad/Al]_S$, while. Sunset Cove showed ranges of 0.14 – 0.54 and 0.10 – 0.32 $[Ad/Al]_{V,S}$, respectively. A large spike in the Port O'Connor core occurred at a depth of about 20 m, suggesting extensive decomposition of vascular plant-derived organic matter.

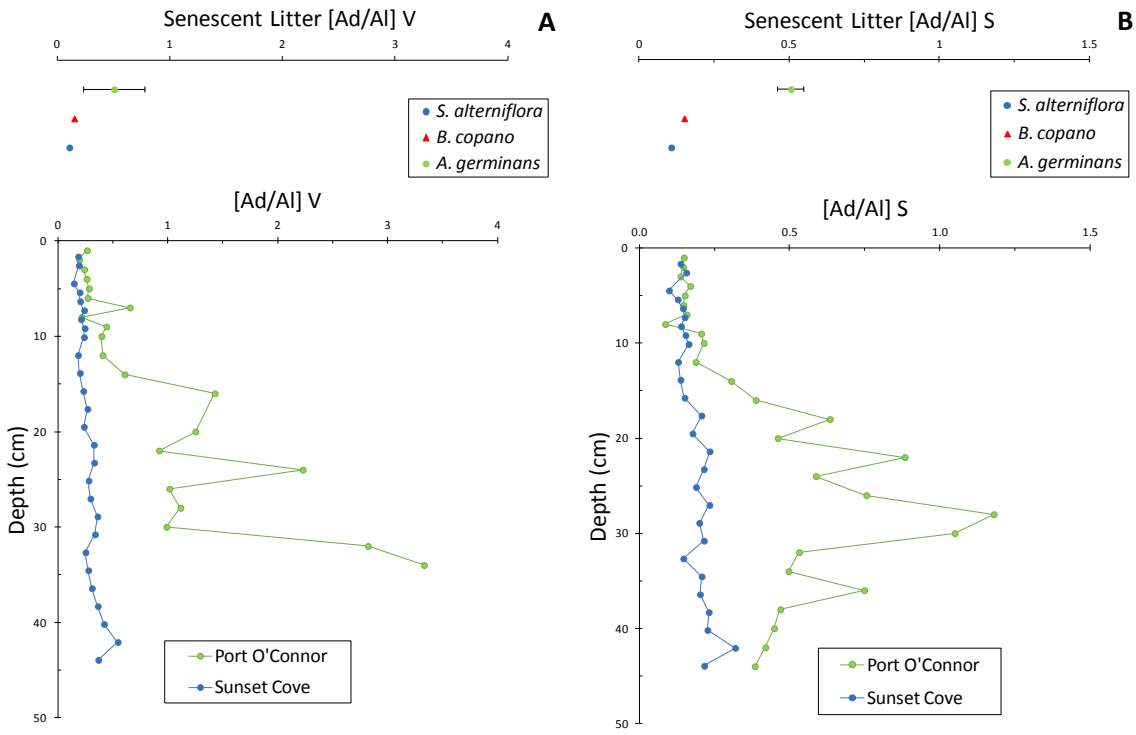

Fig 12. Acid/Aldehyde ([Ad/Al]) ratio, for vanillyl (V) (A) and syringyl (S) (B) for Port O'Connor (*A. germinans)* vs. Sunset Cove (*S. alterniflora*), including senescent material

The degradation of the carbohydrate component of both cores was calculated as the ratio of carbon-normalized yields of neutral sugars in senescent litter material and samples.

$$Sugar\ degradation\ index = \frac{Sediment\ Carbohydrates\ Yield\ \%OC}{Senescent\ Litter\ Total\ Yield\ \%OC}$$

The sugar degradation index was < 1.5 in the upper layer s of the cores (Fig. 13) indicating sediment organic matter had similar neutral sugar yields compared to fresh vegetation. Below 10 cm, the index increased to >2 with a maximum value of 40 in the Sunset Cove core. Sugar degradation indices were much higher in the Sunset Cove core than in the Port O'Connor suggesting a much greater degree of decomposition in the *S alterniflora* dominated, Sunset Cove,

sediment core. Comparison of the sugar degradation index against both [Ad/Al] V, and S plots show only very weak correlation between the two compound classes (Figures 12 and 13). Port O'Connor displays higher degrees of degradation in lignin relative to carbohydrates, while Sunset Cove displays more extensive degradation of its sugar component.

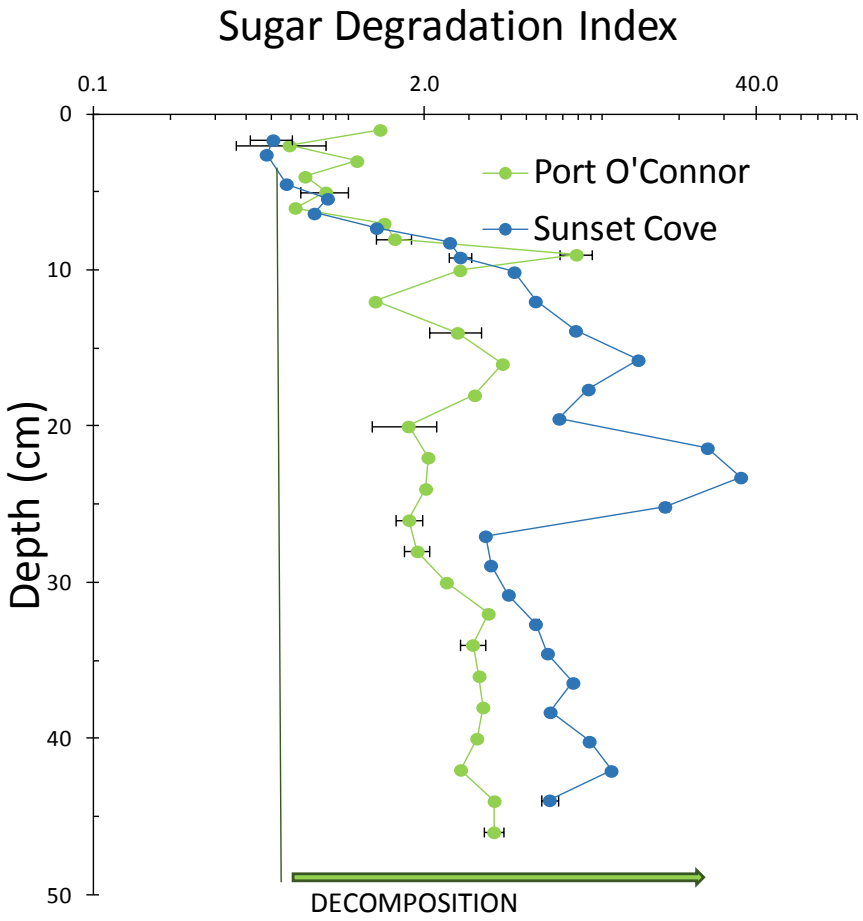

Fig 13. Sugar degradation index for Port O'Connor (*A. germinans)* vs. Sunset Cove (*S. alterniflora*), including senescent material. Error bars show the precision of the analysis calculated from duplicates.

CHAPTER IV

DISCUSSION

4.1 Environmental history of vegetation at Sunset Cove and Port O'Connor

One factor that prompted this study was the observed trend of a northward moving ecotone between *A. germinans* and *S. alterniflora*, along the Texas coastline (Chmura et al., 2003; Comeaux et al., 2012; Giri and Long, 2014). This observed shift from C_4 to C_3, herbaceous to woody plant type is related to a decline in the frequency and intensity of freezes during coastal winters; to which *A. germinans* is relatively intolerant (Chmura et al., 2003; Comeaux et al., 2012; Bianchi et al., 2013). The two selected cores for this study represented each of the two plant types, *Avicennia germinans* (Port O'Connor) and *Spartina alterniflora* (Sunset Cove). Both of these tidal saline plants accumulate soils vertically while they accrete in equilibrium with sea level rise making them efficient tools for the mitigation of rising atmospheric CO_2 concentrations (M^{c}leod et al., 2011; Bianchi et al., 2013). Chmura et al. (2003) observed that while rates of below ground C sequestration for the two plant classes are about the same worldwide (42.6 ± 4 Tg C yr^{-1}) the size of the C pool is much greater in the biomass of *A. germinans* dominant ecosystems with an estimated pool of 5000 ± 400 Tg C worldwide and average soil C density of 0.055 ± 0.004 gcm^{-3}. *S. alterniflora* has an estimated pool of only 430 ± 30 Tg C and average soil C density of 0.039 ± 0.003 gcm^{-3} (Chmura et al., 2003). If the expansion of C_4 woody *A. germinans* continues northward, there is the potential for the tidal saline ecosystems to become even more effective in sequestering C. These habitats need to be preserved and promoted in a world with a continuously increasing need for the mitigation of elevated atmospheric CO_2.

Our analysis of the botanical origin of sedimentary organic matter (SOM) took advantage of specific hemicellulosic carbohydrate compositions in *A. germinans* and *S. alterniflora*. *A. germinans* is characterized by relatively high proportions of Rha and Gal, whereas *S. alterniflora* has higher relative contributions of Xyl. The calculated vegetation index separates both types of vegetation well and, therefore presents a relatively robust tool to follow changes in the composition of the plant community.

The vegetation index clearly shows the dominance of S. alterniflora at the Sunset Cove location and A. germinans at Port O'Connor in the upper sections of the cores, indicating the absence of a change in the dominant plant and ecosystem type over the last 40 years. Both cores also exhibited a progressive shift to higher vegetation index values downcore, which could be interpreted as a shift in vegetation towards *A. germinans* in the Sunset Cove core. The shifts in vegetation indices were well correlated with increasing decomposition as indicated by the sugar degradation index and $[Ad/Al]_{V,S}$ ratios. Rather than a shift in vegetation it is likely that extensive decomposition caused the progressive change of the vegetation index with depth.
It has been observed that neutral sugars are not uniformly distributed among plant polysaccharides, making these ratios sensitive to diagenetic alteration. The mol% Rha was shown to be higher in soil organic matter than fresh plant material (Derrien et al., 2007), and increases during the decomposition of plant litter as a result of incorporation of microbial detritus (Hedges et al., 1994; Opsahl and Benner, 1999) In contrast, Xyl tends to be more reactive than other sugars and the mol% Xyl decreases with decomposition (Opsahl and Benner, 1999).

4.2 Decomposition of Organic Matter

The extensive decomposition of plant material in both cores was indicated by several independent decomposition proxies. The C:N ratio showed a gradual decline with depth consistent with the respiration of OC to CO2 and retention of N by microbes. Two additional proxies, acid/aldehyde ratios of vanillyl (V) and syringyl (S) phenols ($[Ac/Al]_{V,S}$) and the sugar degradation index provided a molecular view on the extent of decomposition of organic matter in sediments. These molecular decomposition indicators tracked the decomposition of different organic constituents of sedimentary OM. The sugar degradation index was sensitive to the polysaccharide component of plants, whereas $[Ac/Al]_{V,S}$ is specific to the lignin component of vascular plants. Both proxies show different sensitivities to the decomposition of respective biopolymers. Selective loss of neutral sugars was observed in highly decomposed vascular plant tissues after loss of >80% of total plant mass (Opsahl and Benner, 1999).

Increasing $[Ac/Al]_{V,S}$ ratios are observed with depth indicating decomposition of the lignin component of litter detritus in both Sunset Cove and Port O'Connor cores. The Port O'Connor core lignin component derived mainly from A. germinans litter exhibited a much higher degree of degradation relative to the lignin contained in the Sunset Cove core. Mangrove leaves contain a high percentage of soluble components that appear to stimulate decomposition of the lignocellulosic component (Lee et al., 1990). Alternatively, the loss of soluble components exposes lignocellulosic detritus to microorganisms attached to leaves potentially enhancing decomposition.

The decomposition of the carbohydrate component of both Port O'Connor and Sunset Cove was calculated via the ratio between senescent litter material %OC and yield (%OC) in carbohydrates (at depth). Both cores show a steady trend of increasing decomposition with depth.

Sunset Cove displays a larger degree of degradation in its carbohydrate component relative to Port O'Connor. The apparent differences in the extent of decomposition of the lignocellulosic component from S. alterniflora and A. germinans is consistent with previous studies. As pointed out by Lee et al. (1990), soluble components of mangroves leaves enhance the decomposition of mangrove-derived lignocellulose. This leads to microbial utilization of cellulose, hemicellulose and lignin at similar rates as indicated by relative constant neutral sugar to phenol ratios in the Port O' Connor (Fig. 9B).

Decomposition studies with S. alterniflora tissue generally show a strong enrichment of lignin due to recalcitrant nature. Given the differences observed between the two cores and their respective dominant plant type it can be concluded that different vegetation leads to storage of different components of plant detritus. Due to the discussed effects of leaching in enhancing degree of decomposition in Mangrove ecosystems OM in Sunset Cove is compositionally different than Port O'Connor.

4.3 Controls of OC Decomposition

Our study tested OC dynamics in only two cores of the two plants types to elucidate key factors controlling OC preservation in coastal ecosystems. Several hypotheses have been offered to explain the persistence of organic matter in soils and sediments. One school of thought concludes that decomposability of OM in soils is a function of molecular composition and structure

(Benner et al., 1990; Hedges et al., 2000; Schmidt et al., 2011). Alternatively, another theory deems decomposition of OM to be controlled by environmental factors including microbial activity and distribution, sorption onto mineral surfaces, sediment size, pore space, etc. (Ekschmitt et al., 2008; Schmidt et al., 2011).

Comparison of the Port O'Connor and Sunset Cove cores displays key differences in the chemical composition of dominant plant types (Fig. 5, 6, and 7). Both cores display enrichment in the carbohydrate components relative to lignin, however the extent of degradation with increasing depth for each component in its respective dominant plant type is varied. Both plant types displayed enrichment in the neutral sugar glucose and the lignin phenol syringyl, whereas relative abundances of other lignin and carbohydrates varied with plant type. Carbohydrates are shown to be more vulnerable to decomposition compared to lignin, which follows the conclusions of Borch (1997) that states that carbohydrates are the more labile of the two compound classes (Valiela et al., 1984; Benner et al., 1990; Borch, 1997). Considering that *S.* alterniflora is more enriched in carbohydrates relative to lignin, the litter is understandably more decomposable leading to the results from the Sunset Cove core. In comparison, *A. germinans* has a much lower ratio of carbohydrates to lignin and therefore a larger degree of resilience to decomposition, reflected in the observed trend from the Port O'Connor core.

With respect to lignin, it has been suggested by Bianchi et al. (2013) that higher concentrations of the more labile cinnamyl phenols will result in selective loss; which could be a contributing factor to the variability in the C pool size for these two plant types (Benner and Opsahl, 2001; Bianchi et al, 2013). *S. alterniflora* plant tissue contains a much higher average concentration of

cinnamyl phenols relative to *A. germinans.* The $[Ad/Al]_{V,S}$ index (Fig. 11) point to a more degraded phenol component in the Port O'Connor core, to be much more degraded relative to the Sunset Cove core. However, it was observed by Benner et al. (1990) that [Ad/Al] ratios should be used with caution with respect to Mangrove plant litter which exhibits very high [Ad/Al] values in leached material due to preferential loss of vanillyl lignin phenols (Benner et al., 1990). This is reflected in the data with elevated ratio values for *A. germinans* senescent material which is also observed in the Port O'Connor core. Benner et al. (1990) also observed that the relative ratios of carbohydrates to lignin remained constant throughout degradation in mangrove ecosystems (Benner et al., 1990); Figure 8(B) supports this observation. These observations display the extensive effects of litter chemistry on overall degradability of tidal saline plant material.

With respect to external factors acting in concert with internal litter composition, rate of sediment accumulation is likely to have a large degree of control over C burial due to its control over oxygen exposure time. Valiela et al. (1984) observed higher degrees of degradation in a litter incubation experiment with *S. alterniflora* when the senescent material was in aerobic conditions (Valeila et al., 1984). Due to the observed differences in vertical accumulation between plant types, oxygen exposure time would be expected to be shorter in *A. germinans* dominant ecosystems due to its higher rate of sediment accumulation relative to *S. alterniflora* (Bianchi et al., 2013; Chmura et al., 2003).

Microbial communities are another external factor controlling extent of decomposition in sediments (Valiela et al., 1984; Benner et al., 1990; Opsahl et al., 1999; Benner et al., 2001).

Although relative activity of these communities fluctuates with temperature, however the relationship between temperature and microbial activity has been shown by Chmura et al. 2003 to only account for <25% of the variation in degradation for *A. germinans* and *S. alterniflora* (Chmura et al., 2003). Valiela et al. (1984) suggested that microbes show preferential uptake of litter rich in N and low in lignin (Valiela et al., 1984). These observations are supported by the collected data, as *A. germinans* litter material is observed to be lower in N and higher in lignin relative to *S. alterniflora*. Preferential degradation of OC in the Sunset Cove core, again supports the relationship between microbial activity and litter composition. It is more likely that the affinity of microbes for specific chemical compounds is what drives extent of degradation in litter material.

Overall, it can be concluded that the compositional makeup of *A. germinans* makes it the less decomposable of the two plant types. OC accumulation is only higher in the Sunset Cove core in the top layers of sediment, where degradation indicators show limited decomposition. However, due to the lower %OC initially input to the sediments the overall amount of OC remaining at depth is similar for both cores. As these plants mature they have been shown to increase in productivity (Bianchi et al., 2013) which allows the conclusion that as these populations grow over time their resilience will result in a large pool of preserved OC in sediments.

4.5 Conclusions

1. There has been no shift in dominant vegetation type in the Sunset Cove core throughout the past 40 years, where the vegetation index is not compromised by degradation.

2. The lignocellulosic component of *S. alterniflora* has different sensitivity to decomposition than the lignocellulosic component of *A. germinans*.
3. The chemical composition of litter material controls the composition and magnitude of OC stored in sediments.
4. If *A. germinans* expansion continues there is the potential for C sequestration efficiency to decline due to the changing composition of OC input to sediments and loss of the more recalcitrant *S. alterniflora* plant tissue.

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