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Vegetative Community and Health Assessment of a Constructed Juncus-Dominated Salt Marsh in The Northern Gulf Of Mexico

Nickolas R. Murphy

VEGETATIVE COMMUNITY AND HEALTH ASSESSMENT OF A CONSTRUCTED *JUNCUS*-DOMINATED SALT MARSH IN THE NORTHERN GULF OF MEXICO

by

Nickolas Murphy

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Approved by:

Dr. Patrick D. Biber, Committee Chair
Dr. Zachary Darnell
Dr. Safra Altman

Dr. Patrick D. Biber Committee Chair	Dr. Robert J. Griffitt Director of School	Dr. Karen S. Coats Dean of the Graduate School

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ABSTRACT

Deer Island is a coastal habitat which provides a buffer from storm and flood damage as well as shore-line stabilization to the mainland of Biloxi, Mississippi. A third of the land has been lost since 1850, largely driven by tropical storm and hurricane impacts as well as sea level rise. The United States Army Corps of Engineers and Mississippi Department of Marine Resources have targeted the shores of the island as sites for restoration using beneficial use dredged material, and two sites of differing age have since been planted with *Spartina alterniflora, Juncus roemerianus, Uniola paniculata, S. patens,* and *Panicum amarum*. Ecological assessment and monitoring of this restoration project was completed by measuring elevation, soil condition, vascular plant diversity, biomass, and the stable isotopes $\delta^{13}C$ and $\delta^{15}N$ from *Spartina alterniflora* tissues. Additionally, sea level rise was projected at the two constructed sites under three scenarios to assess the sites' vulnerability to rising sea levels. The constructed sites were found to have a diverse array of salt marsh and sand-berm vegetation, but function of the salt marsh in terms of root production and sediment organic carbon deposition remained underdeveloped when compared to the natural reference site. All sites were found to be vulnerable to sea level rise except under the lowest sea level rise scenario. Further monitoring should be completed to observe the development of ecological functions at these constructed marshes.

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CHAPTER I - INTRODUCTION

Coastal environments are home to many types of organisms; their interactions form diverse biological communities that supply ecosystem services, which are received by humans on both regional and global scales. Salt marshes, mangroves, and coral reefs are the classic types of coastal habitats and each support global biodiversity to varying degrees. Approximately 950,000 ha of the Gulf of Mexico coastline is emergent salt marsh, which supports a variety of plants, invertebrates, birds, mammals, and fish (Engle 2011). In the state of Mississippi (MS), there are approximately 60’000 ha of coastal marshes (Eleuterius 1973). Plants in salt marshes are, like in many ecosystems, are the primary source of habitat and photosynthetic resources for higher trophic levels. Both global and regional human activity has increased the rate of loss of these valuable, but vulnerable coastal habitats. State and national agencies have attempted to offset these losses and facilitate the formation of new wetlands by implementing various wetland restoration and enhancement mitigation strategies across the United States. However, restoration projects are costly and often executed in unique environments, and thus require habitat-specific planning and post-construction assessment to better inform and advise future restoration efforts to maximize return on investment.

1.1 Salt marsh community structure

Salt marsh habitat is provided by the emergent and submerged vegetation which occur in the interface between brackish or saline open water and the upland dunes and forests. This interface exhibits zonation, which is a classic salt marsh phenomenon influenced by both biotic and abiotic factors (Eleuterius and Eleuterius 1979, Packham and Willis 1997, Emery et al. 2001, Bockelmann et al. 2002). Zonation in northern Gulf of Mexico salt marshes is marked by three distinct vegetation zones described by Eleuterius (1972): 1) 0.0 – 0.54 meters above mean sea level (MAMSL) elevation zone dominated by the smooth cordgrass, *Spartina alterniflora* Loisel, 2) 0.54 – 0.7 MAMSL dominated by the black needlerush, *Juncus roemerianus* Scheele, and 3) zone greater than 0.7 MAMSL with an amalgam of grasses, herbaceous and woody plants. These vegetation zones occur along an elevation gradient; at lower elevations waterlogging and saline water exclude most plant species, and higher in the marsh platform abiotic stress is replaced by competition between species for nutrients, space, and light.

Waterlogged soils present challenges from sulfide toxicity, loss of oxygen to roots, and lower soil nitrogen concentrations via denitrification (Adam 1990). The effects of salinity range from reduced water uptake to injured cells. *S. alterniflora* can colonize and dominate the lower, more frequently inundated parts of the marsh due to adaptations to cope with the abiotic stress. Key adaptations seen in *S. alterniflora* are: 1) porous aerenchyma cells that allow air to diffuse into the roots, 2) localized ion storage to dead and senescing tissues, and 3) production of dimethylsulfoniopropionate (DMSP), a molecule which may improve resistance to sulfide toxicity (Adam 1990). These adaptations can be found across many salt marsh plants, and the prevalence of them in *S. alterniflora* makes this grass a prime competitor in the lower marsh, but the adaptations are metabolically costly. In the *J. roemerianus* dominated zone, there is still stress from waterlogging, however, it is less impactful as inundation is more infrequent than in the lower *S. alterniflora* dominated zone. In this mid-marsh zone, it is likely that *J. roemerianus* outcompetes *S. alterniflora* where it can tolerate waterlogging and salinity. This trend follows in the high-marsh zone where halophytic, rapidly growing grasses such as *Distichlis spicata* L. (saltgrass) and *Spartina patens* (Aiton) Muhl. (salt marsh hay) tend to flourish under conditions with low risk of waterlogging. The diversity of marsh vegetation and zonation promotes a variety of sub-habitat conditions that enhance the capacity of ecosystem services provided by these coastal habitats.

1.2 Salt marsh ecosystem services

Salt marsh vegetation provides numerous ecosystem services to the surrounding environment (Barbier et al. 2011, Engle 2011). Perhaps the most apparent ecosystem service is the habitat provided by salt marshes to invertebrates, juvenile fish, birds, and small mammals. Gulf coast marshes are places of refuge for *Farfantepenaeus aztecus* (brown shrimp), *Litopenaeus setiferus* (white shrimp), and *Uca* spp. (fiddler crabs) as they are able to burrow during the day and reduce visibility to predatory fish, *Callinectes sapidus (blue crab)*, and birds (Zimmerman et al. 2002) . Juvenile and small fish such as *Fundulus* spp. (Gulf killifish) use salt marshes to evade predation from blue crabs and birds (Weisberg et al. 1981). Juvenile blue crabs utilize the salt marsh edge as a place to feed on epiphytic algae, amphipods, and as they mature begin to consume more animal tissues (Zimmerman et al. 2002, Llewellyn and Peyre 2011) .

The marsh periwinkle, *Littorina irrorata*, cultivates fungi on the stems of *S. alterniflora* and *J. roemerianus* by creating scars on the alive tissues and feeding on the fungi that grows on the senesced material (Silliman and Zieman 2001). Mussels are found associated with the rhizosphere in the low and mid marsh zones and filter feed during high tide (Silliman and Zieman 2001). There are many types of birds that live and feed in coastal wetlands, common ones are *Rallus crepitans* (clapper rails), *Sternula antillarum* (least tern), *Ammospiza maritima* (seaside sparrows), and *Ardea herodias*, (great blue heron) (Rush et al. 2009, Burger 2017). These birds build nests using the salt marsh vegetation and feed on the invertebrates and insects that live in the marshes. Aside from aiding in development of transient fish and invertebrates, organic material assimilated in coastal salt marshes often escapes into surrounding waters, supplying nutrients for phyto- and zooplankton, further supporting the marine food web in a way described as the "outwelling" hypothesis (Odum 1980).

Marshes can improve water quality by trapping sediment, thereby reducing turbidity. Further, marshes have been shown to reduce the impacts of wastewater by filtering nitrogen (N) and phosphorous (P) through the roots, capturing 35% and 71%, respectively (Merrill and Cornwell 2000, Engle 2011). Standing vegetative biomass plays a role in reducing the impacts of storm surge by reducing wave amplitude and slowing the water velocity. Boesch et al. (2006) describe the frictional resistance applied by vegetation to storm waves as a cause for implementing wetland loss management to increase the resilience of coastal communities to storms and hurricanes. Wetlands provide ecosystem services that accumulate over time such as carbon sequestration, where plants store carbon in plant biomass and soils after photosynthesis, assimilation, and burial (Mcleod et al. 2011, Davis et al. 2015). Carbon (C) sequestration, also termed "blue carbon" is valuable to the globe as it serves as a carbon sink by burying atmospheric CO_2, which is a source of climate change, sea level rise (SLR), and ocean acidification. In order to offset losses of valuable ecosystem services provided by wetlands, restoration and enhancement projects are typically implemented

1.3 Wetland loss and restoration

Wetland loss is the result of a combination of factors which can be summarized as the balance between accretion and submergence (Turner 1990; Turner 1997; Herbert et al. 2016; Wu et al. 2017). A stable salt marsh is one that has a higher net accretion of soils via deposition of sediment organic matter on the marsh substrate. Submergence is a combination of all factors that raise the water level relative to the marsh surface, such as eustatic SLR and subsidence of the land due to sediment compaction or crustal down-warping (Turner 1990). Anthropogenic impacts on wetlands are widespread, in particular, climate change, altered sediment supply, and coastal development has led to the loss of valuable salt marshes and marine wetlands (Turner 1990; Turner 1997). On the current trajectory, salt marshes are expected to be reduced by 20-45% by the end of the 21st century, and in the Northern Gulf of Mexico, estimated wetland loss has reached 0.86% per year (Kirwan and Megonigal 2013). Die-off of salt marsh vegetation, even in short episodes, can lead to rapid subsidence, erosion, and diminished sediment deposition rates accelerating further wetland loss. Salt marsh vegetation is shown to be resilient to wetland loss under favorable conditions of sediment supply, and this is driven in part by vegetative growth rates in both the rhizosphere and canopy (Kruczynski 1982, Kirwan and Megonigal 2013). As eustatic and relative SLR increases, it is likely that coastal habitats will become increasingly vulnerable to these changes, and there seems to be a threshold where the rate of relative SLR can be greater than that the wetland vegetation can sustain, resulting in devastating wetland loss (Wu et al. 2017)

Construction of coastal wetlands in the United States began in the late 20th century and has become even more prominent today as the state of coastal wetlands has gotten increasingly dire. Restoration in the northern Gulf of Mexico can include de-novo construction of lost marsh platforms using fill or dredged sediments, thin-layer placement in existing marsh platforms, or construction of soil islands and cheniers that form a localized sediment source over time. Depending on the desired habitat, restoration project managers can opt to either plant the material with target vegetation or allow the site to be naturally colonized in hopes that natural ecological succession will follow a desired trajectory (Mitsch and Wilson 1996, Zedler and Callaway 1999, 2000, Craft et al. 2002, 2003, Herbert et al. 2016). The success of constructed wetlands is determined by management's specific goals for the project, but factors that contribute to this success have been studied heavily. To ensure adequate colonization of wetlands by planted vegetation, the factors that must be considered are: 1) elevation, 2) planting density, 3) planting material (e.g., seeds, transplanted plugs, rhizomes), 4) physical and chemical sediment characteristics, and 5) fertilizer usage. These factors have varying purposes in wetland construction, but their core necessity is that they are required to ensure an adequate cost:benefit ratio to project managers and funding sources. The role of elevation in wetland restoration is apparent from the studying of salt marsh plant distribution done by various authors (Eleuterius and Eleuterius 1979, Woerner and Hackney 1997, Bockelmann et al. 2002, Silvestri et al. 2005). Planting density depends on how the risks of physical stress compare to stress from competition. In forested ecosystems, restoration managers tend to recommend sparse planting density to minimize competition for space and light, however, in wetland ecosystems, where the plants will see wave impacts, it is more viable to increase planting density so that plants can facilitate the growth of other individuals through positive interactions (Silliman et al. 2015). The starting material for transplanting salt marsh vegetation varies among species, but bareroot plants are appropriate for transplanting most grasses, according to the United States Department of Agriculture (USDA 2010). Physical sediment characteristics such as texture, porosity, and bulk density can affect the aeration of the rhizosphere and thereby influence rhizobacteria and root growth (Mendelssohn and Morris 2002, Mavrodi et al. 2018). Fertilizers which add inorganic N and P are often applied and can shorten the period between planting and establishment of transplanted vegetation (Broome et al. 1988).

Restoration projects that construct *Spartina*-dominated marshes have been well explored in the past, (Woodhouse Jr 1979, Webb and Newling 1984, LaSalle et al. 1991, Taniguchi 1996, Zedler and Callaway 2000, Craft et al. 2002, Lang 2012) but *Juncus*-dominated marshes are not as common (Sparks et al. 2013, 2015). Assessment of salt marsh restoration projects should be done to observe the site's progress towards pre-established goals, however, specific and time-oriented goals are often missing from project proposals. In these cases, effective criteria for assessing constructed wetlands often include collecting data concerning plant community diversity, biomass, and soil organic content (SOC) and comparing them to a natural reference site over time (Zedler and Callaway 2000).

1.4 Development of restored/constructed sites

The rate at which community composition, biomass, and sediment characteristics change post-construction varies among projects, but there are common trends (Cammen 1975, Earhart and Jr. 1983, Webb and Newling 1984, Zedler and Callaway 1999, Craft et al. 2002, 2003, Herbert et al. 2016, Ebbets et al. 2019). Biomass is often measured in both the canopy and rhizosphere. The canopy of restored marshes are typically comparable to a natural reference site within 2-5 years, while root biomass can take upwards of 15 years (Woodhouse 1979; Webb and Newling 1984; Broome et al. 1988; Craft et al. 2002, 2003). Soil organic matter increases over time as accumulated detritus is exported into the soil and becomes buried; as anaerobic conditions increase with depth, organic matter decomposition is further reduced. This carbon pool typically develops to natural levels in restored sites after 3-5 years, but this can take more than 10 years in some cases (Cammen 1975, Craft et al. 2003, Herbert et al. 2016).

Usage of stable isotopes $\delta^{13}C$ and $\delta^{15}N$ in studying food webs has become prominent into the 21st century. Rezek et al. (2017) used $\delta^{13}C$ and $\delta^{15}N$ values to examine the basal resources of a restored salt marsh and found that shrimp rely on *S. alterniflora* detritus where it was more abundant than suspended particulate organic matter. A similar analysis done by Llewellyn and Peyre (2011) showed that restored marshes can provide trophic support after 5 years by comparing trophic levels of blue crabs associated with marshes of varying ages. Llewellyn and Peyre (2011) further call for more baseline information on trophic support in restored wetlands in order to improve the usage of stable isotopes in restoration assessment, which can add another level of functional equivalency to the assessment criteria presented thus far.

Restoration of a wetland is intertwined with the succession of species and substrate change to a desired endpoint (Luken 1990). Restoration can best achieve a desired community structure by manipulating the factors that control succession on a local scale such as seed supply, substrate changes, elevation, and nutrients (Walker et al. 2007). Restoration managers can aid plant community structure development by reducing the use of old soils which are low in phosphorus (Wardle 2004). If old soils must be used, they can be restored by finding a balance between sufficient fertilization that promotes succession and excessive fertilization that favors strong competitors, which could reduce biological diversity and inhibit further succession. Succession within salt marshes is driven by 1) competition among plant species within the middle and high marsh zones (Johnson 1997), 2) storm events which can displace diverse communities and enable invasive species to take hold (Garbutt and Wolters 2008), and 3) conversion of high marsh to low marsh due to SLR (Choi et al. 2001). Community composition in restored marshes should have similar stages of primary succession to a reference site, however, the path of succession can be delayed by disturbances such as tropical storms and hurricanes. The path of succession in a restored salt marsh is sometimes used as an indicator of the marshes progress towards a reference site, however, inclusion of other indicators (e.g., structural, functional, landscape) can add in the assessment of a restored site's ecological function (Petchey and Gaston 2006, Almeida et al. 2016, Taddeo and Dronova 2018).

Restored marsh characteristics such as coverage, species richness, and biomass vary in developmental trajectory with geomorphic position, tidal range, salinity, and soil classification (Craft et al. 2003) creating additional complexity in determining restoration trajectory. Due to the relative infancy of coastal marsh restoration and the rarity of long-term monitoring of restoration projects, there is a shortage of data concerning the development of a single site over a time-period greater than fifteen years (Craft et al. 2002, 2003, Suding 2011). Plant coverage of restored marshes can develop to reference levels as quickly as one year when planted with vegetation, or can take up to five years if a site is left only to naturally recruit plant species (Walker et al. 2007). Development of belowground biomass, which plays a role in carbon sequestration and marsh sustainability (Darby and Turner 2008, Kulawardhana et al. 2015, Wu et al. 2017), varies among species as there are species-specific adaptations to abiotic stressors such as salinity sulfide-toxicity (Bradley and Morris 1990, Mendelssohn and Morris 2002). Metabolic demand in producing root biomass is also species-specific (De La Cruz and Hackney 1977, Morris and Bradley 1999, Windham 2001). Due to the lack of long-term data on the trajectory of constructed salt marshes, it is imperative that projects are monitored for their progress to inform future efforts to restore similar systems and in the long-term inform on the resilience of these systems to climate change and SLR.

1.5 Threat of sea level rise to coastal wetlands

Sea level rise presents a real current and future threat to natural and constructed coastal wetlands that will accelerate as ice-sheets continue to melt as a result of global climate change. While SLR has remained relatively stable over the past 6,000 years at around 2mm/year, numerous studies have predicted that global SLR will accelerate anywhere from 8-21 mm/year during the 2050-2100 time period, depending on the Intergovernmental Panel on Climate Change (IPCC) emission scenarios (Donoghue 2011). This acceleration will result in global SLR between 0.5-2.0 m higher than present by the end of 2100 (Donoghue 2011). There is a large degree of uncertainty in predictions of climate change and SLR as there is a significant dependence on changes in government policy on the global scale, however, the resilience to SLR of coastal wetlands in Mississippi is predicted to diminish at a threshold of around 11.9 mm/year by 2050 (Wu et al. 2017). Wu et al. (2017) constructed a SLR resilience model at the Grand Bay National Estuarine Reserve in the northern Gulf of Mexico, which accounted for accretion rate, erosion rate, and biomass. The model presents a useful prediction for the fate of northern Gulf of Mexico wetlands and highlights the value of biomass and sedimentation in those systems.

Louisiana contains 37% of the estuarine herbaceous marshes in the continental United States and has lost 4,876 km2 of land between 1932 and 2010 (Couvillion et al. 2011, Glick et al. 2013). Between 30 to 59% of wetland loss in Louisiana is attributed to the creation of canals and navigational channels, which have altered water flow and thereby reduced delivery of nourishing sediment to the Mississippi Deltaic Plain (Boesch et al. 1994). Diversion of sediment supply from the Deltaic Plain to the Gulf of Mexico has disrupted the feedbacks among coastal marsh productivity, organic matter accumulation, sedimentation rates, and maintenance of elevation (Boesch et al. 1994, Kirwan and Guntenspergen 2012, Glick et al. 2013). Eustatic SLR, in addition to reduced accretion of the marsh platform, presents a real threat to Louisiana wetlands over the next century as modelled by Glick et al. (2013). Glick et al. (2013) suggests that changes in sediment supply to the Atchafalaya river delta are needed to reduce wetland loss even under their lowest sea level rise scenario of 3.1mm/yr. Land loss in Louisiana is a complicated issue for coastal resource managers, and adaptive management practices will be needed to mitigate further loss of Louisiana's ecologically valuable marshes and swamps.

Similarly, the Sacramento-San Joaquin Delta and San Francisco Bay have been shown to have severe SLR-related challenges, which could impact the infrastructure and ecological resources of the areas if not actively managed (Stralberg et al. 2011, Luoma et al. 2015). The Sacramento-San Joaquin Delta, which provides much of the water supply for California, is vulnerable to prolonged drought, flooding from atmospheric rivers, and displacement of native species as climate change and coastal infrastructure impact the region (Luoma et al. 2015). Stralberg et al. (2011) presented a model for San Francisco Bay where higher sediment concentration (e.g., upwards of 300 mg/L) was needed to sustain the tidal salt marshes with *D. spicata* and *S. patens* (Wasson and Woolfolk 2011), which are vulnerable to wetland loss (Brophy et al. 2019). Stralberg et al. (2011) concluded that active treatment of coastal marshes in San Francisco Bay with beneficial-use material could improve marsh sustainability under SLR.

Sea level rise is a necessary factor to consider in assessing the long-term success of constructed wetlands as the longevity of these constructed sites will surely be affected. The role of belowground biomass in accreting elevation is apparent and the development of the rhizosphere in constructed wetlands is pertinent to their long-term stability. Analyzing the stability of restoration projects is critical for restoration managers in the northern Gulf of Mexico, as sites are vulnerable to strong storm and hurricane events and SLR. Assessing the threat of these sites being destroyed or having loss in function is important as these projects are often high-cost and undergo some degree of scrutiny by the tax-paying public.

1.6 Objectives

This thesis is among the first studies assessing the progress of two beneficial-use projects on Deer Island, MS. Deer Island Multi-Year Restoration (DIMR) 1 and DIMR2 were constructed in 2005 and 2016, respectively, on the northeastern shore of Deer Island, MS. Aside from the study of DIMR1 by Lang (2012), no assessment of the vegetative community composition and health of these sites has been completed to date. To that aim, my thesis has 5 objectives for the assessment of DIMR1 and DIMR2:

1) Measure physical sediment characteristics and elevation of the two constructed sites and natural reference marsh to understand potential drivers of development and plant community composition.

2) Gather an inventory of the plant community and compare vegetation diversity among the two restored sites and natural reference site to assess the success of planted vegetation and/or natural recruitment of salt marsh and dune vegetation.

3) Compare developmental factors such as above- and below-ground biomass and sediment organic content among the constructed sites and natural reference site to visualize the trajectory of the constructed sites towards Deer Island's natural marsh community.

4) Measure the $\delta^{13}C$, $\delta^{15}N$ and C:N composition of *S. alterniflora* tissues to provide baseline data for future food-web analysis of Deer Island.

5) Examine the threat of SLR to DIMR1 and DIMR2 to visualize how vulnerable the sites are to changing future sea level.

CHAPTER II - METHODS

2.1 Study sites

The study took place on two constructed sites of differing ages and a natural reference marsh on Deer Island, MS (Figure 1). The constructed sites, DIMR1 and DIMR2, are adjacent to each other and joined by a containment dike which protects the inner developing marsh platforms from erosion (Figure 2). The method used in the construction of the DIMR sites was the utilization of beneficial-use of dredge material obtained from the maintenance dredging of commercial shipping channels in the nearby area. This dredged sediment was piped or barged to the restoration site and deposited inside a sandy containment berm, allowed to dewater, and planting was encouraged to further stabilizing the fine sedimentary material

Figure 1. Location of Deer Island, MS in the Mississippi Sound (Source - Google Earth 2017).

The 10+ y constructed site, DIMR1, was constructed in 2004 with dredged sediments sourced from Biloxi Bay channel and is 18-hectare in size. The 10+ y constructed site was first planted in spring 2005 with commercially purchased *J. roemerianus* (13,440), *S. alterniflora* (15,400) and *S. patens* (17,920) within the containment dike. Following extensive dike failure during and after hurricane Katrina (Aug 2005), additional sediments and a more expansive containment feature was constructed during 2010-2011. Further planting of *J. roemerianus, S. alterniflora*, *S. patens*, *P. amarum*, and *U. paniculata* was periodically completed at the 10+ y constructed site from 2008-2011, with the most substantial planting being of 15,000 *J. roemerianus*, 5,000 *S. alterniflora* and 3,000 dune plants in 2008, most of which were subsequently lost due to erosion at the site.

The 2+ y constructed site, DIMR2 is a 16-hectare area constructed from 2015 to 2018 with sediments dredged from multiple sources in Jackson and Harrison Counties in Mississippi. DIMR2 was planted on the eastern third in spring 2016 with commercially purchased *J. roemerianus* (18,836) and *S. alterniflora* (18,836) in the interior high and low marsh zones. On the exterior containment dike, *S. patens* (2,041), *Panicum amarum* Elliot (bitter panicgrass) (2,041), and *Uniola paniculata* L. (seaoats) (4,083) were planted. Additional revegetation of the remainder of this site has occurred largely through natural recruitment since then.

Present-day, the 2+ y and 10+ y constructed sites are both comprised of a low elevation *S. alterniflora* dominated marsh on the dredge-filled soils and a high marsh zone on the containment berm dominated by the planted *S. patens* and a variety of naturally recruited vegetation such as *D. spicata*, *Baccharis halimifolia* L. (eastern baccharis) , and *Sesbania herbacea* (Mill.) McVaugh (bigpod sesbania). The two restored sites were selected due to their differing ages, which made for straightforward observations on constructed salt marsh succession, as well as their close spatial proximity to allow for efficient travel between study sites.

The 100+ y reference marsh is approx. 500 m from the 2+ y and 10+ y constructed sites, separated by an upland dune ridge colonized by the longleaf pine (*Pinus palustris* Mill.) and a variety of shrubs such as *Serenoa repens* W. Bartram (small saw palmetto), and *B. halimifolia*. The natural marsh is entirely comprised of a zone mixed with *J. roemerianus* and *S. alterniflora*, with mussels and fiddler crab burrows frequently found in the soft, muddy sediment. The natural marsh was selected as a reference site for its proximity to the constructed sites as well as it's elevation and plant community composition, which makes it an ideal candidate for gauging the progress of the constructed sites towards being comparable to Deer Island's natural marsh footprint.

2.2 Field sampling design

Sampling was conducted over five seasons: spring 2017, fall 2017, spring 2018, fall 2018, and spring 2019. In the spring 2017 sampling season, two 100 m long replicate transects were established at each study site with approximately 250 m between starting points (Table 1). In the fall 2017 sampling season and onward, an additional 100 m replicate transect was added in the middle of the original two transects for additional sampling of community diversity, resulting in 125 m between transects (Figure 3). Starting points at the 2+ y and 10+ y constructed sites were established randomly along the containment dike which lies on the northern part of the site. The starting points were retained across sampling seasons. At each sampling effort, the transects were ran haphazardly from the starting point, generally in the same direction, resulting in similar transect orientation with different ending points across each sampling season. The orientation of transects at the 2+ y and 10+ y constructed sites were North to South, which captured the dynamic changes in elevation and plant community composition through the sites. Transects at the reference marsh were unable to have the same orientation as those at the constructed sites due to small bodies of water which frequently occurred across the site. The reference transects were oriented Northwest to Southeast, yet the difference in orientation had relatively little impact on our findings due to the site being relatively uniform in elevation and plant community composition. One-hundred and sixty-five discrete elevation points were measured by a Topographic Mapping RTK GPS (Trimble R8) on 4/7/2017 and 8/23/2018, and converted to a contour map (Spheroid: GRS_1980, Coordinate system: GCS_North_American_1983) in ArcGIS.

Figure 2. Deer Island, MS with constructed salt marshes of differing ages and a natural reference marsh outlined. 1: DIMR2, 2+ years old. 2: DIMR1, 10+ years old. 3: natural reference site, 100+ years old. Transects are oriented to capture changes in salt marsh vegetation with change in elevation along the transects.

Table 1. Summary of sample design at a single site observed in this study.

	Spring 2017	Fall 2017	Spring 2018	Fall 2018	Spring 2019
Quadrat transects	2	2	2	2	2
Point-intercept transects	0	3	3	3	3
Biomass cores taken	Yes	Yes	Yes	Yes	Yes
Sediment cores taken	No	Yes	Yes	Yes	Yes

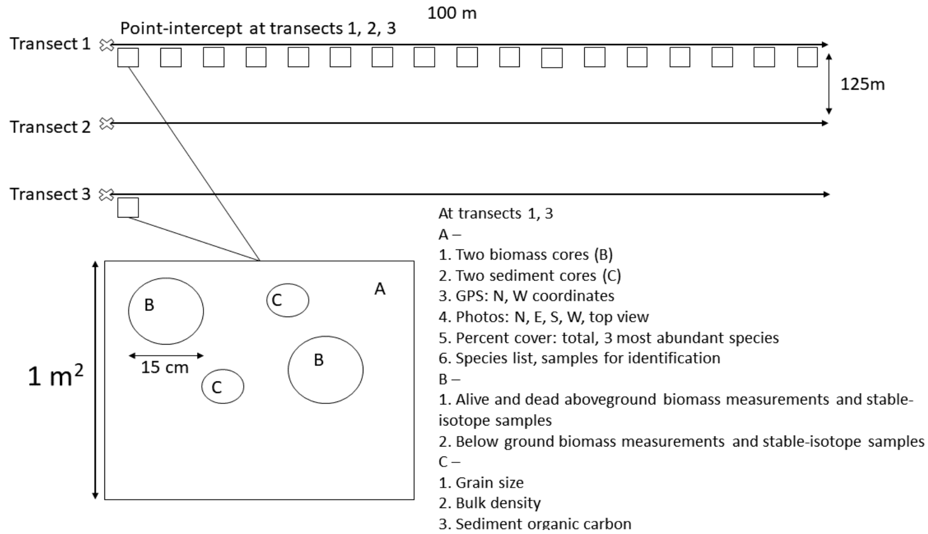

Figure 3. Layout of sample design at a single site observed in this study, not to scale.

2.3 Field sampling

2.3.1 Vegetation diversity and cover

To obtain diversity and species richness estimates by site the Point-Intercept method, described in Caratti (2006), was employed along all transects at 1 m intervals for the fall 2017, spring 2018, fall 2018, and spring 2019 sampling seasons (Table 1). This method involves visually observing and recording the presence or absence of plants at a point along a transect, where a plant is considered present if any structure of the plant intercepts the point in any fashion. In all five seasons, along the original two transects, 1 m^2 quadrats were used to estimate percent cover of species (Figure 3). Quadrats were spaced haphazardly at approximately 20-40 m intervals and placed within representative areas of each marsh zone (low-, mid-, high-marsh, and dunes). Plants were identified in the field to the species level by personnel experienced in identifying plants that occur in the northern Gulf of Mexico. For any unidentified plants one or more voucher specimens were returned to the laboratory for identification using appropriate field guides (Correll and Johnston 1970, Radford et al. 1983, Clewell 1985). The percent cover of the three most abundant species present in the 1 m^2 quadrat was estimated visually by a minimum of two personnel.

2.3.2 Vegetation biomass

In the selected quadrats, two replicate vegetation biomass cores (15 cm diameter x ~30 cm depth of the rhizosphere) of plant species of interest (*S. alterniflora, J. roemerianus, S. patens, D. spicata*) were taken for measurements of canopy and rhizosphere biomass. *S. alterniflora* were later used in δ^{13}C and δ^{15}N stable isotope analysis (see below).

2.3.3 Sediment characteristics

In the selected quadrats, two near surface sediment samples (depth 5-10 cm within the rhizosphere zone) and a deeper sediment samples (20-30 cm, below the rhizosphere zone) were collected from the same hole as the vegetation biomass core. Sampling replication for sediment core samples was two shallow and two deep sediment cores per 1 m^2 quadrat. To get a clean sediment sample, a 50ml syringe with the bottom end cut off was inserted into the sediment, the core was extracted, and the syringe was capped off using a #6 rubber stopper. The sediment sample was extracted from within the plant rhizosphere zone by pushing the syringe at right angles and parallel to the sediment surface into the adjacent root zone on the side-wall of the hole made after the biomass core had been removed. All sediment samples were stored on ice in a cooler until returned to the lab, where they were stored in the refrigerator and processed as soon as possible.

2.4 Laboratory analysis

2.4.1 Physical and chemical sediment characteristics

Sediment cores were subsampled for two different analyses, where the measurement derived from each analysis was representative of the sediment collected from the corresponding vegetation biomass core collected from a quadrat. The subsamples were: 1) a 2.5 mL subsample used for sediment organic content (% organic) via loss on ignition (LOI) and sediment bulk density (g/cm^3), and 2) the remainder of the amount of the total collected sediment (approx. 15 mL), used for grain size analysis. Following subsampling, the sediment subsamples were dried at 70° C until moisture was lost from the sediments. All subsamples were weighed after drying. The mass (g) of the dried 2.5 mL subsamples was considered the sediment bulk density (g/cm^3). The same subsample was then combusted in a furnace (Thermolyne 62700) at 500° C for 4 h, removing any organic matter within the sample (LacCore 2013). The combusted subsample was weighed to obtain the ash-free dry weight (AFDW) which was then subtracted from the pre-combustion weight to estimate the loss of organic matter from ignition. The amount of material lost on ignition was compared to the original amount of pre-combustion (inorganic + organic) material to calculate the percent of organic content of the soil sample (% organic) (LacCore 2013). The subsamples taken for grain-size analysis were wet-sieved over No. 10 (=2 mm, coarse), No. 35 (= 0.5 mm, fine sand), and No. 230 (= 0.063 mm, very-fine sand) mesh sieves according to Folk and Ward (1957). Following sieving, each grain size was stored in pre-weighed aluminum tins and dried in a drying oven at 70° C until all moisture was lost. The tins containing each grain size were then weighed. This measurement for each grain size was summed and then subtracted from the pre-sieving mass to obtain the amount of silt and clay material lost during the sieving process. The mass of each grain size was then divided by the total amount of the sediment to obtain % coarse material, % fine sand, % very fine sand, and % silt/clay.

2.4.2 Vegetation biomass and stable isotope analysis

Vegetative biomass cores taken from the field were promptly washed to remove sediment and debris from the above-ground material (AGM) and below-ground material (BGM) compartments. Using shears, biomass cores were then separated into species-specific tissue compartments consisting of the AGM portion (stems, leaves, and flowering structures) and the BGM portion (roots). The AGM portion was then separated into alive and dead portions based on color, where green material was considered alive and brown or dark material was considered dead. Following separation, all tissue compartments were placed in previously weighed aluminum tins and allowed to dry in a drying oven at 70º C for a minimum of 3 days to remove all moisture from the tissues. After drying, each tissue compartment was weighed on a balance and the mass (g) was recorded. The BGM, AGM separated as separate live and dead canopy compartments were ground in a Wiley mill to pass a # 40 mesh sieve. Each ground sample was stored in a labelled 20 ml glass scintillation vial. For select tissue samples of *S. alterniflora* only, a 3-4 mg subsample of the ground material was then packaged into a 4 x 6 mm tin capsule and analyzed by a Thermo Delta V Advantage stable isotope ratio mass spectrometer coupled to a Costech elemental analyzer via a Conflo IV interface, producing measurements of C, N, and δ^{13}C and δ^{15}N.

2.5 Data analysis

2.5.1 Statistical programming

All analyses were done in R version 3.5.1 (R Core Team 2018). The Bray-Curtis distance matrix, Analysis of Similarity (ANOSIM), and non-metric multidimensional scaling (nMDS) ordination plot were created and performed using 'vegan' version 2.5.3 (Oksanen et al. 2018). The indicator species analysis was performed using 'indicspecies' version 1.7.6 (Caceres and Legendre 2009). The two-way analysis of variance (ANOVA) with type HC3 robust standard errors was performed with 'car' version 3.0.2 (Fox and Weisberg 2011). Tukey's Honest Significant Difference (HSD) tests were performed using 'agricolae' version 1.3.0 (Mendiburu 2017). All figures were created using 'ggplot2' version 3.1.0 (Wickham 2016).

2.5.2 Elevation and sediment characteristics

Elevation measured by a Topographic Mapping RTK GPS was interpolated with Universal Kriging using the 3D analyst toolbox in ArcMAP (ver. 10.4.1) with no transformation and constant trend removal. A one-way ANOVA was used to test for any significant ($p < 0.05$) difference in the mean elevation of each site, which may contribute to any differences found in plant community assemblages.

A two-way ANOVA was used to examine site and season differences in sediment bulk density, which can be used as a proxy for compaction of soil has been shown to impede root growth. Two-way ANOVA, with site and season as factors, was used to test differences in SOC. Sediment cores, which were sieved and separated into coarse sand, fine sand, very-fine sand, and silt/clay were also tested for significant site and season differences using a two-way ANOVA. Tukey's HSD was used following any significant site differences ($p < 0.05$) to identify significant comparisons between sites (Tukey 1949). Following any significant ($p < 0.05$) main or interaction effects, Tukey's HSD test was used to identify contrasts that may have contributed to a significant effect (Tukey 1949).

2.5.3 Vegetation diversity

Point-Intercept and quadrat-based cover observations were used to calculate two measurements of relative percent cover of plant species, subsequently used for calculating species richness, alpha- and beta-diversity, respectively. Percent cover was estimated from point-intercept transects by taking the total number of observations of a species along a transect and dividing it by the total number of observations of all species along the transect. Transects were grouped by site and percent cover estimated by point-intercept was used to calculate the Shannon-Wiener Index of Diversity (Eq. 1) and Simpson's Index of Diversity (Eq. 2) to assess the species richness and evenness of each site. A Bray-Curtis dissimilarity matrix was created using percent cover from quadrats following Eq. 3 to estimate the beta diversity across sites.

$$H' = -\sum_{i=1}^{S} p_i \ln p_i$$

Equation 1. Derived equation for Shannon-Wiener Index of Diversity. The index of diversity if *H*', *S* is species richness, and *p*i is the relative proportion of each species within the community (Peet 1974).

$$D = 1 - \frac{\sum_{i=1}^{S} n_i(n_i - 1)}{N(N-1)}$$

Equation 2. Simpson's Index of Diversity where D is the diversity index value, n^i is the number of observations of i^{th} species, and N is total number of all species (Greenberg 1956).

$$BC_{ij} = 1 - \frac{2C_{ij}}{Si_i + S_j}$$

Equation 3. Bray-Curtis' index of dissimilarity. C_{ij} is the sum of the lesser values for only those species in common between quadrats. S_i and S_j are the total number of species observed at both sites.

The hypothesis that there is no dissimilarity (distance, R) in the vegetative community assemblages among constructed and reference sites, none greater than to be expected by chance alone, was tested with ANOSIM (permutations = 5,000) using a Bray-Curtis dissimilarity matrix created from the quadrat-based cover-observations at α = 0.05. Following the suggestion of the 'vegan' package (Oksanen et al. 2018) metaMDS() function, the dissimilarity matrix was Wisconsin Double standardized and square-root transformed before ordination. Ordination was done via nMDS and the centroids for each site were plotted with one standard deviation ellipses to allow for inferences on what differences exist among sites.

As elevation is known to play a role in salt marsh zonation, indicator species analysis was used to gain insight on how elevation may play a role in the vegetative community assemblage of the constructed and reference sites. The analysis was performed separately for each site by partitioning the sites into elevation ranges. Elevation ranges were estimated from the kriging performed in the Elevation and Sediment Characteristics section and were coded as being either Low- (0.0 – 0.54 MAMSL), Mid- (0.54 – 0.76 MAMSL), or High-marsh (> 0.76 MAMSL) following the classifications of salt marsh elevation zones by Eleuterius and Eleuterius (1979). The indicator species analysis tested the hypothesis that the observed indicator value for each species is no different from the indicator value generated through random permutations ($\alpha = 0.05$).

2.5.4 Biomass development

Two-way analysis of variance (ANOVA), with site and season as factors, was used to test differences in biomass compartments (alive, dead, and below) to assess whether constructed marshes of varying age were comparable to a natural reference marsh in key developmental indicators of constructed salt marshes. The two-way ANOVA for belowground biomass utilized type HC3 White's heteroscedasticity-corrected coefficient covariance matrix robust standard errors to overcome outliers and heteroscedasticity (White 1980). This was beneficial as White's HC3 standard errors perform best in sample sizes less than $n = 250$ (White 1980, Long and Ervin 2000). Following any significant ($p < 0.05$) main or interaction effects, Tukey's HSD test was used to identify contrasts that may have contributed to a significant effect (Tukey 1949).

2.5.5 Stable isotopes

Stable isotopes $\delta^{13}C$ and $\delta^{15}N$, percent C, percent N, and C:N values measured from *S. alterniflora* tissues were compared separately with a one-way ANOVA for site effects. The analysis was performed for each tissue compartment (alive, dead, and below) for each variable.

2.5.6 Sea level rise model

To approximate the longevity of the constructed and reference sites in the presence of Highest, Intermediate-high (~RCP 8.5), and Intermediate-Low (~RCP 4.5) SLR scenarios presented in Parris et al. (2012), a static model of the inundation of the marsh platform over time was created using Eq. 4 for change in sea level from the U.S. Army Corps of Engineers technical letter 1100-2-1 (Dalton 2014). MAMSL was calculated for every quadrat from 2019 to 2100 by subtracting the rise in sea level from the calculated MAMSL from the year prior, averaged by site, and plotted to observe the change in MAMSL over time for each site under the given SLR. The percent of quadrats above sea level was calculated for each site at the years 2025, 2050, 2075, and 2100.

$$E(t_2) - E(t_1) = 0.0017(t_2 - t_1) + b(t_2^2 - t_1^2)$$

Equation 4. where t_1 is the time between 2018 (the start year chosen to measure SLR) and 1992, t_2 is the time which we wish to measure SLR. The coefficient b takes on the values 2.71E-5, 8.71E-5, and 1.56E-4 for the Intermediate-Low, Intermediate-High, and Highest SLR scenarios, respectively (Parris et al. 2012, Wu et al. 2017).

CHAPTER III - RESULTS

3.1 Environmental characteristics

3.1.1 Elevation

A total of 165 elevation points were measured with a Topographic Mapping RTK GPS at the constructed and reference site. The 10+ year constructed site had the highest mean elevation at 0.76 MAMSL and was the most dynamic with a range of 0.80 MAMSL (Table 2). The youngest constructed site was at an average elevation of 0.54 MAMSL and was the least dynamic with a 0.35 MAMSL range. The constructed sites were both higher than the reference site, which was at an average elevation of 0.27 MAMSL and a range of 0.50 MAMSL (Table 2). A one-way ANOVA showed these mean elevation differences were significant ($p < 0.05$, Table A.1). A post-hoc Tukey's HSD showed the sites to be in different statistical groups with the 100+ y reference site < 2+ y constructed site < 10+ y constructed site (Table A.2).

Table 2. Mean and range elevation at constructed and reference salt marsh sites on Deer Island, MS, measured in meters above mean sea level (MAMSL). Superscripts denote significant groupings (p < 0.05) calculated by Tukey's HSD.

Site	Mean Elevation (MAMSL)	Range (MAMSL)	n
2+ y constructed^{b}	0.54	0.35	56
10+ y constructed^{a}	0.76	0.80	55
100+ y reference^{c}	0.27	0.50	54

3.1.2 Bulk density

A total of 129 sediment cores were collected over four seasons from both constructed marshes and the natural reference marsh. A two-way ANOVA for bulk density showed significant site ($F = 23.88$, $p < 0.001$) and season ($F = 2.99$, $p = 0.03$, Table A.3). The sediment cores from the 10+ y constructed site were the densest and ranged from 1.12 g/cm^3 in Spring 2018 to 1.21 g/cm^3 in Fall 2017 (Table 3, Figure 4). Sediment bulk density at the 2+ y constructed site was similar to the 10+ y constructed site (p = 0.97, Table A.4) and ranged from 1.02 g/cm^3 in Spring 2018 to 1.21 g/cm^3 in Spring 2019 (Figure 4). Sediment bulk density at the 100+ y reference marsh varied significantly (p < 0.001, Table A.4) from the two constructed sites and had the least dense sediment cores, which ranged from 0.44 g/cm^3 in Fall 2018 to 1.09 g/cm^3 in Spring 2019. Sediment bulk density also varied significantly by season (p = 0.03, Table A.5), where Spring 2019 had the highest bulk density averaged across groups at 1.19 g/cm^3.

3.1.3 Grain size

Differences in grain size were mostly seen in the very-fine sand and silt/clay portions (Figure 5). The two-way ANOVA for coarse sand showed no significant site or season effect (Table A.6). Fine sand showed no significant variation among sites, however there was significant variation between seasons. Fine sand was significantly lower in Spring 2018 than Fall 2018 (p = 0.01, Table A.7) at 3.75% and 10.25%, respectively (Table 2). Fall 2017 and Spring 2019 showed no significant difference in fine sand when compared to Spring 2018 and Fall 2018 (Table A.5). The constructed sites were similar in both very-fine sand (p = 0.64) and silt/clay portions (p = 0.27), with both having significantly higher very-fine sand than the reference site (Table A.4). Cores collected during Spring 2019 had the highest amount of very fine sand for all sites however there was no significant season effect (p = 0.12, Table A.8). The 100+ y reference site had significantly (p = 0.002) higher silt/clay than the 10+ y constructed site but was similar (p = 0.1) to the 2+ y constructed site (Table A.4).

Table 3. Summary table of mean + SE sediment bulk density, organic content, and grain size portions by percent of core, sieved into coarse sand, fine sand, very fine sand, and silt/clay at constructed and reference salt marshes. Superscripts denote significant ($p < 0.05$) groupings from Tukey's HSD.

	Bulk Density (g/cm^3)	SOC (%LOI)	Coarse Sand (%)	Fine Sand (%)	Very Fine Sand (%)	Silt/Clay (%)
Fall 2017						
2+ y constructed	1.20 (0.08)^{a}	2.14 (0.55)^{b}	0.00	3.47 (0.99)	72.88 (10.34)^{a}	23.65 (9.65)^{ab}
10+ y constructed	1.23 (0.09)^{a}	1.50 (0.37)^{c}	3.84 (2.44)	10.00 (3.64)	74.77 (3.14)^{a}	11.39 (4.78)^{b}
100+ y reference	0.82 (0.19)^{b}	10.00 (2.34)^{a}	0.79 (0.79)	1.99 (0.91)	58.42 (9.23)^{b}	38.80 (8.40)^{a}
Spring 2018						
2+ y constructed	1.02 (0.10)^{a}	5.22 (0.94)^{b}	0.00	3.05 (1.12)	52.96 (10.80)^{a}	43.99 (11.18)^{ab}
10+ y constructed	1.12 (0.07)^{a}	1.66 (0.42)^{c}	0.00	3.20 (1.42)	72.87 (11.98)^{a}	23.93 (12.27)^{b}
100+ y reference	0.62 (0.10)^{b}	14.53 (1.99)^{a}	0.00	4.88 (0.98)	37.27 (7.25)^{b}	57.94 (8.19)^{a}
Fall 2018						
2+ y constructed	1.15 (0.15)^{a}	5.29 (1.44)^{b}	0.22 (0.22)	7.58 (3.49)	67.05 (11.47)^{a}	25.15 (9.93)^{ab}
10+ y constructed	1.13 (0.06)^{a}	2.41 (0.54)^{c}	2.14 (1.26)	8.43 (3.92)	72.16 (8.83)^{a}	17.26 (9.07)^{b}
100+ y reference	0.44 (0.06)^{b}	14.83 (1.57)^{a}	1.91 (1.76)	14.29 (4.50)	47.52 (7.32)^{b}	36.29 (6.91)^{a}
Spring 2019						
2+ y constructed	1.21 (0.10)^{a}	4.11 (1.17)^{b}	0.00 (0.00)	3.26 (1.04)	75.29 (6.26)^{a}	21.45 (6.78)^{ab}
10+ y constructed	1.21 (0.15)^{a}	2.32 (0.84)^{c}	0.93 (0.65)	5.22 (1.61)	74.96 (8.56)^{a}	18.89 (7.72)^{b}
100+ y reference	1.09 (0.19)^{b}	11.05 (2.27)^{a}	0.51 (0.51)	12.61 (2.11)	62.89 (4.18)^{b}	23.99 (3.62)^{a}

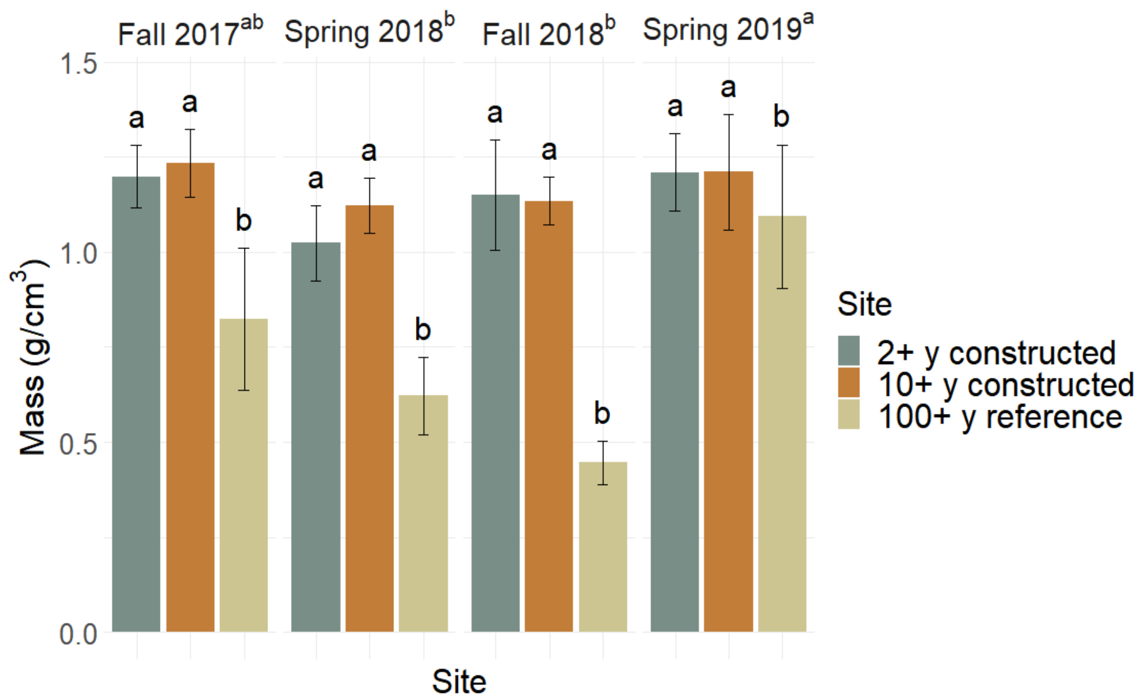

Figure 4. Mean + SE sediment bulk density at constructed salt marshes and a natural reference marsh across sampling seasons. Letters denote significant ($p < 0.05$) groupings by Tukey's HSD. Note: no sediment samples were collected in Spring 2017.

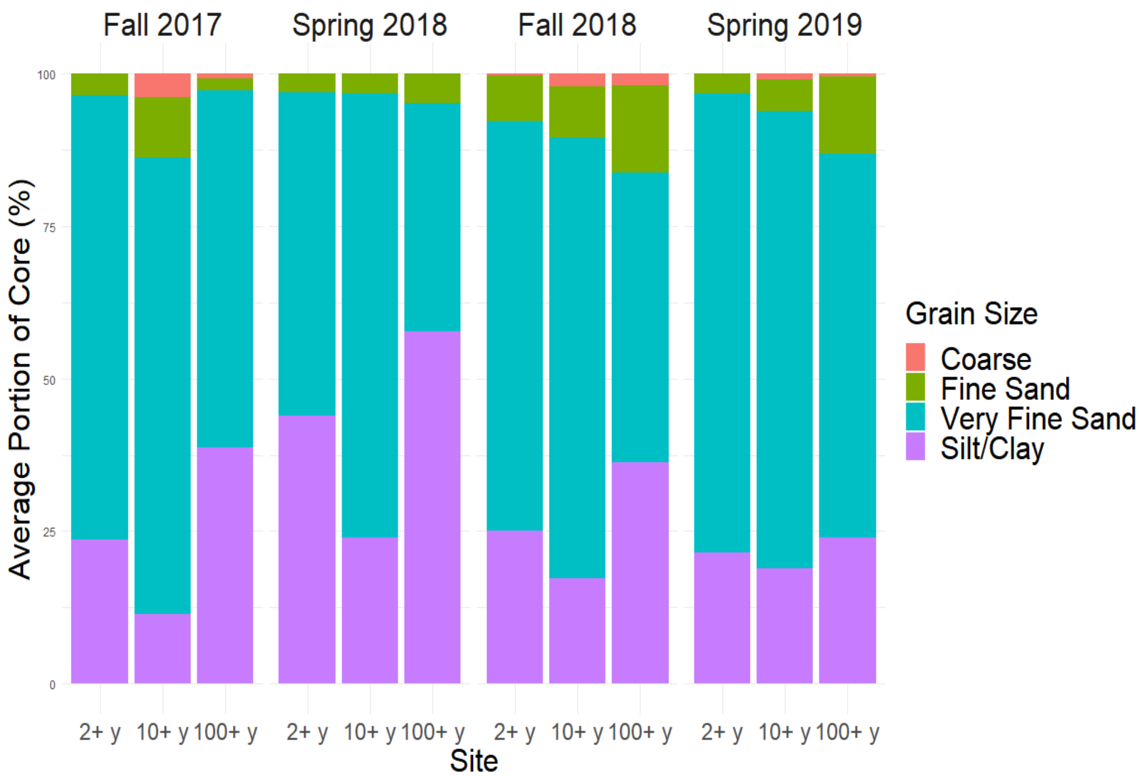

Figure 5. Mean grain size composition (>2 mm = coarse, >0.5 mm = fine sand, >0.063 mm = very-fine sand) of sediment cores from two constructed salt marshes of differing age and a natural reference site.

3.1.4 Soil organic carbon

Soil organic carbon (SOC) followed a similar trend to what was observed in the bulk density values, where there was a significant difference between sites ($F = 79.78$, $p < 0.001$, Table A.10) and the reference site had consistently more soil organic carbon than both constructed sites (Figure 6, Table A.3). All sites were statistically grouped separate from each other, the contrasts can be found in Table A.3. SOC ranged at the 100+ y reference site from 10.00% in Fall 2017 to 14.83% in Fall 2018 (Table 2). The 10+ y constructed site had the lowest overall SOC with 1.5 % in Fall 2017. The 2+ y constructed site seemed to show development between sampling seasons with 2.14 % in Fall 2017 to 5.29 % in Fall 2018. SOC measured in Fall 2017 was significantly ($p < 0.05$)

lower than Spring 2018 – Fall 2018 for all sites but was similar (p = 0.84) to the Spring 2019 season (Table A.5).

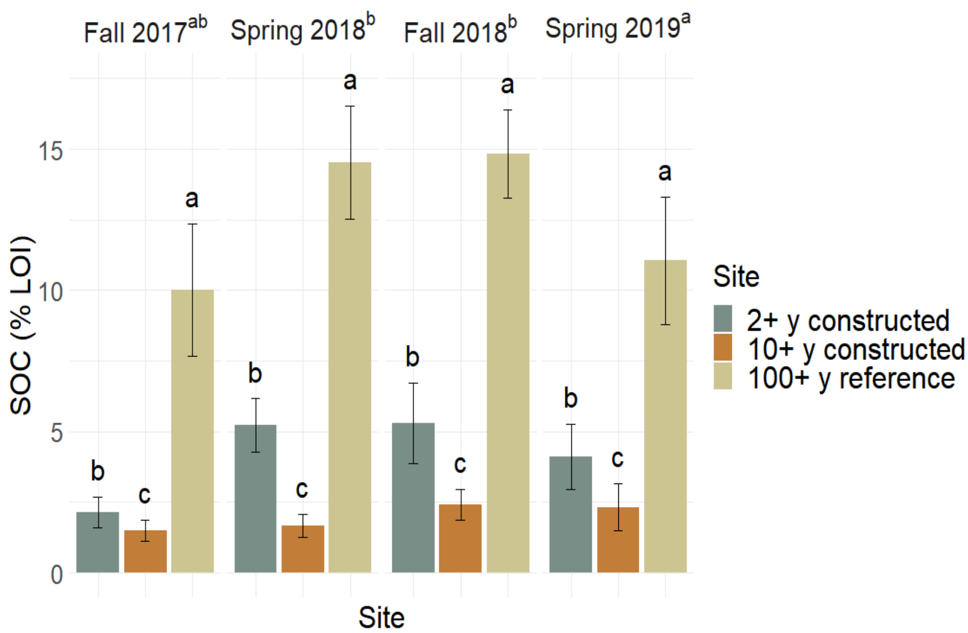

Figure 6. Mean + SE sediment organic carbon (% loss on ignition) at constructed salt marshes and a natural reference marsh across sampling seasons. Letters denote significant ($p < 0.05$) groupings by Tukey's HSD. Note: no sediment samples were collected in Spring 2017.

3.2 Vegetation diversity and cover

3.2.1 Richness and alpha diversity

A comprehensive list of species observed from the point-intercept and the percent cover/biomass quadrats can be found in Tables B.1 and B.2. There were 37 total species found across the survey period from fall 2017 to spring 2019. The 10+ y constructed site had the highest species richness at n = 32. The 2+ y constructed site had half of the amount of species as the 10+ y constructed site at n = 16. The 100+ y reference marsh had the least amount of species at n = 5. Seasonally, there were discrepancies between the quadrat and point-intercept species richness measurements, but site differences are apparent.

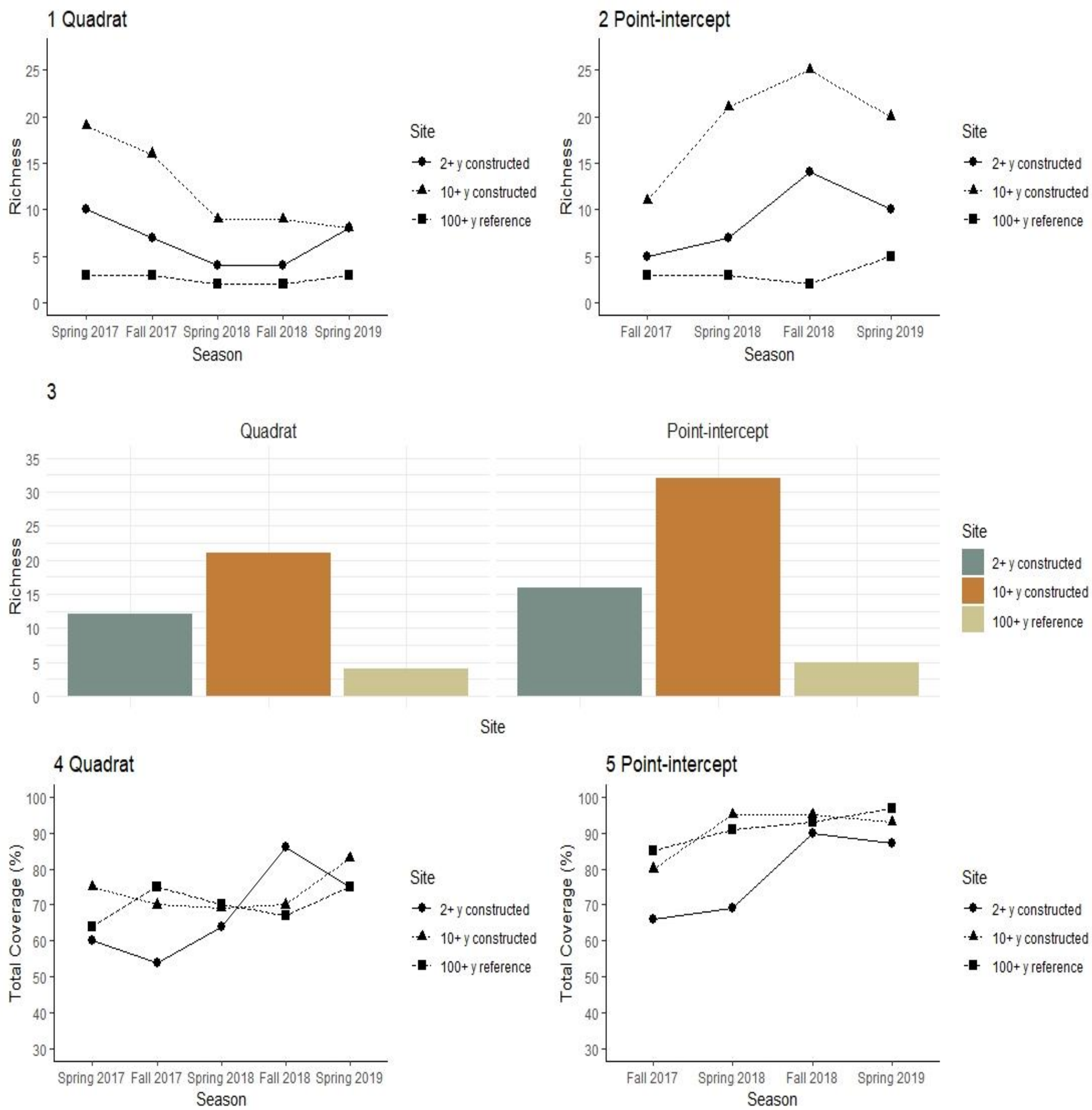

Figure 7. Species richness by season at two constructed marshes of differing ages and a natural reference marsh measured with quadrat (1) and point-intercept (2) sampling. (3) Cumulative species richness by site measured from quadrat and point-intercept sampling across all sampling seasons. The total percent coverage of vegetation at the constructed sites and reference marsh using (4) quadrat and (5) point-intercept sampling, respectively.

The 10+ y constructed site had the highest species richness across both sampling methods. The maximum species richness at the 10+ y constructed site was observed at n = 19 in Spring 2017 when using quadrat sampling and at n = 25 in Fall 2018 when using point-intercept sampling (Figure 7, Tables B.31 and B.32). The maximum species richness at the 2+ y constructed site was observed at n = 10 in Spring 2017 when using quadrat sampling and at n = 15 in Fall 2018 when using point-intercept sampling (Figure 7). The maximum species richness at the 100+ y reference marsh when using quadrat sampling was observed at n = 3 in Spring 2017, Fall 2017, and Spring 2019. The maximum species richness observed at the 100+ y reference marsh was n = 5 during Spring 2019 when using point-intercept sampling (Figure76).

The 2+ y constructed site showed a steady development of total vegetative coverage when measured by both quadrat and point-intercept sampling. Quadrat sampling showed the 2+ y constructed site increased from 54% total vegetative coverage in Fall 2017 to 86% total vegetative coverage in Fall 2018 (Figure 7). Point-intercept sampling similarly showed an increase from 66% total vegetative coverage in Fall 2017 to 90% in Fall 2018. Total vegetative coverage at both the 10+ y constructed site and 100+ y reference marsh was relatively consistent over time, regardless of sampling method. The 10+ y constructed varied from 69% in Spring 2018 to 83% in Spring 2019 when using quadrat sampling. Point-intercept sampling at the 10+ y constructed site showed that coverage varied from 80% in Fall 2017 to 95% in Fall 2018. The 100+ y reference site ranged from 64% in Spring 2017 to 75% in Spring 2019 when using quadrat sampling. When using point-intercept sampling the 100+ y reference marsh ranged from 85% in Fall 2017 to 97% in Spring 2019.

Specific species coverage at a given site during a specific season for each sampling method can be found in tables B.3 – B. 30.Quadrat sampling showed an increase in *S. alterniflora* cover at the 2+ y and 10+ y constructed sites over the entire Spring 2017 – Spring 2019 sampling period. Quadrat sampling showed an increase in *S. patens* cover from 3% to 20% between Spring 2017 – Spring 2019 at the 2+ y constructed site. Point-intercept sampling showed a smaller increase in *S. patens* coverage at the 2+ y constructed site with an increase from 4% in Spring 2017 to 10% in Spring 2019. The 10+ y constructed site fluctuated in *S. patens* cover throughout the quadrat sampling period; it reached a high of 40% in Spring 2018 and a low of 15% in Fall 2018. Point-intercept sampling at the 10+ y constructed site showed *S. patens* coverage at the 10+ y coverage was more consistent; coverage varied from 40% in Fall 2017 to 35% in Spring 2019.

All sites shared commonly found salt marsh plants such as *D. spicata*, *J. roemerianus*, *S. alterniflora*, and *S. patens* (Tables B.1, B.2). The two constructed sites uniquely shared species such as the grasses *Panicum amarum* Elliott (bitter panicgrass) and *Schizachyrium maritimum* (Chapm.) Nash (gulf bluestem). The vine *Vigna luteola* (Jacq.) Benth (hairypod cowpea) was also found in the dry, sandy areas of both constructed sites. *Ruppia maritima* L. (widgeon grass) was found in a submerged portion of the 2+ y constructed site and an unsampled canal at the 10+ y constructed site. Species unique to the 2+ y constructed site were *Panicum repens* L., *Sesuvium portulacastrum* (L.) L. (shoreline seapurslane), and *Uniola paniculata* L. (seaoats). Notable species unique to the 10+ y constructed site are *Baccharis halimifolia* L. (eastern baccharis), *Hydrocotyle bonariensis* Comm. Ex Lam. (largeleaf pennywort), and *Solidago* *sempervirens* L. (seaside goldenrod). Invasive species were absent at 2+ y constructed site and the 100+ y natural reference marsh, but the invasive *Imperata cylindrica* (L.) P. Beauv. (cogongrass) was found at the 10+ y constructed site, albeit in small amounts.

Quadrat sampling, likely due to a decrease in observations across Spring 2017 – Spring 2019 time period, showed mixed results with respect to Shannon-Wiener's H' and Simpson's D diversity indices (Table B.31). In acknowledging this, changes in alpha-diversity measurements through the five seasons of quadrat sampling should be interpreted with caution. Site differences in Shannon's H' and Simpson's D at the study sites when measured with quadrat sampling are apparent, however, it is inconclusive whether or not there is any meaningful change over time. When using both quadrat and point-intercept sampling methods, both the 2+ y and 10+ y constructed sites had higher diversity than the 100+ y reference site (Tables B.31, B.32). The 10+ y constructed site was the most diverse when using quadrat (Shannon's *H*' = 2.24, Simpson's D = 0.85) and point-intercept (Shannon's *H*' = 2.42, Simpson's D = 0.85). The 2+ y constructed site had the second highest diversity rank when using quadrat (Shannon's *H*' = 1.62, Simpson's D = 0.76) and point-intercept (Shannon's *H*' = 1.44, Simpson's D = 0.59) sampling methods. The 100+ y reference marsh had the lowest diversity indices when using quadrat (Shannon's *H*' = 1.14, Simpson's D = 0.67) and point-intercept sampling methods (Shannon's *H*' = 0.85, Simpson's D = 0.56)

3.2.2 Beta-diversity and Indicator Species

Beta-diversity across the 3 sites was quantified by creating a Bray-Curtis dissimilarity matrix from a total of 186 quadrats across all sampling sites and was tested with ANOSIM with the null hypothesis being no distance greater than zero between sites.

All site comparisons showed significant beta-diversity among sites (Table B.33). The 10+ y constructed site and the 100+ y reference marsh were the most dissimilar with an R statistic of 0.39. The 2+ y constructed site was most similar with the 10+ y constructed site (R = 0.11) but was almost as similar with the reference site (R = 0.21). To visualize the differences seen from ANOSIM, 186 points were plotted in ordination space with nMDS (k = 2, stress = 0.08). The centroids and 95% confidence intervals for each site were plotted, and the nMDS reflects the distances measured by ANOSIM (Figure 8). Points from the two constructed sites exhibit wide variability, which can be attributed to the higher elevation ranges and resulting increased plant species diversity within those sites in comparison to the reference marsh, which had both a tight grouping of points as well as a smaller 95% confidence ellipse. In terms of position on the nMDS plot, it is apparent that the 10+ y constructed is more different from the 100+ y reference marsh than it is from the 2+ y constructed site, likely due to the shared dune species observed in the two constructed sites. The position of the 10+ y constructed site's centroid can probably be attributed to the number of unique species found at that site in the higher elevation dune habitat. The location of the centroid for the 2+ y constructed site could seem misleading, however, as there is a high density of points that overlap and lead to the centroid shifting to the right of the plot. This phenomenon can likely be attributed to numerous observations with solely *S. alterniflora* in nearly identical abundances as a result of rapid growth and coalescence after restoration planting in spring 2016.

Figure 8. Non-metric multidimensional scaling plot (k = 2, Stress = 0.08) of vegetative species abundances cover grouped for all five sampling seasons at constructed sites and natural reference site at Deer Island, MS. The larger dots are the centroids with 95% confidence interval ellipses for each group.

Indicator analysis was used to observe associations of salt marsh plant species with elevation ranges (low-, mid-, and high-marsh) at the two constructed and natural reference sites. As expected, *S. alterniflora* was associated with low-marsh elevation at each of the three sites (Tables B.34 – B.36). The association was significant at the 2+ y (IV = 0.76, $p < 0.01$) and 10+ y (IV = 0.68, $p < 0.01$) constructed sites, however, it was insignificant ($p = 0.29$) at the reference site despite a high indicator value of 0.95 (upper-bound = 1). This is likely because the marsh platform at the reference site was uniformly low at an average of 0.27 MAMSL, which led to there simply being a low number of permutations where *S. alterniflora* would have been associated with the mid-marsh elevation ranges. This analysis would improve if different elevation ranges had occurred within the sampled transects at the natural marsh site. *J. roemerianus* was associated with the low-marsh elevation range at the reference site (IV = 0.79), but this association was insignificant ($p = 0.89$). *J. roemerianus* tended to associate with the 0.54 – 0.76 MAMSL mid-marsh range at both constructed sites, although the indicator value was low and insignificant in both cases, likely because of the overall low occurrence of *J. roemerianus* along the sampled transects and constructed sites in general. There was a similar pattern of *S. patens* at the 2+ y constructed where it was significantly associated with the mid-marsh elevation range (IV = 0.59, $p = 0.01$), but at the 10+ y constructed site *S. patens* was insignificantly (IV 0.52, $p = 0.52$) associated with the mid-marsh elevation range. Patterns of *D. spicata* association showed similar discrepancies at all sites, where it was significantly associated with the low-marsh range at the 10+ y constructed site (IV = 0.74, $p < 0.001$), but associations with the mid-marsh range at the 2+ y constructed site (IV = 0.33, $p = 0.35$) and 100+ y reference marsh (IV = 0.30, $p = 0.04$) were contradictory. Another significant pattern was found where *Vigna luteola* was significantly (IV = 0.55, $p = 0.04$) associated with the high-marsh at the 10+ y constructed site.

3.3 Biomass development

Alive-, dead-, and below-ground biomass was measured from a total of 160 biomass cores from all sites and seasons (Figure 9). Alive plant biomass varied significantly among sites ($F = 4.07$, $p = 0.02$) but was comparable across sampling seasons ($F = 1.25$, $p = 0.29$, Table C.2). Site contrasts for alive biomass showed that the 2+ y constructed site was comparable to both the 10+ y constructed site ($p = 0.07$, Table

C.3) and the 100+ y reference marsh ($p = 0.79$). Significant differences in alive plant biomass were found between the 10+ y constructed site and 100+ y reference site ($p = 0.02$). There were no significant site ($F = 2.62$, $p = 0.08$) or season ($F = 1.81$, $p = 0.13$) differences for dead biomass (Table C.4), however, there was a significant site by season interaction ($F = 2.05$, $p = 0.04$). The significant interaction was due to a large increase in dead biomass at the 2+ y constructed site from 487.20 g/cm^3 in Spring 2017 to 1470.09 g/cm^3 in Fall 2017 (Table C.1). Belowground biomass showed significant site ($F = 52.91$, $p < 0.001$) and season effects ($F = 9.57$, $p < 0.001$, Table C.5). The 2+ y and 10+ y constructed sites were similar to each other ($p = 0.90$) but different from the 100+ y reference marsh ($p < 0.001$), suggesting development in the rhizosphere has yet to sufficiently take place. The Spring 2018 and Fall 2018 sampling seasons were the sole pair of seasons that were significantly ($p = 0.04$) different from each other.

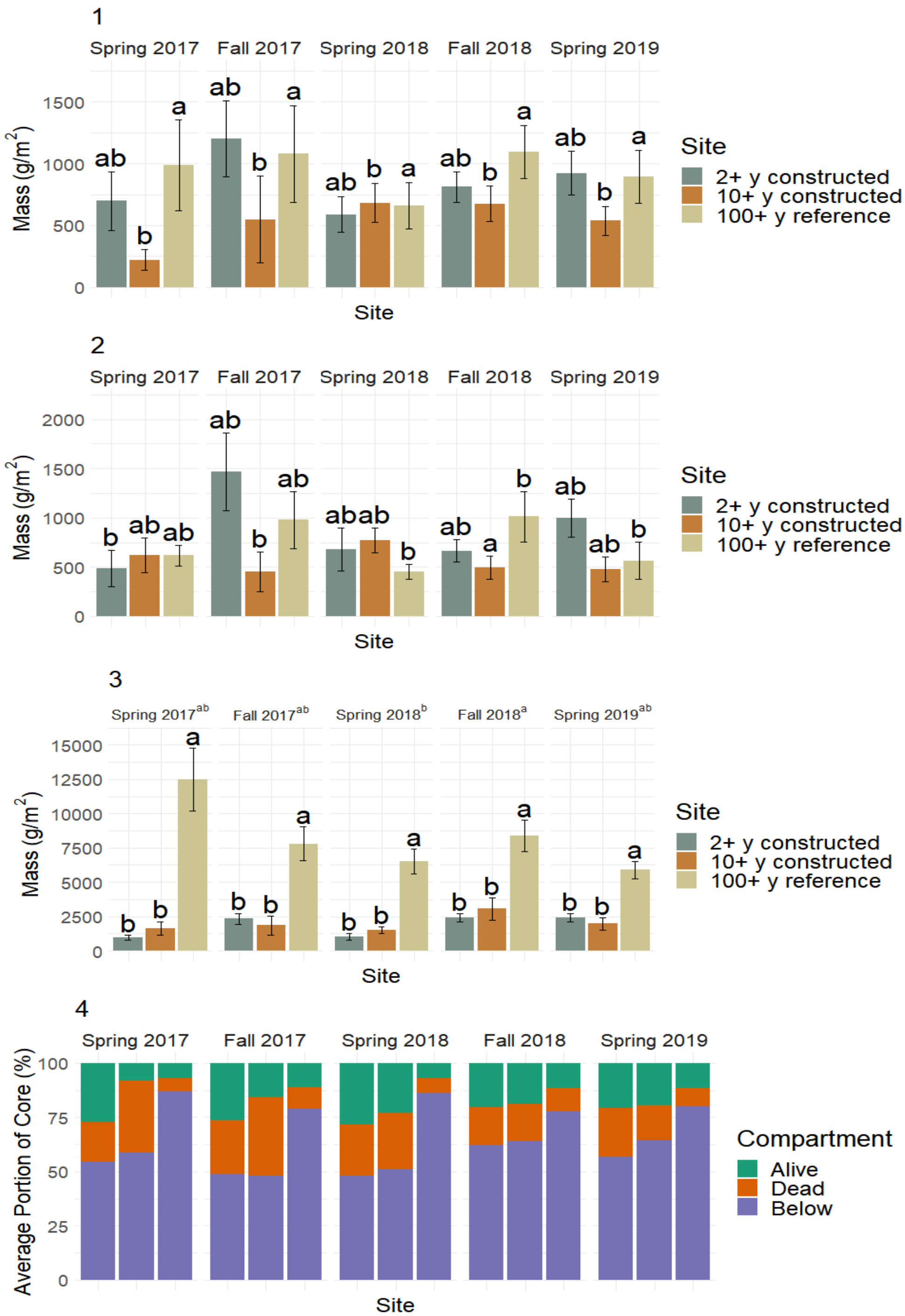

Figure 9. Mean + SE of (1) alive above-, (2) dead above-, (3) below-ground biomass (g/m^2), and (4) average percent of the three compartments in each biomass core at two constructed salt marshes and a natural reference marsh across all five sampling seasons. Letters denote significant ($p < 0.05$) groupings by Tukey's HSD.

3.4 Stable isotope analysis

Stable isotope analysis of live and dead *S. alterniflora* leaves (n = 29) showed that $\delta^{13}C$ varied significantly across sites (Tables D.1 – D.2). The 2+ y constructed site was the lightest isotopically with respect to both $\delta^{13}C$ and $\delta^{15}N$ in alive aboveground tissues at -13.44 ± 0.06‰ $\delta^{13}C$ and 3.63 ± 1.05 ‰ $\delta^{15}N$ (Figure 10). The 2+ y constructed site was significantly different from the 10+ y constructed site ($p < 0.01$) and the 100+ y reference marsh ($p < 0.01$) in $\delta^{13}C$ of alive aboveground tissues (Table D.2). There was a significant site effect for $\delta^{13}C$ in dead *S. alterniflora* tissues ($F = 10.45$, $p < 0.001$, Table D.4), and the 100+ y reference marsh was significantly lower than the 10+ y constructed site, but was not different from the 2+ y constructed site (Table D.2). Roots of *S. alterniflora* (n = 20) showed no significant differences in $\delta^{13}C$ across the three sites (Table D.5). One-way ANOVA of $\delta^{15}N$ of alive aboveground tissues had a significant site effect ($F = 4.88$, $p = 0.02$, Table D.6), but Tukey's HSD showed no specific significant site contrasts. No significant differences were found in $\delta^{15}N$ of dead aboveground and belowground tissues (Tables D.7 – D.8).

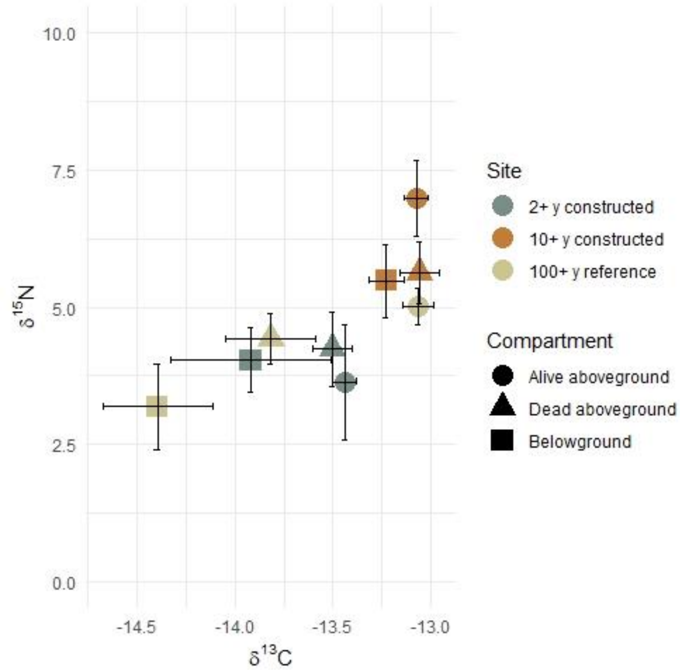

Figure 10. Biplot of mean ± SE $\delta^{13}C$ and $\delta^{15}N$ of S. alterniflora tissues at two constructed sites and a natural reference site on Deer Island, MS.

Carbon content among all sites for all tissue compartments varied between 34% to 41% C but there were no significant sites effects found for all tissue compartments (Tables D.9 – D.11). A significant site effect ($p < 0.05$) was found for N content in all tissue compartments (Tables D.12 – D.14). N-content was always significantly higher in tissues from the 2+ y constructed site (Table D.5). Nitrogen in live tissues at the 2+ y constructed site was an average of 1.93 ± 0.13%, nearly double that of the 100+ y reference marsh at 1.08 ± 0.09%, suggesting a possible depletion of N - availability over time as sediments age and become more organic-rich (Table D.1). The same inference can be drawn from the dead leaves, where the 2+ y constructed site averaged 0.90 ± 0.08% N and the 100+ y reference marsh averaged 0.58 ± 0.04% N. Dead tissues from the 10+ y constructed site fell in between the other two sites at 0.70 ± 0.07% N and statistically grouped intermediate to both the 2+ y constructed site and the 100+ y reference marsh. Nitrogen from the roots exhibited a somewhat different trend, as the 100+ y reference marsh grouped with the 10+ y constructed and the 2+ y constructed sites, which were significantly different from each other (Table D.5).

The difference in N across sites was the main driver of differences in the C:N ratio, as the same pattern was found with respect to statistical groupings. All one-way ANOVAs for C:N ratio in the three tissue compartments showed a significant site effect (Tables D.15 – D.17). In every case, *S. alterniflora* tissues C:N ratios were lower at the 2+ y constructed site than at the other two sites (Figure 11). The average C:N ratio of the live leaves from the 2+ y constructed site was 21.80 ± 2.17 (dimensionless), which differed significantly from the 100+ y reference marsh but was not significant from the 10+ y constructed site. The same groupings applied to the C:N ratio of the dead tissues, however, in the below-ground tissues, the 2+ y and 10+ y constructed sites were different from each other but similar to the 100+ y reference marsh (Table D.5).

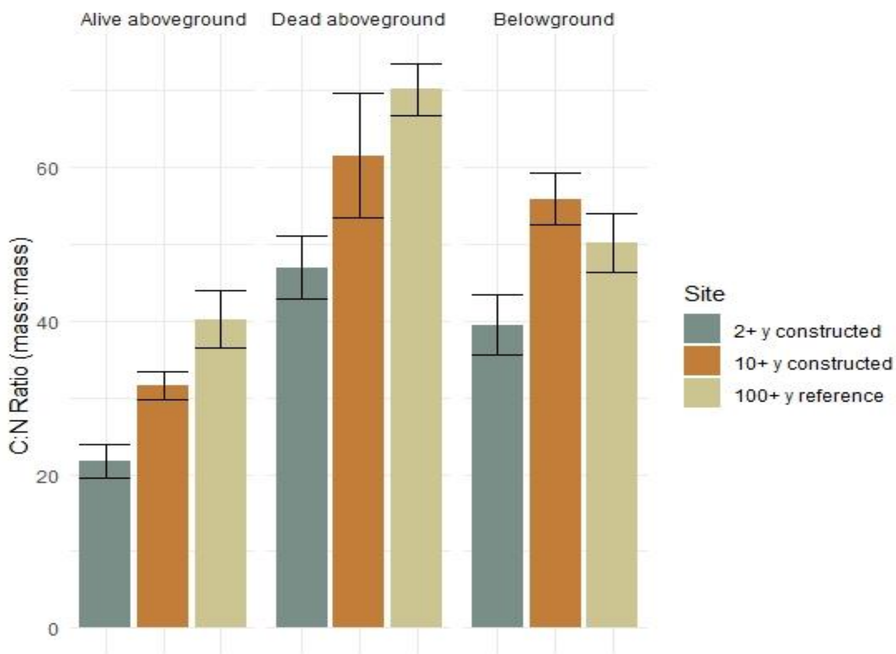

Figure 11. Mean ± SE C:N ratio of *S. alterniflora* tissues at two constructed sites and a natural reference site on Deer Island, MS.

3.5 Sea level rise

To better assess the resiliency of the sites to rising future sea levels, SLR was modelled at the three study sites for Highest, Intermediate-high (~RCP 8.5), and Intermediate-Low (~RCP 4.6) SLR scenarios presented in Parris et al. (2012). All sites remained partially emergent under Intermediate-Low SLR, with the 10+ y and the 2+ y constructed sites the most resilient to low SLR. 98 percent of the 10+ y constructed site remained emergent in 2100 and 93 percent of the 2+ y constructed site remained emergent under low SLR (Table E.1), however, only 3.7 percent of the 100+ y reference marsh remained emergent, making it the most vulnerable to SLR under even the best-case scenario in this study (Figure 12). Under the intermediate SLR scenario, all sites were submerged by 2100. Less than 10 percent of the 2+ y constructed site and the natural 100+ y reference marsh remained emergent by 2075. The 10+ y constructed site retained

76 percent of its land in 2075 under intermediate SLR. The highest SLR scenario resulted in all sites being submerged by 2075. Again, the 100+ y reference marsh was the most vulnerable and was projected to retain only 3 percent of its land in 2050, while 82 percent of the 2+ y constructed site remained emergent and 98% percent of the 10+ y constructed site remained emergent at the same time point given highest SLR.

The average elevation of each site was projected for the three SLR scenarios in Figure 12. Under the lowest SLR projection, the average elevation relative to MSL of the 2+ y and 10+ y constructed sites was reduced by 80 percent and 50 percent respectively but remained above sea level. The 100+ y reference marsh began drop below mean sea level around 2075 under the lowest SLR. The intermediate SLR projection reduced the mean elevation to below sea level in all sites by 2090. The 100+ y reference marsh dropped below 0m at 2050, the 2+ y constructed site will be inundated by 2070, and the 10+ y constructed site was the most resilient to SLR, as the elevation remained positive until around 2085. The highest SLR scenario submerged the 100+ y reference marsh by 2040. The 2+ y constructed site remained above sea level until 2055, while the 10+ y constructed site was resilient until 2065. Figure 13 shows a graphical representation of SLR at the three sites over time under the different SLR scenarios.

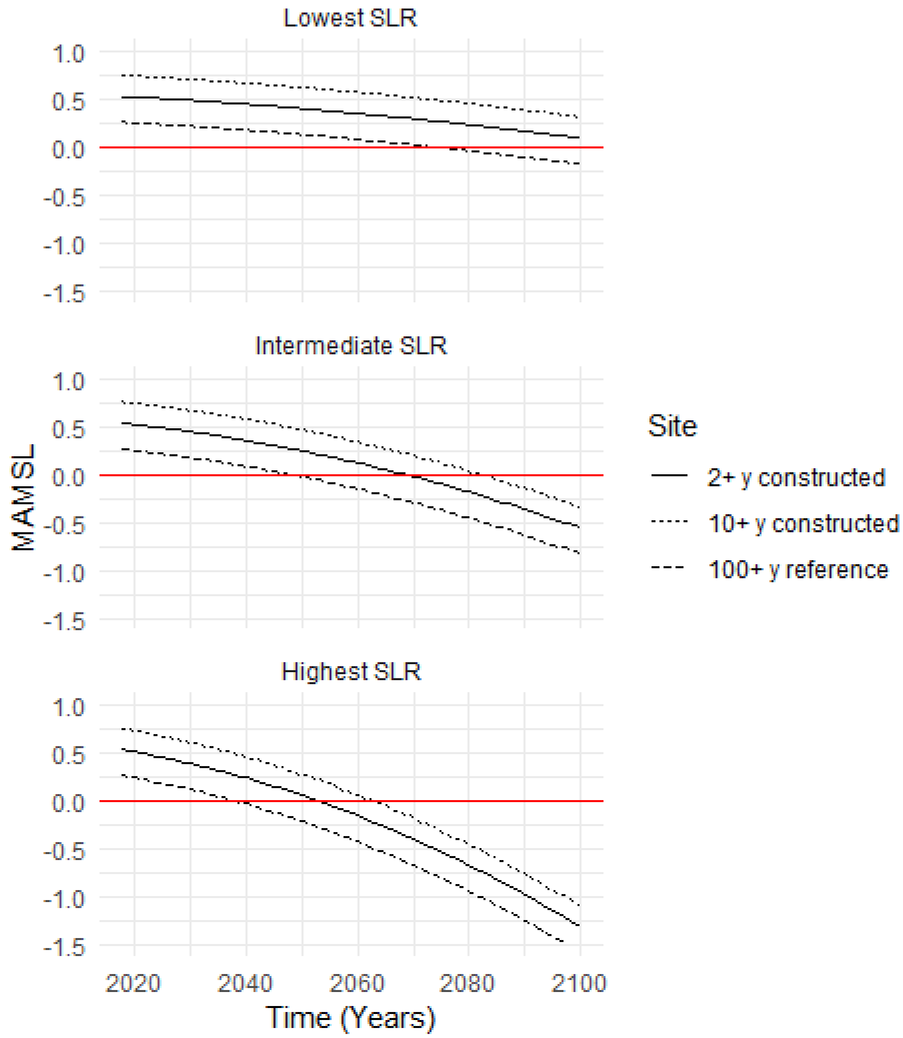

Figure 12. Average elevation in meters above mean sea level at two constructed marshes and a natural reference marsh under low-intermediate SLR (~ RCP 4.5), intermediate SLR (~RCP 8.5), and high SLR from 2019 – 2100.

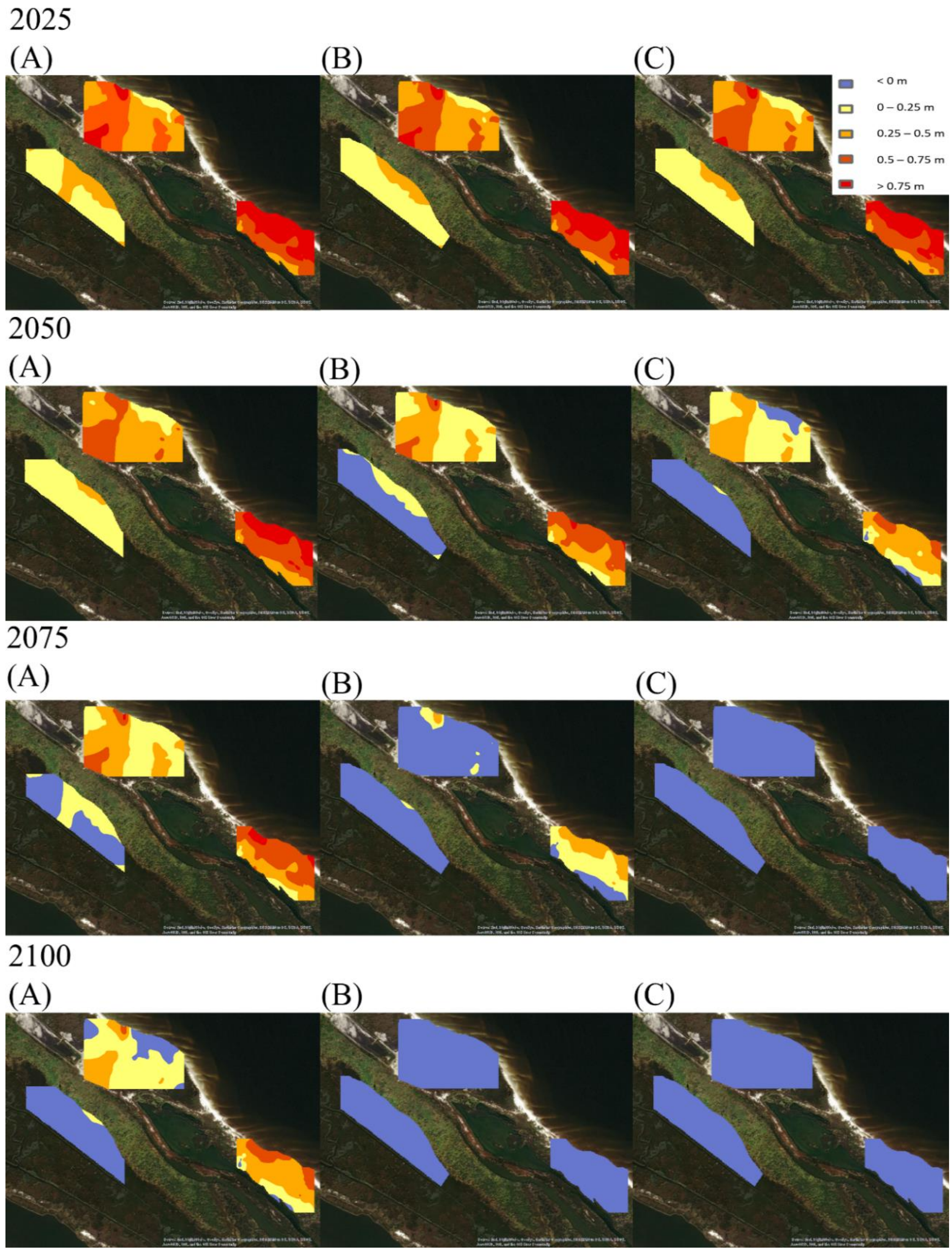

Figure 13. Emergent elevation of two constructed sites and a natural reference marsh over time (2025 – 2100) under (A) the lowest SLR scenario (~RCP4.5), (B) the intermediate SLR scenario (~RCP 8.5), and (C) the highest SLR scenario.

CHAPTER IV - DISCUSSION

4.1 Wetland restoration and site construction

Constructed wetlands are becoming more prevalent as humans attempt to offset the loss of valuable coastal environments. Enhancing coastal wetlands by restoring environments to their natural footprint can lead to regaining ecosystem functions and services, such as water quality improvement, carbon sequestration, storm surge protection, and wildlife habitat. Wetland loss is rampant in the Gulf of Mexico, more so than the watersheds of the Atlantic coastline and the Great Lakes (Stedman and Dahl 2008, Engle 2011). Restoration in the United States has generally targeted *Spartina*-dominated marshes in Texas, Louisiana, and much of the Atlantic coast (Webb and Newling 1984, LaSalle et al. 1991, Taniguchi 1996, Zedler and Callaway 1999). The *Juncus*-dominated marshes of Mississippi and Alabama have gone relatively-unnoticed and thus assessments of marsh restoration in these areas are few and far between (LaSalle 1996, Lang 2012).

The success of marsh restoration should be measured by a project's progress towards goals specifically stated by in the restoration management plan, however, the goals often have little specificity or a timeline for meeting them (Zedler and Callaway 2000). This study measured progress of the two marshes constructed with beneficial-use material on Deer Island by comparing classic developmental indicators such as plant diversity and biomass, as well as SOC. Salt marsh development is only able to take place once vegetation is planted or grown in adequate densities at a proper elevation with soil that is nontoxic and unrestricting of root growth.

The elevation and physical sediment characteristics varied significantly between the constructed and reference sites. The 10+ y constructed site tended to be higher in elevation and have higher portions of larger sand grain sizes than both the 2+ y constructed site and the 100+ y reference marsh. Soil organic content from sediment cores taken from the 10+ y constructed site and the 2+ y constructed site was comparable between the two sites, despite them being more than a decade apart from each other. Grain size has been shown to play a role in the accretion of SOC within salt marsh sediments, as finer sediments tend to accumulate SOC more rapidly (Thomas 2004). SOC at constructed sites tend to be comparable to natural reference sites within a decade (Edwards and Proffitt 2003, Craft et al. 2003), meaning that the 10+ y constructed site is on the upper-limit of the age expectation for this indicator, while the 2+ y constructed site still has time. The differences in SOC at the 10+ y constructed site are likely a reflection of the coarser sediments used to fill the site, which can increase porosity and thereby oxygen exposure, resulting in more rapid decomposition of organic matter at the expense of building the SOC pool (Mavrodi et al. 2018). Despite the relatively lower SOC in samples collected from the constructed sites, the bulk density was significantly higher than the 100+ y reference marsh, even though these variables tend to correlate positively (Avnimelech et al. 2001). Higher sediment bulk density has been shown to correlate with *S. alterniflora* aboveground biomass by DeLaune and Pezeshki (1988). Any relationship between bulk density and belowground biomass is unexplored in *S. alterniflora* or *J. roemerianus* dominated marshes, however, the bulk density and root biomass relationship tends to vary by species (Helliwell et al. 2019, Jones 1983).

Ideal soil conditions for future projects in Mississippi would more closely mimic reference soil conditions to promote development of marsh productivity and community composition. Crawford and Stone (2015) suggest that, during periods of drought, *S. alterniflora* marshes with lower silt content, higher bulk density, and lower water retention are more likely to experience marsh dieback. Silt and clay particles have a greater ability to retain plant nutrients and organic matter due to high surface area and cation exchange capacity (Jackson et al. 2006). Burial of soil organic carbon within *J. roemerianus* marshes is likely enhanced with more frequent tidal inundation, where mineralization is lessened (Steinmuller et al. 2019). Based on the lower SOC at the 10+ y constructed site, it would likely be beneficial for future restoration efforts at Deer Island, MS to acquire more fine sediments as well as ensure that the site is constructed at an elevation that would be more frequently tidally inundated. These modifications to site structure would likely increase ecological functions such as carbon burial and promote a plant community structure more similar to the 100+ y reference site, at least in the portions of the constructed site where *J. roemerianus* and *S. alterniflora* were planted (Woerner and Hackney 1997, Jackson et al. 2006, Wolf et al. 2011, Crawford and Stone 2015, Kulawardhana et al. 2015, Helliwell et al. 2019, Steinmuller et al. 2019).

4.2 Vegetative community composition

Alpha- and beta-diversity were measured at the constructed and reference sites from point-intercept and quadrat sample data, respectively. The measurements hold value as they capture the diversity within specific site and season combinations (alpha diversity) as well as the differences in diversity between sites (beta diversity). The 10+ y constructed site was the highest and most dynamic in elevation, and in turn was the most diverse both in terms of species richness (n = 32) and diversity indices. The relationship between variation in elevation and salt marsh vegetative community composition can also be found at the 2+ y constructed site and the 100+ y reference marsh. the 2+ y constructed site averaged 0.54 MAMSL and had higher species richness (n = 16) than the 100+ y reference marsh which was lower in elevation (0.27 MAMSL) and species richness (n = 5). Abundances of species that the sites had in common were also different. Abundance of *S. alterniflora* at the 2+ y constructed site (cover = 74%) was more similar to the 100+ y reference marsh (cover = 61%) than it was to the 10+ y constructed site (cover = 16%) as a much larger portion of the 2+ y constructed site and the 100+ y reference marsh was below 0.5 MAMSL. *J. roemerianus*, which tends to dominate at higher elevations than the *S. alterniflora* zone, covered 37% of the 100+ y reference marsh but was effectively absent from the constructed sites despite the 18,836 stems planted at the 2+ y constructed site and the 13,440 stems planted at the 10+ y constructed site for reasons that are not yet fully understood. The dune associated grasses, *S. patens* and *D. spicata*, were found to cover 40% and 10% of the 10+ y constructed site, respectively, while less than 10% of the 2+ y constructed site was covered by both of these species. It is likely that over time the 2+ y constructed site could begin to reach species richness comparable to the 10+ y constructed site in the higher elevation zone of the site, as natural recruitment of species can take up to five years (Mitsch and Wilson 1996).

Indicator species analysis was used to assess whether relationships between elevation ranges and salt marsh zonation could be observed in the constructed marshes. Overall the patterns seen between salt marsh species and elevation ranges were as expected, and most deviations from classical salt marsh zonation were statistically insignificant. A notable pattern from the 10+ y constructed site is the significant association of *D. spicata* with the 0 – 0.54 MAMSL zone (IV = 0.74, $p < 0.001$), as it tends to be found bordering the upper limit of the mid-elevation, *J. roemerianus* dominated zone (Eleuterius 1972, Eleuterius and Eleuterius 1979, Hunter et al. 2008). The use of indicator species analysis at the 100+ y reference marsh was uninformative in terms of statistical power, as the bulk of the site is classified by the 0 – 0.54m elevation zone, which impaired the ability of the analysis to compare the observed patterns with the permuted patterns. This analysis could be improved by more extensive sampling of the higher elevation zone that separates the constructed sites from the natural reference marsh.

4.3 Development of biomass in restored and constructed salt marshes

The biomass compartments of vegetation act as proxies for certain ecosystem functions. The above-ground biomass, which is a proxy for the amount of material available to undergo photosynthesis, was comparable across all sites. In addition to primary production, the lush canopy also can act to provide habitat for birds and enhance the site's ability to provide a buffer from storm surge (Farber 1987). An optimal trajectory of marsh biomass over time is unknown as there is a lack of long-term monitoring of a single site. Craft et al. (2003) approximated marsh biomass responses over time by comparing sites of differing ages with similar geomorphic position, tidal range, salinity, and soil classification to overcome the lack of monitoring data. Craft et al. (2003) found that, in North Carolina restored marshes, above-ground biomass can develop in the first 5 years, while root material can develop as fast as 15 years post-construction. Through meta-analysis of 25 restored wetland assessments in the northern Gulf of Mexico, Ebbets et al. (2019) developed a trajectory of above- and below-ground biomass, cover, and SOC. Ebbets et al. (2019) showed that in the first 5 years of development, restored marshes tend to have 25% higher above-ground biomass than reference sites, and in the first 15 years, below-ground biomass was between 44 to 92% lower at restored sites than reference sites. The meta-analysis done by Ebbets et al. (2019), while specific to the northern Gulf of Mexico, lacks source material from the *Juncus*-dominated marshes of Mississippi and Alabama and is comprised of entirely *Spartina*- dominated marshes in Texas and Louisiana. To my knowledge, Lasalle (1996) and Sparks et al. (2015) are the only assessments of restored *J. roemerianus* marshes that measure both above- and below-ground biomass. Lasalle (1996) studied an eight-year-old restored *Juncus*-dominated marsh in Pascagoula, MS and found that above-ground biomass at the restored marsh was comparable to the natural reference marsh, while below-ground biomass was not. Sparks et al. (2015) showed that at a *Juncus*-dominated marsh in Grand Bay National Estuarine Research Reserve in Mississippi, above- and below-ground biomass in full-density planted *J. roemerianus* plots were comparable to reference plots after two years. The site examined in Sparks et al. (2015) was planted with transplanted sods of *J. roemerianus* from a nearby marsh as opposed to transplanted plugs from a nursery like at our study site on Deer Island, MS.

Deer Island, MS is a mainland remnant island, which provides a buffer from tropical storms and hurricanes to nearby Biloxi, MS. It is a prime case for restoration assessment as the construction of marshes with beneficial-use material is relatively uncommon in Mississippi when compared to the likes of Texas and Louisiana (Ebbets et al. 2019). The 2+ y and the 10+ y constructed sites have followed the trajectory of aboveground biomass and cover in constructed marshes shown in previous studies (Webb and Newling 1984, LaSalle 1996, Zedler and Callaway 1999, 2000, Craft et al. 2002, 2003, Sparks et al. 2015, Ebbets et al. 2019). The 10+ y constructed site on Deer Island, MS has yet to develop roots comparable to the natural reference marsh, showing results similar to the analyses in Ebbets et al. (2019). The 2+ y constructed site was less than 5 years old at the time of this study but has root biomass comparable to the 10+ y constructed site, meaning a case could be made for the 2+ y constructed site to have root biomass comparable to the natural reference site by the time it reaches the 15 year mark. Based on the above conclusion on root biomass, it is possible that the 10+ y constructed site could perform poorly in sequestering carbon, as the more coarse sand and poor root development could negatively impact carbon burial (Mcleod et al. 2011). The age of the 10+ y constructed site could potentially be misleading due to the periodic plantings over the past 10+ years, however, the comparability between the 10+ y constructed site and the 2+ y constructed site makes it worrisome that so little root material is present despite more than 10 years of vegetation presence.

The differences in vegetative community assemblage as well as structural differences provide implications for overall biodiversity at the constructed sites (Streever 2000). The elevation, sediment characteristics, and vegetative composition are likely to influence bird, fish, and invertebrate habitat usage. Utilization of beneficial-use material marshes by birds is relatively inconclusive, but a common trend is that bird assemblage varies with both vegetative composition as well as structures commonly found in created marshes, however, this is probably dependent on the type and amount of cover for nest safety and food availability (Burger 2017). Invertebrates and fish depend largely on the ability to evade predation by using vegetation as coverage (Weisberg et al. 1981, Baumann et al. 2018). Further, invertebrate diet often consists of benthic macroalgae, detritus, and alive or senesced leaves, which are abundant in both constructed and natural marshes. These various organic resources form the basis of the marsh trophic web and can be tracked through stable isotope analysis of tissue and sediment samples.

4.4 $\delta^{13}C$ and $\delta^{15}N$ in *S. alterniflora* from constructed marshes

Biogeochemical cycling in salt marshes is driven by interactions between salt marsh organisms (e.g., primary producers, mycorrhizal fungi, rhizobacteria, consumers, decomposers) and their environment. The primary cycles that influence the vegetation primary productivity of salt marsh vegetation are the carbon, nitrogen, sulfur, and phosphorus cycles. The ways that salt marsh plants interact with these biogeochemical cycles are similar at their core, but nuances in physiological processes (e.g., photosynthesis) result in variation of the chemical properties of primary producers' tissues. C_3 and C_4 photosynthesis are the two types of photosynthesis pathways found in salt marsh plants on Deer Island. Differences in these pathways, like the use of phosphoenolpyruvate (PEP) carboxylase in C_4 plants (e.g., *S. alterniflora*) versus the primary use of the enzyme ribulose-1,5-bisphosphate carboxylase oxygenase (RuBisCo) in C_3 plants (e.g., *J. roemerianus*) result in differential fractionation of $\delta^{13}C$. Measuring stable isotopes like $\delta^{13}C$ and $\delta^{15}N$ to assess trophic levels of organisms and identify the base of food chains is ubiquitous in ecological studies today (Haines 1976, Peterson and Fry 1987, Currin et al. 1995). Stable isotope work has been completed on restored oyster reefs (Dillon et al. 2015) and marshes (Llewellyn and Peyre 2011) within the northern Gulf of Mexico and is valuable in tracking the usage of constructed marshes in the future.

Dillon et al. (2015) showed *Crassostrea virginica* (eastern oyster), an economically valuable fishery in the Mississippi Sound, utilized *J. roemerianus*-derived carbon in the form of detritus as well as benthic macro-algae and phytoplankton. Llewellyn and Peyre (2011) showed restored marshes can be, in the matter of trophic support, functionally equivalent to natural marshes within 10 years through stable isotope analysis of *Callinectes sapidus* (blue crab). Further, measuring C and N content of vegetative growth post-planting provides an indicator of relative nutrient availability in constructed marshes. The total C content of *S. alterniflora* at the three study sites were found to be comparable to *S. alterniflora* marshes elsewhere in the United States (White and Howes 1994, Anderson et al. 1997). Stable isotope $\delta^{13}C$ found in *S. alterniflora* tissues showed no major deviation from the accepted values for healthy *S. alterniflora* tissues (Benner et al. 1987). The similarity in $\delta^{13}C$ and C (%) content of *S. alterniflora* tissues at Deer Island suggest that plants at the three sites function similarly in terms of C-fixation through photosynthesis. However, based on the below-ground biomass and sediment organic content, there is little C burial occurring yet at the constructed sites when compared to the natural reference marsh. Carbon sequestered from the atmosphere and buried in wetland sediments is called blue carbon, and storing blue carbon is a major beneficial function of salt marshes and mangroves (Davis et al. 2015). Salt marshes, mangroves, and seagrass beds bury as much as ten times more carbon per unit area than tropical, boreal, and temperate forests (Mcleod et al. 2011, Davis et al. 2015). Assimilation of atmospheric CO_2 into root biomass acts to combat SLR by both increasing resilience (e.g., capturing sediment, raising elevation of marsh platform) and removing CO_2 from the atmosphere, which slows climate change and feeds back to reduced SLR rates (Stralberg et al. 2011, Kirwan and Megonigal 2013, Kulawardhana et al. 2015, Wu et al. 2017).

The differences in $\delta^{15}N$ among the alive and dead tissues at the three study sites are likely due to fluctuations in microbial activity during the decomposition process (Bouillon et al. 2011). The higher N content of *S. alterniflora* tissues from the 2+ y constructed site could be reflected in the apparently higher $\delta^{15}N$ of dead tissues (4.24‰) than alive (3.63‰) (Bouillon et al. 2011). In comparison, both the 10+ y constructed site and the 100+ y reference marsh have live tissues with higher $\delta^{15}N$ than dead tissues. The higher N content in *S. alterniflora* tissues could be a remnant of the fertilization of the soil during planting as well as a reservoir of nitrogen that was present in the beneficial-use material. It is plausible that, based on the decrease in N with increase in the age of the sampled sites, there is a trend that will see a decrease in both the 2+ y constructed site and the 10+ y constructed site nitrogen availability over time to levels comparable to the 100+ y reference marsh as nutrients are flushed/consumed from the system. Herbivores have been shown to prefer N-rich plants by Silliman and Zieman (2001), Silliman and Bertness (2002) and He and Silliman (2015). Due to the high N content of vegetation within the constructed sites, the plant community could facilitate the establishment of herbivores such as crustaceans, gastropods, and insects. Insect grazing in *Juncus* marshes in Grand Bay was found to be a major source of trophic N transfer to the adjacent terrestrial habitats by Sparks and Cebrian (2015) and Montemayor et al. (2017)

Gaining observations on the stable isotope composition of *S. alterniflora* on the island could prove useful in the future as assessment of other resources, such as birds and invertebrates, are completed to understand the trophic usage of the constructed marshes.

Estimates about the trophic level of resident fauna at the study sites on Deer Island would need a more complete picture of the isotopic composition of other potential food sources at the base of the food web, such as tissues of other plant species, detritus, and benthic macroalgae. It would have been valuable to have gained stable isotope measurements for other critical species such as *J. roemerianus* and *S. patens*, however, lack of substantial plant material for comparisons and financial burden of analyzing samples prevented this for this study.

4.5 Resilience of Deer Island to sea level rise

The threat of SLR to coastal systems is relevant in assessing the ability of natural and constructed wetlands to function in the long term (decades to centuries). Some ecosystem services that constructed wetlands can be expected to provide may take decades to develop (Ebbets et al. 2019). It has been discussed in past work that coastal wetlands exhibit resiliency to SLR as plant biomass traps and accretion of sediments on the marsh platform, effectively raising elevation and maintaining the marsh function. Wu et al. (2017) modelled the resiliency of the nearby Grand Bay NERR to SLR and found that factors that contribute to resiliency are sediment deposition, erosion, as well as below- and above-ground biomass. I projected the impact of SLR on the marsh platform of the constructed and reference sites on Deer Island in a simple way with no parameterization outside of the different SLR rates and recognize that there are more physical processes that may contribute to the resilience of marshes to SLR. Based on my study I can infer from Wu et al. (2017) that Deer Island will likely remain resilient if emissions follow the best-case scenario (RCP 2.6) proposed by the IPCC. Under current or higher emission levels, Deer Island has a poor chance of keeping up with SLR and will likely be lost. This is even more so the case at the constructed sites if below-ground biomass production does not reach comparable levels to the natural reference site as maintaining elevation will be difficult without organic matter sequestration. Sediment deposition rates at Deer Island are unknown, therefore, it is unclear how sites will respond with increasing SLR outside of the projection I've created. I could surmise based on the construction strategy of the 10+ y constructed site and the 2+ y constructed site that the marshes within the containment dikes will see low sediment deposition as the dike prevents sediment from both leaving and being deposited on the inner marsh. If these sites fail in accreting elevation and emissions are unchanged or worsen, all sites could be lost as early as 2065 (Figure 11). The vegetative community composition of sites could shift from a mix of low- and high-marsh plants towards a majority of low- to mid-marsh plants, effectively diminishing the biodiversity of plant and bird wildlife alike, as the MAMSL of the marsh platform lowers and abiotic stress from waterlogged soils increases. It is imperative that the future of constructed and natural wetlands take into consideration climate change policy in the coming years and develop mitigation plans for future periods when SLR begins to outpace natural processes that maintain marsh elevation.

CHAPTER V - CONCLUSION

This study was valuable in providing a comparative assessment of two adjacent beneficial-use material filled and planted sites of differing ages, which were planned to create wetlands representative of classical northern Gulf of Mexico salt marshes. The post-construction vegetative assessment at the 2+ y and 10+ y constructed site has shown a mixed bag of successes and areas that leave more to be desired. With regards to the canopy of the constructed marshes, the amount of above-ground material and N content in leaves suggest that the sites are thriving in vegetative growth and can provide habitat for higher trophic level organisms. The overall diversity of the plant community is high, further supporting prior observations that the 2+ y and 10+ y constructed sites can support a diverse community of salt marsh fauna. In the rhizosphere, the amount of below-ground material at the 10+ y constructed site is far below that of the 100+ y reference marsh and is more comparable to the 2+ y constructed site, which was constructed almost a decade later. The amount of SOC from cores collected at the sites also still shows evidence of more aerobic than anaerobic decomposition at the 10+ y constructed site, which is likely due to the higher elevation and more coarse sandier sediments. The 2+ y constructed site overall seems to be developing well in terms of both root biomass and sediment organic deposition, however, the future of the site will need to be monitored for progress to ensure this trajectory is maintained. The study was successful in providing measurements of stable isotopes $\delta^{13}C$ and $\delta^{15}N$ in *S. alterniflora* tissues for reference in future assessments of the constructed sites on Deer Island, MS. The future of Mississippi salt marsh restoration using beneficial-use sediments is going to rely on successful colonization of *J. roemerianus*, whether by planting or natural recruitment, as this species is indicative of a natural northern Gulf of Mexico salt marsh. It is unclear how the plant community and marsh platform will respond to rising sea level as there are many factors that determine the resilience of individual wetlands, but it is apparent that Deer Island is at risk of being submerged or heavily fragmented if CO_2 emissions and SLR are not reduced.

Future assessments at Deer Island could examine: (1) the factors that determine successful transplanting of *J. roemerianus,* (2) the function of the 2+ y and 10+ y constructed sites in filtering nutrients, in particular N, (3) utilization of the sites by herbivorous insects, and (4) sediment deposition within the 2+ y and 10+ y constructed sites. The proposed studies could allow resource managers and the public to better understand how these constructed sites provide ecosystem services as well as aid the reduction of the financial burden imposed by failed plantings.

APPENDIX A - Elevation, bulk density, grain size, and sediment organic content summary and ANOVA tables, and Tukey's HSD comparisons.

Table A.1 Summary One-Way ANOVA table for mean elevation (MAMSL) by site (n = 3).

Source	*df*	SS	MS	*F*	*p*
Site	2	6.638	3.319	239.4	< 0.001
Residuals	162	2.246	0.014		

Table A.2 Tukey's HSD contrasts of elevation measured in meters above mean sea level (MAMSL) at constructed and reference salt marsh sites on Deer Island, MS. * denotes statistical significance.

	Elevation (MAMSL)
Contrast	*p*
2+ y constructed - 10+ y constructed	< 0.001
2+ y constructed - 100+ y reference	< 0.001
10+ y constructed - 100+ y reference	< 0.001

Table A.3 Summary Two-Way ANOVA table for sediment bulk density by site (n = 3) and season (n = 4).

Source	df	SS	MS	*F*	*p*
Site	2	6.14	3.07	23.88	< 0.001*
Season	3	1.15	0.38	2.99	0.03*
Site x Season	6	1.08	0.18	1.39	0.22
Residuals	117	15.04	0.13		

Table A.4 Tukey's HSD contrasts of sediment bulk density, organic content, and grain size portions by percent of core, very fine sand, and silt/clay by site. * denotes statistical significance.

	Bulk Density (g/cm^3)	SOC (%LOI)	Very Fine Sand (%)	Silt/Clay (%)
Contrast	*p*	*p*	*p*	*p*
2+ y constructed - 10+ y constructed	0.97	0.04*	0.64	0.27
2+ y constructed - 100+ y reference	< 0.001*	< 0.001*	0.01*	0.1
10+ y constructed - 100+ y reference	< 0.001*	< 0.001*	0.001*	0.002*

Table A.5 Tukey's HSD contrasts of sediment bulk density, organic content, and grain size portions by percent of core, very fine sand, and silt/clay by season. * denotes statistical significance.

Contrast	Bulk Density (g/cm^3) *p*	SOC (%LOI) *p*	Fine Sand (%) *p*	Silt/Clay (%) *p*
Fall 2017 - Spring 2018	0.14	0.01*	0.89	0.07
Fall 2017 - Fall 2018	0.11	0.005*	0.1	0.98
Fall 2017 - Spring 2019	0.85	0.84	0.97	0.99
Fall 2018 - Spring 2018	0.99	0.99	0.01*	0.14
Fall 2018 - Spring 2019	0.01*	0.07	0.22	0.9
Spring 2018 - Spring 2019	0.02*	0.13	0.64	0.03*

Table A.6 Summary Two-Way ANOVA table for mean percent of coarse sand per sediment core by site (n = 3) and season (n = 4).

Source	df	SS	MS	*F*	*p*
Site	2	29.53	14.77	2.80	0.07
Season	3	28.71	9.57	1.82	0.16
Site x Season	6	27.35	4.56	0.86	0.53
Residuals	53	279.50	5.27		

Table A.7 Summary Two-Way ANOVA table for mean percent of fine sand per sediment core by site (n = 3) and season (n = 4).

Source	df	SS	MS	*F*	*p*
Site	2	214.60	107.31	3.02	0.06
Season	3	377.20	125.72	3.54	0.02*
Site x Season	6	368.60	61.43	1.73	0.13
Residuals	53	1881.40	35.50		

Table A.8 Summary Two-Way ANOVA table for mean percent of very-fine sand per sediment core by site (n = 3) and season (n = 4).

Source	df	SS	MS	F	p
Site	2	6476.59	3238.29	7.61	< 0.01*
Season	3	2569.32	856.44	2.01	0.12
Site x Season	6	1067.47	177.91	0.42	0.86
Residuals	53	22547.82	425.41		

Table A.9 Summary Two-Way ANOVA table for mean percent of silt and clay per sediment core by site (n = 3) and season (n = 4).

Source	df	SS	MS	F	p
Site	2	5410.24	2705.12	6.43	< 0.01*
Season	3	3974.13	1324.71	3.15	0.03*
Site x Season	6	1202.79	200.47	0.48	0.82
Residuals	53	22283.98	420.45		

Table A.10 Summary Two-Way ANOVA table for percent sediment organic content by site (n = 3) and season (n = 4)

Source	df	SS	MS	F	p
Site	2	2801.50	1400.75	79.78	< 0.001*
Season	3	161.30	53.77	3.06	0.03*
Site x Season	6	84.40	14.07	0.80	0.57
Residuals	117	2054.28	17.56		

APPENDIX B - Species lists, coverage, diversity indices, ANOSIM contrasts, and indicator species summary tables.

Table B.1 Species list of salt marsh and dune vegetation observed from point-intercept sampling at two constructed marshes and a natural reference marsh across all sampling seasons.

Species	2+ y constructed	10+ y constructed	100+ y reference
Andropogon virginicus L.		< 1%%	
Baccharis halimifolia L.		2%	
Cyperus spp.		< 1%	
Distichlis spicata (L.) Greene	3%	10%	1%
Eragrostis secundiflora J. Presl		3%	
Eupatorium capillifolium (Lam.) Small	< 1%	< 1%	
Fimbristylis castanea (Michx.) Vahl		2%	
Heterotheca subaxillaris (Lam.) Britton & Rusby		< 1%	
Hydrocotyle bonariensis Comm. Ex Lam.		9%	
Imperata cylindrica (L.) P. Beauv.		< 1%	
Ipomoea imperati (Vahl) Griseb.		< 1%	
Iva frutescens L.	< 1%	1%	
Iva imbricata Walter		< 1%	
Juncus roemerianus Scheele	1%	2%	37%
Limonium carolinianum (Walter) Britton		< 1%	
Phyla nodiflora (L.) Greene		< 1%	
Panicum amarum Elliott	2%	< 1%	
Panicum repens L.	2%		
Paspalum distichum L.	1%	< 1%	
Physalis angustifolia Nutt.		< 1%	
Polypremum procumbens L.		< 1%	

Table B.1 (continued)

Species	2+ y constructed	10+ y constructed	100+ y reference
Proserpinaca intermedia Mack.		< 1%	
Ruppia maritima L.	2%		
Sarcocornia perennis (Mill.) A.J. Scott		< 1%	
Schizachyrium maritimum (Chapm.) Nash	1%	1%	
Schoenoplectus americanus (Pers.) Volkart ex Schinz & R. Keller	< 1%	< 1%	
Schoenoplectus robustus (Pursh) M.T. Strong	< 1%	< 1%	< 1%
Sesbania herbacea (Mill.) McVaugh		< 1%	
Sesuvium portulacastrum (L.) L.	1%		
Solidago sempervirens L.		4%	

Table B.2 Species list of salt marsh and dune vegetation observed from quadrat sampling at two constructed marshes and a natural reference marsh across all sampling seasons.

Species	10+ y constructed	2+ y constructed	100+ y reference
Andropogon virginicus L.	3%		
Symphyotrichum tenuifolium (L.) G.L. Nesom	1%		
Baccharis halimifolia L.	2%	< 1%	
Cyperus spp.	1%		
Distichlis spicata (L.) Greene	8%	9%	1%
Eragrostis secundiflora J. Presl	2%		
Eupatorium capillifolium (Lam.) Small	1%		
Fimbristylis castanea (Michx.) Vahl	5%		
Hydrocotyle bonariensis Comm. Ex Lam.	1%		
Ipomea stolonifera	< 1%		
Iva frutescens L.	2%		
Juncus roemerianus Scheele	2%	1%	36%
Panicum amarum Elliott	%	5%	
Panicum repens L.	%	< 1%	
Paspalum distichum L.	3%	6%	
Polypremum procumbens L.	< 1%		
Schoenoplectus americanus (Pers.) Volkart ex Schinz & R. Keller	1%	1%	
Sesbania herbacea (Mill.) McVaugh	< 1%		
Sesuvium portulacastrum (L.) L.	< 1%	< 1%	
Solidago sempervirens L.	4%		
Spartina alterniflora Loisel.	34%	63%	62%
Spartina patens (Aiton) Muhl.	29%	13%	1%
Uniola paniculata L.		1%	
Vigna luteola (Jacq.) Benth	2%	< 1%	

Spring 2017

Table B.3 Ground coverage of species measured from quadrat sampling at the 2+ y constructed site, from the Spring 2017 sampling season.

Species	Percent Cover	n
Spartina alterniflora	28.09	10
Paspalum distichum	12.65	
Distichlis spicata	5.44	
Panicum amarum	4.41	
Spartina patens	3.53	
Schoenoplectus spp.	0.88	
Panicum repens	0.88	
Vigna luteola	0.29	
Sesuvium portulachastrum	0.15	
Juncus roemerianus	0.15	
No vegetation	43.53	

Table B.4 Ground coverage of species measured from quadrat sampling at the 10+ y constructed site, from the Spring 2017 sampling season.

Species	Percent Cover	n
Spartina patens	21.64	19
Spartina alterniflora	11.9	
Vigna luteola	9.31	
Distichlis spicata	8.1	
Solidago sempivirens	4.91	
Schizachyrium maritimum	4.14	
Paspalum distichum	3.79	
Fimbristylis castanea	3.36	
Baccharis halimifolia	1.47	
Symphyotrichum tenuifolium	1.38	
Eupatorium capillifolium	1.21	
Juncus roemerianus	1.21	
Hydrocotyle bonariensis	0.69	
Cyperus spp.	0.52	
Iva frutescens	0.52	
Sesbania herbacea	0.35	
Sesuvium portulachastrum	0.35	
Eragrostis secundiflora	0.09	
Polypremum procumbens	0.09	
No vegetation	24.97	

Table B.5 Ground coverage of species measured from quadrat sampling at the 100+ y reference marsh, from the Spring 2017 sampling season.

Species	Percent Cover	n
Spartina alterniflora	34.77	3
Juncus roemerianus	27.84	
Distichlis spicata	0.91	
No vegetation	36.48	

Fall 2017

Table B.6 Ground coverage of species measured from quadrat sampling at the 2+ y constructed site, from the Fall 2017 sampling season.

Species	Percent Cover	n
Spartina alterniflora	42.50	7
Spartina patens	5.42	
Distichlis spicata	3.88	
Panicum amarum	1.11	
Sesuvium portulachastrum	0.42	
Juncus roemerianus	0.42	
Paspalum distichum	0.14	
No vegetation	46.11	

Table B.7 Ground coverage of species measured from quadrat sampling at the 10+ y constructed site, from the Fall 2017 sampling season.

Species	Percent Cover	n
Spartina alterniflora	34.95	16
Spartina patens	14.28	
Fimbristylis castanea	5.25	
Iva frutescens	2.5	
Schoenoplectus spp.	2.25	
Baccharis halimifolia	2.1	
Solidago sempivirens	1.38	
Eragrostis secundiflora	1.35	
Paspalum distichum	1.25	
Vigna luteola	1.25	
Hydrocotyle bonariensis	0.88	
Cyperus spp.	0.5	
Eupatorium capillifolium	0.33	
Distichlis spicata	0.13	
Sesbania herbacea	0.1	
Panicum amarum	0.05	
No vegetation	31.45	

Table B.8 Ground coverage of species measured from quadrat sampling at the 100+ y reference marsh, from the Fall 2017 sampling season.

Species	Percent Cover	n
Spartina alterniflora	47.07	3
Juncus roemerianus	29.57	
Distichlis spicata	0.11	
No vegetation	23.25	

Spring 2018

Table B.9 Ground coverage of species measured from quadrat sampling at the 2+ y constructed site, from the Spring 2018 sampling season.

Species	Percent Cover	n
Spartina alterniflora	50	4
Spartina patens	7.86	
Schoenoplectus spp.	2.14	
Panicum amarum	2.14	
No vegetation	37.86	

Table B.10 Ground coverage of species measured from quadrat sampling at the 10+ y constructed site, from the Spring 2018 sampling season.

Species	Percent Cover	n
Spartina patens	40	9
Spartina alterniflora	24.67	
Eragrostis angustiflora	1.25	
Vigna luteola	0.67	
Baccharis halimifolia	0.67	
Iva frutescens	0.67	
Distichlis spicata	0.5	
Hydrocotyle bonariensis	0.4	
Schoenoplectus spp.	0.17	
No vegetation	31	

Table B.11 Ground coverage of species measured from quadrat sampling at the 100+ y reference marsh, from the Spring 2018 sampling season.

Species	Percent Cover	n
Spartina alterniflora	53.33	2
Juncus roemerianus	16.67	
No vegetation	30.00	

Fall 2018

Table B.12 Ground coverage of species measured from quadrat sampling at the 2+ y constructed site, from the Fall 2018 sampling season.

Species	Relative Percent Cover	n
Spartina alterniflora	47.50	4
Spartina patens	20.83	
Distichlis spicata	13.33	
Panicum amarum	5.00	
No vegetation	13.34	

Table B.13 Ground coverage of species measured from quadrat sampling at the 10+ y constructed site, from the Fall 2018 sampling season.

Species	Relative Percent Cover	n
Spartina alterniflora	27.50	9
Distichlis spicata	17.33	
Spartina patens	15.40	
Eragrostis secundiflora	3.17	
Vigna luteola	2.50	
Hydrocotyle bonariensis	1.67	
Ipomea stolonifera	0.42	
Baccharis halimifolia	0.17	
Cyperus spp.	0.17	
No vegetation	31.67	

Table B.14 Ground coverage of species measured from quadrat sampling at the 100+ y reference marsh, from the Fall 2018 sampling season.

Species	Relative Percent Cover	n
Spartina alterniflora	41.67	2
Juncus roemerianus	25.00	
No vegetation	33.33	

Spring 2019

Table B.15 Ground coverage of species measured from quadrat sampling at the 2+ y constructed site, from the Spring 2019 sampling season.

Species	Relative Percent Cover	n
Spartina alterniflora	34.38	8
Spartina patens	20.62	
Distichlis spicata	10.00	
Uniola paniculata	4.38	
Juncus roemerianus	2.50	
Panicum amarum	1.88	
Baccharis halimifolia	0.62	
Vigna luteola	0.12	
No vegetation	25.50	

Table B.16 Ground coverage of species measured from quadrat sampling at the 10+ y constructed site, from the Spring 2019 sampling season.

Species	Relative Percent Cover	n
Spartina alterniflora	36.00	8
Spartina patens	20.00	
Juncus roemerianus	12.00	
Eragrostis secundiflora	7.00	
Solidago sempivirens	3.00	
Baccharis halimifolia	2.00	
Hydrocotyle bonariensis	2.00	
Fimbristylis castanea	1.00	
No vegetation	17.00	

Table B.17 Ground coverage of species measured from quadrat sampling at the 100+ y reference marsh, from the Spring 2019 sampling season.

Species	Relative Percent Cover	n
Spartina alterniflora	40	3
Juncus roemerianus	32.5	
Distichlis spicata	1.25	
No vegetation	26.25	

Fall 2017

Table B.18 Ground coverage of species measured from point-intercept sampling at the 2+ y constructed site, from the Fall 2017 sampling season.

Species	Relative Percent Cover	n
Spartina alterniflora	45.15	5
Spartina patens	5.34	
Panicum amarum	2.91	
Distichlis spicata	2.43	
Paspalum distichum	0.97	
No Vegetation	43.20	

Table B.19 Ground coverage of species measured from point-intercept sampling at the 10+ y constructed site, from the Fall 2017 sampling season.

Species	Relative Percent Cover	n
Spartina patens	41.92	11
Spartina alterniflora	13.64	
Distichlis spicata	6.06	
Hydrocotyle bonariensis	5.56	
Solidago sempervirens	3.54	
Baccharis halimifolia	3.03	
Eragrostis secundiflora	1.52	
Fimbristylis castanea	1.52	
Juncus roemerianus	1.52	
Schizachyrium maritimum	1.01	
Iva frutescens	0.51	
No Vegetation	20.20	

Table B.20 Ground coverage of species measured from point-intercept sampling at the 100+ y reference marsh, from the Fall 2017 sampling season.

Species	Relative Percent Cover	n
Spartina alterniflora	52.86	3
Juncus roemerianus	31.90	
Distichlis spicata	0.48	
No Vegetation	14.76	

Spring 2018

Table B.21 Ground coverage of species measured from point-intercept sampling at the 2+ y constructed site, from the Spring 2018 sampling season.

Species	Relative Percent Cover	n
Spartina alterniflora	54.10	7
Panicum repens	4.59	
Spartina patens	3.93	
Panicum amarum	1.64	
Schizachyrium maritimum	1.64	
Uniola paniculata	1.64	
Vigna luteola	0.66	
No Vegetation	31.80	

Table B.22 Ground coverage of species measured from point-intercept sampling at the 10+ y constructed site, from the Spring 2018 sampling season.

Species	Relative Percent Cover	n
Spartina patens	41.47	21
Spartina alterniflora	20.29	
Distichlis spicata	12.06	
Fimbristylis castanea	5.00	
Hydrocotyle bonariensis	4.71	
Eragrostis secundiflora	2.06	
Vigna luteola	1.47	
Imperata cylindrica	1.18	
Schizachyrium maritimum	1.18	
Solidago sempervirens	1.18	
Andropogon virginicus	0.88	
Baccharis halimifolia	0.59	
Physalis angustifolia	0.59	
Symphyotrichum tenuifolium	0.29	
Eupatorium capillifolium	0.29	
Iva frutescens	0.29	
Juncus roemerianus	0.29	
Limonium carolinianum	0.29	
Lippia nodiflora	0.29	
Polypremum procumbens	0.29	
Typha domingensis	0.29	
No Vegetation	5	

Table B.23 Ground coverage of species measured from point-intercept sampling at the 100+ y reference marsh, from the Spring 2018 sampling season.

Species	Relative Percent Cover	n
Spartina alterniflora	64.35	4
Juncus roemerianus	24.64	
Distichlis spicata	1.74	
Spartina patens	0.29	
No Vegetation	8.99	

Fall 2018

Table B.24 Ground coverage of species measured from point-intercept sampling at the 2+ y constructed site, from the Fall 2018 sampling season.

Species	Relative Percent Cover	n
Spartina alterniflora	61.74	14
Spartina patens	9.28	
Distichlis spicata	5.80	
Panicum amarum	2.32	
Paspalum distichum	2.03	
Vigna luteola	2.03	
Sesuvium portulachastrum	1.16	
Eupatorium capillifolium	0.87	
Juncus roemerianus	0.87	
Ruppia maritima	0.87	
Schoenoplectus robustus	0.87	
Iva frutescens	0.29	
Schoenoplectus americanus	0.29	
Uniola paniculata	0.29	
No vegetation	11.30	

Table B.25 Ground coverage of species measured from point-intercept sampling at the 10+ y constructed site, from the Fall 2018 sampling season.

Species	Relative Percent Cover	n
Spartina patens	32.18	25
Spartina alterniflora	13.10	
Hydrocotyle bonariensis	8.97	
Distichlis spicata	8.28	
Vigna luteola	6.90	
Eragrostis secundiflora	3.68	
Iva frutescens	2.99	
Solidago sempivirens	2.76	
Juncus roemerianus	2.53	
Sesbania herbacea	2.07	
Symphyotrichum tenuifolium	1.84	
Schoenoplectus robustus	1.61	
Ipomea imperati	1.15	
Baccharis halimifolia	0.92	
Paspalum distichum	0.92	
Andropogon virginicus	0.69	
Fimbristylis castanea	0.69	
Proserpinaca intermedia	0.69	
Eupatorium capillifolium	0.46	
Lippia nodiflora	0.46	
Sarcocornia perennis	0.46	
Schizachyrium maritimum	0.46	
Cyperus spp.	0.23	
Panicum amarum	0.23	
Schoenoplectus americanus	0.23	
No vegetation	5.52	

Table B.26 Ground coverage of species measured from point-intercept sampling at the 100+ y reference marsh, from the Fall 2018 sampling season.

Species	Relative Percent Cover	n
Spartina alterniflora	47.97	2
Juncus roemerianus	45.53	
No vegetation	6.50	

Spring 2019

Table B.27 Ground coverage of species measured from point-intercept sampling at the 2+ y constructed site, from the Spring 2019 sampling season.

Species	Relative Percent Cover	n
Spartina alterniflora	65.00	10
Spartina patens	9.12	
Ruppia maritima	5.29	
Distichlis spicata	1.76	
Juncus roemerianus	1.47	
Schoenoplectus robustus	1.18	
Uniola paniculata	0.88	
Panicum amarum	0.59	
Paspalum distichum	0.59	
Sesuvium portulachastrum	0.59	
No vegetation	13.53	

Table B.28 Ground coverage of species measured from point-intercept sampling at the 10+ y constructed site, from the Spring 2019 sampling season.

Species	Relative Percent Cover	n
Spartina patens	34.96	20
Spartina alterniflora	13.18	
Hydrocotyle bonariensis	10.89	
Distichlis spicata	8.88	
Solidago sempivirens	6.02	
Juncus roemerianus	4.01	
Baccharis halimifolia	2.29	
Eragrostis secundiflora	2.29	
Schizachyrium maritimum	2.29	
Imperata cylindrica	1.72	
Fimbristylis castanea	1.15	
Eupatorium capillifolium	0.86	
Schoenoplectus americanus	0.86	
Schoenoplectus robustus	0.86	
Ipomea imperati	0.57	
Iva frutescens	0.57	
Iva imbricata	0.57	
Heterotheca subaxillaris	0.29	
Lippia nodiflora	0.29	
Vigna luteola	0.29	
No vegetation	7.16	

Table B.29 Ground coverage of species measured from point-intercept sampling at the 100+ y reference marsh, from the Spring 2019 sampling season.

Species	Relative Percent Cover	n
Spartina alterniflora	58.97	5
Juncus roemerianus	35.56	
Distichlis spicata	1.22	
Schoenoplectus robustus	0.30	
Spartina patens	0.30	
No vegetation	3.65	

Table B.30 Diversity indices and species richness calculated from quadrat sampling at two constructed marshes and a natural reference marsh across the Spring 2017, Fall 2017, Spring 2018, Fall 2018, and Spring 2019 sampling seasons.

	Shannon-Wiener Index (*H*)	Simpson's Index	Richness
Spring 2017			
2+ y constructed	1.47	0.68	10
10+ y constructed	2.24	0.85	19
100+ y reference	0.75	0.51	3
Fall 2017			
2+ y constructed	0.78	0.36	7
10+ y constructed	1.65	0.69	16
100+ y reference	0.68	0.48	3
Spring 2018			
2+ y constructed	0.67	0.33	4
10+ y constructed	0.97	0.54	9
100+ y reference	0.55	0.36	2
Fall 2018			
2+ y constructed	1.37	0.69	4
10+ y constructed	1.63	0.77	9
100+ y reference	1.08	0.65	2
Spring 2019			
2+ y constructed	1.62	0.76	8
10+ y constructed	1.74	0.78	8
100+ y reference	1.14	0.67	3

Table B.31 Diversity indices and species richness calculated from point-intercept sampling at two constructed marshes and a natural reference marsh from the Fall 2017, Spring 2018, Fall 2018, and Spring 2019 sampling seasons.

	Shannon-Wiener Index (*H*)	Simpson's Index	Richness
Fall 2017			
2+ y constructed	0.76	0.35	5
10+ y constructed	1.6	0.68	11
100+ y reference	0.69	0.48	3
Spring 2018			
2+ y constructed	0.84	0.36	7
10+ y constructed	1.8	0.74	21
100+ y reference	0.69	0.42	3
Fall 2018			
2+ y constructed	1.44	0.59	14
10+ y constructed	2.42	0.85	25
100+ y reference	0.89	0.56	2
Spring 2019			
2+ y constructed	1.24	0.55	10
10+ y constructed	2.22	0.83	20
100+ y reference	0.89	0.52	5

Table B.32 Results of ANOSIM comparisons of Bray-Curtis dissimilarity across two constructed marshes and a natural reference marsh. * Denotes significant difference.

Comparisons	Distance (R)	Significance
2+ y x 10+ y	0.11	< 0.001*
2+ y x 100+ y	0.21	< 0.001*
10 + y x 100 + y	0.39	< 0.001*

Table B.33 Results of indicator species analysis of salt marsh plants at the 2+ y constructed site within low and intermediate elevation zones. 1 denotes an association of a species with the respective elevation zone at the 2+ y constructed site.

Species	0 - 0.54 MAMSL	0.54 - 0.76 MAMSL	IV	*p*
Spartina patens	0	1	0.59	< 0.01*
Paspalum distichum	0	1	0.34	0.23
Vigna luteola	1	0	0.27	0.36
Baccharis halimifolia	1	0	0.21	0.42
Distichlis spicata	0	1	0.33	0.35
Schoenoplectus americanus	1	0	0.24	0.47
Spartina alterniflora	1	0	0.76	< 0.01*
Panicum amarum	0	1	0.43	0.14
Panicum repens	0	1	0.25	0.51
Sesuvium portulacastrum	0	1	0.35	0.14
Juncus roemerianus	0	1	0.33	0.33
Uniola paniculata	0	1	0.17	1.00

Table B.34 Results of indicator species analysis of salt marsh plants at the 10+ y constructed site within low, intermediate, and high elevation zones. 1 denotes an association of a species with the respective elevation zone at the 10+ y constructed site. * denotes a significant association of a species with the respective elevation zone at the 10+ y constructed site.

Species	0 - 0.54 MAMSL	0.54 - 0.76 MAMSL	> 0.76 MAMSL	IV	*p*
Spartina patens	0	1	0	0.52	0.52
Paspalum distichum	0	1	0	0.20	0.77
Vigna luteola	0	0	1	0.55	0.04*
Baccharis halimifolia	0	0	1	0.29	0.72
Eragrostis secundiflora	0	0	1	0.45	0.05
Sesbania herbacea	0	0	1	0.24	0.51
Cyperus spp.	0	0	1	0.30	0.34
Hydrocotyle bonariensis	0	1	0	0.28	0.70
Iva frutescens	0	1	0	0.34	0.21
Distichlis spicata	1	0	0	0.69	< 0.001*
Schoenoplectus americanus	0	1	0	0.21	0.46
Schoenoplectus robustus	1	0	0	0.33	0.13
Eragrostis secundiflora	0	0	1	0.39	0.15
Fimbristylis castanea	0	1	0	0.35	0.43
Spartina alterniflora	1	0	0	0.68	< 0.01*
Panicum amarum	0	1	0	0.21	0.46
Sesuvium portulacastrum	0	1	0	0.21	0.48
Juncus roemerianus	0	1	0	0.30	0.20
Solidago sempivirens	0	1	0	0.27	0.83
Schizachyrium maritimum	0	1	0	0.20	0.87
Symphyotrichum tenuifolium	0	1	0	0.21	0.48
Polypremum procumbens	0	0	1	0.17	1.00
Ipomea stolonifera	0	0	1	0.17	1.00

Table B.35 Results of indicator species analysis of salt marsh plants at the 100+ y natural reference site within low and intermediate elevation zones. 1 denotes an association of a species with the respective elevation zone at the 100+ y natural reference site.

Species	0 - 0.54 MAMSL	0.54 - 0.76 MAMSL	IV	*p*
Distichlis spicata	0	1	0.30	0.04*
Spartina alterniflora	1	0	0.95	0.29
Juncus roemerianus	1	0	0.79	0.89

APPENDIX C - Biomass summary table, ANOVA summary tables, and Tukey's HSD comparisons.

Table C.1 Summary table for mean ± SE alive, dead, and below biomass at the two constructed sites and natural reference marsh. Significant groupings from Tukey's HSD are in superscripts.

Site	Alive (g/m^2)	Dead (g/m^2)	Below (g/m^2)
Spring 2017			
2+ y constructed	696.9 (234.83)^{ab}	487.2 (184.39)^{b}	946.25 (179.24)^{b}
10+ y constructed	222.02 (80.90)^{b}	622.21 (177.46)^{ab}	1865.93 (493.57)^{b}
100+ y reference	987.44 (365.76)^{a}	618.41 (104.64)^{ab}	12501.86 (2284.11)^{a}
Fall 2017			
2+ y constructed	1200.4 (306.16)^{ab}	1470.09 (393.21)^{a}	2562.981(373.02)^{b}
10+ y constructed	549.44 (350.22)^{b}	451.13 (199.29)^{ab}	2780.73 (804.29)^{b}
100+ y reference	1080.12 (391.07)^{a}	977.66 (289.18)^{ab}	7793.91 (1235.29)^{a}
Spring 2018			
2+ y constructed	589.79 (145.51)^{ab}	678.34 (218.35)^{ab}	1023.06 (236.95)^{b}
10+ y constructed	682.32 (158.98)^{b}	769.13 (126.69)^{ab}	1502.6 (262.67)^{b}
100+ y reference	660.28 (189.81)^{a}	451.08 (77.59)^{ab}	6535.21 (905.88)^{a}
Fall 2018			
2+ y constructed	811.02 (122.58)^{ab}	665.56 (116.74)^{ab}	2424.72 (293.3)^{b}
10+ y constructed	674.56 (144.26)^{b}	494.17 (119.54)^{ab}	3063 (820.18)^{b}
100+ y reference	1095.59 (391.07)^{a}	1010.72 (256.68)^{ab}	8391.18 (1134.06)^{a}
Spring 2019			
2+ y constructed	923.88 (174.39)^{ab}	1000.18 (192.39)^{ab}	2426.23 (289.40)^{b}
10+ y constructed	537.53 (118.25)^{b}	477.54 (125.98)^{ab}	1981.74 (446.65)^{b}
100+ y reference	895.39 (212.85)^{a}	563.46 (190.33)^{ab}	5893.51 (627.85)^{a}

Table C.2 Summary Two-Way ANOVA table for alive biomass by site (n = 3) and season (n = 5).

Source	df	SS	MS	*F*	*p*
Site	2	4.05×10^{6}	2.03×10^{6}	4.07	0.02*
Season	4	2.49×10^{6}	6.23×10^{5}	1.25	0.29
Site x Season	8	2.82×10^{6}	3.53×10^{5}	0.71	0.68
Residuals	145	7.21×10^{7}	4.97×10^{5}		

Table C.3 Tukey's HSD contrasts of alive-aboveground and belowground biomass by site. * denotes statistical significance.

	Alive Biomass g/m^2	Below Biomass g/m^2
Contrast	*p*	*p*
2+ y constructed - 10+ y constructed	0.07	0.90
2+ y constructed - 100+ y reference	0.79	< 0.001*
10+ y constructed - 100+ y reference	0.02*	< 0.001*

Table C.4 Summary Two-Way ANOVA table for dead biomass by site (n = 3) and season (n = 5).

Source	df	SS	MS	*F*	*p*
Site	2	2.29×10^{6}	1.15×10^{6}	2.62	0.08
Season	4	3.17×10^{6}	7.92×10^{5}	1.81	0.13
Site x Season	8	7.18×10^{6}	8.97×10^{5}	2.05	0.04*
Residuals	145	6.35×10^{8}	4.38×10^{5}		

Table C.5 Summary Two-Way ANOVA table for belowground biomass by site (n = 3) and season (n = 5).

Source	df	*F*	*p*
Site	2	52.91	< 0.001*
Season	3	9.57	< 0.001*
Site x Season	6	1.79	0.08
Residuals	111		

Table C.6 Tukey's HSD contrasts of belowground biomass by season. * denotes statistical significance.

	Below Biomass g/m^2
Contrast	*p*
Spring 2017 - Fall 2017	0.99
Spring 2017 - Spring 2018	0.42
Spring 2017 - Fall 2018	0.88
Spring 2017 - Spring 2019	0.58
Fall 2017 - Spring 2018	0.76
Fall 2017 - Fall 2018	0.58
Fall 2017 - Spring 2019	0.88
Spring 2018 - Fall 2018	0.04*
Spring 2018 - Spring 2019	0.99
Fall 2018 - Spring 2019	0.09

Appendix D - ANOVA summary tables for tissue C and N concentration and isotope data

Table D.1 Summary table for mean ± SE $\delta^{13}C$, $\delta^{15}N$, C, N, and C:N ratio of *S. alterniflora* tissues from two restored marshes and a natural reference site.

	‰ $\delta^{13}C$	‰ $\delta^{15}N$	Carbon (%)	Nitrogen (%)	Atomic C:N (mass:mass)
Alive aboveground					
2+ year constructed	-13.44 (0.06)[a]	3.63 (1.05)	39.80 (0.42)	1.93 (0.13)[a]	21.80 (2.17)[b]
10+ year constructed	-13.07 (0.06)[b]	6.99 (0.69)	39.65 (0.46)	1.28 (0.06)[b]	31.59 (1.74)[ab]
100+ year reference	-13.06 (0.08)[b]	5.03 (0.33)	40.30 (0.71)	1.08 (0.09)[b]	40.19 (3.76)[a]
Dead aboveground					
2+ year constructed	-13.50 (0.10)[ab]	4.24 (0.68)	39.13 (0.82)	0.90 (0.08)[a]	46.94 (4.14)[b]
10+ year constructed	-13.06 (0.10)[a]	5.62 (0.56)	40.14 (0.80)	0.70 (0.07)[ab]	61.54 (8.17)[ab]
100+ year reference	-13.73 (0.23)[b]	4.56 (0.46)	40.83 (0.93)	0.58 (0.04)[b]	70.94 (3.36)[a]
Belowground					
2+ year constructed	-13.92 (0.41)	4.04 (0.59)	34.32 (2.21)	0.91 (0.08)[a]	39.47 (3.95)[b]
10+ year constructed	-13.23 (0.09)	5.48 (0.66)	37.37 (1.28)	0.68 (0.03)[b]	55.87 (3.31)[a]
100+ year reference	-14.40 (0.28)	3.19 (0.77)	37.53 (0.97)	0.77 (0.07)[ab]	50.16 (3.87)[ab]

Table D.2 Tukey's HSD contrasts of alive aboveground, dead aboveground, and belowground $\delta^{13}C$, N (%), and atomic C:N (mass:mass) by site.

	Alive $\delta^{13}C$	Dead δ13C	Alive N (%)	Dead N (%)	Below N (%)	Alive Atomic C:N (mass:mass)	Dead Atomic C:N (mass:mass)	Below Atomic C:N (mass:mass)
Contrast	*p*	*p*	*p*	*p*	*p*	*p*	*p*	*p*
2+ y constructed - 10+ y constructed	< 0.01*	0.06	< 0.001*	0.2	0.04*	0.05	0.15	< 0.001*
2+ y constructed - 100+ y reference	< 0.01*	0.50	0.34	0.04*	0.57	< 0.001*	0.02*	0.17
10+ y constructed - 100+ y reference	0.99	0.02*	< 0.001*	0.66	0.32	0.09	0.54	0.45

$\delta^{13}C$ content

Table D.3 Summary One-way ANOVA table for $\delta^{13}C$ in alive *Spartina alterniflora* tissues by site (n = 3)

Source	df	SS	MS	*F*	*p*
Site	2	0.9064	0.4532	10.45	< 0.001*
Residuals	26	1.1279	0.0434		

Table D.4 Summary One-way ANOVA table for $\delta^{13}C$ in dead *Spartina alterniflora* tissues by site (n = 3)

Source	df	SS	MS	*F*	*p*
Site	2	1.315	0.6577	5.255	0.02*
Residuals	18	2.253	0.1252		

Table D.5 Summary One-way ANOVA table for $\delta^{13}C$ in *Spartina alterniflora* root tissues by site (n = 3)

Source	df	SS	MS	*F*	*p*
Site	2	32.59	16.29.	2.773	0.09
Residuals	19	111.64	5.876		

$\delta^{15}N$ content

Table D.6 Summary One-way ANOVA table for $\delta^{15}N$ in alive *Spartina alterniflora* tissues by site (n = 3)

Source	df	SS	MS	*F*	*p*
Site	2	53.49	26.744	4.883	0.02*
Residuals	26	142.41	5.477		

Table D.7 Summary One-way ANOVA table for $\delta^{15}N$ in dead *Spartina alterniflora* tissues by site (n = 3)

Source	df	SS	MS	*F*	*p*
Site	2	7.3	3.649	1.194	0.33
Residuals	18	55	3.056		

Table D.8 Summary One-way ANOVA table for $\delta^{15}N$ in *Spartina alterniflora* root tissues by site (n = 3)

Source	df	SS	MS	*F*	*p*
Site	2	20.13	10.063	3.43	0.05
Residuals	19	55.73	2.933		

C content

Table D.9 Summary One-way ANOVA table for C (%) in alive *Spartina alterniflora* tissues by site (n = 3)

Source	df	SS	MS	*F*	*p*
Site	2	2.2	1.101	0.374	0.69
Residuals	26	76.56	2.945		

Table D.10 Summary One-way ANOVA table for C (%) in dead *Spartina alterniflora* tissues by site (n = 3)

Source	df	SS	MS	*F*	*p*
Site	2	10.45	5.226	0.962	0.40
Residuals	18	97.76	5.431		

Table D.11 Summary One-way ANOVA table for C (%) in *Spartina alterniflora* root tissues by site (n = 3)

Source	df	SS	MS	*F*	*p*
Site	2	47.70	23.84	1.175	0.33
Residuals	19	385.60	20.29		

N content

Table D.12 Summary One-way ANOVA table for N (%) in alive *Spartina alterniflora* tissues by site (n = 3)

Source	df	SS	MS	*F*	*p*
Site	2	3.98	1.9882	20.56	< 0.001*
Residuals	26	2.51	0.0937		

Table D.13 Summary One-way ANOVA table for N (%) in dead *Spartina alterniflora* tissues by site (n = 3)

Source	df	SS	MS	*F*	*p*
Site	2	0.37	0.1841	3.958	0.04*
Residuals	18	0.84	0.0465		

Table D.14 Summary One-way ANOVA table for N (%) in *Spartina alterniflora* root tissues by site (n = 3)

Source	df	SS	MS	*F*	*p*
Site	2	0.2162	0.1081	3.683	0.04*
Residuals	19	0.56	0.0294		

C:N content

Table D.15 Summary One-way ANOVA table for C:N in alive *Spartina alterniflora* tissues by site (n = 3)

Source	df	SS	MS	*F*	*p*
Site	2	1693	846.50	11.5	< 0.001*
Residuals	26	1914	73.60		

Table D.16 Summary One-way ANOVA table for C:N in dead *Spartina alterniflora* tissues by site (n = 3)

Source	df	SS	MS	*F*	*p*
Site	2	2107	1053.40	5.034	0.02
Residuals	18	3767	209.30		

Table D.17 Summary One-way ANOVA table for C:N in *Spartina alterniflora* root tissues by site (n = 3)

Source	df	SS	MS	*F*	*p*
Site	2	1089	554.30	5.739	0.04*
Residuals	19	1802	94.90		

APPENDIX E - Sea level rise summary table

Table E.1 Percent of marsh platform remaining emergent (above water) under low (~RCP 4.5), intermediate (~RCP 8.5), and high SLR scenarios at two constructed marshes and a natural reference marsh.

	2025	2050	2075	2100
Low				
2+ year constructed	100%	100%	100%	83%
10+ year constructed	100%	100%	98%	98%
100+ year reference	100%	100%	31%	4%
Intermediate				
2+ year constructed	100%	100%	7%	0%
10+ year constructed	100%	98%	76%	0%
100+ year reference	100%	22%	2%	0%
High				
2+ year constructed	100%	83%	0%	0%
10+ year constructed	100%	98%	0%	0%
100+ year reference	100%	4%	0%	0%

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