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Vegetative Community Diversity Assessment of a Beneficial Use Salt Marsh Restoration

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ABSTRACT

Restoration of lost marsh platforms can include de-novo construction using dredged sediments. Two restoration projects constructed with beneficial use material in 2004 and 2015 were planted with native vegetation in the anticipation they would function similarly to adjacent *Juncus roemerianus* (Black needlerush) dominated salt marshes. Planted *J. roemerianus*, however, failed to establish and exists sparsely in the restored marsh. To better understand potential sources of failure, this study assessed vascular plant diversity in Spring and Fall of 2017 through 2019 and demonstrated establishment of both planted and naturally recruited vegetation. The two constructed sites were found to have higher species richness and plant diversity (Shannon-Wiener H', and Simpson's D) than the natural reference marsh, in part due to the higher elevations of the two constructed sites. The plant diversity metrics indicated the two beneficial use restorations did not meet the species composition of the reference marsh. Further monitoring should be conducted to observe the species development over the long term with considerations given to the potential future marsh change.

Key Words

Salt marsh; beneficial-use; sediment organic content; diversity; Black needlerush

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Conflicts of interest/Competing interests – The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest

Availability of data and material – Data are freely available in Murphy (2020), and/or upon request to the corresponding author on reasonable request (Patrick.biber@usm.edu).

Code availability - Not applicable

Authors' contributions – NM and PB contributed to the study conception and design. Material preparation, data collection and analysis were performed by NM who then wrote the thesis on which this manuscript is based. PB edited and updated the relevant portions of that thesis to create this manuscript. All authors read and approved the final manuscript.

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INTRODUCTION

Coastal wetland losses from anthropogenic impacts are widespread due to altered sediment supply and hydrology, coastal development, and recently climate change and accelerated sea level rise and frequency of large storm events (Turner 1990, 1997; Herbert et al. 2016; Wu et al. 2017, 2020). Salt marshes in the U.S. are expected to be reduced by 20-45% by the end of the 21st century, and in the northern Gulf of Mexico (GoM), wetland loss is rampant (Stedman and Dahl 2008; Engle 2011; Kirwan and Megonigal 2013). To offset these losses, construction of coastal wetlands in the United States began in the late 20th century and has become more prominent today as a management action. Restoration of coastal wetlands in the northern GoM can include de-novo construction of lost marsh platforms using fill or dredged sediments, thin-layer placement in existing marsh platforms, or construction of dredged material 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 newly placed material with target vegetation or allow the site to be naturally colonized (Mitsch and Wilson 1996; Zedler and Callaway 1999, 2000; Craft et al. 2002, 2003; Herbert et al. 2016).

Many salt marsh distribution studies have highlighted the role of elevation in wetland restoration (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 coastal wetlands, where the plants will experience wave impacts, it can be important for managers to consider increased planting density so that plants can facilitate the growth of other individuals through positive interactions (Silliman et al. 2015). Although the starting material for transplanting salt marsh vegetation varies among species, bareroot plants are appropriate for transplanting most grasses (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 successful establishment of transplanted vegetation (Broome et al. 1988). The speed at which vegetation diversity and standing stock biomass change post-construction varies among projects and depends on successional processes (Cammen 1975; Earhart and Garbish. 1983; Webb and Newling 1984; Zedler and Callaway 1999; Craft et al. 2002, 2003; Herbert et al. 2016; Ebbets et al. 2019).

Restoration success of a constructed wetland is intertwined with the succession of species resulting in differences in diversity over time (Luken 1990; Mossman et al. 2012). Restored marsh characteristics such as vegetation coverage and plant species richness, can vary with geomorphic position, tidal range, salinity, and soil classification (Craft et al. 2003). 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 to naturally recruit plant species (Walker et al. 2007).-Species 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 sediment compaction, subsidence, and relative SLR (Choi et al. 2001). Community composition in successfully restored marshes should have similar diversity to a reference site, however, 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 reference site conditions (Petchey and Gaston 2006; Almeida et al. 2016; Taddeo and Dronova 2018).

Coastal restoration in the United States has generally targeted *Spartina alterniflora* Loisel. (Smooth cordgrass) dominated marshes in Texas, Louisiana, and along much of the Atlantic coast (Webb and Newling 1984; LaSalle et al. 1991; Taniguchi 1996; Zedler and Callaway 1999). Restoration projects that assess vegetation diversity trends in constructed *Spartina*-dominated marshes have, therefore, been well explored in the past (Woodhouse 1979; Webb and Newling 1984; LaSalle et al. 1991; Taniguchi 1996; Zedler 2000; Craft et al. 2002; Lang 2012). However, reports from restored *Juncus roemerianus* Scheele (Black needlerush) dominated marshes, in particular in the northern GoM, are under-reported and thus there are relatively few assessments of marsh restoration with this species (LaSalle 1996; Lang 2012; Sparks et al. 2013, 2015). Effective criteria for assessing constructed wetlands often include collecting data concerning plant community diversity and then comparing them to a natural reference site over time (Zedler and Callaway 2000).

This manuscript is among the first studies documenting the restoration progress of *Juncus*-dominated marshes at two beneficial use sites. Assessment of the vegetation community composition of these two restored sites was compared against an adjacent natural reference marsh using a space for time substitution with three objectives:

- 1) Understand potential drivers of plant community composition and development by measuring site elevations.
- 2) Assess the success of planted vegetation versus natural recruitment by inventorying the plant community and comparing vegetation diversity among sites.
- 3) Determine the successional trajectory of the constructed sites towards the natural marsh community by comparing species diversity change over time.

MATERIALS AND METHODS

Study sites

This study took place on two constructed sites of differing ages and a natural reference marsh on Deer Island, Mississippi (MS). Deer Island Multi-Year Restoration (DIMR) 1 and DIMR2 were constructed in 2004 and 2015, respectively, on the northeastern shore of Deer Island, MS (Figure 1). The constructed sites, DIMR1 and DIMR2, are adjacent to each other and joined by a sand containment dike that protects the inner developing marsh platforms from erosion. At both sites a shallow sandy containment berm was constructed and filled with beneficial use materials from the maintenance dredging placed at numerous discharge locations within the containment area to create variation in the finished elevation and habitat diversity (Roth et al. 2012). The western end of the containment berm was initially left open to promote circulation but subsequently filled in over time.

The 2+ year 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. The eastern third of the DIMR2 site was planted in spring 2016 with commercially purchased *J. roemerianus* (18,836 plants) and *S. alterniflora* (18,836 plants) in the interior high and low marsh zones. On the exterior containment dike, *Spartina patens* (Aiton) Muhl. (saltmarsh hay - 2,041 plants), *Panicum amarum* Elliott (bitter panicgrass - 2,041 plants), and *Uniola paniculata* L. (seaoats - 4,083 plants) (Biber 2020). Additional revegetation of the remainder of this site has occurred largely through natural recruitment since then.

The 10+ year constructed site, DIMR1, was constructed in 2004 with dredged sediments sourced from Biloxi Bay channel and is 18 hectares in size (MDMR 2010; Gerhardt Smith et al. 2015) and is highlighted as a successful example of the Engineering with Nature™ (EWN) program by the U.S. Army Corps of Engineers. The 10+ yr constructed site was first planted in

spring 2005 with field harvested *J. roemerianus* (13,440 plants), commercially purchased *S. alterniflora* (15,400 plants) and *S. patens* (17,920 plants) 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 (Lang 2012; Roth et al. 2012). Further planting of *J. roemerianus*, *S. alterniflora*, *S. patens*, *P. amarum*, and *U. paniculata* was periodically completed at the 10+ yr 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 (Biber 2011), with natural recruitment of vegetation since then. It should be noted that the age of the 10+ yr constructed site could potentially be misleading due to the periodic sediment renourishment and supplemental plantings over the past 10+ years to address ongoing erosion problems.

The 100+ year reference marsh is approx. 500 m from the 2+ yr and 10+ yr constructed sites, separated by an upland dune ridge colonized by *Pinus palustris* Mill. (longleaf pine) and a variety of shrubs such as *Serenoa repens* W. Bartram (small saw palmetto), and *Baccharis halimifolia* L. (eastern baccharis). 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 (Peterson et al. 2007; Wright et al. 2013).

Field sampling and elevation

Sampling was conducted over six seasons: spring and fall 2017, spring and fall 2018, and spring and fall 2019 (Table 1). During the initial spring 2017 sampling season, two 100 m long replicate transects were established at each study site with approximately 250 m between starting points. 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 improved sampling of community diversity using the point-intersect method, resulting in 125 m between transects. Starting points at the 2+ yr and 10+ yr constructed sites were established randomly along the containment dike which lies on the northern part of the site; at the 100+ yr site the starting point was located at the mid to high marsh transition (Figure 1). The starting points were retained across all sampling seasons. At each sampling effort, the transects were ran from the starting point, generally in the same compass direction, resulting in similar transect orientations with different ending points across each sampling season.

Finally, for collection of high-resolution site-specific elevation data, one-hundred and sixty-five discrete elevation points were measured by a Real Time Kinematic GPS (Trimble R8) in April 2017 and August 2018 (Table 1) and converted to an elevation contour map (Spheroid: GRS_1980, Coordinate system: GCS_North_American_1983) in ArcGIS (Esri, Redlands, CA). Elevation data points were interpolated with Universal Kriging using the 3D analyst toolbox in ArcMAP (ver. 10.4.1) with no transformation and constant trend removal. Tidal range in the region is between 0.3 and 0.6m with a single high and low tide in a 24hr time span. The natural sites was regularly inundated at high tide, whereas the two restored sites had limited tidal exchange and are mostly groundwater fed. Salinities range from brackish to 25 ppt depending on season and freshwater inputs.

Vegetation diversity and percent cover

In five seasons, along the outer two transects, 1 m² quadrats were used to estimate percent cover of plant species (Table 1). 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). The percent cover of the three most abundant species in the 1 m² quadrat was estimated visually by a minimum of two personnel experienced in identifying plants that occur in the northern GoM. To obtain additional resolution on along transect vegetation diversity and plant species richness estimates the Point-Intercept method described in Caratti (2006), was employed along all three transects at 1 m intervals for the fall 2017, spring 2018, fall 2018, and spring 2019 sampling seasons (Table 1). For any unidentified plants in either the quadrat-based or point-intercept sampling, 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).

Point-Intercept and quadrat-based percent cover observations were used to calculate separate measurements of relative percent cover of each plant species observed. 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. The two independent percent cover estimates were then used for calculating species richness, alpha- and beta-diversity, respectively for each site and season. Transects were grouped by site and percent cover estimated by the point-intercept method and 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 as estimates of alpha diversity for each site. A Bray-Curtis index of dissimilarity (Eq. 3) was created using percent cover from the quadrat-based samples 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).

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

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.

Statistical analyses

All analyses were run in R version 3.5.1 (R Core Team 2018), figures were created using 'ggplot2' version 3.1.0 (Wickham 2016). Vegetation diversity analyses using 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). Vegetative community dissimilarity (distance, R) among constructed and reference sites was tested with ANOSIM (permutations = 5,000) using a Bray-Curtis dissimilarity matrix created from the quadrat-based cover-observations at $\alpha = 0.05$. The dissimilarity matrix was Wisconsin Double standardized and square-root transformed before ordination, following the

guidance in the ‘vegan’ package (Oksanen et al. 2018). Ordination was done via nMDS and the centroids for each site were plotted with one standard deviation ellipses to allow for inferences on whether differences exist among sites.

Indicator species analysis was performed using ‘indicspecies’ version 1.7.6 (Caceres and Legendre 2009) to gain insight on how elevation may play a role in the vegetative 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 on the elevation data 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 ($\alpha = 0.05$). Finally, one-way analysis of variance (ANOVA) was used to test for any significant ($p < 0.05$) difference in the mean elevation of each site, which may have contributed to any differences found in plant community assemblages.

RESULTS

Elevation

The 10+ year constructed site had the highest mean elevation at 0.76 meters above mean sea level (MAMSL) and widest range of 0.80 MAMSL (Table 2). The 2+ yr constructed site was at an average elevation of 0.54 MAMSL and the narrowest range at 0.35 MAMSL. The constructed sites were both higher than the 100+ yr 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 for each site (Table S1).

Vegetation Diversity

(1) Richness and alpha diversity

There were 37 total plant species found across the survey period from spring 2017 to spring 2019. The 10+ yr constructed site had the highest species richness at $n = 32$. The 2+ yr constructed site had half the species at $n = 16$. The 100+ yr reference marsh had the least number of species at $n = 5$. A comprehensive list of species observed from the point-intercept and the

quadrats can be found in Table 3. At the time of sampling, the 2+ yr and 10+ yr constructed sites were 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*, *B. halimifolia*, and *Sesbania herbacea* (Mill.) McVaugh (Bigpod sesbania). The 100+ yr natural marsh was dominated almost exclusively by *S. alterniflora* and *J. roemerianus*. All sites shared commonly found salt marsh plants such as *D. spicata*, *J. roemerianus*, *S. alterniflora*, and *S. patens*. The two constructed sites uniquely shared species such as the grasses *P. amarum* 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+ yr constructed site and an unsampled canal at the 10+ yr constructed site. Species unique to the 2+ yr constructed site were *Panicum repens* L., *Sesuvium portulacastrum* (L.) L. (Shoreline seapurslane), and *U. paniculata*. Notable species unique to the 10+ yr constructed site are *B. halimifolia*, *Hydrocotyle bonariensis* Comm. Ex Lam. (Largeleaf pennywort), and *Solidago sempervirens* L. (Seaside goldenrod). Invasive species were absent at 2+ yr constructed site and the 100+ yr natural reference marsh, but the invasive *Imperata cylindrica* (L.) P. Beauv. (Cogon grass) was found at the 10+ yr constructed site, albeit in small amounts (Table 3).

The maximum species richness at the 2+ yr 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 (Fig. 2, Table 4). The maximum species richness at the 10+ yr 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 (Fig. 2). The maximum species richness at the 100+ yr reference marsh when using quadrat sampling was $n = 3$ in Spring 2017, Fall 2017, and Spring 2019 and $n = 5$ during Spring 2019 when using point-intercept sampling (Table 4).

Changes in alpha-diversity, Shannon-Wiener H' and Simpson's D , measured at the study sites through the five seasons are similar to the species richness trends (Table 4). When using both quadrat and point-intercept sampling methods, both the 2+ yr and 10+ yr constructed sites had higher diversity than the 100+ yr reference site (Table 4). The 2+ yr constructed site had the second highest diversity 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 10+ yr

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 $D = 0.85$) data. Finally, the 100+ yr 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 $D = 0.56$).

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

(2) *Beta-diversity and Indicator Species*

Beta-diversity across the three sites was quantified by creating a Bray-Curtis dissimilarity matrix from a total of 186 quadrats across all sampling sites and five seasons. The matrix 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 S2). The 10+ yr constructed site and the 100+ yr reference marsh were the most dissimilar with an R statistic of 0.39. The 2+ yr constructed site was most similar with the 10+ yr constructed site ($R = 0.11$) but was almost as similar with the reference site ($R = 0.21$). To visualize the differences seen in the ANOSIM, the 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 (Fig. 3).

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 S3 – S5). The association was significant at the 2+ yr (IV = 0.76, $p < 0.01$) and 10+ yr (IV = 0.68, $p < 0.01$) constructed sites, however, it was insignificant ($p = 0.29$) at the 100+ yr 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. *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 (Tables S3 – S5). There was a similar pattern of *S. patens* at the 2+ yr constructed where it was significantly associated with the mid-marsh elevation range (IV = 0.59, $p = 0.01$), but at the 10+ yr constructed site *S. patens* was insignificantly (IV 0.52, $p = 0.52$) associated with the mid-marsh elevation range.

DISCUSSION

Constructed wetlands are becoming more prevalent as humans attempt to offset the loss of these 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. The success of a marsh restoration project should be measured by progress towards goals specifically stated in the restoration management plan associated with the project, however, those goals often have little specificity or a timeline (Zedler and Callaway 2000). This study measured progress of two marshes constructed with beneficial use material in the northern GoM by comparing plant diversity as indicators of site succession and ecosystem developmental.

At Deer Island, the elevation and sediment characteristics (Murphy 2020) varied significantly between the constructed and reference sites. The 10+ yr constructed site tended to be higher in elevation and had higher bulk density, higher sand content, and lower sediment organic content than either the 2+ yr constructed site and the 100+ yr reference marsh (Murphy 2020). Salt marsh habitat typically exhibits zonation by elevation, which is influenced by both

biotic and abiotic factors (Eleuterius and Eleuterius 1979; Packham and Willis 1997; Emery et al. 2001; Bockelmann et al. 2002), thereby influencing species composition. Zonation in northern GoM salt marshes is marked by three distinct vegetation zones described previously by Eleuterius (1972): (1) 0.0 – 0.54 meters above mean sea level (MAMSL) elevation zone dominated by the smooth cordgrass, *S. alterniflora*, (2) 0.54 – 0.7 MAMSL dominated by the black needlerush, *J. roemerianus*, and (3) zone greater than 0.7 MAMSL with a combination of grasses dominated by salt marsh hay, *S. patens* and saltgrass, *D. spicata*, among other herbaceous and even woody plants. These vegetation zones occur along an elevation gradient; at lower elevations waterlogging and saline water inundation exclude most plant species, and higher in the marsh platform abiotic stress is replaced by competition between species for nutrients, space, and light.

Alpha- and beta-diversity were measured at the constructed and reference sites from point-intercept and quadrat sample data, respectively. The measurements captured the diversity within specific site and season combinations (alpha diversity) as well as the differences in diversity between sites (beta diversity). Alpha diversity and species richness decline with elevation. The 10+ yr constructed site had the highest (0.76 MAMSL) and widest range in elevation, and in turn was the most diverse both in terms of species richness ($n = 32$) and two diversity indices. The 2+ yr constructed site averaged 0.54 MAMSL and had higher species richness ($n = 16$) and diversity than the 100+ yr reference marsh, which was lower in elevation (0.27 MAMSL) and species richness ($n = 5$). The nMDS plot (Fig. 3) illustrates these differences, with points from the two constructed sites showing wide variability, which can be attributed to the higher elevation ranges and resulting increased plant species diversity within those sites in comparison to the lower elevation 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 also apparent that the 10+ yr constructed site is more different from the 100+ yr reference marsh than it is from the 2+ yr constructed site, likely due to the shared dune species observed in the two constructed sites. The location of the 10+ yr 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+ yr constructed site could be 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.

Relative abundances of species that the sites had in common measured as percent cover using point-intercept sampling were also different. Abundance of *S. alterniflora* at the 2+ yr constructed site (cover = 74%) was more similar to the 100+ yr reference marsh (cover = 61%) than it was to the 10+ yr constructed site (cover = 16%), in part because a much larger portion of the 2+ yr constructed site and the 100+ yr reference marsh was below 0.5 MAMSL in the low-marsh zone most suited for *S. alterniflora* dominance. *Juncus roemerianus*, which tends to dominate at mid-marsh elevations, covered 37% of the 100+ yr reference marsh but was effectively absent from the two constructed sites for reasons that are not yet fully understood, this despite the 18,836 stems planted at the 2+ yr constructed site and the 13,440 stems planted at the 10+ yr constructed site. The high-marsh and dune associated grasses, *S. patens* and *D. spicata*, were found to cover 40% and 10% of the 10+ yr constructed site, respectively, while less than 10% of the lower elevation 2+ yr constructed site was covered by both of these species. It is likely that over time the 2+ yr constructed site could begin to reach species richness comparable to the 10+ yr 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 documented in the constructed marshes. Overall the patterns seen between salt marsh species and elevation ranges were as expected from the literature on the northern GoM (Eleuterius 1972; Eleuterius and Eleuterius 1979; Hunter et al. 2008), and most deviations from this classical salt marsh zonation were statistically insignificant. The use of indicator species analysis at the 100+ yr reference marsh was uninformative in terms of statistical power, as most 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.

The 2+ yr and the 10+ yr constructed sites have followed the trajectory of cover in constructed marshes reported in these 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). Both the 2+ yr and 10+ yr constructed sites have yet to equilibrate to species diversity

comparable to the natural reference marsh. The differences in vegetative community assemblage may provide implications for overall floral and faunal biodiversity at the two constructed sites (Streever 2000). The elevation and vegetative composition are likely to influence bird, fish, and invertebrate habitat usage (Weisberg et al. 1981; Burger 2017; Baumann et al. 2018). Furthermore, the construction design of these two beneficial-use sites included a sand berm that fully enclosed the periphery to contain the dredged sediments (Roth et al. 2012). The lack of channels that could allow regular tidal flushing and provide aquatic organisms access to the interior low elevation areas capable of supporting marsh vegetation reduces the potential habitat value. A prior study by Lang (2012) documented groundwater flow across the DIMR1 site originating from the natural marsh to the south and percolating to the northeast following the elevation contours of the original bathymetry prior to site construction. Much of the standing water in the interior of the constructed sites is likely recharged from this groundwater, subsurface hydraulic tidal influence through the coarse sand berm, and rainwater. The salt marsh vegetation composition and distribution at the DIMR1 site was potentially influenced by the lower soil salinity, which has also been noted in other studies (Klijn and Witte 1999; Zedler 2000; Lang 2012)

CONCLUSIONS

This study provides the first comparative assessment of two adjacent beneficial-use sites of differing ages, which were planted to create *Juncus*-dominated wetlands representative of northern GoM salt marshes. The post-construction vegetative assessment at the 2+ yr and 10+ yr constructed site has shown mixed success, with *J. roemerianus* mostly absent from the vegetation community. The overall diversity of the plant community is high in the two restored sites compared to the reference site, supporting prior observations that the 2+ yr and 10+ yr constructed sites can support a diverse community of salt marsh flora. The future of salt marsh restoration using beneficial use sediments in the northern GoM is going to require successful colonization of *J. roemerianus*, whether by planting or natural recruitment, as this species is indicative of a natural marsh in this region.

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Tables

Table 1. Summary of sampling conducted at each site over the six seasons observed in this study.

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

Table 2. Mean and range of 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+ yr constructed ^b	0.54	0.35	56
10+ yr constructed ^a	0.76	0.80	55
100+ yr reference ^c	0.27	0.50	54

635 Table 3. Species list and percent occurrence of salt marsh and dune vegetation observed from
636 quadrat (Q) and point-intercept (PI) sampling at two constructed marshes and a natural reference
637 marsh across four sampling seasons.

Species	2+ yr		10+ yr		100+ yr	
	Q	PI	Q	PI	Q	PI
<i>Andropogon virginicus</i> L.			3	<1		
<i>Baccharis halimifolia</i> L.	<1	<1	2	2		
<i>Cyperus</i> spp.			1	<1		
<i>Distichlis spicata</i> (L.) Greene	9	4	8	7		1
<i>Eragrostis secundiflora</i> J. Presl			2	3		
<i>Eupatorium capillifolium</i> (Lam.) Small		<1	1	<1		
<i>Fimbristylis castanea</i> (Michx.) Vahl			5	2		
<i>Heterotheca subaxillaris</i> (Lam.) Britton & Rusby				<1		
<i>Hydrocotyle bonariensis</i> Comm. Ex Lam.			1	8		
<i>Imperata cylindrica</i> (L.) P. Beauv.				<1		
<i>Ipomoea imperati</i> (Vahl) Griseb.		<1	<1	<1		
<i>Iva frutescens</i> L.		<1	2	1		
<i>Iva imbricata</i> Walter				<1		
<i>Juncus roemerianus</i> Scheele	1	1	2	2	36	37
<i>Limonium carolinianum</i> (Walter) Britton				<1		
<i>Phyla nodiflora</i> (L.) Greene				<1		
<i>Panicum amarum</i> Elliott	5	2		<1		
<i>Panicum repens</i> L.	<1	1				
<i>Paspalum distichum</i> L.	6	2	3	<1		
<i>Physalis angustifolia</i> Nutt.				<1		
<i>Polypremum procumbens</i> L.			<1	<1		
<i>Proserpinaca intermedia</i> Mack.				<1		
<i>Ruppia maritima</i> L.		2				
<i>Sarcocornia perennis</i> (Mill.) A.J. Scott				<1		
<i>Schizachyrium maritimum</i> (Chapm.) Nash		<1		2		
<i>Schoenoplectus americanus</i> (Pers.) Volkart ex Schinz & R. Keller	1	<1	1	<1		
<i>Schoenoplectus robustus</i> (Pursh) M.T. Strong		<1		<1		<1
<i>Sesbania herbacea</i> (Mill.) McVaugh			<1	<1		
<i>Sesuvium portulacastrum</i> (L.) L.	<1	1	<1			
<i>Solidago sempervirens</i> L.			4	4		
<i>Spartina alterniflora</i> Loisel.	63	75	34	16	62	61
<i>Spartina patens</i> (Aiton) Muhl.	13	9	29	42	1	<1
<i>Symphyotrichum tenuifolium</i> (L.) G.L. Nesom			1			
<i>Uniola paniculata</i> L.	1	<1				
<i>Vigna luteola</i> (Jacq.) Benth	<1	1	2	4		
TOTAL	100	100	100	100	100	100

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Table 4. Diversity indices and species richness calculated from quadrat (Q) and point-intercept (PI) sampling at two constructed marshes and a natural reference marsh across five sampling seasons.

	Shannon-Wiener (H')		Simpson's (D)		Richness	
	Q	PI	Q	PI	Q	PI
Spring 2017						
2+ yr constructed	1.47	-	0.68	-	10	-
10+ yr constructed	2.24	-	0.85	-	19	-
100+ yr reference	0.75	-	0.51	-	3	-
Fall 2017						
2+ yr constructed	0.78	0.76	0.36	0.35	7	5
10+ yr constructed	1.65	1.60	0.69	0.68	16	11
100+ yr reference	0.68	0.69	0.48	0.48	3	3
Spring 2018						
2+ yr constructed	0.67	0.84	0.33	0.36	4	7
10+ yr constructed	0.97	1.80	0.54	0.74	9	21
100+ yr reference	0.55	0.69	0.36	0.42	2	3
Fall 2018						
2+ yr constructed	1.37	1.44	0.69	0.59	4	14
10+ yr constructed	1.63	2.42	0.77	0.85	9	25
100+ yr reference	1.08	0.89	0.65	0.56	2	2
Spring 2019						
2+ yr constructed	1.62	1.24	0.76	0.55	8	10
10+ yr constructed	1.74	2.22	0.78	0.83	8	20
100+ yr reference	1.14	0.89	0.67	0.52	3	5

Figures

Fig. 1 Location of transects sampled on 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: REF is the natural reference site, 100+ years old

Fig. 2 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. The total percent coverage of vegetation at the constructed sites and reference marsh using (4) quadrat and (5) point-intercept sampling, respectively

Fig. 3 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

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Figure 1

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Figure 2

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Figure 3

Supplementary Information

Table S1. One-Way ANOVA table for mean elevation (MAMSL) by site (n = 3).

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

Table S2. Results of ANOSIM comparisons of Bray-Curtis dissimilarity across two constructed marshes and a natural reference marsh. * Denotes significant difference at alpha = 0.05.

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 S3. Results of indicator species analysis of salt marsh plants at the 2+ yr constructed site within low and intermediate elevation zones. 1 denotes an association of a species with the respective elevation zone at the 2+ yr constructed site. * denotes a significant association of a species with the respective elevation zone at the 2+ yr constructed site.

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

Table S4. Results of indicator species analysis of salt marsh plants at the 10+ yr constructed site within low, intermediate, and high elevation zones. 1 denotes an association of a species with the respective elevation zone at the 10+ yr constructed site. * denotes a significant association of a species with the respective elevation zone at the 10+ yr constructed site.

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

Table S5. 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. * denotes a significant association of a species with the respective elevation zone at the 100+ yr constructed site.

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

Figures

Figure 1

Location of transects sampled on 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: REF is the natural reference site, 100+ years old Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.

Figure 2

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. The total percent coverage of vegetation at the constructed sites and reference marsh using (4) quadrat and (5) point-intercept sampling, respectively

Figure 3

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

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [SupplementaryInformation.docx](#)