



Restoration Affects Sexual Reproductive Capacity in a Salt Marsh

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Abstract

Plant sexual reproduction is an important driver of plant community maintenance, dispersal, and recovery from disturbance. Despite this, sexual reproduction in habitats dominated by clonally spreading perennial species, such as salt marshes, is often ignored. Communities dominated by long-lived perennial species can still depend on sexual reproduction for recolonizing large disturbed patches or for establishing in new patches, such as restored sites. We investigated the influence of restoration and elevation on flowering phenology, potential seed and seedling production, and insect flower damage of the dominant salt marsh grass, *Spartina alterniflora*, in reference and restored marshes in southeastern Louisiana, USA. We additionally tested whether elevation gradients or soil parameters could explain differences in sexual reproduction between sites. We demonstrate that sediment-slurry amendment restoration may not affect flowering phenology or insect flower damage at ecologically relevant levels, but that restoration activity increases sexual reproductive output at the patch scale. Restoration activity affected reproductive dynamics more often than changes in elevation alone. Restoration of subsiding salt marsh habitat by altering the soil environment may increase sexual reproductive capacity of these wetlands.

Keywords Salt marsh · *Spartina alterniflora* · Sediment amendment restoration · Sexual reproduction · Seed germination · Flower phenology

Introduction

Plant sexual reproduction is driven by complex interactions between abiotic (Stephenson 1981), biotic (e.g., Bertness et al. 1987) and temporal (Wolkovich et al. 2014) factors. Sexual reproduction encompasses a plant community's investment into sexual units, the dispersal of those units, their successful

germination and establishment, and the timing of reproductive activity. Understanding these varied facets of sexual reproduction is crucial to understanding community reassembly after disturbance, the potential reproductive capacity of habitat patches, and species' ability to expand through seed. The influence of environmental drivers on sexual reproduction is especially salient in restoration settings, as managers sometimes have the ability to control key environmental parameters in the design or management of restoration projects.

In salt marshes, plant communities are often dominated by long-lived perennial species that are capable of vegetative reproduction. Long-lived perennial species are thought to depend heavily on vegetative reproduction compared to sexual reproduction, especially in aquatic or stressful habitats (Grace 1993; Silvertown 2008). For example, *Spartina alterniflora* salt marsh communities typically do not have a viable seed bank (Hartman 1988), as seeds are only viable for 1 year (Mooring et al. 1971). Biotic factors, such as flower predation, can also hinder sexual reproductive efforts in salt marshes (Bertness et al. 1987). The assumption that sexual reproduction is unimportant for long-lived salt marsh perennials may not, however, be warranted, especially for recolonizing large disturbed patches (reviewed in Jones et al. 2016) or expanding into new areas (McCormick et al. 2010; Kettenring &

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Whigham 2018). Furthermore, *S. alterniflora* salt marshes, in particular, have higher local genetic diversity than would be expected without sexual reproduction (Richards et al. 2004; Travis et al. 2004; Travis and Hester 2005), although somatic mutations (Barrett 2015) may play a role in driving clonal genetic diversity.

Salt marshes are highly valuable ecosystems (Barbier et al. 2011) that have been the focus of restoration activity, as they are often degraded by human activities (e.g., climate change, conversion to agriculture, urbanization; Gedan et al. 2009). Sediment-slurry amendment is a common restoration technique in salt marshes in the Gulf of Mexico and can lead to rapid habitat restoration (Mendelssohn and Kuhn 2003; Stagg and Mendelssohn 2010). Slurry amendments raise marsh platform elevations by adding sediment (often to a subsided marsh), reducing flooding frequency and associated edaphic stressors. Despite studies investigating restored salt marsh community development (e.g., Wolters et al. 2008; Duarte et al. 2015; Derksen-Hooijberg et al. 2018), little is known about how restoration with sediment-slurry amendment (hereafter restoration) alters target species' sexual reproduction. This is partially because the reproductive biology of target plants is not well studied in salt marshes (but see Fang 2002). Restored salt marsh sites offer the opportunity to study salt marsh sexual reproduction along extended hydrologic gradients in the field, as they are typically higher in elevation than nonrestored marshes. This opportunity is also a challenge, as disentangling direct effects (e.g., decreased inundation stress) from indirect effects (e.g., reduced competition) of restoration activity is difficult. By understanding the underlying drivers of sexual reproductive function in restored and nonrestored salt marshes, restoration practitioners may be able to manage salt marsh systems for increased reproductive capacity. Additionally, the extended hydrologic gradient available in restored marshes may shed light on how stress gradients structure sexual reproduction in perennial species more broadly.

To better understand the drivers of salt marsh sexual reproduction, we investigated the effects of restoration activity and hydrology on several facets of reproduction in *S. alterniflora* dominated salt marshes in coastal Louisiana, USA. We hypothesized that (1) restoration activity would increase sexual reproductive capacity by decreasing inundation-related stressors and (2) decreased inundation stress at restored sites would be a result of higher elevations after sediment-slurry amendment.

Methods

Experimental Design and Study Area Description

To test our hypotheses, we measured responses to sediment-slurry amendment restoration activity along elevation

gradients in the field. We established plots in *S. alterniflora*-dominated salt marshes in southeastern Louisiana (Fig. 1a) at four sites in summer 2016: two reference marshes (Fig. 1b, c; 29.17° N, 90.25° W), one 2-year old restored marsh (hereafter “young restored” Fig. 1d; 29.13° N, 90.22° W), and one 14-year old restored marsh (hereafter “old restored” Fig. 1e; 29.18° N, 90.24° W). At each site, three replicate transects were established along elevation gradients that corresponded to inundation, as each site was tidally well connected (sites ranged from 5 to 60% flooded; Jones, unpublished data). Along each transect, 3–5 plots were established from low to high elevation (Fig. 1b–e; Table A1). The two reference sites were combined for all analyses. The experimental design therefore consists of three restoration treatments (reference, young restored, old restored) along elevation gradients (15 total treatment combinations), sub-replicated three times using within-site transects (45 total plots).

Sites were located within 8 km of each other (Fig. 1a), and experienced diurnal micro-tides (0.37 m tide range; NOAA 2016). All restored sites were restored using sediment-slurry amendment, which adds a watery sediment slurry to the top of subsided marsh platforms (Mendelssohn and Kuhn 2003). Additionally, the young restored site was planted with “Vermilion” *S. alterniflora*, as is common in Louisiana wetland restoration practice. The old restored site did not have successful plantings of “Vermilion” *S. alterniflora* (C. Stagg, personal communication). Restored sites were on average higher in elevation than reference sites, but transects were established to ensure elevation overlap between treatments (reference 0.09–0.24 m NAVD88, young restored 0.20–0.23 m NAVD88, old restored 0.21–0.32 m NAVD88). Plots were established using real-time kinematic surveying (RTK; Trimble R-8 Receiver & TSC3 Controller, Trimble Inc., Sunnyvale, CA) in NAVD88 reference geoid 12A. Each plot contained a large PVC pole marking the center of a 2-m² circle. Soil and standing vegetation conditions at each site were quantified in September 2016, and plots were sampled for flowering phenology, reproductive effort, germination rate, and insect flower damage throughout the sexual reproductive period in August–December 2016.

Soil and Vegetation Conditions

We characterized edaphic conditions at each site in September 2016 using several methods. All soil parameters were sampled along the same elevation contours as vegetative plots (Table A1); soil samples were taken between existing vegetative plots to avoid altering plant response. Soil redox potentials were measured at 10-cm depth (Orion ORP Electrode 9179BN; Thermo Fisher Scientific Inc., Waltham, MA, USA). Soil bulk density was calculated via dry weight to volume (Blake and Hartge 1986) from soil cores. Cores were collected using a 5 cm diameter soil corer; each 20 cm length

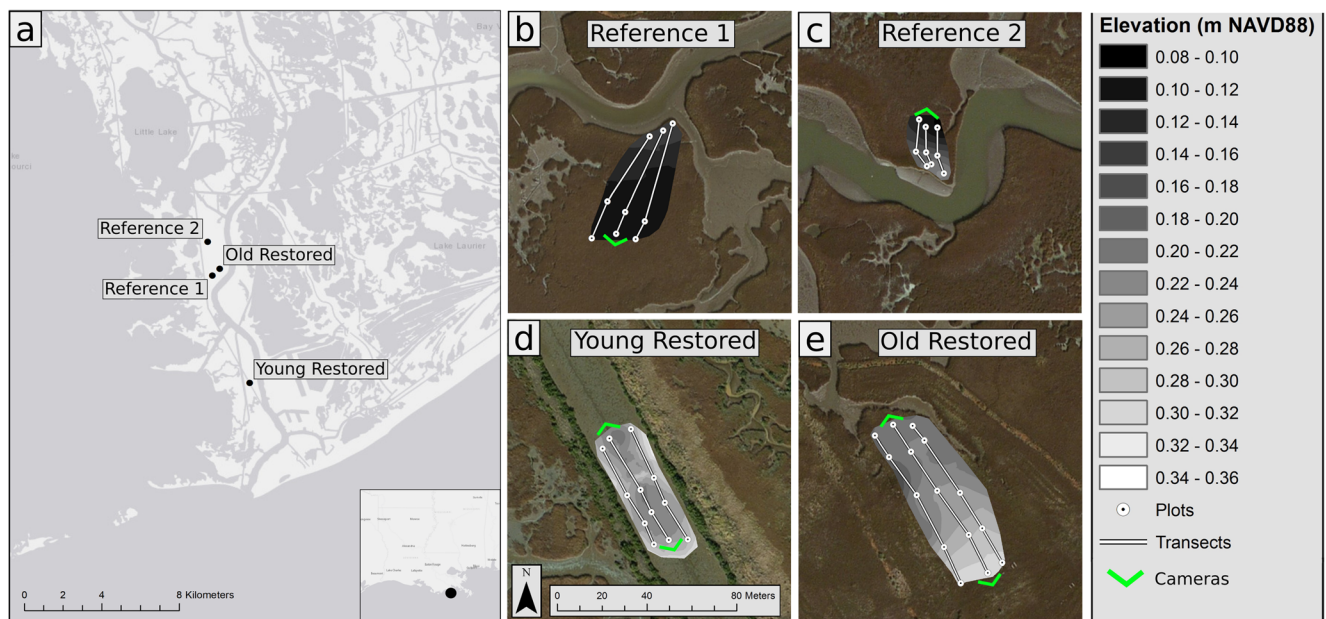


Fig. 1 Site locations **a** within the Gulf of Mexico and the Port Fourchon area. Sites were located along existing elevation gradients at **b** reference site 1, **c** reference site 2, **d** young restored site, and **e** old restored site

core was dried at 60 °C to constant mass and weighed. Redox potential and bulk density were measured twice at each elevation in each site, for a total of 6–10 samples per site. Porewater was sampled using sippers inserted to 10 cm and was immediately frozen upon return to the lab. Porewater was collected twice at each elevation at each site, for a total of 6–10 samples per site. A subset of porewater samples from the highest and lowest elevations at each site was vacuum-filtered and sent to Louisiana State University to determine concentrations of nitrate/nitrite and ammonium.

Standing vegetation was characterized at each site in September 2016. Average plant height was measured in each plot, and live and dead stem densities were calculated using 0.25 m × 0.25 m quadrats in each plot.

Flowering Phenology

We used time-lapse cameras to capture true-color images of all sites during the flowering season (Brinno TLC200 PRO, Brinno Inc., Taipei City, Taiwan; 112° field of view). Near-term remote sensing approaches for measuring phenology are used to measure a variety of phenology responses (e.g., Richardson et al. 2009), and time-lapse cameras have been successfully deployed for targeted phenological investigations (Bater et al. 2011). Each restoration treatment contained two cameras (Fig. 1), installed at approximately 1.5 m in height (Fig. A1) and were set to record an image every minute during daylight hours. Cameras were aligned so that each camera captured approximately the same amount of marsh in each image at each of the study sites.

To quantify the transition from nonflowering to peak flowering at each site, we ran a supervised classification on a representative picture from each morning (after sunrise but before the sun was high enough to cast shadows on the marsh) from mid-August to mid-October for each camera installation, using Trainable Weka Segmentation in the Fiji distribution of ImageJ software (Schindelin et al. 2012; Arganda-Carreras et al. 2017). This classification assigned image pixels into plant, background, PVC, and flower classes and was trained using an image taken from peak flowering at each camera location (Fig. A2). Days with rain, fog, or other visual impairments (e.g., bird feces) obscuring the camera lens were omitted from analysis. The area of each image classified as flowers was quantified by summing all pixels in the flower class, and that sum was divided by the area classified as marsh plus flowers to determine flowering intensity. Flowering intensity was adjusted for analysis so that nonflowering values were centered on 0 and peak flowering values centered on 1. This allowed standardized comparisons between sites.

Reproductive Effort

Spartina alterniflora inflorescences are 10–40-cm-long panicles that contain between 3 and 30 spikes, each with 10–50 spikelets (Mobberley 1953; Fang 2002). Spikelets produce at most one seed, representing the basic unit of sexual reproduction. On the Gulf Coast, *S. alterniflora* starts to elongate stems in preparation for flowering in late August, flowering (visible anthers or pistils) starts and peaks in September and continues

into October, and seed maturation occurs from late October into December (Fang 2002; Jones, personal observation). We quantified reproductive effort throughout the flowering season in 2016 by counting flowering culms in each plot, counting the number of spikes in three haphazardly chosen panicles per plot, and counting total spikelets per spike in three mid-inflorance spikes per plot. Number of spikes per inflorescence and number of spikelets per spike were averaged together for each plot before analysis. We present here data representing peak reproductive output, recorded on October 18, 2016, after peak flowering. Percent flowering stems is therefore cumulative and is a measure of percent live stems that flowered in the 2016 season, not percent stems currently flowering. Potential seed production (total spikelets) is presented at the stem (spikelets stem⁻¹) and patch (spikelets m⁻²) scales.

Germination Rate

We collected seeds in early December 2016 from every elevation at each site. Transects were combined so each elevation and site is represented by a single seed collection of several thousand seeds, for 15 total collections. Inflorescences were clipped in the field and bagged, taking care to clip only mature inflorescences. Inflorescences were stored dry at 4 °C for 1 month until processing. In the lab, inflorescences were hand threshed to separate readily detached seeds for use in a germination assay. After threshing, we stored seeds according to the successful method of Mooring et al. (1971): submerged in 30 ppt seawater (Instant Ocean Sea Salt, Spectrum Brands, Blacksburg, VA) at 2–3 °C for 3 months. Seawater prevents fungal growth without undermining final germination rates (Mooring et al. 1971) and was changed every month. After removal from seawater, seeds were lightly rinsed with deionized water and allowed to germinate in moist conditions at a constant 20 °C. Germinated seeds from each collection were counted and removed over a 6-week period. To estimate the total number of seeds in each seed collection, collection-specific dry weight to seed count ratios were multiplied by the total dry weight of seeds from each collection. The number of total germinated seeds per collection was then divided by total seeds harvested to calculate germination rates. We use germination per spikelet, not germination per viable seed, as in Daehler and Strong (1994). *Spartina alterniflora*, like many other plants (Stephenson 1981), does not invest energy into every potential seed and some potential seeds are aborted before becoming viable; by estimating germination rates per spikelet instead of germination rates per viable seed, we are quantifying potential seedling production or reproductive capacity. Potential seedling production (total spikelets × germination rate) is presented at the stem and patch scales.

Insect Flower Damage

During peak flowering in October, we recorded spikelet damage caused by insects. Plots were examined visually and an average percent damage of spikelets (number of spikelets with damage/total number of spikelets) was estimated for each plot. Spikelets were considered damaged when obvious herbivory was observed, including chew marks, drilling, and damage to spike or spikelets. Using pictures, tissue samples, field notes, and literature (Bertness et al. 1987; Slater and Baranowski 1990), we determined the most likely insect(s) responsible for flower damage.

Statistical Analyses

All data were initially analyzed using a regression framework in R statistical software to test relationships among elevation, restoration treatment, and response variables (R Core Team 2016). All reported comparisons had a *p* value of *P* < 0.05; full model statistics are presented in Table A2, and all Tukey post hoc comparisons when restoration treatment was significant are in Table A3. For all analyses, residuals were evaluated for model assumptions. One outlier was removed for porewater NO_x analysis, but for all other analyses outliers, if present, were retained.

Phenological transitions recorded with near-surface remote sensing can be successfully modeled using logistic functions (Richardson et al. 2006); we therefore used a simple sigmoidal regression to analyze phenological data, following the general sigmoidal approach outlined in Feher et al. (2017). Data for each restoration treatment were analyzed separately, with two cameras per restoration treatment. The following equation was used:

$$y(x) = \frac{a}{1 + e^{-\left(\frac{x-c}{b}\right)}}$$

where *y* = flowering intensity, *x* = Julian day, *a* = peak flowering, *b* = the growth rate, and *c* = the midpoint of flowering transition. We used nonlinear least squares to fit sigmoidal curves in R (function *nls*; R Core Team 2016) and to quantify flowering transition midpoints. To determine flowering onset, time of peak flowering, and length of flowering transition, the second derivative of each restoration treatment's sigmoidal function was calculated and solved for local maxima and minima (similar to Osland et al. 2014). All terms were significant for each site type (*P* < 0.001).

Finally, to investigate the drivers of significant responses between restoration treatments, we regressed plant and soil covariates such as soil redox potential, plant height, and stem density on response variables in a regression framework. Generalized least squares (package

“nlme”; Pinheiro et al. 2016) was used to assess the relationship between potential seed production and plant height to address heterogeneous variance; error in this model was allowed to exponentially increase with larger values of plant height (Zuur et al. 2009).

Results

Soil and Vegetation Conditions

Soil conditions varied between restoration treatments (Table 1). Average redox potential was lower in reference sites than the young restored site, with the old restored site intermediate ($P < 0.05$; Table 1). Bulk density differed between sites in a similar fashion, although higher elevations additionally had higher bulk densities ($P < 0.05$; Table A2). Nitrate and nitrite levels did not vary between sites or along the elevation gradient ($P > 0.05$; Table A2). There were between-site differences in ammonium but the differences were not significant ($P = 0.07$; Table A2).

Vegetation metrics varied between restoration treatments and by elevation (Table 1). Average plant height increased with elevation, and the young restored site had taller plants than other sites on average ($P < 0.05$; Tables A2–3). Live stem density did not differ between restoration treatments but increased with elevation (Table A2). Dead stem density did not vary between restoration treatments or along elevation gradients (Table A2).

Flowering Phenology

Patches in the young restored site began flowering before other sites, but the transition into peak flowering happened within the same month, September, for all sites (Fig. 2). The onset of flowering, midpoint of the flowering transition, and peak flowering were all 4–8 days earlier in the young restored site compared to the other sites (Table 2). Additionally, a 95% confidence interval of the modeled midpoint of the flowering transition for the young restored site did not overlap other sites (Fig. 2). After peak flowering, sites continued flowering until

approximately mid-October, when they transitioned into seed maturation.

Reproductive Effort

Restoration treatment and elevation both influenced sexual reproductive effort, although restoration effects were more common (Tables A2–3). Plots at higher elevations had slightly more potential seeds per stem (Table A2). Additionally, the young restored site had nearly twice as many potential seeds per stem as other sites ($P < 0.05$; 381 ± 70 young restored, 189 ± 42 old restored, 193 ± 27 reference; all values presented are means \pm standard error). Plant height mediated restoration treatment and elevation effects on potential seed production (Fig. 3), as restored sites (especially the young restored site) typically had taller stems which produced more potential seeds. For every 10 cm taller, a plant produced 33 more potential seeds. There was no relationship between elevation, restoration treatment, or plant height with the number of spikelets per spike ($P > 0.05$; 28 ± 2.2), or the structure of the spike itself.

The percentage of flowering stems increased with elevation and was higher in reference sites than restored sites ($P < 0.05$; $22 \pm 7\%$ reference sites, $11 \pm 3\%$ old restored, $5 \pm 2\%$ young restored). As stem density increased, percent flowering culms decreased (Fig. 4) until approximately 300 stems per m^2 .

While restoration treatment influenced potential seed production of individual culms (Fig. 3, Tables A2–3), there was no pattern in potential seed production at the patch scale (Fig. 5, Table A2). Restored plots with tall, fecund stems also had low overall flowering culm density. Similarly, there was no relationship between potential seed production and plant height or stem density at the patch scale ($P > 0.05$).

Germination Rate

Elevation did not influence germination rates, but restoration treatment did; the young restored site had higher germination rates than reference sites while the old restored site had intermediate germination rates (Tables A2–3; 0.1

Table 1 Soil and vegetation conditions across all sites. Values are means \pm standard error. Significant differences between restoration treatments (Tukey post hoc $P < 0.05$) denoted with letters in parentheses

	Reference	Old restored	Young restored
Redox potential (mV)	-118 ± 11 (a)	-103 ± 17 (ab)	-34 ± 13 (b)
Bulk density ($g\ cm^{-3}$)	0.24 ± 0.01 (a)	0.37 ± 0.07 (ab)	0.52 ± 0.03 (b)
Porewater NO_2/NO_3 (μM)	4.3 ± 1.2 (ns)	4.6 ± 0.8 (ns)	3.8 ± 1.0 (ns)
Porewater NH_4 (μM)	265 ± 59 (ns)	264 ± 150 (ns)	35 ± 15 (ns)
Average plant height (cm)	58.3 ± 6.8 (a)	68.2 ± 5.8 (a)	94.0 ± 15.4 (b)
Live stem density ($N\ m^{-2}$)	13 ± 6 (ns)	13 ± 3 (ns)	17 ± 8 (ns)
Dead stem density ($N\ m^{-2}$)	17 ± 4 (ns)	17 ± 6 (ns)	14 ± 6 (ns)

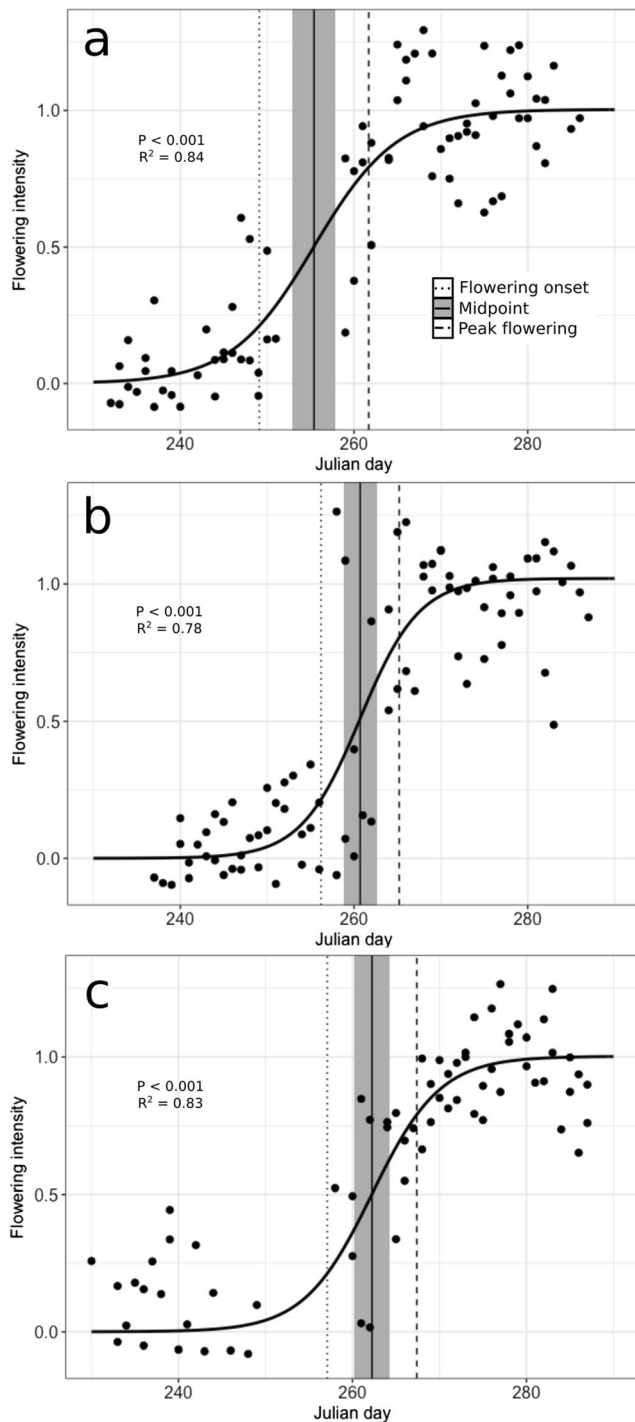


Fig. 2 Transition from no flowering to peak flowering over time in *S. alterniflora* using sigmoidal regressions at **a** young restored, **b** old restored, and **c** reference sites. Gray boxes are 95% confidence intervals around the model midpoint

$\pm 0.1\%$ reference, $4 \pm 2\%$ old restored, $5 \pm 2\%$ young restored). Maximum potential seedlings was higher in both restored sites than reference sites at the individual stem scale (Tables A2–3; 0.2 ± 0.2 reference, 8 ± 3 old restored, 21 ± 6 young restored) and patch (Tables A2–3; 7 ± 4

reference, 189 ± 88 old restored, 308 ± 142 young restored) scales. Additionally, the young restored site had higher maximum potential seedling production than the old restored site at the individual stem scale, but not at the patch scale (Tables A2–3). Given maximum seed production and similar germination rates observed here, restored marshes could potentially create 28 to 46 times more seedlings than reference marshes (Fig. 5).

Insect Flower Damage

Elevation did not influence flower damage, but the young restored marsh had higher damage than other sites (Tables A2–3; $54 \pm 13\%$ reference, $41 \pm 8\%$ old restored, $83 \pm 12\%$ young restored). There was no relationship between flower damage and potential seed production ($P > 0.05$). We did not quantify seed predator abundance in this study, but *Ischnodemus conicus* was found in great abundance at all sites within flower heads (Jones, personal observation), and some flower damage matched that described for sap-sucking insects (e.g., *Prokelisia* spp. Bertness, Wise & Ellison 1987). *Orchelimum* sp. was found at sites less frequently, but many spikelets had chewing damage to the ovules (Jones, personal observation), matching grasshopper seed predation (Bertness, Wise & Ellison 1987; Sparks & Cebrian 2015).

Discussion

Restoration activity weakly impacted flowering onset and insect flower damage, but restoration altered *S. alterniflora* sexual reproductive dynamics by increasing reproductive capacity. These effects were not simply a result of decreased flooding from higher marsh platforms. Our results suggest altered soil environments and/or plant demography from restoration activity may ultimately drive higher sexual reproductive capacity in restored sites.

Flowering Phenology

Despite the same macro-climate forcings and day lengths, restoration activity affected plant flowering phenology. The young restored site sporadically flowered as early as July, and flowering onset occurred 1 week earlier than other sites. First-flowering plants in the young restored site had decreased cross-pollination with the broader *S. alterniflora* community, but this 1-week delay did not totally prevent cross-pollination with later-flowering culms (Somers and Grant 1981) and did not translate into reduced reproductive capacity. Plants in the northern Gulf of Mexico flower between September and November (Eleuterius and Caldwell 1984), and 85% of plants flower in September (Fang et al. 2004b), matching our findings for all sites.

Table 2 Flowering phenology sigmoidal regression parameters across all sites

	Reference	Old restored	Young restored
Julian day of flowering onset	257.1	256.2	249.1
Julian day of transition midpoint	262.2	260.7	255.4
Julian day of peak flowering	267.4	265.2	261.7
Length of transition (days)	10.3	9.0	12.6
Growth rate	3.9	3.4	4.8

It is unclear what is driving earlier flowering in the youngest restored site. Despite broad knowledge of macro-climatic drivers of flowering phenology, there is a paucity of work on nontemperature environmental control on plant phenology (Wolkovich et al. 2014). Crosby et al. (2015) did find differences in flowering phenology based on local-scale hydrologic regimes in New England *S. alterniflora*, but responses were inconsistent. In the current study, it is difficult to distinguish edaphic characteristics from potential genetic differences between sites; patches of the young restored site were planted with a specific accession of *S. alterniflora* (Fine and Thomassie 2000) that may have an altered phenology compared to other accessions, even though the planted accession is native to Louisiana. It is not known how well the initial outplantings survived in the portion of the site that we sampled here, but this particular genotype is common in restoration plantings in Louisiana and throughout the northern Gulf of Mexico. Although altered phenology in the current study did

not lead to reduced reproductive capacity, the long-term genetic impacts of phenological mismatch are unclear; genetic typing of each site over time is necessary to fully disentangle phenological drivers and impacts.

Insect Flower Damage

Restoration treatment did alter insect flower damage, but high damage in the young restored site did not translate into reduced reproductive capacity. Consumers from planthoppers to grasshoppers are noted as *S. alterniflora* flower predators along the Atlantic and West Coasts of the USA, but data from the Gulf Coast are scarce (but see Sokolov et al. 2018). This lack of regional data is problematic: *Spartina alterniflora* salt marsh herbivores may vary regionally (Gulf Coast *Orchelimum* sp. and *I. conicus*, present study; Northern Atlantic *Conocephalus* sp. and *Prokelisia* sp., Bertness et al. 1987; West Coast *Prokelisia* spp., Daehler and Strong 1994),

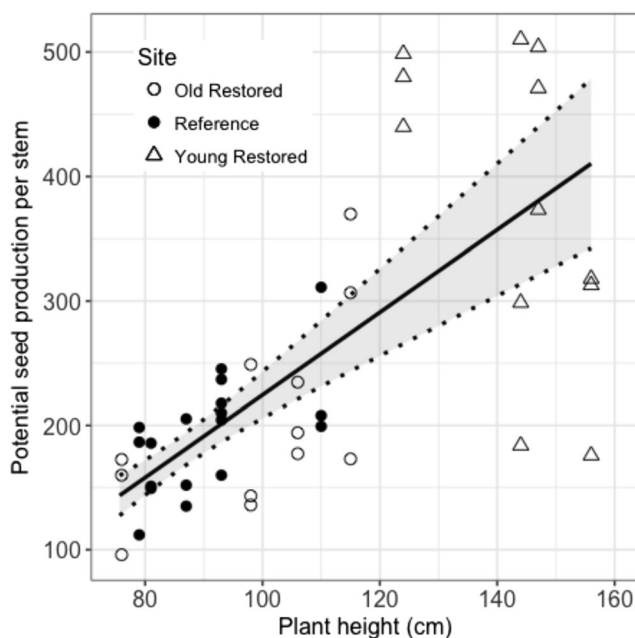


Fig. 3 Relationship between plant height and potential seed production per stem across all sites. Solid line is GLS regression fit (potential seed production per stem = $3.3 \times \text{plant height (cm)} - 107.5$; exponential error variance; $P < 0.0001$) and dotted line is 95% confidence interval

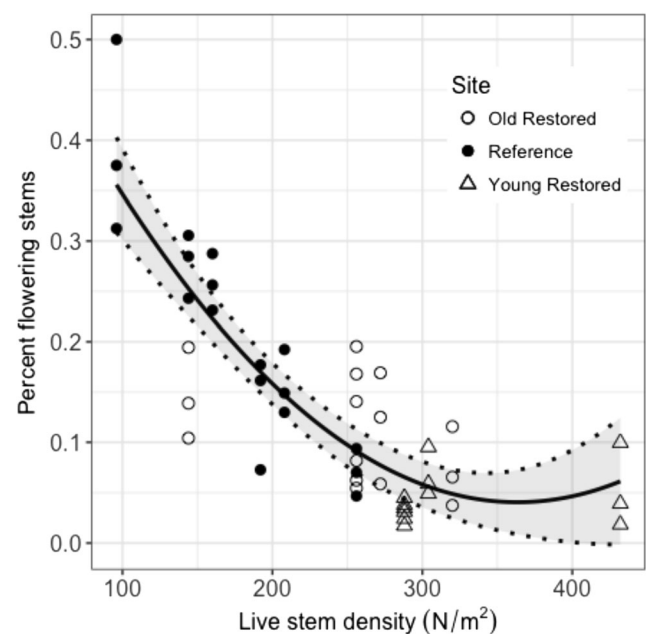


Fig. 4 Relationship between live stem density and percent flowering stems across all sites. Solid line is quadratic regression fit (percent flowering stems = $-0.003 \times \text{live stem density (N/m}^2\text{)} + 0.000004 \times \text{live stem density (N/m}^2\text{)}^2 + 0.6$; $P < 0.001$, $R^2 = 0.72$) and dotted line is 95% confidence interval

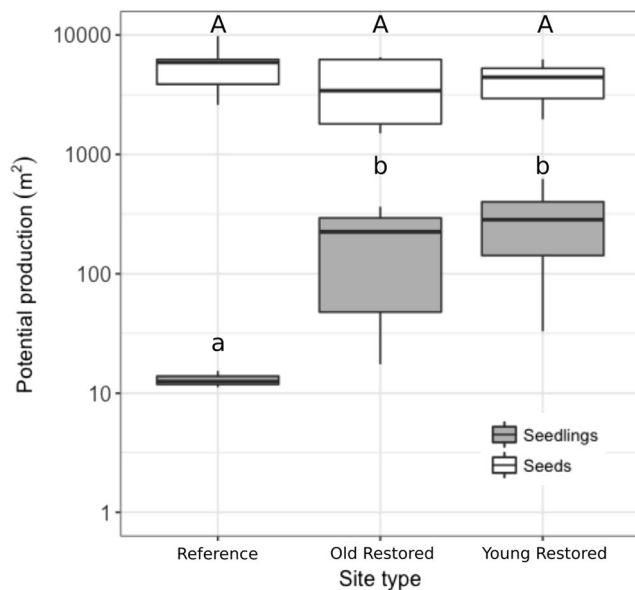


Fig. 5 Potential seed and seedling production at the patch scale across all sites. Note log-scale on y-axis. Boxplot boxes span the lower and upper data quartiles, with the center line the median ($n=9-15$). Boxplot whiskers go out as far as $1.5 \times$ the interquartile range. Uppercase letters represent significantly different groups for potential seed production, and lowercase letters represent significantly different groups for potential seedling production (Tukey post hoc $P < 0.05$)

and herbivore pressure on vegetation may vary latitudinally. Low latitudes may have higher chewing insect herbivore pressure than high latitudes, while sap-sucking insects are still relatively understudied (Pennings et al. 2009). In Rhode Island, Bertness et al. (1987) and Bertness and Shumway (1992) found flower damage averaging $\sim 50\%$ in *S. alterniflora*, comparable to this study. We did not find evidence of increased herbivore pressure at our low latitude site, except in the young restored site. It may be that the young restored site harbored a different insect community or had higher chewing insect populations than other sites, but this is speculative. As the sites in the current study differed in the relative resources available to flower predators, we cannot simply assume that higher flower damage in the young restored site is from a larger population of insects. Regardless, high insect damage did not translate to reduced reproductive capacity in our measured metrics. Further study of insect population sizes, community dynamics, and predation intensity are warranted in subtropical salt marshes.

Reproductive Effort

Decreasing hydrologic stress, both from increases in elevation and alteration of soil conditions by restoration, was associated with increased salt marsh sexual reproductive capacity. Potential seedling production was higher at both the stem and patch scale in restored sites but did not vary by elevation

alone. As potential seedling production is a function of both the total plant investment in potential seeds and the viability or germination success of those seeds, it integrates several processes into one metric. In the present study, potential seedling production was higher in restored sites primarily due to increased germination rates, as the number of potential seeds did not differ between sites.

Germination rate increases could be the result of an increase in plant investment in seeds (or a concomitant decrease in seed abortion), or an increase in the viability of filled seeds (those seeds that have been invested in). In *S. alterniflora*, the fraction of total potential seeds that the plant actually invests energy into is typically low, but filled seeds often have high viability (Callaway and Josselyn 1992; Biber and Caldwell 2008). The proportion of filled seeds to total seeds, or seed set, can vary due to environmental factors associated with latitude (0–80%, Liu et al. 2016), which may explain the wide variability in reported seed set values for *S. alterniflora*: $\sim 50\%$ (Fang et al. 2004a), $< 50\%$ (Biber and Caldwell 2008), 37% (Somers and Grant 1981), and 5% (Callaway and Josselyn 1992).

Alternatively, genetic diversity may be influencing seed viability directly, as has been demonstrated in *Phragmites australis*, another clonal wetland plant. In *P. australis* on the Atlantic Coast of the USA, increased genetic diversity increases seed viability (Kettenring and Whigham 2009); patch size can also increase seed viability (Kettenring et al. 2010), likely due to larger patches containing higher genetic diversity (Kettenring et al. 2011). Travis et al. (2004) found low genetic diversity in young *S. alterniflora* marshes; high self-fertilization in these young populations would presumably lead to lower seed viability as in *P. australis*. Because seed set can vary with environmental conditions, and viability of filled seeds is typically high, we hypothesize that differences in germination rates between sites reported here are driven primarily by differences in seed set. As we did not explicitly test seed viability, the proportion of filled seeds, or genetic diversity, more data is needed to address this hypothesis.

Potential Drivers of Restoration Effects: Altered Soil Conditions

Salt marsh reproductive capacity was enhanced by decreasing flooding stress as predicted, but elevation alone could not predict reproductive capacity, despite being a good proxy for hydrologic regime in these sites. Increased reproductive potential, therefore, was not simply due to higher marsh platforms at restored sites. Restored sites did have higher bulk density and higher soil redox potentials compared to nonrestored sites, which influence multiple biogeochemical pathways important to plant energy balance. When soils become reduced due to excessive flooding, nitrate uptake kinetics are disrupted in *S. alterniflora* (Bradley and Morris 1991).

High sulfide levels in anoxic soils can also negatively impact *S. alterniflora* vegetative growth (Mendelssohn and McKee 1988; Bradley and Morris 1990). These facts suggest that altered soil environments in restored sites reduced flooding-related stress compared to non-restored sites at the same elevation.

Ultimately, less stressful conditions in restored sites may have driven differences in the energy available for plants to allocate to sexual reproduction. As environmental conditions become more stressful, plant tissue maintenance becomes more costly, thus reducing investment in sexual reproduction and/or asexual reproduction or growth (Grime 1977; Obeso 2002). There is evidence that environmental conditions may influence reproductive success in *S. alterniflora* in the field. Crosby et al. (2015) found increased flowering in creekbank habitats compared to more stressful interior patches in some instances in New England marshes. Clones growing higher in the intertidal had higher germination (4.2%) than those in more flooded environments (1.7%) (Daehler and Strong 1994). Drought conditions may also lead to reduced germination (Mooring et al. 1971) and seed set (Biber and Caldwell 2008). These potential relationships are complicated by uncertainties surrounding how clonal plants allocate resources between sexual and asexual reproduction among individual ramets, especially along stress gradients.

Potential Drivers of Restoration Effects: Altered Plant Demography

We hypothesize that plant demography may also be important in structuring sexual reproduction in *S. alterniflora* salt marshes. While both restored sites likely had more energy available for plants to allocate towards sexual reproduction from ameliorated soil conditions, the young restored site additionally had much taller stems and higher stem density than other sites. It is unclear what is driving tall, robust growth in the young restored site, but similar reports from young restored sites in Louisiana suggest nutrient pulses may be important (Slocum et al. 2005). Slocum et al. (2005) detected short-lived nutrient pulses in the first 3 years post-restoration; the young restored site in the present study was under 3 years old, and a nutrient pulse was not detected in soil porewater subsamples of NO_2/NO_3 or NH_4 (Table 1). We used a small subset of samples for nutrient analyses, but the only potential trend we observed was for the young restored site to have highly reduced levels of NH_4 . Plants may have already taken up an initial nutrient pulse, fueling the tall robust stems we recorded.

The genetic history of each site may underpin differences in plant demography. The young restored site was planted with NRCS Golden Meadow “Vermilion” plants, which is a native Louisiana accession developed by the USDA (Fine and

Thomassie 2000). This accession is planted in nearly every *S. alterniflora* habitat restoration project in Louisiana and is therefore inextricably linked to restoration activity in this region. It is unclear how much natural colonization has occurred at this site, but if “Vermilion” grows taller than other native Louisiana accessions, the higher potential seed production per stem values at the young restored site may be due to this genetic signature. As “Vermilion” is known as a poor seed producer (Fine and Thomassie 2000), if genotype was a dominant driver for reproductive capacity in this site we would expect to see low reproduction, the opposite pattern we observed. Additionally, if the site was dominated by a single accession, this would reduce genetic variability which can reduce viability in clonal wetland plants (e.g., Kettenring et al. 2011). Although we conclude that genotype is a weak predictor of reproductive capacity in these sites, genotyping of each site will be necessary to separate environmental and genetic signatures in driving demographic and reproductive changes.

Conclusions

We present here evidence that restoration activity can increase salt marsh sexual reproduction, by decreasing flooding-related stress. Notably, soil conditions at restored sites had reduced flooding-related stress even when located at the same elevation as nearby nonrestored sites. Soil environmental characteristics may therefore be linchpins of salt marsh sexual reproduction, by influencing plant energy allocation. Monitoring and controlling soil environmental characteristics beyond elevation and percent time flooded in wetland restoration may thus be an effective method to increase sexual reproduction of target species and may increase sexual reproduction for more than a decade post restoration. Plant demography also affected sexual reproduction at the individual stem scale and was likely influenced by genetic choices implicitly made in restoration planting decisions, highlighting the need to consider genetic diversity in restoration plantings.

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