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Salinity and simulated herbivory influence *Spartina alterniflora* traits and defense strategy

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Abstract

Sea-level rise is expected to push saline waters into previously fresher regions of estuaries, and higher salinities may expose oligohaline marshes to invertebrate herbivores typically constrained by salinity. The smooth cordgrass, *Spartina alterniflora* (syn. *Sporobolus alterniflorus*), can defend itself against herbivores in polyhaline marshes, however it is not known if *S. alterniflora*'s defense varies along the mesohaline to oligohaline marsh gradient in estuaries. I found that *S. alterniflora* from a mesohaline marsh is better defended than plants from an oligohaline marsh, supporting the optimal-defense theory. Higher salinity treatments lowered carbon content, C:N, and new stem biomass production, traits associated with a tolerance strategy, suggesting that salinity may mediate the defense response of *S. alterniflora*. Further, simulated herbivory increased the nitrogen content and decreased C:N of *S. alterniflora*. This indicates that grazing may increase *S. alterniflora* susceptibility to future herbivory via improved forage quality. Simulated herbivory also decreased both belowground and new stem biomass production, highlighting a potential pathway in which herbivory can indirectly facilitate marsh loss, as *S. alterniflora* biomass is critical for vertical accretion and marsh stability under future sea-level rise scenarios.

Keywords: tolerance, resistance, plant-defense strategy, functional traits, salt marsh

Introduction

Tidal marshes are responsible for ecosystem services that contribute to human well-being including carbon sequestration, erosion control, and nutrient cycling (DeGroot et al. 2012; Costanza et al. 2014). Tidal marshes occur along natural salinity gradients within estuaries and are typically categorized by their salinity regime (e.g. oligohaline – 0 to 5 ppt, mesohaline – 5 to 18 ppt, and polyhaline – 18 to 30 ppt) (Odum 1988; Montagna et al. 2013). In the Chesapeake Bay region, accelerated sea-level rise is a threat to tidal marshes (Najjar et al. 2010). Average sea-level rise in this region is ~3.80 mm yr^{-1}, which is 3-4 times higher than the global mean of ~0.98 mm yr^{-1} (Sallenger et al. 2012; Boon & Mitchell 2015). A marsh's ability to keep pace with sea-level rise depends on sediment size and supply (Kirwan et al. 2010), and vegetation stem density and biomass production, both above- and belowground (Leonard & Luther 1995; Elsey-Quirk & Unger 2018). Marsh vegetation is responsible for regulating the process of vertical accretion, as plant stems trap sediments above ground and accumulate organic matter below ground (Kirwan & Megonigal 2013), thus building marsh elevation and keeping pace with rising seas.

In addition to sea-level rise, tidal marshes are threatened by intense herbivory (Gedan et al. 2009; He & Silliman 2016; Angelini et al. 2018). Many of the invertebrate herbivores implicated in runaway consumption (e.g. the marsh periwinkle, *Littoraria irrorata*: Silliman et al. 2005; the purple marsh crab, *Sesarma reticulatum*: Holdredge et al. 2009) are physiologically limited to mesohaline and polyhaline marshes (Staton & Felder 1992; Henry et al. 1993) and are not found in oligohaline marshes. As sea-level rise pushes saline waters into oligohaline marshes, invertebrate herbivores may follow, increasing the vulnerability of these marshes to herbivory. In some instances, runaway herbivory can remove vegetation from large spatial areas and transition the marsh to a mudflat (Holdredge et al. 2009; Vu et al. 2017), intensifying marsh susceptibility to drowning; however, despite extreme herbivory, marshes persist. This may be due in part to how plants respond to herbivory pressure. Thus, understanding how marsh plant traits change in response to herbivory provides direct insight into one aspect of marsh resilience.

The palatability of plant tissue can control the rate of herbivory (Siska et al. 2002; Salgado & Pennings 2005), and thus susceptibility to grazing. Following herbivory, many plants can induce changes to their chemical, structural, and morphological traits to mitigate damage and deter further grazing (Ito & Sakai 2009; Burghardt & Schmitz 2015), which in combination with constitutive traits, can decrease herbivore consumption and vegetation removal. Alterations in both constitutive and induced traits define the two primary plant defense strategies: tolerance and resistance. Plants can tolerate herbivory by increasing above and belowground biomass production to compensate for mass lost to herbivores (Mauricio et al. 1997; Burghardt & Schmitz 2015). Alternatively, plants can resist herbivore attack by producing chemical and/or structural defenses to decrease palatability and deter future grazing (Mauricio et al. 1997; Burghardt & Schmitz 2015). Depending on factors such as environmental conditions or herbivore abundance, these strategies may or may not be mutually exclusive (Mauricio et al. 1997; Więski & Pennings 2014).

My overarching goal was to compare plant defense response to simulated herbivory between plants from a mesohaline and oligohaline marsh and to test the hypothesis that salinity can influence plant defense responses. In North Atlantic estuaries, salinity and elevation are key determinants of the vegetative community. The ‘low marsh’ (below mean high water) of oligohaline marshes typically has high plant diversity, whereas the low marsh of polyhaline marshes is dominated by monotypic stands of the smooth cordgrass, *Spartina alterniflora* (syn.

Sporobolus alterniflorus) (Perry & Atkinson 1997). In the Chesapeake Bay region, *S. alterniflora* grows along the natural salinity gradient of estuaries and is found in both oligohaline and mesohaline marshes. Thus, it was selected as the focal species of this study. Previous work on *S. alterniflora* defense response has been focused in polyhaline marshes alone (Pennings et al. 1998; Hendricks et al. 2011; Long et al. 2011; Sieg et al. 2013; Long & Porturas 2014; Więski & Pennings 2014). It is unclear, however, whether a pattern in *S. alterniflora* defense exists along the mesohaline to oligohaline marsh gradient, and if so, how this response may be influenced by increasing salinities anticipated with sea-level rise.

Optimal-defense theory predicts that the probability or incidence of herbivore attack may determine the extent of a plants' defense response (Herms & Mattson 1992; Ito & Sakai 2009). In wetlands, the type of herbivory varies along the natural estuarine salinity gradient. Both oligohaline and mesohaline marshes suffer from vertebrate (e.g. avian and/or mammalian) and insect herbivory (Crain 2008). In addition to vertebrate and insect herbivores, mesohaline marshes also have high abundances of other invertebrate herbivores (e.g. crustaceans and/or mollusks), which are not typically found in oligohaline marshes (Crain 2008; Sutter et al. 2019). Although not explicitly tested in marshes, the type of herbivore inflicting damage may influence plant defense strategy. For example, terrestrial grasses follow a tolerance strategy in response to mammalian herbivores (Frank & McNaughton 1993) and marine macroalgae follow a resistance strategy in response to invertebrate grazing (Cronin & Hay 1996). If this pattern holds true for tidal marshes, I would expect *S. alterniflora* from the oligohaline marsh, where vertebrate herbivory is prevalent, to follow a tolerance strategy. In contrast, I would expect *S. alterniflora* from the mesohaline marsh, which suffers more from invertebrate herbivory, to more closely align with a resistance strategy. Additionally, although the exact age of these marshes is unknown, the underlying strata indicate that the mesohaline marsh may be much older than the oligohaline marsh (Hobbs 2009), thus, length of exposure to herbivory may also influence plant defense. Therefore, I expected plant defense response to be greater in the mesohaline marsh which has a longer history of herbivory and a wider variety of herbivores than the oligohaline marsh.

Salinity may also mediate plant defense response, as increased salinity can restrict growth and germination in *Spartina* spp. (Alberti et al. 2010; Daleo et al. 2015; Infante-Izquierdo et al. 2019), as well as inhibit compensatory growth in response to herbivory (Long & Porturas 2014). This is particularly important for plants following a tolerance strategy in which compensatory growth is the primary mechanism of defense. Further, salinity directly affects plant tissue stoichiometry (MacTavish & Cohen 2017; Sutter et al. 2019). Therefore, I expected that *S. alterniflora* in high salinity treatments, regardless of collection site, would have lower carbon content due to decreased photosynthesis and carbon assimilation (MacTavish & Cohen 2017; Sutter et al. 2019) and higher nitrogen content caused by increased osmolyte production (Munns 2002; Sutter et al. 2019), both of which contribute to lower C:N.

A trait-based approach was used to quantify the defense response of *S. alterniflora*. For example, if *S. alterniflora* were to follow a resistance strategy, I expected to see increased tissue phenolic concentrations and decreased protein content. Phenolics can lower plant palatability and serve as deterrence against herbivore grazing (Dorenbosch & Bakker 2011; Zhang et al. 2019), as well as play a role in primary metabolism or UV protection (Close & McArthur 2002; Neilson et al. 2013). Herbivores forage for proteins to meet metabolic demands (Cebrian et al. 2009), so plants may decrease the concentration of proteins to deter further herbivory. In contrast, if *S. alterniflora* were following a tolerance strategy, I expected to see higher biomass (new stem and/or belowground) and carbon content, with lower C:N. Elevated biomass production indicates compensatory growth (Long & Porturas 2014), with both carbon content and C:N influenced by biomass.

Materials and Methods

Collection Sites

Spartina alterniflora was collected from two marshes within the York River Estuary (Virginia, USA; Figure 1a), a tributary of the Chesapeake Bay. There are two herbivores of concern in the York River: the marsh periwinkle, *Littoraria irrorata*, and the purple marsh crab, *Sesarma reticulatum*. These herbivores have been implicated in the large-scale die-off of U.S. Atlantic polyhaline marshes (Silliman et al. 2005; Bertness et al. 2014). Their distribution in the York River is currently limited to mesohaline and polyhaline marshes, although they are expected to move into oligohaline marshes as sea-level rise pushes saline waters up-estuary. Sweet Hall marsh (37.566087, -76.882472, hereafter 'oligohaline marsh') is near the head of the York River (Figure 1b, circle), has average salinities of 0-3 ppt (VECOS database) and does not have a population of either herbivore (Wittyngham, *personal observation*). In contrast, Taskinas Creek marsh (37.416330, -76.715054, hereafter 'mesohaline marsh') is located mid-estuary in the York River (Fig. 1b, triangle), has average salinities of 6-14 ppt (VECOS database) and has known populations of both *L. irrorata* (average density of ~44 snails per m^2; Failon et al. 2020) and *S. reticulatum* (unknown density; Wittyngham, *personal observation*). Although there are physical differences between the two marshes (e.g., sediment composition and hydrology), the goal of this study was not to make inferences about the marshes themselves, but rather to draw comparisons between *S. alterniflora* that have experienced different levels of salinity and herbivory.

Mesocosm Set-up & Maintenance

In the summer of 2017, roots and shoots of *S. alterniflora* were collected from each marsh. All plants were collected within one meter of the marsh edge using a trowel. Individual collected shoots were at least 0.5 meters apart to minimize collecting ramets from the same clone. Roots and rhizomes were kept intact to minimize the impact of collection and transport to the Virginia Institute of Marine Science (VIMS). Two of the *S. alterniflora* stems from each marsh were planted in an 11-liter nursery pot containing a 90:10 potting mix to sand mixture. Each pot was suspended in a 19-liter bucket. Following planting, each stem was tagged with a unique colored zip tie and one of five salinity treatments (0, 6, 14, 19, or 26 ppt) was randomly assigned to each replicate bucket, with 5 replicates per treatment. Salinity treatments of 0, 6, and 14 ppt are based on average salinities at the oligohaline and mesohaline collection sites (VECOS database), and treatments of 19 and 26 ppt were used to capture salinities expected with future sea-level rise. Each mesocosm was mechanically tidal following the methods of MacTavish & Cohen (2014), and programmed tidal cycles followed the natural semidiurnal tides of the Chesapeake Bay region. Water was collected directly from the York River (salinity ~17-20 ppt) via a flow-through seawater system and salinity was augmented to high treatment levels (19 and 26 ppt) through the addition of Instant Ocean salts or to low treatment levels (0, 6, and 14 ppt) by adding tap water from a garden hose. Reservoir bucket salinity was measured using a handheld YSI ProDSS multiparameter water quality meter and was changed once every three days to avoid algal growth and to maintain nutrient and dissolved oxygen levels. After approximately three weeks of acclimation, one of two *S. alterniflora* stems from each marsh within each mesocosm was randomly assigned a clipped treatment to simulate herbivory. Moving from the base of the stem upward, every other leaf was clipped at the ligule with garden shears. This pattern of mechanical herbivory maximized the possibility of eliciting a response within *S. alterniflora* tissues, while leaving enough remaining aboveground biomass for trait analyses. Clipping was repeated every two weeks to mimic chronic herbivory while still allowing for plant growth.

After two months of simulated herbivory and three months of salinity treatments, the experiment ended. At this point aboveground biomass of the original planted shoot was separated from belowground biomass and new clonal stems (produced by asexual rhizomatous growth) at the sediment surface. All belowground biomass and new stems were washed in an outdoor sieve (1 mm^2 mesh) to remove sediments. New stems were then sorted by stem of origin and separated from belowground biomass. All aboveground biomass was placed in plastic, resealable bags and held in a -80°C freezer to await further processing. All belowground biomass was placed into pre-weighed foil packets and dried in a drying oven at 60°C for twelve days, and dry masses were recorded.

Plant-Trait Analysis

Aboveground biomass was lyophilized and ground to a fine powder using a mini Wiley Mill fitted with a 40-mesh sieve. Samples were run on a FlashEA CHN elemental analyzer for carbon and nitrogen analysis and values were calculated using an Acetanilide standard curve. Total soluble protein content was measured using a modified Bradford assay (Wittyngham et al. 2019) in which 1mL of 1M NaOH was added to 5 mg of pulverized plant matter and incubated at 4°C for 24 hours for extraction. Following incubation, samples were centrifuged at 60G for 15 minutes and 30 µL of the supernatant was placed in sterile centrifuge tubes. 1.5 mL of

Coomassie reagent was added to each sample and allowed to incubate at room temperature for 20 minutes. Absorbance was read at 595 nm and compared to a bovine serum albumin (BSA) standard curve. All samples and standards were run in duplicate. Total phenolic concentrations were measured using a modified Folin-Ciocalteu protocol (Wittyngham et al. 2019). Three successive extractions (70%, 70%, 100% MeOH) were conducted on 100mg of pulverized plant matter. All three extracts were combined and a 150µL aliquot was added to a sterile centrifuge tube. 150µL of Folin reagent was added to each tube and mixed for two minutes. 800 µL of 0.5M sodium bicarbonate ($NaHCO_3$) was added to stop the reaction, and then samples were incubated at room temperature for 20 minutes to allow for color development. Absorbance was measured at 760 nm and compared to a ferulic acid standard curve.

Statistical Analysis

Statistical analysis was conducted in R (R Core Team, 2019). All responses were tested for normality and homogeneity of variance, and those which did not meet these assumptions were transformed using Box-Cox transformations or were log transformed. Multiple hypotheses were tested for each response using generalized linear models. All models were evaluated with model selection, and Akaike Information Criterion corrected for small sample size (AICc) and weights were used to assess best fit. Any model with a weight greater than 0.1 was assessed further using the *anova* function. For all models, salinity was treated as a continuous fixed factor, with simulated herbivory treatment and site as categorical fixed factors. Additional covariates for some models included: initial aboveground biomass, new stem biomass, and nitrogen content. Initial biomass and new stem biomass were added to account for a possible nutrient dilution effect, as seen in other studies (Grant et al. 2014; Luo et al. 2019). Significance was set at an alpha of 0.05.

Results

Table 1 outlines all models tested, best model fit, AICc values, and weights for all response variables. There were no significant interactions between variables, therefore additive models were used for all responses.

Spartina alterniflora from the mesohaline marsh had higher carbon content (p=0.007; Fig. 2A) and subsequently higher C:N (p=0.04; Fig. 3A) than *S. alterniflora* from the oligohaline marsh. As salinity increased, carbon content tended to decrease (p=0.063; Fig. 2B), with the highest carbon content at a salinity of 0 ppt, and lower carbon content in treatments of 14, 19, and 26 ppt (Fig. 2B). In contrast, nitrogen content significantly increased as salinity increased (p=0.02; Fig. 4A), with the highest nitrogen content at 26 ppt, and the lowest nitrogen content at 0 ppt (Fig. 4A). These results for carbon and nitrogen content lead to an overall decline in C:N with increasing salinity (p=0.005; Fig. 3B). Simulated herbivory via clipping tended to elevate tissue nitrogen content (p=0.08; Fig. 4B) and lower C:N (p=0.002; Fig. 3C).

Contrary to expectations, there were no effects of collection site, salinity, or clipped treatments on protein and phenolic content. The only significant predictor of protein content was nitrogen content, which had a positive, linear effect (p=0.005; Online Resource 1). Additionally, phenolic concentrations had a significantly positive linear relationship with new stem biomass production (p=0.01; Online Resource 2). Although new stem biomass tended to increase as salinity increased from 6 ppt to 19 ppt (p=0.05; Fig. 5A), there were no significant differences in new stem biomass production between salinity treatments. Interestingly, new stem biomass was significantly lower in clipped treatments when compared to controls ($p=1.36x10^{-6}$; Fig. 5B). *Spartina alterniflora* from the mesohaline marsh produced more belowground biomass (p=0.0006; Fig. 6A) than the oligohaline *S. alterniflora* and clipped treatments produced less belowground biomass than controls (p=0.04; Fig. 6B).

Discussion

I expected that simulated herbivory via clipping would elicit either a resistance (e.g., higher phenolic concentrations and lower protein content) or a tolerance (e.g., increased carbon, C:N and biomass production) defense response in *S. alterniflora*, as seen in previous polyhaline marsh studies (Johnson & Jessen 2008; Long et al. 2011; Sieg et al. 2013). Contrary to these expectations, I found no signs of a resistance strategy, as clipping had no effect on the phenolic or protein content of *S. alterniflora*. In addition, clipping did not elicit a tolerance strategy in *S. alterniflora*, as clipped plants had significantly lower C:N and biomass (both belowground and new stem) production than controls, and clipping had no effect on carbon content.

There is some evidence, however, that clipped treatments may have stimulated a defense response in *S. alterniflora* not captured by my measured response variables. When resources are limited, there is a trade-off between growth and defense, and therefore a decline in growth may indicate an investment of resources in anti-herbivore compounds (Coley et al. 1985; Basey & Jenkins 1993). In my study, clipped treatments decreased both belowground and new stem biomass. Although some phenolics can serve as chemical defense against herbivores in *S. alterniflora* (Sieg et al. 2013), other anti-herbivore compounds such as lignin (Buchsbaum et al. 1986), fiber (Buchsbaum et al. 1984), and silica (Massey et al. 2007) were not measured in this study and may have been induced by clipping, resulting in lower biomass production. In addition to these variables, direct measures of tissue toughness should also be included in future studies to better understand their role in herbivore deterrence (Pennings et al. 1998).

Aside from compensatory biomass production, other traits such as resource allocation, plant morphology, phenological changes, and increased photosynthetic capacity can indicate a tolerance defense response (Stowe et al. 2000; Tiffin 2000). In my study, clipped *S. alterniflora* tissues had significantly higher nitrogen content than controls. I expected this nitrogen pool to be used for protein synthesis, as I found a positive linear relationship between these variables. Despite these expectations, there was no effect of clipping on protein content, therefore the increased nitrogen could have been used for other functions, such as chlorophyll production. This would increase the photosynthetic capacity of clipped plants, an indicator of a tolerance response (Tiffin 2000).

Based on the optimal-defense theory, I anticipated that *S. alterniflora* collected from a mesohaline marsh would be better defended against herbivory than plants collected from an oligohaline marsh (Optimal-Defense Theory: Rhoads 1979; Herms & Mattson 1992; Stamp 2003), as mesohaline marshes have a higher diversity of herbivores and incidence of attack (Crain 2008; Sutter et al. 2019). Additionally, Hobbs (2009) found that although the surface sediments of both the mesohaline and oligohaline marsh used in my study are from the Quaternary period, the underlying strata of the mesohaline marsh is from the Tertiary period, indicating that this marsh may be older and thus have a longer history of herbivory. Carbon content, C:N, and belowground biomass were higher in *S. alterniflora* collected from the mesohaline marsh than the oligohaline marsh. Carbon content and C:N are measures of structural complexity and belowground biomass production provides insights into allocation patterns, all of which indicate a tolerance response (Stowe et al. 2000; Tiffin 2000). These results support the optimal-defense theory and my hypothesis that *S. alterniflora* collected from the mesohaline marsh is more defended than plants from the oligohaline marsh.

These differences in carbon content, C:N, and belowground biomass production between mesohaline and oligohaline marshes may ultimately be driven by salinity. In a similar mesocosm study, there was a significant effect of salinity (0 to 3 ppt) on *S. alterniflora* stoichiometry, with the highest C:N, carbon, and nitrogen content in 0 ppt treatments (Sutter et al. 2015). My results follow similar patterns for carbon and C:N, with both variables declining as salinity increases, regardless of collection site. I found an opposing pattern for nitrogen, with elevated salinity leading to higher nitrogen content. This follows my expectations, as increased nitrogen content may be needed to synthesize osmolytes to combat osmotic stress with higher salinities (Munns 2002; Sutter et al. 2019). There was no effect of collection site on nitrogen content, however the declines in carbon and C:N are more pronounced for *S. alterniflora* from the oligohaline marsh, indicating that plants from the mesohaline marsh may be more resilient to the effects of salinity.

Lastly, I hypothesized that *S. alterniflora* in high salinity treatments would follow a resistance strategy rather than a tolerance strategy, as salinity can inhibit compensatory growth in other *Spartina* spp. (*Spartina densiflora*: Alberti et al. 2010, Daleo et al. 2015, Infante-Izquierdo et al. 2019; *Spartina foliosa*: Long & Porturas 2014; *Spartina maritima*: Infante-Izquierdo et al. 2019). If *S. alterniflora* were opting for a resistance strategy instead of a tolerance strategy, I expected increased phenolic concentrations accompanied by declines in protein content, carbon content, and C:N. Although there was no effect of salinity on protein content or phenolic concentrations, *S. alterniflora* in higher salinity treatments had lower carbon content and C:N. This further indicates that perhaps my measured variables did not fully capture a defense response in *S. alterniflora* and that future studies should include additional functional traits.

It's important to note that the use of clipping to simulate herbivory may serve as a caveat to this study, as mimicked herbivory is not always a perfect surrogate for natural herbivory

(Strauss & Agrawal 1999). Different herbivores graze *S. alterniflora* in distinctive ways (e.g. phloem-sucking by *Prokelesia marginata*; radula-scraping & fungal cultivation by *Littoraria irrorata*; clipping and shredding by *Sesarma reticulatum*), so it can also be difficult to determine which herbivore to mimic. Further, the direct removal of aboveground biomass via clipping may have altered the photosynthetic capacity of *S. alterniflora*, potentially influencing production. In addition, my study focused on *S. alterniflora* from only one mesohaline marsh and one oligohaline marsh. Similar future studies should examine plants from multiple marshes to examine these concepts further. Lastly, there are potential drawbacks to applying the classic dichotomy of resistance versus tolerance strategies to tidal marshes. Although each of the traits measured in my study have been used repeatedly to quantify these strategies in the literature, my results demonstrate that factors other than herbivory (e.g. collection site and salinity) can elicit changes in plant traits. I suggest that use of this framework can be important to draw comparisons between ecosystems, such as wetlands versus terrestrial grasslands, however results should be interpreted with caution.

Overall, this study provides insight into *S. alterniflora*'s ability to defend itself against herbivore attack and informs our understanding of marsh resilience against sea-level rise. Through the process of vertical accretion, *S. alterniflora* plays a key role in elevation maintenance and marsh vulnerability to sea-level rise (Morris et al. 2002; Kirwan & Megonigal 2013). Herbivory on *S. alterniflora* can remove large patches of vegetation, impacting marsh stability and contributing to marsh loss (Gedan et al. 2009; He & Silliman 2016; Angelini et al. 2018). Both herbivory and salinity are drivers of vegetation diversity and stem density within tidal marshes, and thus can influence accretion capacity (Morris et al. 2002; Elsey-Quirk & Unger 2018). My results indicate that *S. alterniflora* exposed to herbivory may have higher forage quality (e.g. increased nitrogen content, decreased C:N), presenting a positive feedback between herbivory and vegetation die-off.

In addition, higher salinities expected with sea-level rise may actually increase the probability of future attack from herbivores via improved forage quality (e.g. increased nitrogen content, decreased carbon content and C:N). *Spartina alterniflora* from the mesohaline marsh had enhanced tolerance traits when compared to its oligohaline counterpart, demonstrating that these marshes may be more resilient to herbivory. As sea level rises and pushes saline waters into fresher regions of estuaries, invertebrate herbivores previously constrained by salinity may establish in oligohaline marshes, potentially increasing their vulnerability to runaway herbivory and thus sea-level rise.

Finally, a decline in *S. alterniflora* biomass production caused by herbivory may ultimately decrease the marsh’s ability to vertically accrete and keep pace with sea-level rise, as this process is a function of belowground organic matter accumulation and sediment deposition, which is controlled, in part, by stem density (Elsey-Quirk & Unger 2018). Although these results present a pathway to marsh loss, many marshes remain intact, despite the presence of herbivores. This study highlights that though widespread marsh loss from herbivory can occur (Silliman et al. 2005; Davidson & de Rivera 2010; Bertness et al. 2014), this may not be a universal response. Thus, in addition to biotic interactions, ecologists must also consider the geomorphic (e.g. sediment supply) and biogeochemical (e.g. carbon storage in peat) feedbacks that contribute to marsh stability.

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Table 1. Model selection for each response variable. Bolded model indicates best fit based on AICc and weight. Explanatory variables with an asterisk (*) indicate significance. Response variables with two asterisks (**) were log transformed to meet assumptions and those with three asterisks (***) were transformed with Box-Cox. (Abbreviations for explanatory variables: Sa = Salinity, Cl = Clipping, Si = Site, IAB = Initial Aboveground Biomass, NSB = New Stem Biomass, N = Nitrogen).

Response Variable	Explanatory Variables	k	AICc	Weight
Carbon	Sa + Cl	4	258.5234	< 0.01
	Sa + Cl + Si	5	253.1361	< 0.01
	Sa + Cl + Si + IAB	6	254.4296	< 0.01
	Sa* + Cl + Si* + IAB + NSB	**7**	**241.2353**	**0.99**
Nitrogen**	**Sa* + Cl***	**4**	**4.425692**	**0.58**
	Sa + Cl + Si	5	5.802641	0.29
	Sa + Cl + Si + IAB	6	7.976806	< 0.01
	Sa + Cl + Si + IAB + NSB	7	10.140312	< 0.01
C:N	Sa + Cl	4	466.4417	< 0.01
	Sa + Cl + Si	5	466.2139	< 0.01
	Sa + Cl + Si + IAB	6	468.6781	< 0.01
	Sa* + Cl* + Si* + IAB + NSB	**7**	**438.3512**	**0.99**
Protein**	Sa + Cl	4	-35.84847	< 0.01
	Sa + Cl + Si	5	-33.72827	< 0.01
	Cl + N*	**4**	**-42.04862**	**0.48**
	Sa + Cl + N	5	-40.08086	0.18
	Cl + N + NSB	5	-40.90411	0.27
	Sa + Cl + Si + IAB + N	7	-36.32555	< 0.01
	Sa + Cl + Si + IAB + N + NSB	8	-34.43539	< 0.01
Phenolics**	Sa + Cl	4	43.01449	< 0.01
	Sa + Cl + Si	5	45.16761	< 0.01

	Cl + N	4	42.30521	< 0.01
	Sa + Cl + N	5	44.68106	< 0.01
	Cl + N + NSB*	**5**	**28.21158**	**0.93**
	Sa + Cl + Si + IAB + N	7	48.19093	< 0.01
	Sa + Cl + Si + IAB + N + NSB	8	33.54844	< 0.01
New Stem Biomass***	**Sa* + Cl***	**4**	**161.3125**	**0.62**
	Sa + Cl + Si	5	162.8990	0.28
	Sa + Cl + Si + IAB	6	165.1308	< 0.01
Belowground Biomass***	Sa + Cl	4	188.0034	< 0.01
	Sa + Cl* + Si*	**5**	**177.6864**	**0.99**

Figures

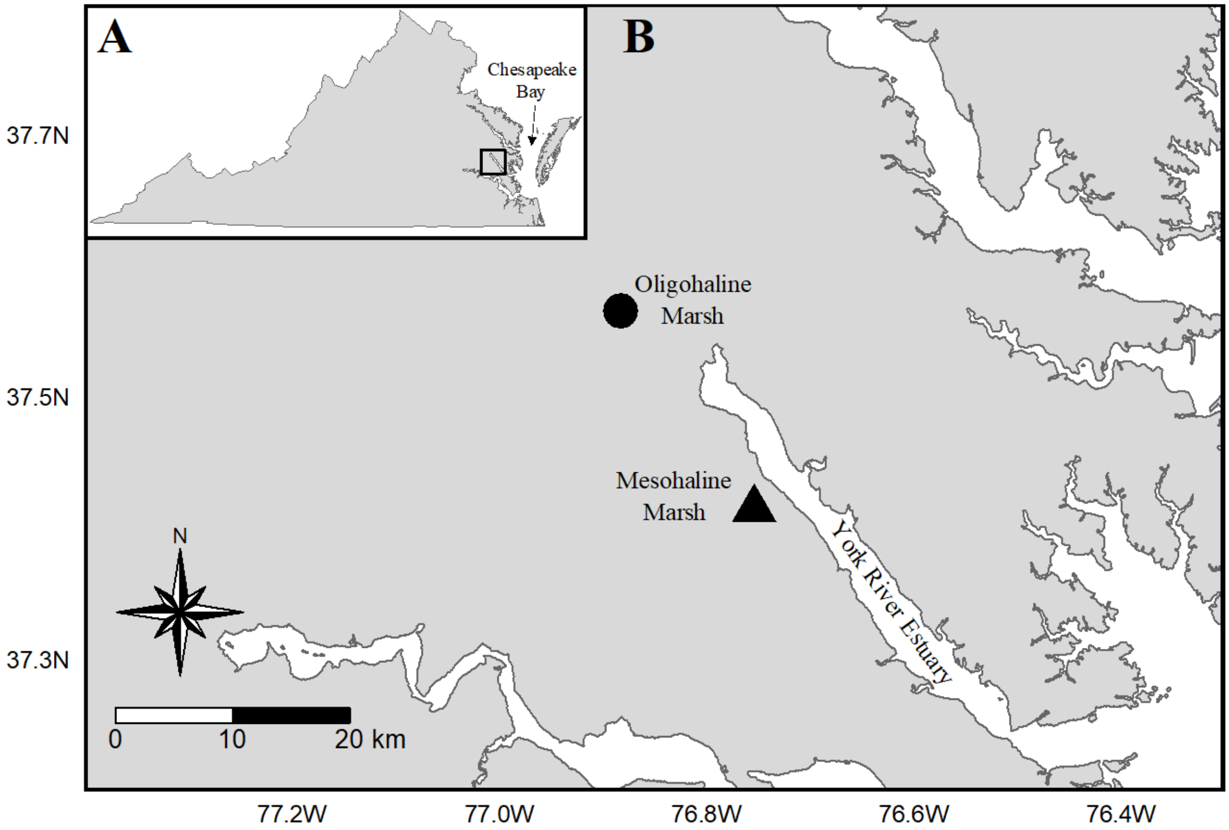


Fig. 1 A) Inset map of the state of Virginia, U.S.A. Boxed area indicates study region. B) Enlarged map of study region along the York River Estuary. The circle represents the oligohaline marsh (Sweet Hall) and the triangle the mesohaline marsh (Taskinas Creek)

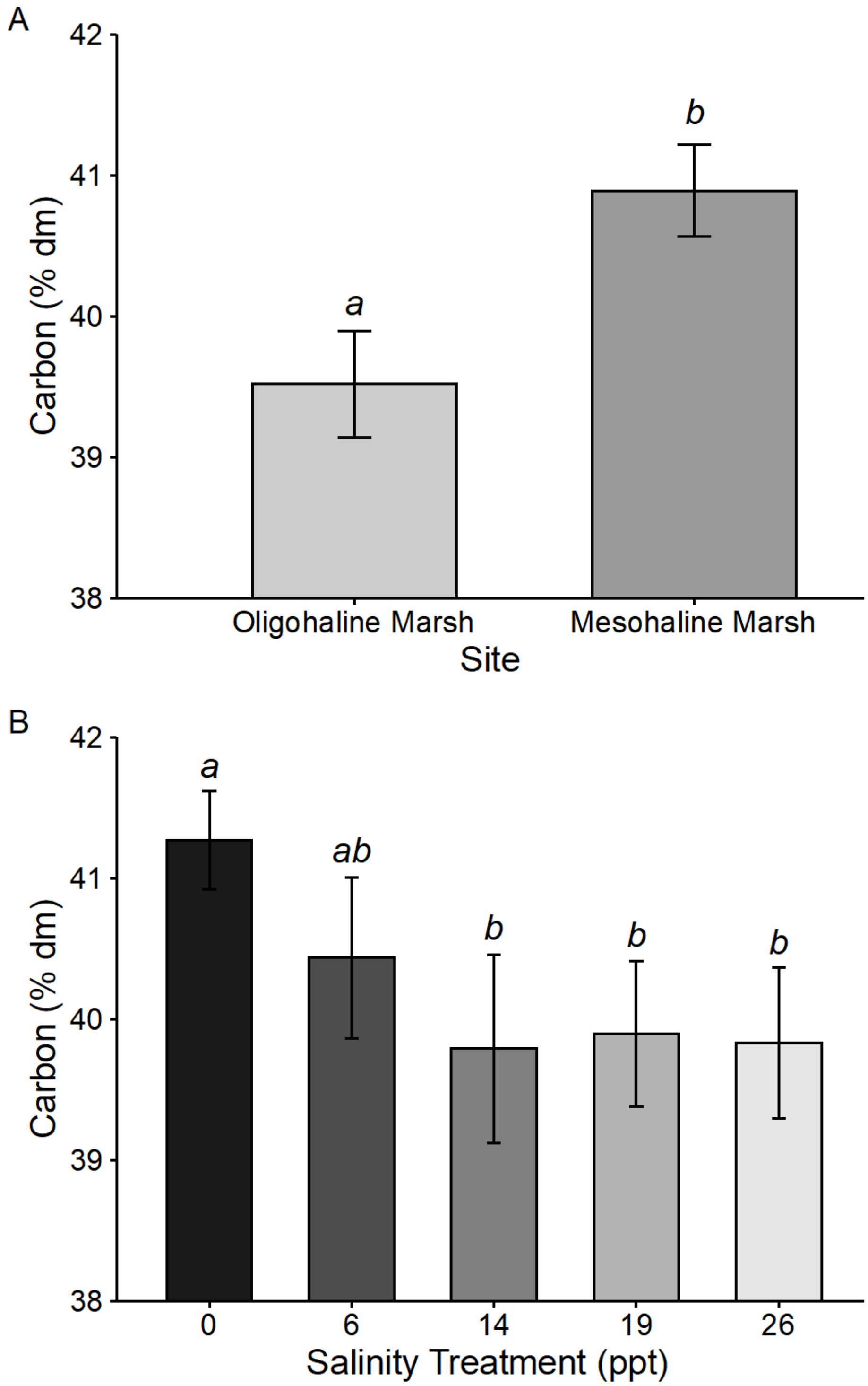


Fig. 2 Mean carbon content (percent dry mass) of *S. alterniflora* tissues A) by collection site (oligohaline or mesohaline marsh) and B) subjected to one of five salinity treatments (0, 6, 14, 19, or 26 ppt). Italicized letters above bars indicate significance between treatments. Error bars represent standard error

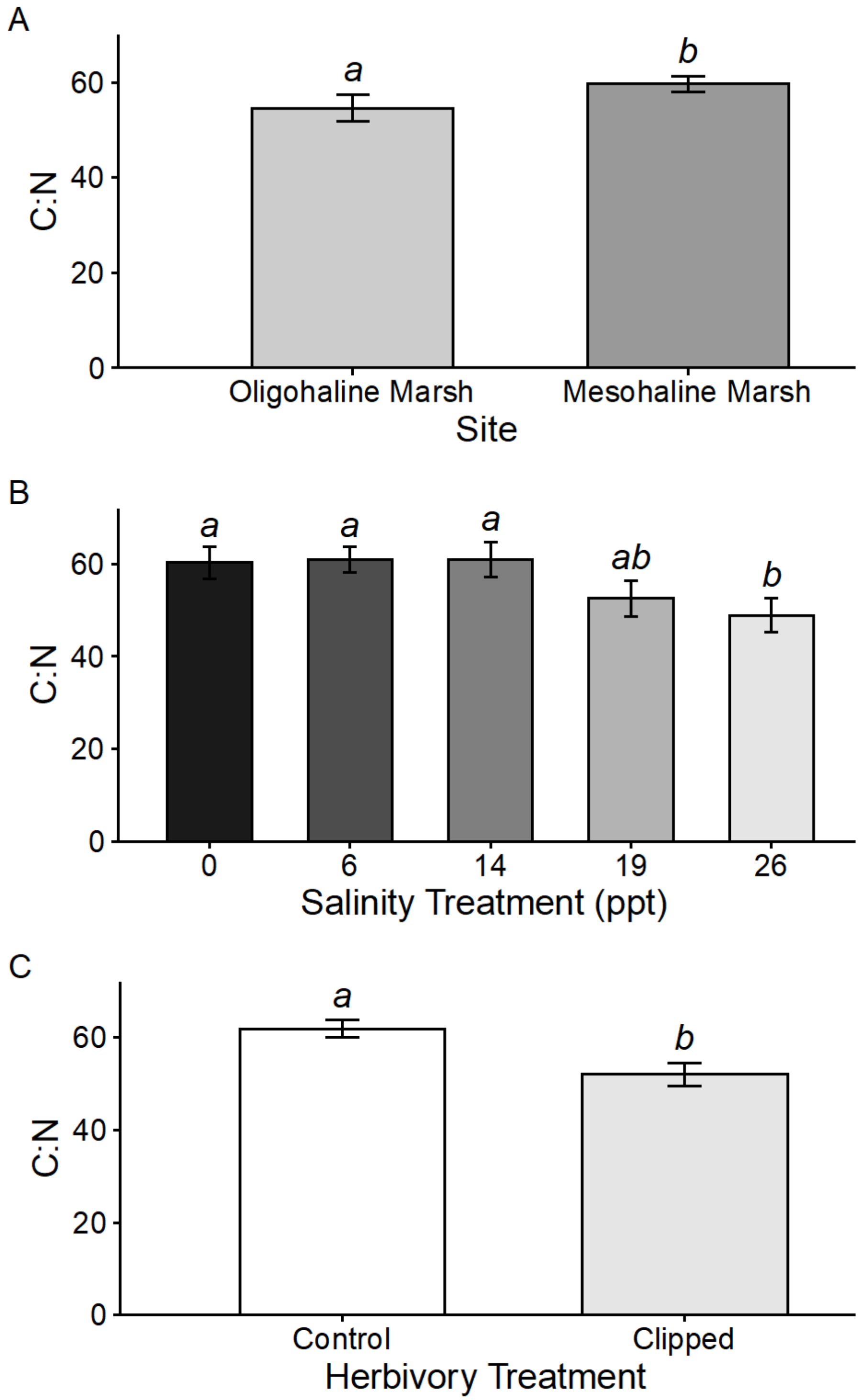


Fig. 3 Mean C:N molar ratios of *S. alterniflora* tissues A) by collection site (oligohaline or mesohaline marsh), B) subjected to one of five salinity treatments (0, 6, 14, 19, or 26 ppt), and C) by simulated herbivory treatment (control or clipped). Italicized letters above bars indicate significance between treatments. Error bars represent standard error

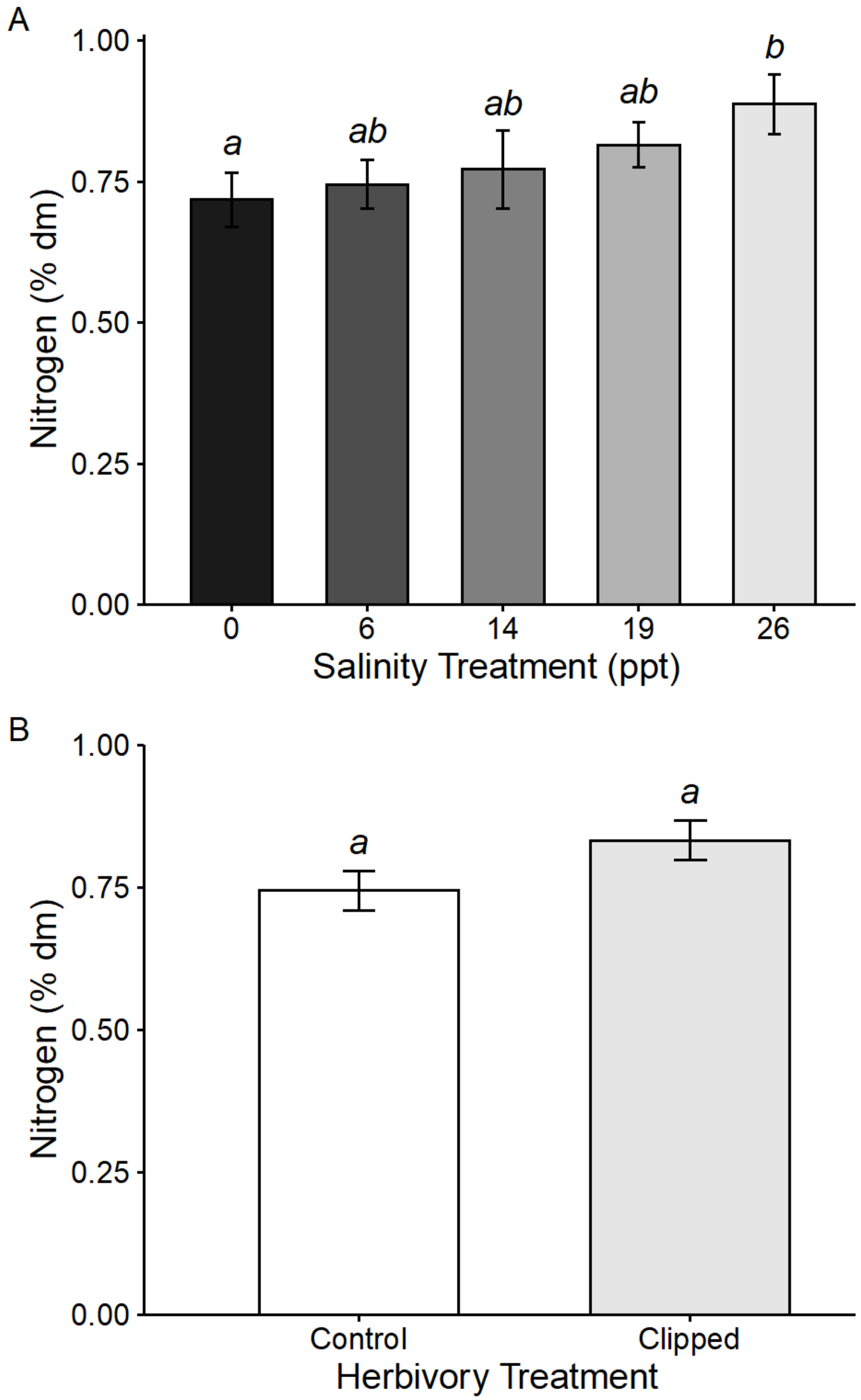


Fig. 4 Mean nitrogen content (percent dry mass) of *S. alterniflora* tissues A) subjected to one of five salinity treatments (0, 6, 14, 19, or 26 ppt) and B) by simulated herbivory treatment (control or clipped). Italicized letters above bars indicate significance between treatments. Error bars represent standard error

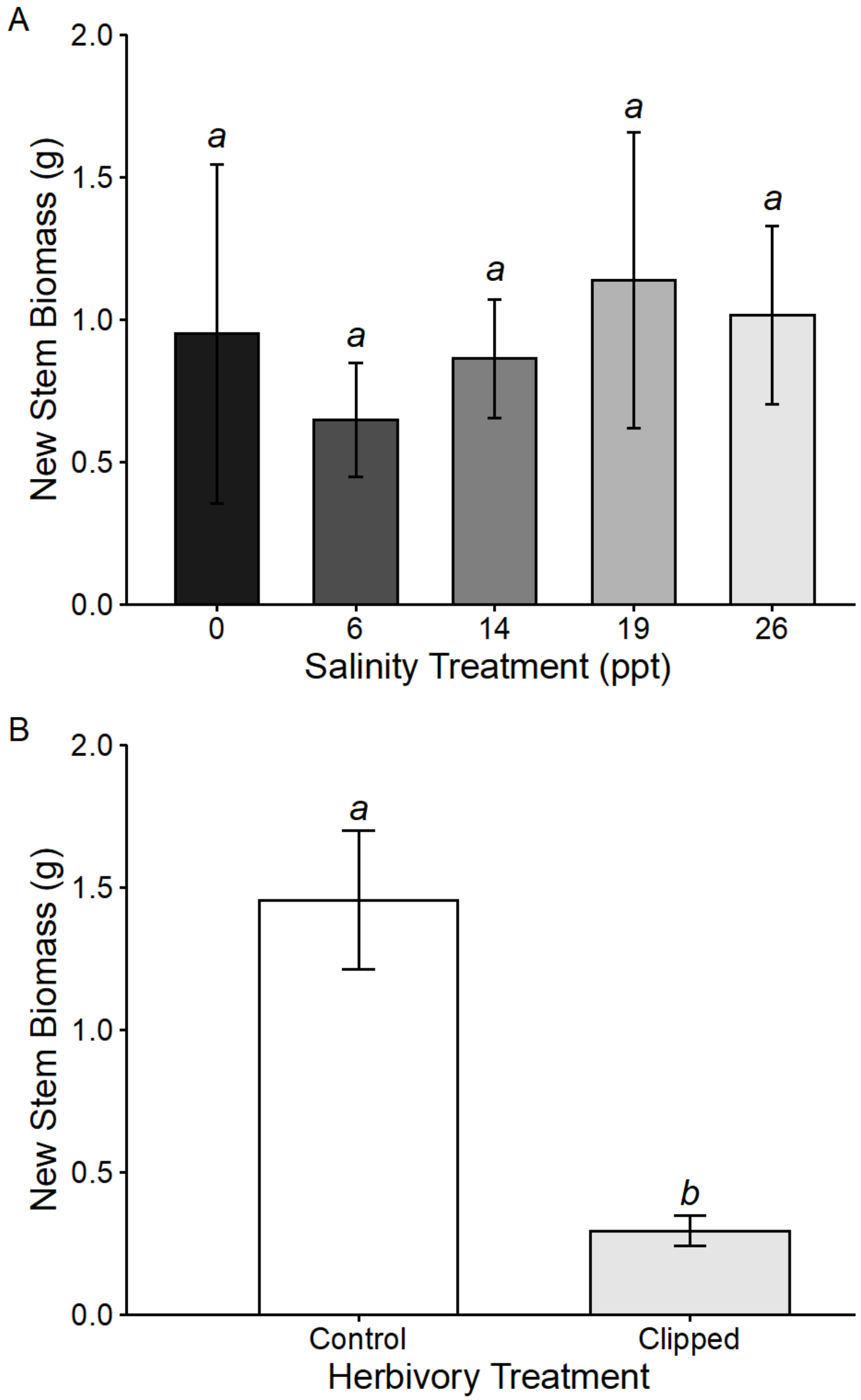


Fig. 5 Mean dry biomass (grams) of new stems produced by *S. alterniflora* A) subjected to one of five salinity treatments (0, 6, 14, 19, or 26 ppt) and B) by simulated herbivory treatment (control or clipped). Italicized letters above bars indicate significance between treatments. Error bars represent standard error

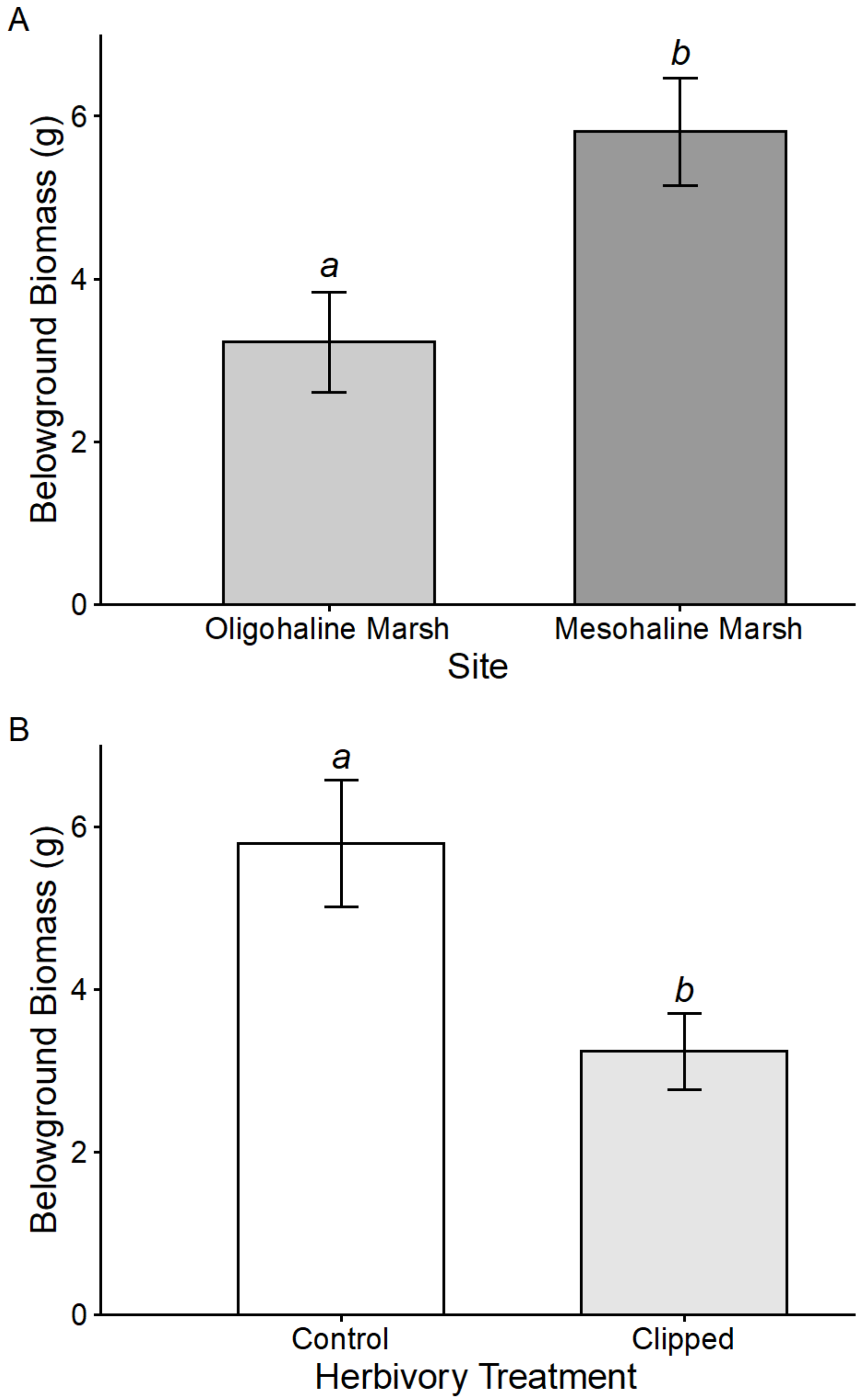
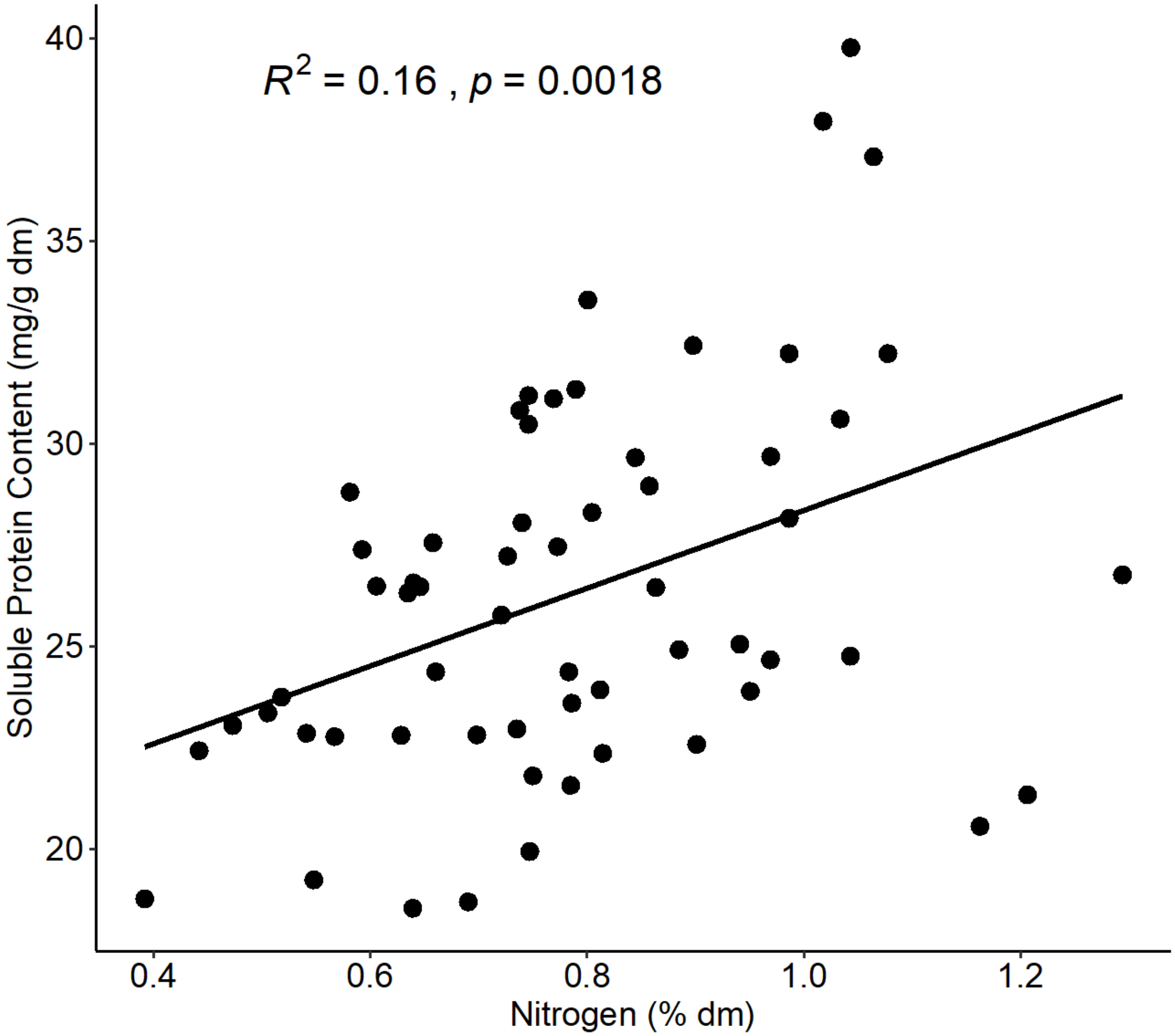
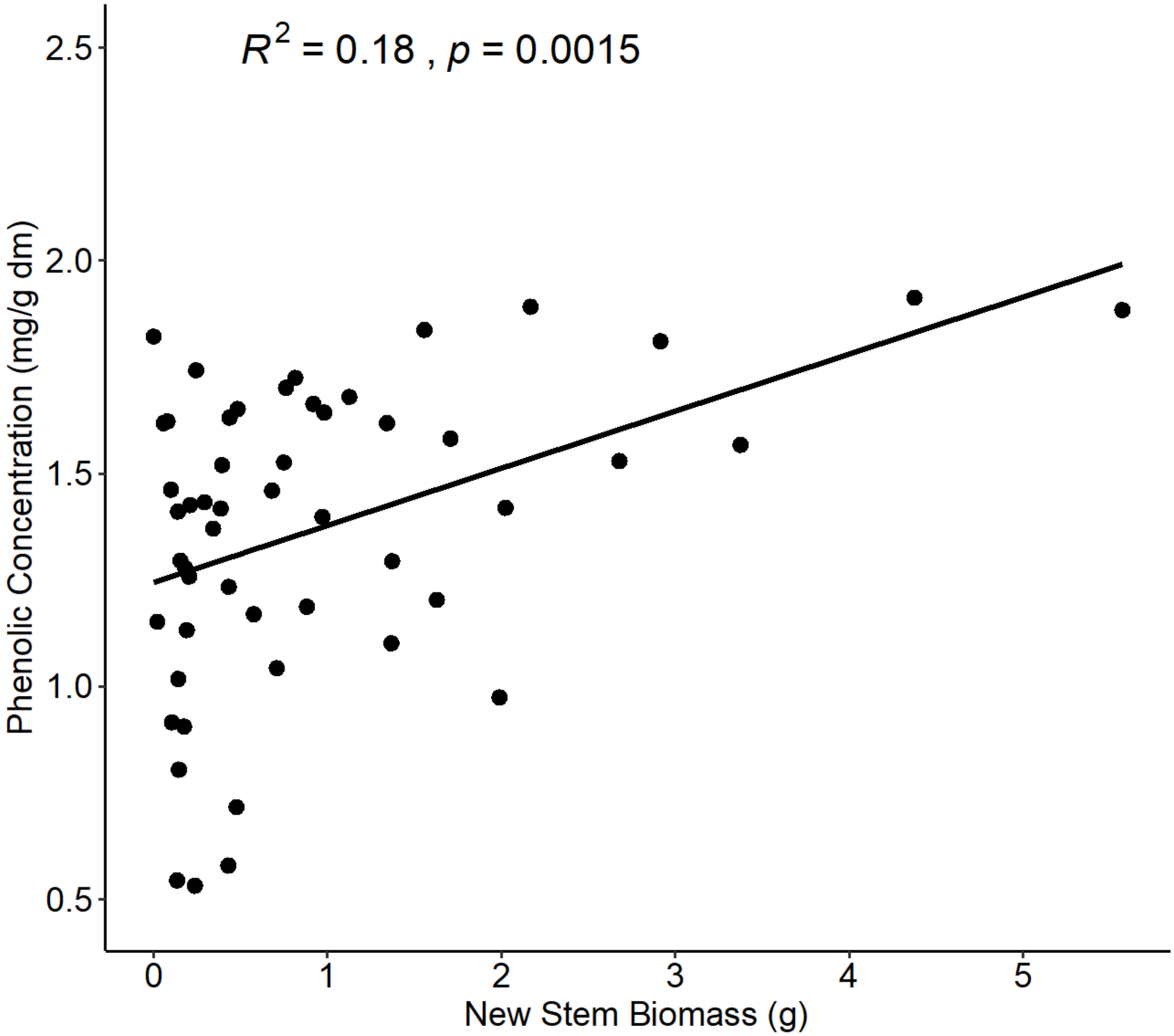


Fig. 6 Mean dry belowground biomass (grams) of *S. alterniflora* A) by collection site (oligohaline or mesohaline marsh) and B) by simulated herbivory treatment (control or clipped). Italicized letters above bars indicate significance between treatments. Error bars represent standard error

Electronic Supplementary Material



ESM 1 Mean soluble protein content (milligrams/gram dry mass) of *S. alterniflora* tissues across nitrogen content (percent dry mass). Trend line represents smoothed linear regression line



ESM 2 Mean phenolic concentrations (milligrams/gram dry mass) of *S. alterniflora* tissues across new stem biomass (grams). Trend line represents smoothed linear regression line