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

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6 **Abstract**

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

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

23

24 **Introduction**

25 Tidal marshes are responsible for ecosystem services that contribute to human well-being
26 including carbon sequestration, erosion control, and nutrient cycling (DeGroot et al. 2012;
27 Costanza et al. 2014). Tidal marshes occur along natural salinity gradients within estuaries and
28 are typically categorized by their salinity regime (e.g. oligohaline – 0 to 5 ppt, mesohaline – 5 to
29 18 ppt, and polyhaline – 18 to 30 ppt) (Odum 1988; Montagna et al. 2013). In the Chesapeake
30 Bay region, accelerated sea-level rise is a threat to tidal marshes (Najjar et al. 2010). Average
31 sea-level rise in this region is $\sim 3.80 \text{ mm yr}^{-1}$, which is 3-4 times higher than the global mean of
32 $\sim 0.98 \text{ mm yr}^{-1}$ (Sallenger et al. 2012; Boon & Mitchell 2015). A marsh's ability to keep pace
33 with sea-level rise depends on sediment size and supply (Kirwan et al. 2010), and vegetation
34 stem density and biomass production, both above- and belowground (Leonard & Luther 1995;
35 Elsey-Quirk & Unger 2018). Marsh vegetation is responsible for regulating the process of
36 vertical accretion, as plant stems trap sediments above ground and accumulate organic matter
37 below ground (Kirwan & Megonigal 2013), thus building marsh elevation and keeping pace with
38 rising seas.

39 In addition to sea-level rise, tidal marshes are threatened by intense herbivory (Gedan et
40 al. 2009; He & Silliman 2016; Angelini et al. 2018). Many of the invertebrate herbivores
41 implicated in runaway consumption (e.g. the marsh periwinkle, *Littoraria irrorata*: Silliman et
42 al. 2005; the purple marsh crab, *Sesarma reticulatum*: Holdredge et al. 2009) are physiologically
43 limited to mesohaline and polyhaline marshes (Staton & Felder 1992; Henry et al. 1993) and are
44 not found in oligohaline marshes. As sea-level rise pushes saline waters into oligohaline
45 marshes, invertebrate herbivores may follow, increasing the vulnerability of these marshes to
46 herbivory. In some instances, runaway herbivory can remove vegetation from large spatial areas

47 and transition the marsh to a mudflat (Holdredge et al. 2009; Vu et al. 2017), intensifying marsh
48 susceptibility to drowning; however, despite extreme herbivory, marshes persist. This may be
49 due in part to how plants respond to herbivory pressure. Thus, understanding how marsh plant
50 traits change in response to herbivory provides direct insight into one aspect of marsh resilience.

51 The palatability of plant tissue can control the rate of herbivory (Siska et al. 2002;
52 Salgado & Pennings 2005), and thus susceptibility to grazing. Following herbivory, many plants
53 can induce changes to their chemical, structural, and morphological traits to mitigate damage and
54 deter further grazing (Ito & Sakai 2009; Burghardt & Schmitz 2015), which in combination with
55 constitutive traits, can decrease herbivore consumption and vegetation removal. Alterations in
56 both constitutive and induced traits define the two primary plant defense strategies: tolerance and
57 resistance. Plants can tolerate herbivory by increasing above and belowground biomass
58 production to compensate for mass lost to herbivores (Mauricio et al. 1997; Burghardt &
59 Schmitz 2015). Alternatively, plants can resist herbivore attack by producing chemical and/or
60 structural defenses to decrease palatability and deter future grazing (Mauricio et al. 1997;
61 Burghardt & Schmitz 2015). Depending on factors such as environmental conditions or
62 herbivore abundance, these strategies may or may not be mutually exclusive (Mauricio et al.
63 1997; Więski & Pennings 2014).

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

70 *Sporobolus alterniflorus*) (Perry & Atkinson 1997). In the Chesapeake Bay region, *S.*
71 *alterniflora* grows along the natural salinity gradient of estuaries and is found in both oligohaline
72 and mesohaline marshes. Thus, it was selected as the focal species of this study. Previous work
73 on *S. alterniflora* defense response has been focused in polyhaline marshes alone (Pennings et al.
74 1998; Hendricks et al. 2011; Long et al. 2011; Sieg et al. 2013; Long & Porturas 2014; Więski &
75 Pennings 2014). It is unclear, however, whether a pattern in *S. alterniflora* defense exists along
76 the mesohaline to oligohaline marsh gradient, and if so, how this response may be influenced by
77 increasing salinities anticipated with sea-level rise.

78 Optimal-defense theory predicts that the probability or incidence of herbivore attack may
79 determine the extent of a plants' defense response (Herms & Mattson 1992; Ito & Sakai 2009).
80 In wetlands, the type of herbivory varies along the natural estuarine salinity gradient. Both
81 oligohaline and mesohaline marshes suffer from vertebrate (e.g. avian and/or mammalian) and
82 insect herbivory (Crain 2008). In addition to vertebrate and insect herbivores, mesohaline
83 marshes also have high abundances of other invertebrate herbivores (e.g. crustaceans and/or
84 mollusks), which are not typically found in oligohaline marshes (Crain 2008; Sutter et al. 2019).
85 Although not explicitly tested in marshes, the type of herbivore inflicting damage may influence
86 plant defense strategy. For example, terrestrial grasses follow a tolerance strategy in response to
87 mammalian herbivores (Frank & McNaughton 1993) and marine macroalgae follow a resistance
88 strategy in response to invertebrate grazing (Cronin & Hay 1996). If this pattern holds true for
89 tidal marshes, I would expect *S. alterniflora* from the oligohaline marsh, where vertebrate
90 herbivory is prevalent, to follow a tolerance strategy. In contrast, I would expect *S. alterniflora*
91 from the mesohaline marsh, which suffers more from invertebrate herbivory, to more closely
92 align with a resistance strategy. Additionally, although the exact age of these marshes is

93 unknown, the underlying strata indicate that the mesohaline marsh may be much older than the
94 oligohaline marsh (Hobbs 2009), thus, length of exposure to herbivory may also influence plant
95 defense. Therefore, I expected plant defense response to be greater in the mesohaline marsh
96 which has a longer history of herbivory and a wider variety of herbivores than the oligohaline
97 marsh.

98 Salinity may also mediate plant defense response, as increased salinity can restrict growth
99 and germination in *Spartina* spp. (Alberti et al. 2010; Daleo et al. 2015; Infante-Izquierdo et al.
100 2019), as well as inhibit compensatory growth in response to herbivory (Long & Porturas 2014).
101 This is particularly important for plants following a tolerance strategy in which compensatory
102 growth is the primary mechanism of defense. Further, salinity directly affects plant tissue
103 stoichiometry (MacTavish & Cohen 2017; Sutter et al. 2019). Therefore, I expected that *S.*
104 *alterniflora* in high salinity treatments, regardless of collection site, would have lower carbon
105 content due to decreased photosynthesis and carbon assimilation (MacTavish & Cohen 2017;
106 Sutter et al. 2019) and higher nitrogen content caused by increased osmolyte production (Munns
107 2002; Sutter et al. 2019), both of which contribute to lower C:N.

108 A trait-based approach was used to quantify the defense response of *S. alterniflora*. For
109 example, if *S. alterniflora* were to follow a resistance strategy, I expected to see increased tissue
110 phenolic concentrations and decreased protein content. Phenolics can lower plant palatability and
111 serve as deterrence against herbivore grazing (Dorenbosch & Bakker 2011; Zhang et al. 2019),
112 as well as play a role in primary metabolism or UV protection (Close & McArthur 2002; Neilson
113 et al. 2013). Herbivores forage for proteins to meet metabolic demands (Cebrian et al. 2009), so
114 plants may decrease the concentration of proteins to deter further herbivory. In contrast, if *S.*
115 *alterniflora* were following a tolerance strategy, I expected to see higher biomass (new stem

116 and/or belowground) and carbon content, with lower C:N. Elevated biomass production indicates
117 compensatory growth (Long & Porturas 2014), with both carbon content and C:N influenced by
118 biomass.

119

120 **Materials and Methods**

121 *Collection Sites*

122 *Spartina alterniflora* was collected from two marshes within the York River Estuary
123 (Virginia, USA; Figure 1a), a tributary of the Chesapeake Bay. There are two herbivores of
124 concern in the York River: the marsh periwinkle, *Littoraria irrorata*, and the purple marsh crab,
125 *Sesarma reticulatum*. These herbivores have been implicated in the large-scale die-off of U.S.
126 Atlantic polyhaline marshes (Silliman et al. 2005; Bertness et al. 2014). Their distribution in the
127 York River is currently limited to mesohaline and polyhaline marshes, although they are
128 expected to move into oligohaline marshes as sea-level rise pushes saline waters up-estuary.
129 Sweet Hall marsh (37.566087, -76.882472, hereafter ‘oligohaline marsh’) is near the head of the
130 York River (Figure 1b, circle), has average salinities of 0-3 ppt (VECOS database) and does not
131 have a population of either herbivore (Wittyngham, *personal observation*). In contrast, Taskinas
132 Creek marsh (37.416330, -76.715054, hereafter ‘mesohaline marsh’) is located mid-estuary in
133 the York River (Fig. 1b, triangle), has average salinities of 6-14 ppt (VECOS database) and has
134 known populations of both *L. irrorata* (average density of ~44 snails per m²; Failon et al. 2020)
135 and *S. reticulatum* (unknown density; Wittyngham, *personal observation*). Although there are
136 physical differences between the two marshes (e.g., sediment composition and hydrology), the
137 goal of this study was not to make inferences about the marshes themselves, but rather to draw

138 comparisons between *S. alterniflora* that have experienced different levels of salinity and
139 herbivory.

140 *Mesocosm Set-up & Maintenance*

141 In the summer of 2017, roots and shoots of *S. alterniflora* were collected from each
142 marsh. All plants were collected within one meter of the marsh edge using a trowel. Individual
143 collected shoots were at least 0.5 meters apart to minimize collecting ramets from the same
144 clone. Roots and rhizomes were kept intact to minimize the impact of collection and transport to
145 the Virginia Institute of Marine Science (VIMS). Two of the *S. alterniflora* stems from each
146 marsh were planted in an 11-liter nursery pot containing a 90:10 potting mix to sand mixture.
147 Each pot was suspended in a 19-liter bucket. Following planting, each stem was tagged with a
148 unique colored zip tie and one of five salinity treatments (0, 6, 14, 19, or 26 ppt) was randomly
149 assigned to each replicate bucket, with 5 replicates per treatment. Salinity treatments of 0, 6, and
150 14 ppt are based on average salinities at the oligohaline and mesohaline collection sites (VECOS
151 database), and treatments of 19 and 26 ppt were used to capture salinities expected with future
152 sea-level rise. Each mesocosm was mechanically tidal following the methods of MacTavish &
153 Cohen (2014), and programmed tidal cycles followed the natural semidiurnal tides of the
154 Chesapeake Bay region. Water was collected directly from the York River (salinity ~17-20 ppt)
155 via a flow-through seawater system and salinity was augmented to high treatment levels (19 and
156 26 ppt) through the addition of Instant Ocean salts or to low treatment levels (0, 6, and 14 ppt) by
157 adding tap water from a garden hose. Reservoir bucket salinity was measured using a handheld
158 YSI ProDSS multiparameter water quality meter and was changed once every three days to avoid
159 algal growth and to maintain nutrient and dissolved oxygen levels. After approximately three
160 weeks of acclimation, one of two *S. alterniflora* stems from each marsh within each mesocosm

161 was randomly assigned a clipped treatment to simulate herbivory. Moving from the base of the
162 stem upward, every other leaf was clipped at the ligule with garden shears. This pattern of
163 mechanical herbivory maximized the possibility of eliciting a response within *S. alterniflora*
164 tissues, while leaving enough remaining aboveground biomass for trait analyses. Clipping was
165 repeated every two weeks to mimic chronic herbivory while still allowing for plant growth.

166 After two months of simulated herbivory and three months of salinity treatments, the
167 experiment ended. At this point aboveground biomass of the original planted shoot was separated
168 from belowground biomass and new clonal stems (produced by asexual rhizomatous growth) at
169 the sediment surface. All belowground biomass and new stems were washed in an outdoor sieve
170 (1 mm² mesh) to remove sediments. New stems were then sorted by stem of origin and separated
171 from belowground biomass. All aboveground biomass was placed in plastic, resealable bags and
172 held in a -80°C freezer to await further processing. All belowground biomass was placed into
173 pre-weighed foil packets and dried in a drying oven at 60°C for twelve days, and dry masses
174 were recorded.

175

176 *Plant-Trait Analysis*

177 Aboveground biomass was lyophilized and ground to a fine powder using a mini Wiley
178 Mill fitted with a 40-mesh sieve. Samples were run on a FlashEA CHN elemental analyzer for
179 carbon and nitrogen analysis and values were calculated using an Acetanilide standard curve.
180 Total soluble protein content was measured using a modified Bradford assay (Wittingham et al.
181 2019) in which 1mL of 1M NaOH was added to 5 mg of pulverized plant matter and incubated at
182 4°C for 24 hours for extraction. Following incubation, samples were centrifuged at 60G for 15
183 minutes and 30 µL of the supernatant was placed in sterile centrifuge tubes. 1.5 mL of

184 Coomassie reagent was added to each sample and allowed to incubate at room temperature for
185 20 minutes. Absorbance was read at 595 nm and compared to a bovine serum albumin (BSA)
186 standard curve. All samples and standards were run in duplicate. Total phenolic concentrations
187 were measured using a modified Folin-Ciocalteu protocol (Wittyngham et al. 2019). Three
188 successive extractions (70%, 70%, 100% MeOH) were conducted on 100mg of pulverized plant
189 matter. All three extracts were combined and a 150 μ L aliquot was added to a sterile centrifuge
190 tube. 150 μ L of Folin reagent was added to each tube and mixed for two minutes. 800 μ L of
191 0.5M sodium bicarbonate (NaHCO₃) was added to stop the reaction, and then samples were
192 incubated at room temperature for 20 minutes to allow for color development. Absorbance was
193 measured at 760 nm and compared to a ferulic acid standard curve.

194

195 *Statistical Analysis*

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

208

209 **Results**

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

213 *Spartina alterniflora* from the mesohaline marsh had higher carbon content ($p=0.007$;
214 Fig. 2A) and subsequently higher C:N ($p=0.04$; Fig. 3A) than *S. alterniflora* from the oligohaline
215 marsh. As salinity increased, carbon content tended to decrease ($p=0.063$; Fig. 2B), with the
216 highest carbon content at a salinity of 0 ppt, and lower carbon content in treatments of 14, 19,
217 and 26 ppt (Fig. 2B). In contrast, nitrogen content significantly increased as salinity increased
218 ($p=0.02$; Fig. 4A), with the highest nitrogen content at 26 ppt, and the lowest nitrogen content at
219 0 ppt (Fig. 4A). These results for carbon and nitrogen content lead to an overall decline in C:N
220 with increasing salinity ($p=0.005$; Fig. 3B). Simulated herbivory via clipping tended to elevate
221 tissue nitrogen content ($p=0.08$; Fig. 4B) and lower C:N ($p=0.002$; Fig. 3C).

222 Contrary to expectations, there were no effects of collection site, salinity, or clipped
223 treatments on protein and phenolic content. The only significant predictor of protein content was
224 nitrogen content, which had a positive, linear effect ($p=0.005$; Online Resource 1). Additionally,
225 phenolic concentrations had a significantly positive linear relationship with new stem biomass
226 production ($p=0.01$; Online Resource 2). Although new stem biomass tended to increase as
227 salinity increased from 6 ppt to 19 ppt ($p=0.05$; Fig. 5A), there were no significant differences in
228 new stem biomass production between salinity treatments. Interestingly, new stem biomass was
229 significantly lower in clipped treatments when compared to controls ($p=1.36 \times 10^{-6}$; Fig. 5B).

230 *Spartina alterniflora* from the mesohaline marsh produced more belowground biomass

231 (p=0.0006; Fig. 6A) than the oligohaline *S. alterniflora* and clipped treatments produced less
232 belowground biomass than controls (p=0.04; Fig. 6B).

233 **Discussion**

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

242 There is some evidence, however, that clipped treatments may have stimulated a defense
243 response in *S. alterniflora* not captured by my measured response variables. When resources are
244 limited, there is a trade-off between growth and defense, and therefore a decline in growth may
245 indicate an investment of resources in anti-herbivore compounds (Coley et al. 1985; Basey &
246 Jenkins 1993). In my study, clipped treatments decreased both belowground and new stem
247 biomass. Although some phenolics can serve as chemical defense against herbivores in *S.*
248 *alterniflora* (Sieg et al. 2013), other anti-herbivore compounds such as lignin (Buchsbaum et al.
249 1986), fiber (Buchsbaum et al. 1984), and silica (Massey et al. 2007) were not measured in this
250 study and may have been induced by clipping, resulting in lower biomass production. In addition
251 to these variables, direct measures of tissue toughness should also be included in future studies to
252 better understand their role in herbivore deterrence (Pennings et al. 1998).

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

262 Based on the optimal-defense theory, I anticipated that *S. alterniflora* collected from a
263 mesohaline marsh would be better defended against herbivory than plants collected from an
264 oligohaline marsh (Optimal-Defense Theory: Rhoads 1979; Herms & Mattson 1992; Stamp
265 2003), as mesohaline marshes have a higher diversity of herbivores and incidence of attack
266 (Crain 2008; Sutter et al. 2019). Additionally, Hobbs (2009) found that although the surface
267 sediments of both the mesohaline and oligohaline marsh used in my study are from the
268 Quaternary period, the underlying strata of the mesohaline marsh is from the Tertiary period,
269 indicating that this marsh may be older and thus have a longer history of herbivory. Carbon
270 content, C:N, and belowground biomass were higher in *S. alterniflora* collected from the
271 mesohaline marsh than the oligohaline marsh. Carbon content and C:N are measures of structural
272 complexity and belowground biomass production provides insights into allocation patterns, all of
273 which indicate a tolerance response (Stowe et al. 2000; Tiffin 2000). These results support the
274 optimal-defense theory and my hypothesis that *S. alterniflora* collected from the mesohaline
275 marsh is more defended than plants from the oligohaline marsh.

276 These differences in carbon content, C:N, and belowground biomass production between
277 mesohaline and oligohaline marshes may ultimately be driven by salinity. In a similar mesocosm
278 study, there was a significant effect of salinity (0 to 3 ppt) on *S. alterniflora* stoichiometry, with
279 the highest C:N, carbon, and nitrogen content in 0 ppt treatments (Sutter et al. 2015). My results
280 follow similar patterns for carbon and C:N, with both variables declining as salinity increases,
281 regardless of collection site. I found an opposing pattern for nitrogen, with elevated salinity
282 leading to higher nitrogen content. This follows my expectations, as increased nitrogen content
283 may be needed to synthesize osmolytes to combat osmotic stress with higher salinities (Munns
284 2002; Sutter et al. 2019). There was no effect of collection site on nitrogen content, however the
285 declines in carbon and C:N are more pronounced for *S. alterniflora* from the oligohaline marsh,
286 indicating that plants from the mesohaline marsh may be more resilient to the effects of salinity.

287 Lastly, I hypothesized that *S. alterniflora* in high salinity treatments would follow a
288 resistance strategy rather than a tolerance strategy, as salinity can inhibit compensatory growth in
289 other *Spartina* spp. (*Spartina densiflora*: Alberti et al. 2010, Daleo et al. 2015, Infante-Izquierdo
290 et al. 2019; *Spartina foliosa*: Long & Porturas 2014; *Spartina maritima*: Infante-Izquierdo et al.
291 2019). If *S. alterniflora* were opting for a resistance strategy instead of a tolerance strategy, I
292 expected increased phenolic concentrations accompanied by declines in protein content, carbon
293 content, and C:N. Although there was no effect of salinity on protein content or phenolic
294 concentrations, *S. alterniflora* in higher salinity treatments had lower carbon content and C:N.
295 This further indicates that perhaps my measured variables did not fully capture a defense
296 response in *S. alterniflora* and that future studies should include additional functional traits.

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

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

313 Overall, this study provides insight into *S. alterniflora*'s ability to defend itself against
314 herbivore attack and informs our understanding of marsh resilience against sea-level rise.
315 Through the process of vertical accretion, *S. alterniflora* plays a key role in elevation
316 maintenance and marsh vulnerability to sea-level rise (Morris et al. 2002; Kirwan & Megonigal
317 2013). Herbivory on *S. alterniflora* can remove large patches of vegetation, impacting marsh
318 stability and contributing to marsh loss (Gedan et al. 2009; He & Silliman 2016; Angelini et al.
319 2018). Both herbivory and salinity are drivers of vegetation diversity and stem density within
320 tidal marshes, and thus can influence accretion capacity (Morris et al. 2002; Elsey-Quirk &
321 Unger 2018). My results indicate that *S. alterniflora* exposed to herbivory may have higher

322 forage quality (e.g. increased nitrogen content, decreased C:N), presenting a positive feedback
323 between herbivory and vegetation die-off.

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

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

342

343

344

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355

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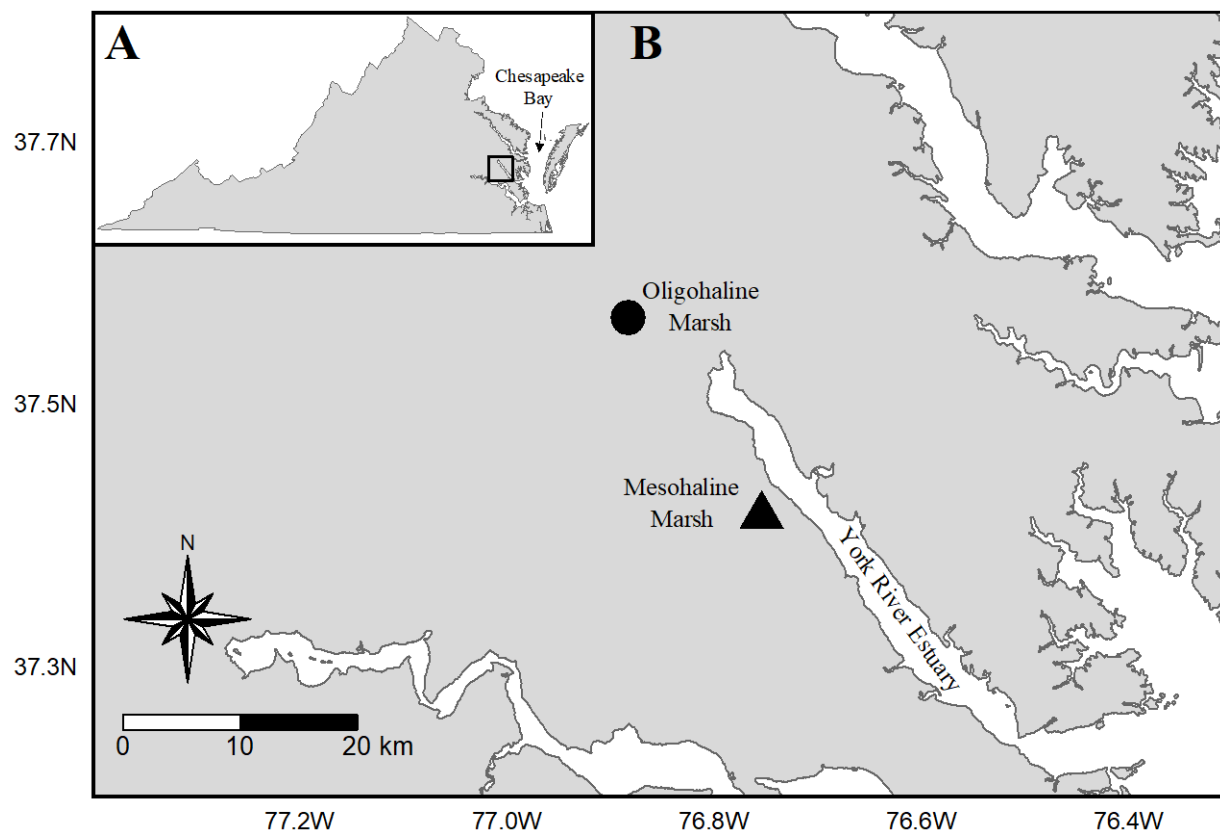
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547 **Table 1.** Model selection for each response variable. Bolded model indicates best fit based on
548 AICc and weight. Explanatory variables with an asterisk (*) indicate significance. Response
549 variables with two asterisks (**) were log transformed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

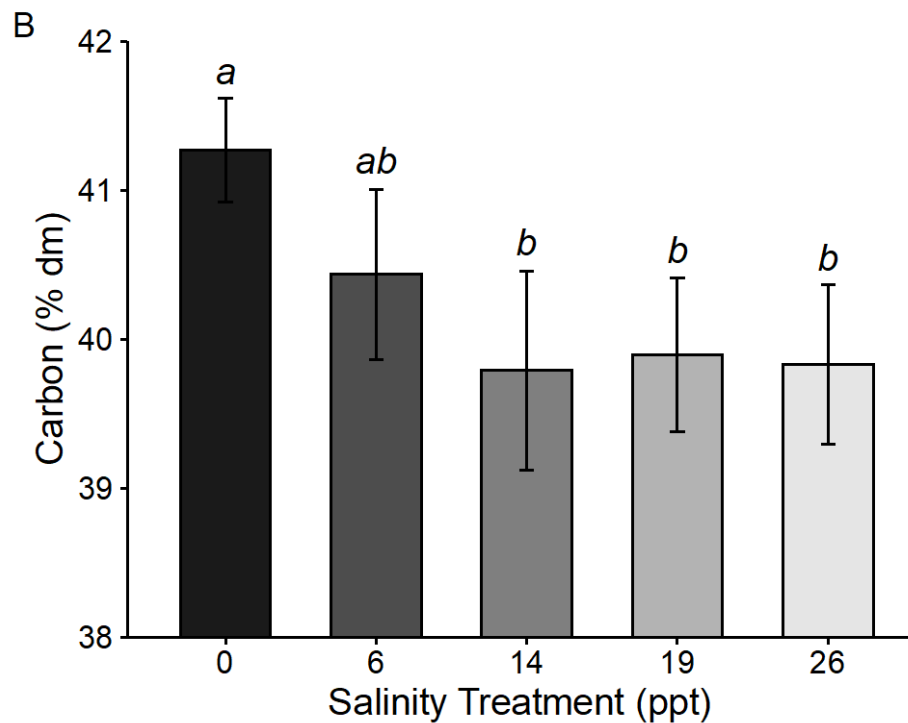
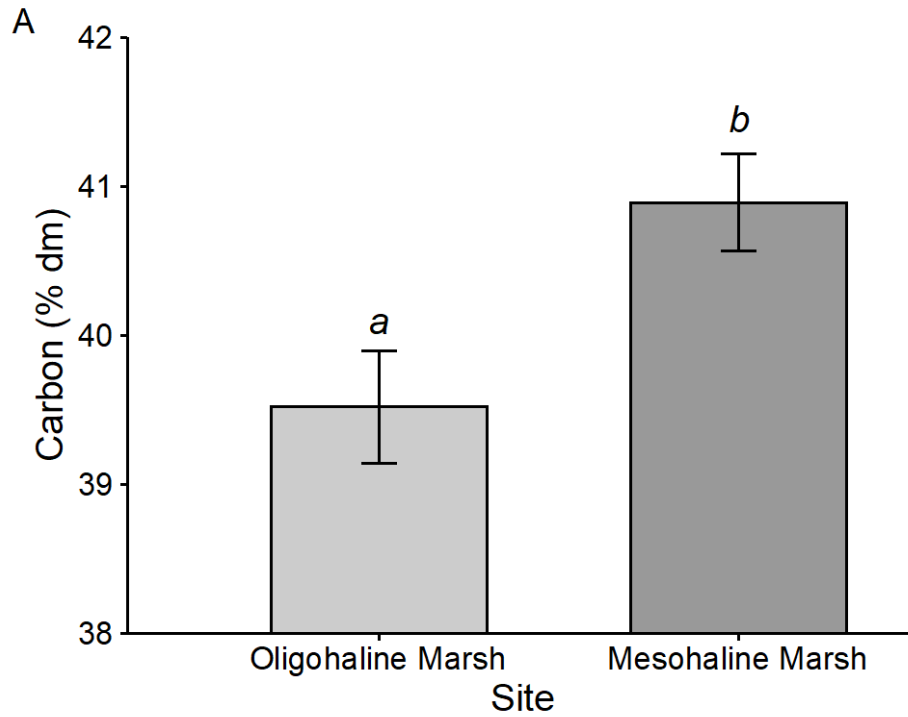
Response Variable	Explanatory Variables	k	AICc	Weight
Carbon	Sa + Cl	4	258.5234	< 0.01
	Sa + Cl + Si	5	253.1361	< 0.01
	Sa + Cl + Si + IAB	6	254.4296	< 0.01
	Sa* + Cl + Si* + IAB + NSB	7	241.2353	0.99
Nitrogen**	Sa* + Cl*	4	4.425692	0.58
	Sa + Cl + Si	5	5.802641	0.29
	Sa + Cl + Si + IAB	6	7.976806	< 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

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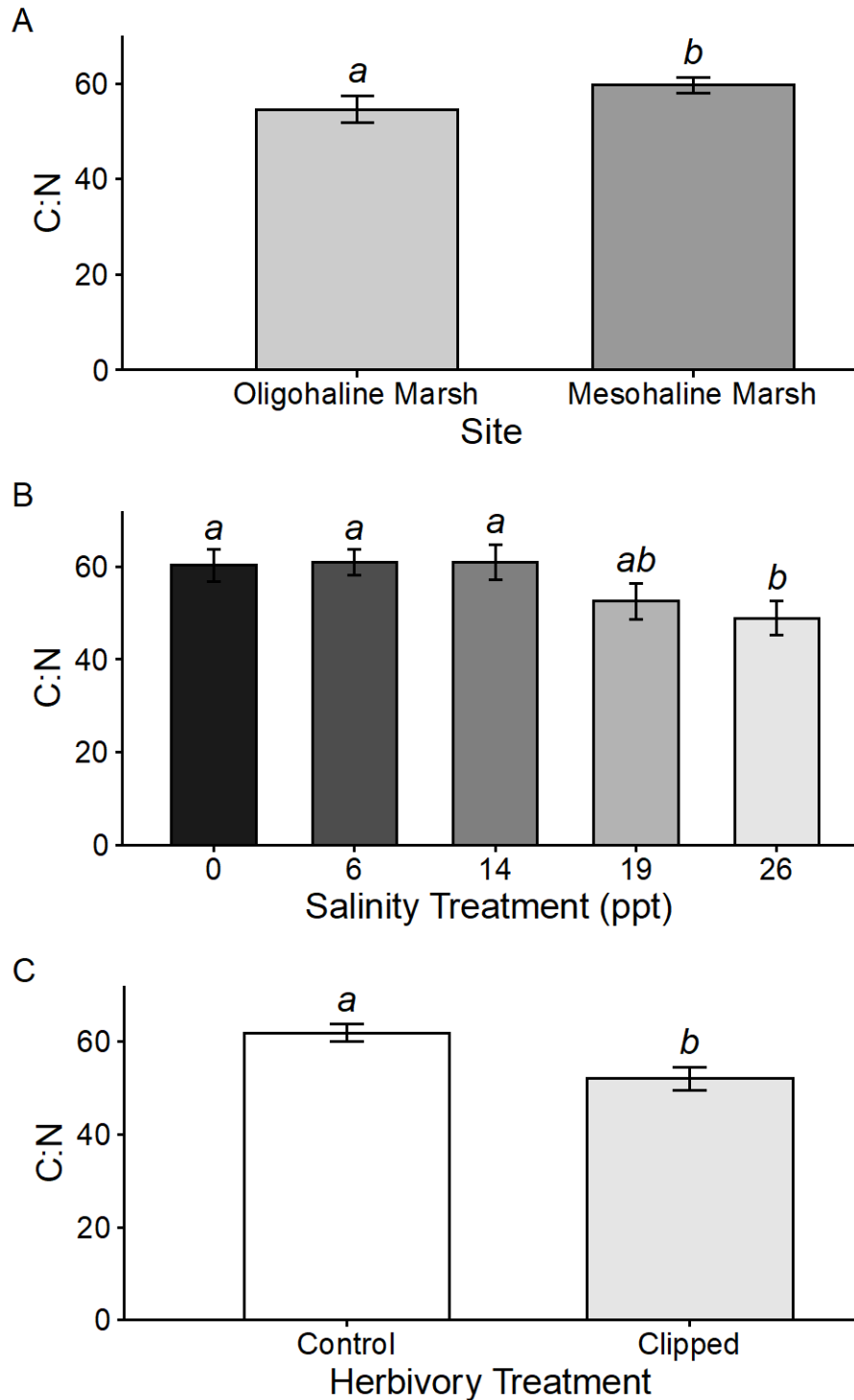


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585 **Fig. 1** A) Inset map of the state of Virginia, U.S.A. Boxed area indicates study region. B)
586 Enlarged map of study region along the York River Estuary. The circle represents the oligohaline
587 marsh (Sweet Hall) and the triangle the mesohaline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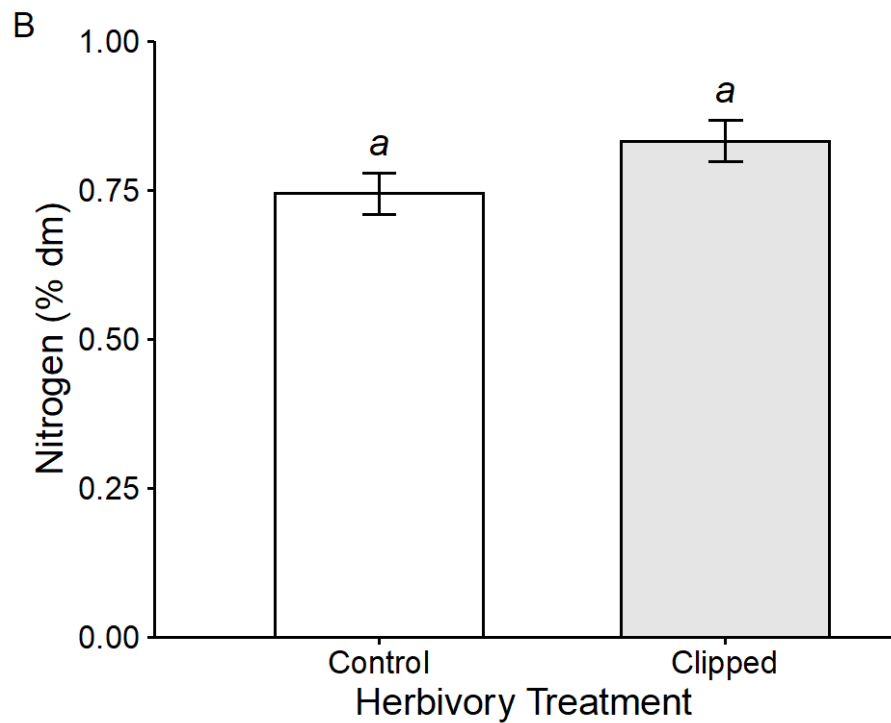
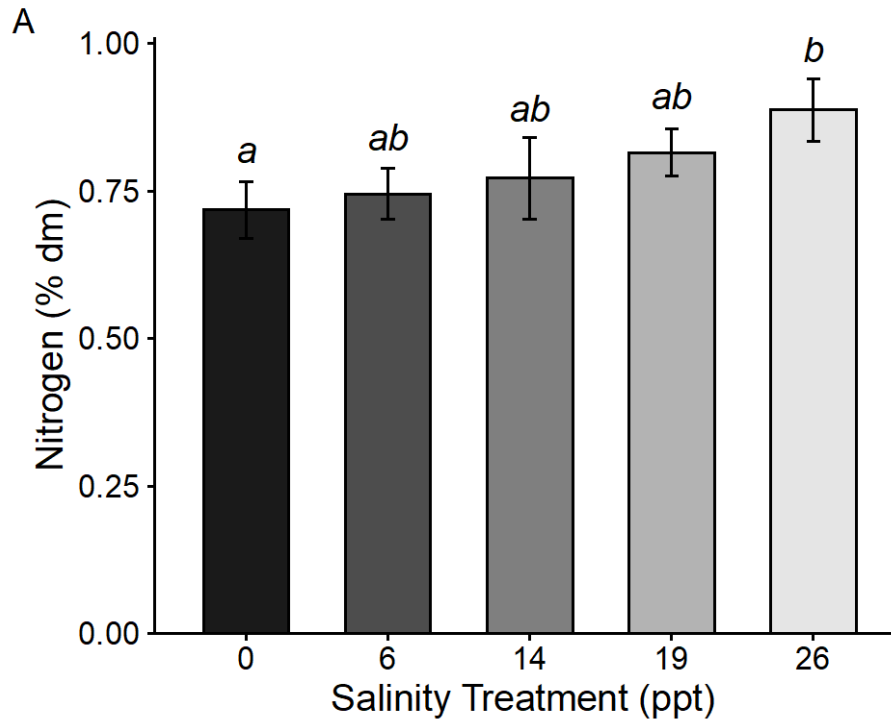


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 591 **Fig. 2** Mean carbon content (percent dry mass) of *S. alterniflora* tissues A) by collection site
 592 (oligohaline or mesohaline marsh) and B) subjected to one of five salinity treatments (0, 6, 14,
 593 19, or 26 ppt). Italicized letters above bars indicate significance between treatments. Error bars
 594 represent standard error

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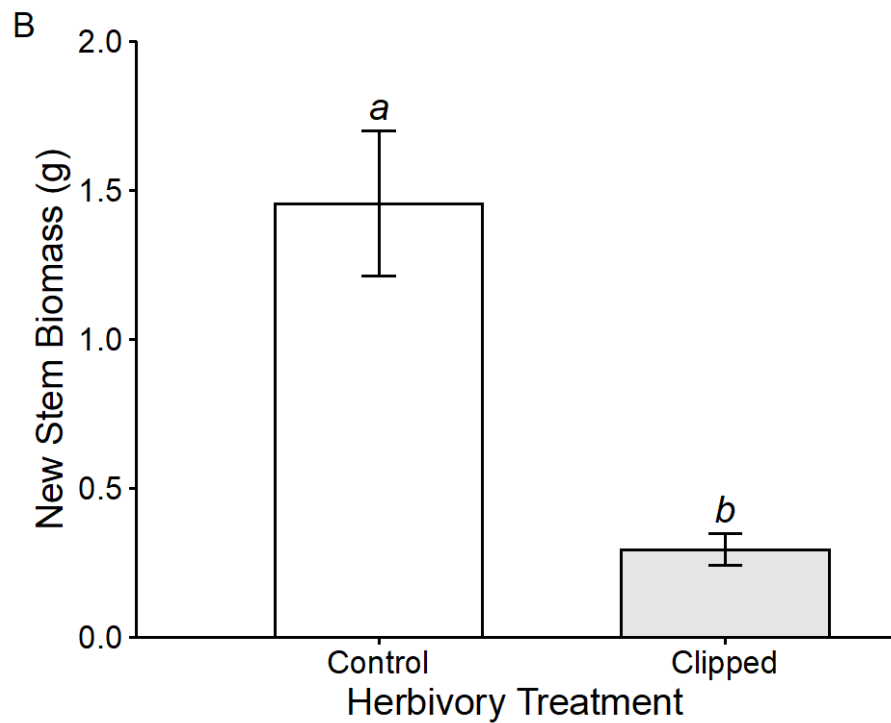
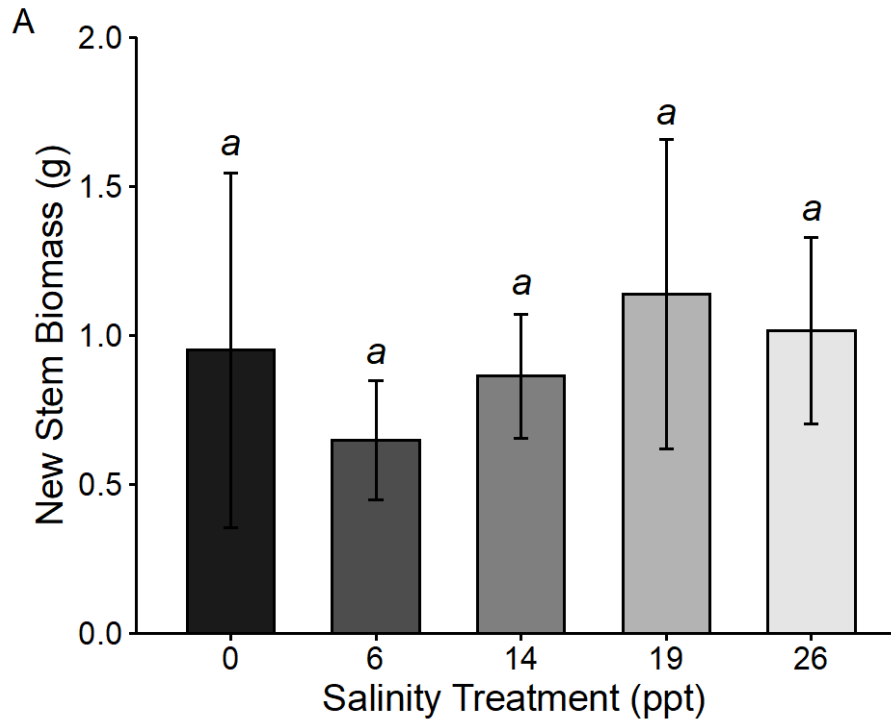


597 **Fig. 3** Mean C:N molar ratios of *S. alterniflora* tissues A) by collection site (oligohaline or
 598 mesohaline marsh), B) subjected to one of five salinity treatments (0, 6, 14, 19, or 26 ppt), and
 599 C) by simulated herbivory treatment (control or clipped). Italicized letters above bars indicate
 600 significance between treatments. Error bars represent standard error
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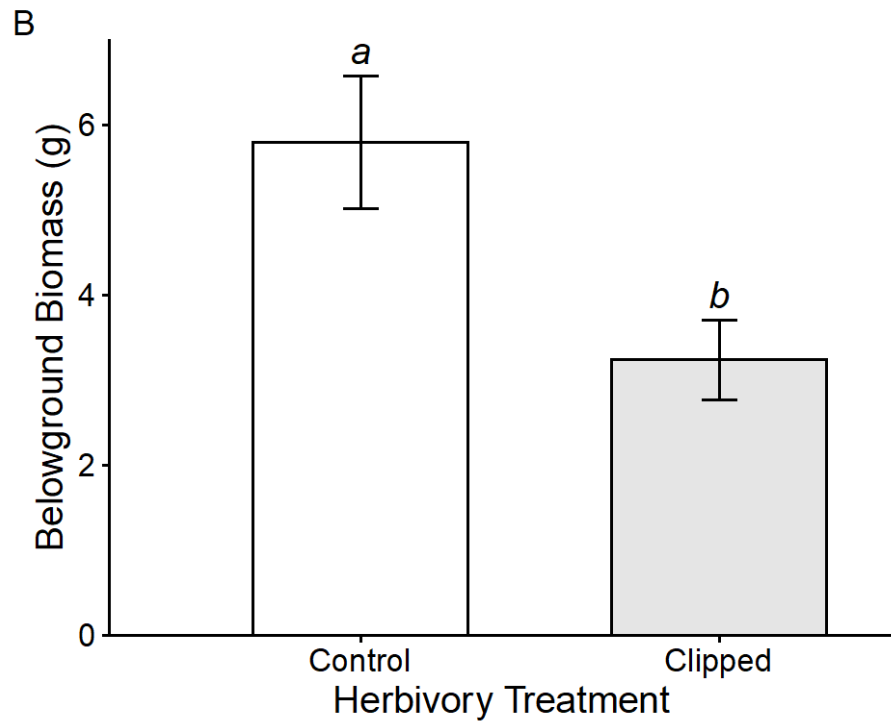
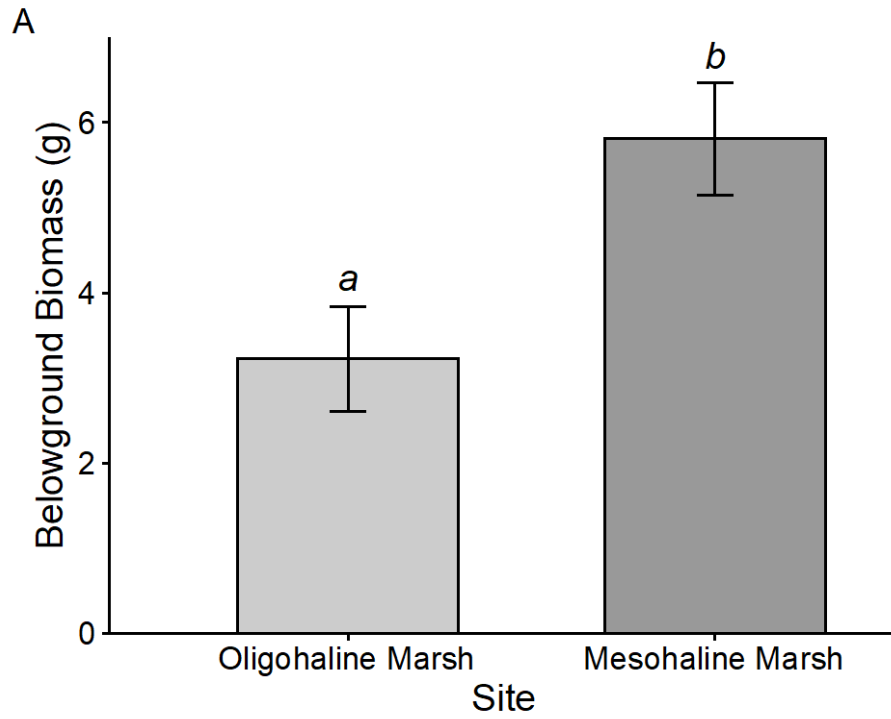
604 **Fig. 4** Mean nitrogen content (percent dry mass) of *S. alterniflora* tissues A) subjected to one of
 605 five salinity treatments (0, 6, 14, 19, or 26 ppt) and B) by simulated herbivory treatment (control
 606 or clipped). Italicized letters above bars indicate significance between treatments. Error bars
 607 represent standard error
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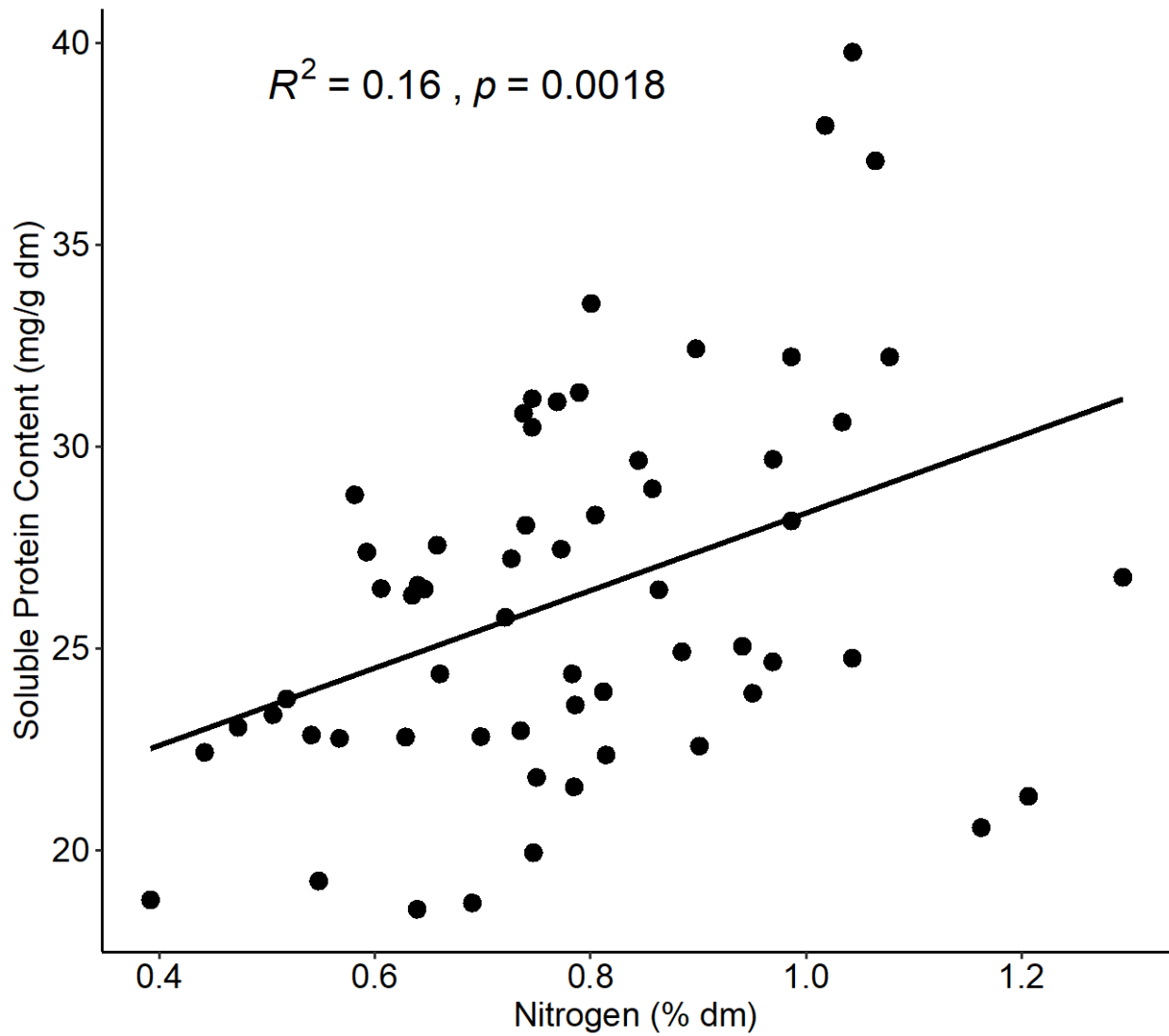
611 **Fig. 5** Mean dry biomass (grams) of new stems produced by *S. alterniflora* A) subjected to one
 612 of five salinity treatments (0, 6, 14, 19, or 26 ppt) and B) by simulated herbivory treatment
 613 (control or clipped). Italicized letters above bars indicate significance between treatments. Error
 614 bars represent standard error
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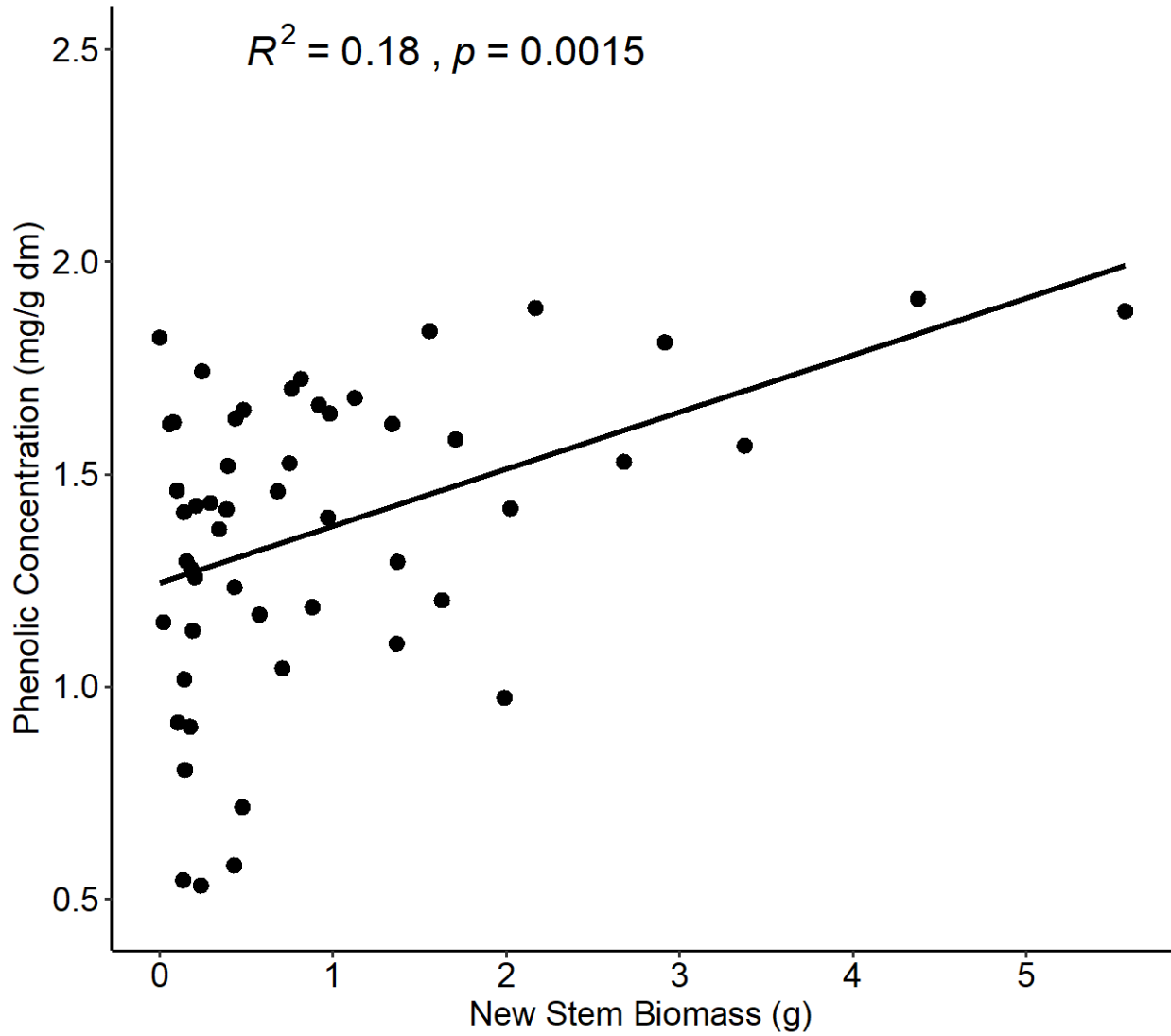


618 **Fig. 6** Mean dry belowground biomass (grams) of *S. alterniflora* A) by collection site
 619 (oligohaline or mesohaline marsh) and B) by simulated herbivory treatment (control or clipped).
 620 Italicized letters above bars indicate significance between treatments. Error bars represent
 621 standard error
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628 **ESM 1** Mean soluble protein content (milligrams/gram dry mass) of *S. alterniflora* tissues across
629 nitrogen content (percent dry mass). Trend line represents smoothed linear regression line
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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