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Spartina Alterniflora Defense Against Herbivory

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Spartina alterniflora Defense Against Herbivory

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In Partial Fulfillment

of the Requirements for the Degree of

Doctor of Philosophy

by

Serina Sebilian Wittyngham

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APPROVAL PAGE

This dissertation is submitted in partial fulfillment of

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This Ph.D. is dedicated to my grandmother, Gloria Sebilian, who provided unfailing and unconditional support, despite not fully understanding what I do for a living. Conversations with her were critical in developing my science communication skills and helping me not take myself too seriously.

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AUTHOR'S NOTE

Chapters II, III, and IV of this dissertation have been prepared as manuscripts for publication in peer-reviewed journals. Thus, these chapters were written in the first-person, singular (Chapter II) and plural (Chapter III, IV) to reflect co-author contributions when appropriate. Chapter IV will not be submitted in its current form, but will be edited and submitted in the near future.

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Chapter II

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Chapter III

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Chapter IV

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ABSTRACT

Plants can alter their chemical, structural (‘resistance strategy’), or morphological traits (‘tolerance strategy’) to deter or mitigate herbivore damage. Developed in terrestrial ecosystems, plant defense theory provides a testable framework for evaluating drivers of plant trait variation and defense strategy selection. Yet, it has rarely been tested in coastal vegetated ecosystems, where intense grazing can denude large spatial areas and disrupt ecosystem services and functioning. Through the lens of plant defense theory, this dissertation examines abiotic and biotic control on traits and defense in the tidal marsh plant, *Spartina alterniflora*, and assesses their influence on further grazing and herbivore distribution. As a foundation species, *Spartina* regulates how marshes keep pace with sea-level rise, thus herbivore removal of *Spartina* directly affects marsh resilience. In mesocosms, I manipulated salinity and simulated herbivory on brackish and freshwater *Spartina* to evaluate the growth-rate hypothesis and the optimal defense theory (Chapter II). Simulated herbivory reduced tolerance traits and brackish *Spartina* was better defended than freshwater *Spartina*, supporting these hypotheses. Elevated salinity caused greater variation in freshwater *Spartina* traits, suggesting climate-driven saltwater intrusion may not affect brackish marshes, but could mediate freshwater *Spartina* response to herbivory. In mesocosms, I assessed how nutrient enrichment affected *Spartina* defense against grazing from the marsh periwinkle, *Littoraria irrorata* (‘resource-availability hypothesis’ or RAH) (Chapter III). Trait variation was assessed across plant age (original versus clonal new stems), which can influence traits in terrestrial plants. Nutrients promoted tolerance traits while decreasing constitutive resistance, supporting the RAH. Newer stems had higher tolerance and resistance traits, implying they are better defended than older stems. Neither nutrient availability nor plant age stimulated *Littoraria* consumption in feeding assays, suggesting nutrient loading will not intensify top-down control, and may increase vertical accretion through enhanced tolerance traits. Lastly, I examined if Spartina traits influenced consumer fronts created by the purple marsh crab, *Sesarma reticulatum* (Chapter IV). Despite causing marsh die-off in New England, in southern marshes, including Virginia, the *Sesarma* front is moving inland, allowing tall-form *Spartina* to revegetate and prevent marsh loss. Others hypothesized that sediment characteristics, abiotic conditions, and predation pressure drive this movement inland. Here, I tested if *Spartina* palatability, nutritional quality, and accessibility also act as a driver, as plant traits can determine herbivore distribution in terrestrial ecosystems. A caging study then evaluated if *Sesarma* grazing directly shapes *Spartina* traits. Intense predation pressure in the low marsh and enhanced *Spartina* forage quality in the high marsh were the only significant predictors of *Sesarma* front movement. Grazing from *Sesarma* affected short- and tall-form *Spartina* differently. Herbivory increased palatability and reduced short-form *Spartina*’s ability to mitigate damage, while having little effect on tall-form *Spartina*. Thus, higher constitutive defense in tall-form and increased palatability of short-form *Spartina* further propagate the *Sesarma* front inland. Overall, this dissertation demonstrates that plant traits can influence ecosystem resilience, directly through biomass production, and indirectly by shaping herbivore distribution, and should be considered when assessing how coastal vegetated ecosystems are affected by climate change and anthropogenic disturbance.

Spartina alterniflora Defense Against Herbivory

CHAPTER I

Introduction

Plant Defense Theory

To avoid predation, mobile prey can outrun their predators or seek refuge. Prey that are sessile, however, such as plants, are unable to escape their predators. Instead, plants can alter their chemical, structural, and morphological traits to influence palatability and nutritional quality, ultimately deterring or mitigating damage from herbivores. These trait alterations define the two primary plant defense strategies: tolerance and resistance. Plants can *tolerate* herbivory by reallocating biomass or resources, stimulating above- and belowground biomass production, and/or increasing photosynthetic capacity to compensate for mass lost to herbivores (Mauricio et al. 1997; Burghardt & Schmitz 2015; Thomas et al. 2017). Alternatively, plants can *resist* herbivore attack by producing chemical and/or structural defenses to decrease palatability and forage quality and deter further grazing (Mauricio et al. 1997; Burghardt & Schmitz 2015). Plants employ these strategies in ecosystems worldwide (e.g., tropical forests: Coley et al. 2005, Endara et al. 2017; deciduous forests: Loughnan & Williams 2019; savanna grasslands: Bryant et al. 1989; rice fields: Horgan et al. 2018; seagrasses: Vergés et al. 2008, Hernán et al. 2019, Hernán et al. 2021; salt marshes: Hendricks et al. 2011, Long et al. 2011, Sieg et al. 2013, Long & Porturas 2014, Więski & Pennings 2014). Historically, ecologists focused on resistance as the only defense strategy (Nuñez-Farfán et al. 2007), however there is growing support for tolerance as a secondary strategy (Vergés et al. 2008; Long & Porturas 2014; Więski & Pennings 2014; Freitas et al. 2016; Horgan et al. 2018; Hernán et al. 2019), and an increasing need for studies evaluating both strategies in tandem (Strauss & Agrawal 1999; Leimu et al. 2006). For the remainder of this dissertation, the term “defense” is used to indicate both tolerance and resistance strategies unless otherwise specified.

Plant defense response is quantified through the measurement of plant traits. For this dissertation, biomass production (aboveground, belowground, new stem growth), carbon content, C:N ratios, chlorophyll content (*a*, *b*, total), plant stem height, and stem diameter were all considered "tolerance traits", as they can help plants mitigate damage from grazing (Hernán et al. 2019). Total phenolic concentrations, total soluble protein content, nitrogen content, biogenic silica, and neutral detergent fiber content were all considered "resistance traits" as they directly influence plant palatability and forage quality, which can shape herbivore preference or prevent further damage (Pennings et al. 1998; Massey et al. 2007; Hernán et al. 2019). Thus, directional changes in tolerance and resistance traits were used to quantify a tolerance or resistance defense response. Further, each of these plant traits can be constitutive (i.e., inherent to the plant regardless of herbivore presence) or induced (i.e., produced in response to grazing) (Karban & Baldwin 1997; Garcia et al. 2021).

Drivers of Plant Trait Change & Defense Response

Previous work on plant defense theory has generated several hypotheses to explain the underlying mechanisms driving plant antiherbivore defenses (e.g., growth-rate hypothesis: Coley et al. 1985; growth-differentiation-balance hypothesis: Herms & Mattson 1992; resource-availability hypothesis: Coley et al. 1985; limiting-resource model: Wise & Abrahamson 2008; plant-apparency theory: Feeny 1976; compensatory-growth continuum: Maschinksi & Whitham 1989; plant-vigor hypothesis: Price 1991; optimal-defense theory: Rhoads 1979). These hypotheses are founded on three major themes: 1) a trade-off between growth and chemical or structural defense, in which a plant can either increase growth or increase chemical and structural defenses (i.e., tolerance vs. resistance), as there is limited evidence that these strategies are not mutually exclusive (Mauricio et al. 1997; Cipollini et al. 2014), 2) a trade-off in constitutive versus induced defense response, in which plants either have high constitutive defenses or high inducible defenses (Kempel et al. 2011), and 3) a trade-off between abiotic and biotic control on plant defense strategy (Coley et al. 1985). In general, this last trade-off posits that either resource availability (e.g., nutrients) or prevalence of herbivory determines which defense strategy a plant follows and the magnitude of that defense. In addition to resource availability and herbivore pressure, plant trait change can be influenced by other abiotic (e.g., salinity: Sutter et al. 2019; temperature: Wittyngham et al. 2019; drought: Angelini et al. 2018) or biotic (e.g., competition: Sutter et al. 2015; plant age: Funk et al. 2021, Henn & Damschen 2021) factors.

Plant Defense Theory in the Marine Realm

Plant defense theory has been primarily generated and tested in terrestrial ecosystems, and only applied to marine ecosystems on a few occasions. Vergés et al. (2008) found that the tropical seagrass, *Posidonia oceanica*, followed a tolerance strategy (i.e., ‘compensation’) in response to simulated fish herbivory, although trait responses varied with increasing herbivory pressure. In the same seagrass species, Hernán et al. (2019) found that nutrient enrichment decreased constitutive resistance traits, and that changes in plant traits influenced herbivore preference in subsequent feeding assays. In a similar study on the temperate seagrass species, *Zostera marina*, Hernán et al. (2021) found that latitudinal variation in defense was mediated by nutrient availability, and that plants were able to tolerate moderate levels of herbivory but showed declines in defensive traits in response to high grazing pressure.

Additionally, there are several studies focused on macroalgae-herbivore relationships, although they were examined through the lens of resistance only. The consensus is that chemical defenses vary across latitude (Sotka & Hay 2002) and among herbivore and macroalgal species (Duffy & Hay 1991; Cronin & Hay 1996A; Cronin & Hay 1996B). Combined, this body of work also highlights that despite artificial conditions created by feeding assays, there is insight to be gained in examining specific traits (e.g., secondary metabolites) and their ability to control the consumption rate and preference of herbivores.

This previous work applied plant defense theory to submerged macrophytes and benthic macroalgae, however, few studies have evaluated the defenses of emergent vegetation. Thus, the overarching goal of my dissertation is to apply these concepts of plant defense theory to plant-herbivore interactions in tidal marshes. Marshes are an ideal study system for this research because they have lower herbivore and vegetation diversity than other ecosystems (Noto & Shurin 2017), eliminating these variables as confounding factors. Further, previous work on plant defense within marshes is focused on either tolerance or resistance independently (tolerance: Johnson & Jessen 2008; Long & Porturas 2014; Więski & Pennings 2014; Freitas et al. 2016; resistance: Pennings et al. 1998; Hendricks et al. 2011; Long et al. 2011; Sieg et al. 2013), and few have examined induced and constitutive defense (Long et al. 2011; Salgado & Pennings 2005) or abiotic control on defense directly (Long & Porturas 2014).

Tidal Marshes

Tidal marshes occur at the land-sea interface and are most often classified by their salinity regime (oligohaline, hereafter 'freshwater marsh': 0-5 ppt, mesohaline, hereafter 'brackish marsh': 5-18 ppt, and polyhaline, hereafter 'salt marsh': 19-30 ppt; Odum 1988). They provide critical ecosystem services and processes such as storm buffering (Arkema et al. 2013), nutrient cycling (de Groot et al. 2012; Bulseco et al. 2019), and carbon sequestration (Mcleod et al. 2011; Smith et al. 2022). One of the largest threats to tidal marshes in the U.S. mid-Atlantic region is accelerated sea-level rise (Kirwan et al. 2010), which is 3-4 times higher than the global average (Sallenger et al. 2012; Boon et al. 2018). To keep up with sea-level rise, marshes must either migrate to higher ground or accrete (build up) vertically. When uninhibited by human-made barriers, marshes can migrate upland (Kirwan et al. 2016), however, the rate and ability of the marsh to vertically accrete remains a point of concern. Assuming adequate sediment supply (Coleman et al. 2020), saltmarsh plants regulate accretion rates through a series of ecogeomorphic feedbacks. Aboveground, plant stems and leaves attenuate waves at high tide, allowing sediment to settle from the water column to the marsh surface, building elevation (Kirwan & Megonigal 2013). Belowground, plant roots and rhizomes contribute to organic matter accumulation, which expands the sediment surface, further increasing elevation (Kirwan & Megonigal 2013). Thus, plants are critical for marsh persistence.

Both brackish and salt marshes along the U.S. Atlantic coast are dominated by the smooth cordgrass, *Spartina alterniflora* (hereafter '*Spartina*'). *Spartina* is present in lower densities in freshwater marshes as it is competitively excluded by salt-intolerant species (Sutter et al. 2015). However, one projected outcome of climate-driven sea-level rise is salinity intrusion into previously fresh regions (Weston et al. 2011). Accordingly, we would expect current freshwater marshes to convert to brackish and then salt marshes over time, with their plant communities ultimately dominated by *Spartina*. The primary herbivores in Virginia marshes are the purple marsh crab, *Sesarma reticulatum*, and the marsh periwinkle, *Littoraria irrorata*. Runaway or unconstrained consumption by these herbivores, which can remove vegetation in large areas, is a potential driver of tidal marsh loss via decreases in vertical accretion capacity (He & Silliman 2016; Angelini et al. 2018). However, despite the presence and high densities of these herbivores, salt marshes persist. This suggests that plant defense response (i.e., altered plant traits) to herbivory may contribute to marsh resilience against runaway consumption. Thus, understanding how *Spartina* can defend itself against grazers may provide direct insight into marsh stability.

Dissertation Overview & Structure

This dissertation has three experimental chapters with two overarching goals: 1) evaluate three plant-defense hypotheses in the context of tidal marshes: the growth-rate hypothesis (Chapter II), the optimal-defense theory (Chapter II), and the resource-availability hypothesis (Chapter III), and 2) test how plant palatability, nutritional quality, and accessibility shape herbivore distribution and consumer front impacts in salt marshes (Chapter IV). Chapter V summarizes findings from each chapter and the broader implications of these results, as well as comments on potential directions for future studies.

Chapter II: Salinity and simulated herbivory influence Spartina alterniflora *traits and defense strategy*

The growth-rate hypothesis states there is a trade-off between plant growth and investment in anti-herbivore defenses (i.e., tolerance and resistance are mutually exclusive, Coley et al. 1985; Basey & Jenkins 1993), while the optimal-defense theory predicts that constitutive defense will be high when the probability of herbivore attack is high, otherwise resources are allocated to inducible defenses when the incidence of herbivory is low (Rhoads 1979; Herms & Mattson 1992; Stamp 2003). Both of these hypotheses have been evaluated in other ecosystems (growth-rate hypothesis: *reviewed in* Basey & Jenkins 1993 & Stamp 2003;

optimal-defense theory: *reviewed in* Stamp 2003 & Schuman & Baldwin 2016), however there are few instances in which they have been applied to tidal marshes. There are no studies evaluating the optimal-defense theory, and the two studies conducted in tidal marshes found support for the growth-rate hypothesis (Minden & Kleyer 2015; Qiao et al. 2018). As herbivore identity and density vary along the salinity gradient associated with tidal marshes, understanding how probability of attack (optimal-defense theory) influences how plants defend themselves (growth-rate hypothesis) is critical for assessing tidal marsh stability and resilience.

To evaluate these hypotheses, I conducted a factorial mesocosm experiment testing the effects of five salinity treatments (0, 6, 14, 19, 26 pp) and simulated herbivory (clipped or unclipped) on *Spartina* collected from a freshwater marsh and from a brackish marsh. It was expected that simulated herbivory would either increase *Spartina* growth and photosynthetic capacity (tolerance strategy) or stimulate chemical and structural compound production (resistance strategy), supporting the growth-rate hypothesis. Further, if the optimal-defense theory applied, I expected *Spartina* from the brackish marsh to have higher constitutive defense than freshwater *Spartina*, as brackish marshes have a higher density and diversity of herbivores, and thus a greater probability of attack. Lastly, I tested if salinity mediated trait variation in *Spartina* to assess how chronic versus pulse salinity events associated with climate change may shape *Spartina* defense response.

Chapter III: Resource availability and plant age drive defense against herbivory in salt marshes

The resource-availability hypothesis (a sub-theory of the growth-rate hypothesis) states the nature and magnitude of plant defense response is limited by the resources available (Coley et al. 1985; Endara & Coley 2011). This hypothesis asserts that in low resource environments, plant growth is slow, therefore constitutive defense is high (resistance strategy). In contrast, when resources are abundant, plant growth is fast, and constitutive defense is low (tolerance strategy). Although recently tested in seagrass ecosystems (Hernán et al. 2019; Hernán et al. 2021), the resource-availability hypothesis has only been applied once to freshwater and brackish marsh plant species (Rejmánková 2016) and has never been applied to salt marshes, which are threatened by anthropogenic nutrient loading (Deegan et al. 2012). Excess nutrient availability can convert these typically nutrient limited ecosystems ('low-resource environment') into eutrophic ecosystems ('high-resource environment'), with feedback to plant defense strategy selection. Further, chronic herbivory in salt marshes can remove large areas of saltmarsh plants, sometimes leading to ecosystem state change (Gedan et al. 2009; He & Silliman 2016; Williams & Johnson 2021). Thus, I expected elevated nutrient availability to switch *Spartina* defense response from constitutive resistance to induced tolerance, supporting the resource-availability hypothesis. To test this hypothesis, I conducted a factorial mesocosm experiment manipulating resource availability (i.e., nutrient addition via fertilizer amendments) and herbivory from the marsh periwinkle, *L. irrorata*. At the end of the experiment, the plant traits and defense response of originally-planted stems and clonally-grown new stems were analyzed to assess how plant age influenced trait variation, a pattern never tested in marshes, but recently established in the terrestrial literature (Cope et al. 2020; Funk et al. 2021; Henn & Damschen 2021). A feeding assay then evaluated if altered traits or plant age influenced subsequent *L. irrorata* grazing.

Chapter IV: Predation pressure and plant traits drive consumer fronts in salt marshes

This chapter builds on my findings from Chapters II and III and evaluates whether altered plant traits and defense strategy can exert control over the distribution of *S. reticulatum* consumer fronts in salt marshes on the Eastern Shore of Virginia. These fronts are responsible for marsh die-off in many instances (Holdredge et al. 2009; Coverdale et al. 2013; Schultz et al. 2016; Szura et al. 2017; Vu et al. 2017). However, in some cases, the front is moving directionally inland (Hughes et al. 2009; Pettengill et al. 2018; Vu & Pennings 2021), allowing tall-form *Spartina* to revegetate near the creek edge. This revegetation allows the marsh to continue to vertically accrete, preventing further loss. Previous work suggests that sediment characteristics (Bertness et al. 2009), abiotic conditions (Vu & Pennings 2021), and predation pressure (Altieri et al. 2012) are responsible for driving the front inland. Despite their ability to shape herbivore preference and distribution in terrestrial systems (oak trees: Feeny 1970; poplar & dogwood trees: Dudt & Shure 1994; alder & willow trees: Ikonen et al. 2002; birch trees: Muiruri et al. 2019; agricultural crops: Godinho et al. 2020; evergreen forests: Martini et al. 2022; broadleaf dock: Ohsaki et al. 2022), plant traits have never been tested as a driver of consumer fronts in salt marshes. Thus, in this chapter, I test each of these drivers concurrently, and test the novel hypothesis that *Spartina* palatability, nutritional quality, and accessibility further contribute to shaping *S. reticulatum* distribution and front movement. Additionally, through a manipulative caging experiment, I tested whether direct grazing by *S. reticulatum* can alter the plant traits of *Spartina*, with feedback to front movement and salt marsh resilience.

Significance

Each plant defense strategy can have differential impacts on marsh resilience. A tolerance strategy may benefit vertical accretion via increases in above- and belowground biomass production, enhancing sediment capture and organic matter accumulation. In contrast, a resistance strategy, in which *Spartina* bolsters its existing tissues in lieu of biomass production, may reduce grazing pressure, but still lose some vertical accretion capacity. Thus, it is critical to assess how abiotic (i.e., salinity, nutrient availability) and biotic (i.e., herbivory) factors influence plant traits and defense response to evaluate marsh stability under future climate change. Further, if plant palatability and nutritional quality influence the distribution and impacts of herbivores in salt marshes, this would present a novel pathway by which plants exert bottom-up control on consumer fronts, with feedback to hydrological and geomorphological processes that can shape marsh resilience.

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CHAPTER II

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 150 µL aliquot was added to a sterile centrifuge tube. 150 µL 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 ($p=1.36\times10^{-6}$; 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

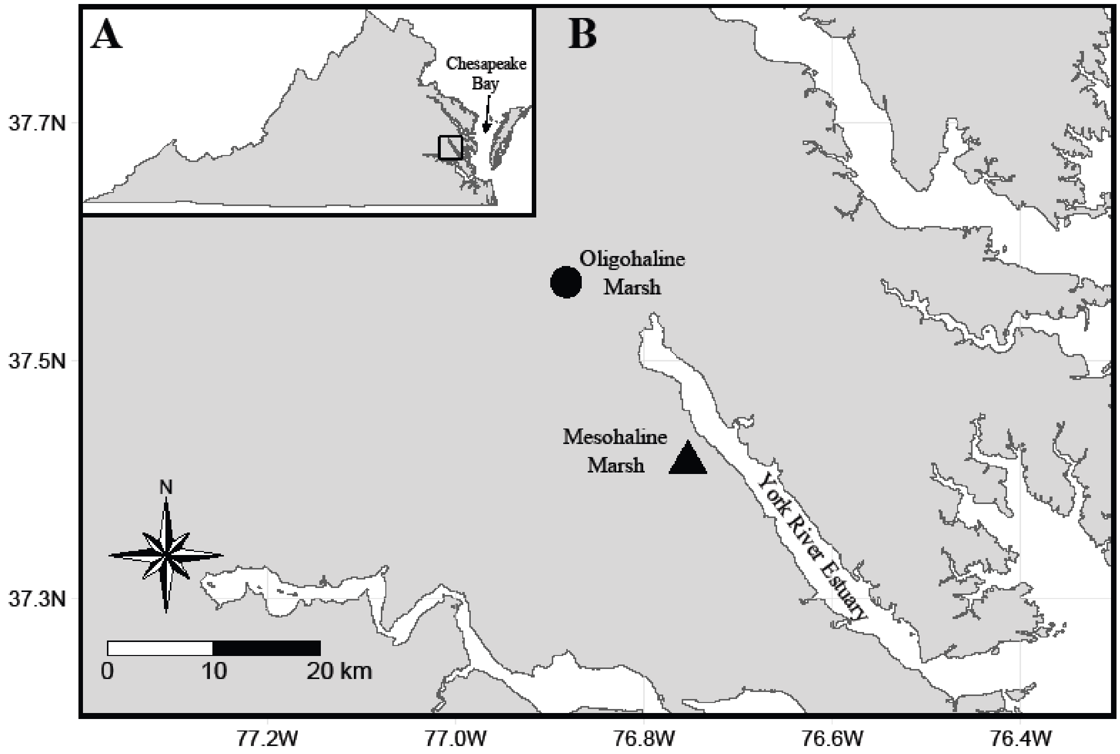


Fig. 1 A) Inset map of the state of Virginia, U.S.A. Boxed area indicates study region. B) Enlarged map of study region along the York River Estuary. The circle represents the oligohaline marsh (Sweet Hall) and the triangle the mesohaline marsh (Taskinas Creek).

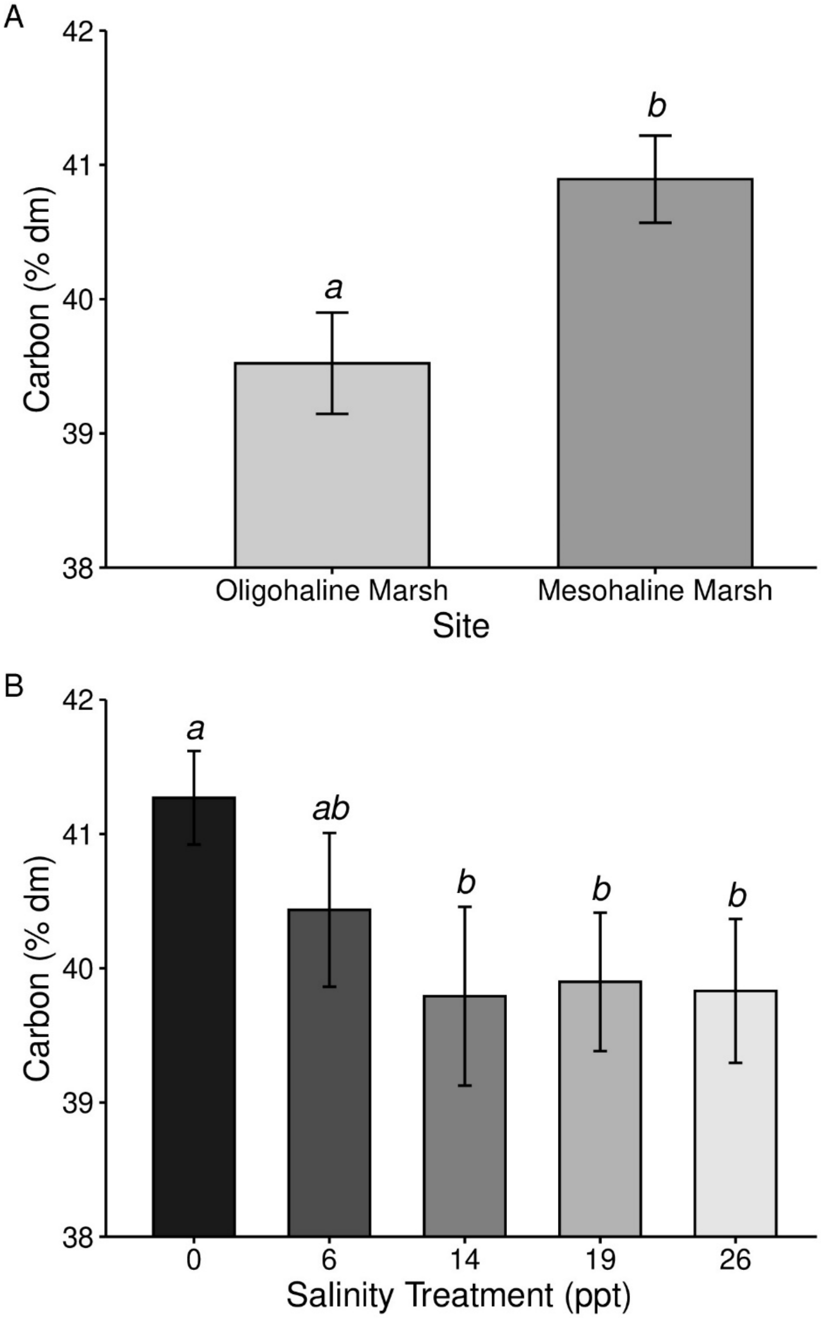


Fig. 2 Mean carbon content (percent dry mass) of *S. alterniflora* tissues A) by collection site (oligohaline or mesohaline marsh) and B) subjected to one of five salinity treatments (0, 6, 14, 19, or 26 ppt). Italicized letters above bars indicate significance between treatments. Error bars represent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

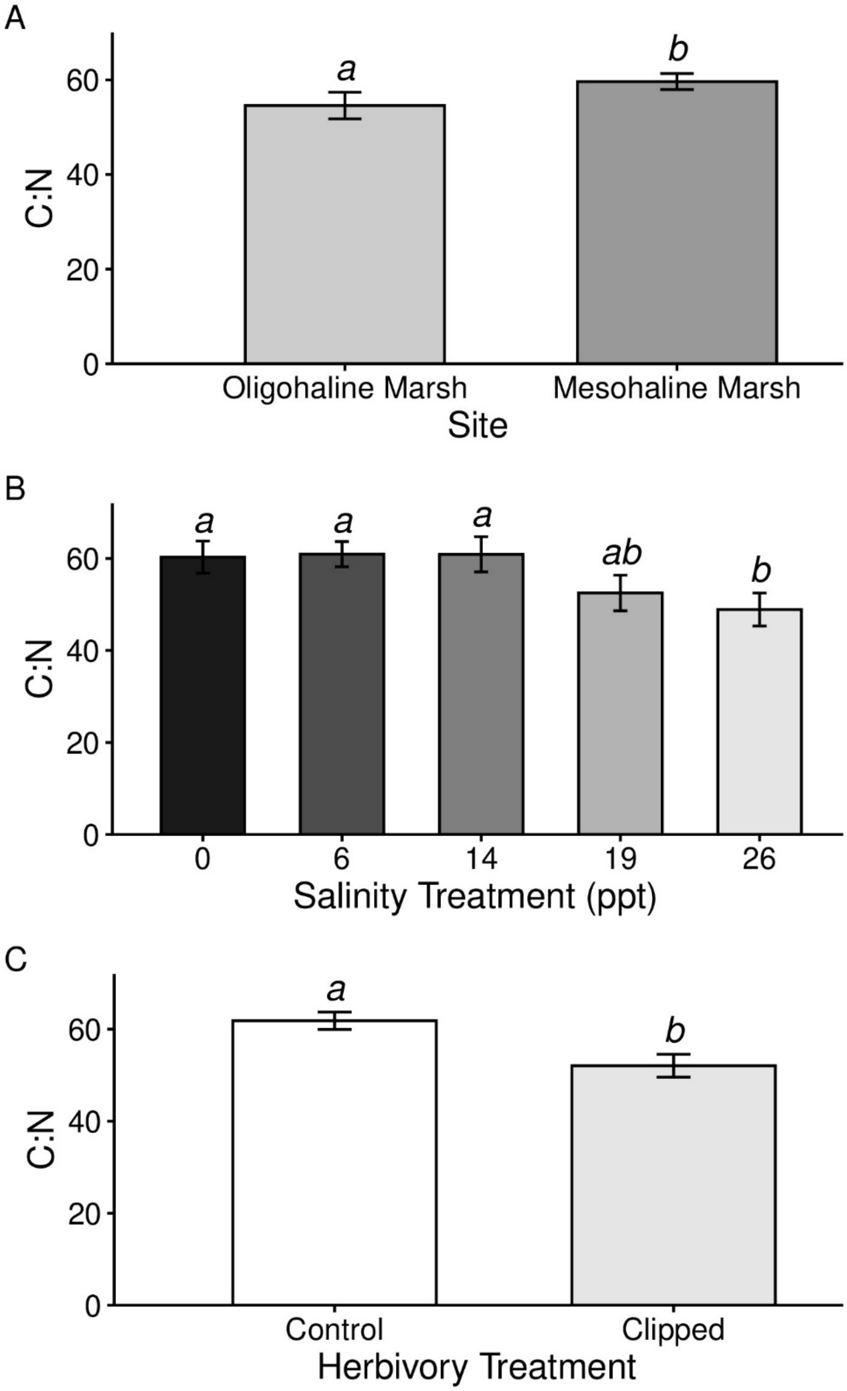


Fig. 3 Mean C:N molar ratios of *S. alterniflora* tissues A) by collection site (oligohaline or mesohaline marsh), B) subjected to one of five salinity treatments (0, 6, 14, 19, or 26 ppt), and C) by simulated herbivory treatment (control or clipped). Italicized letters above bars indicate significance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

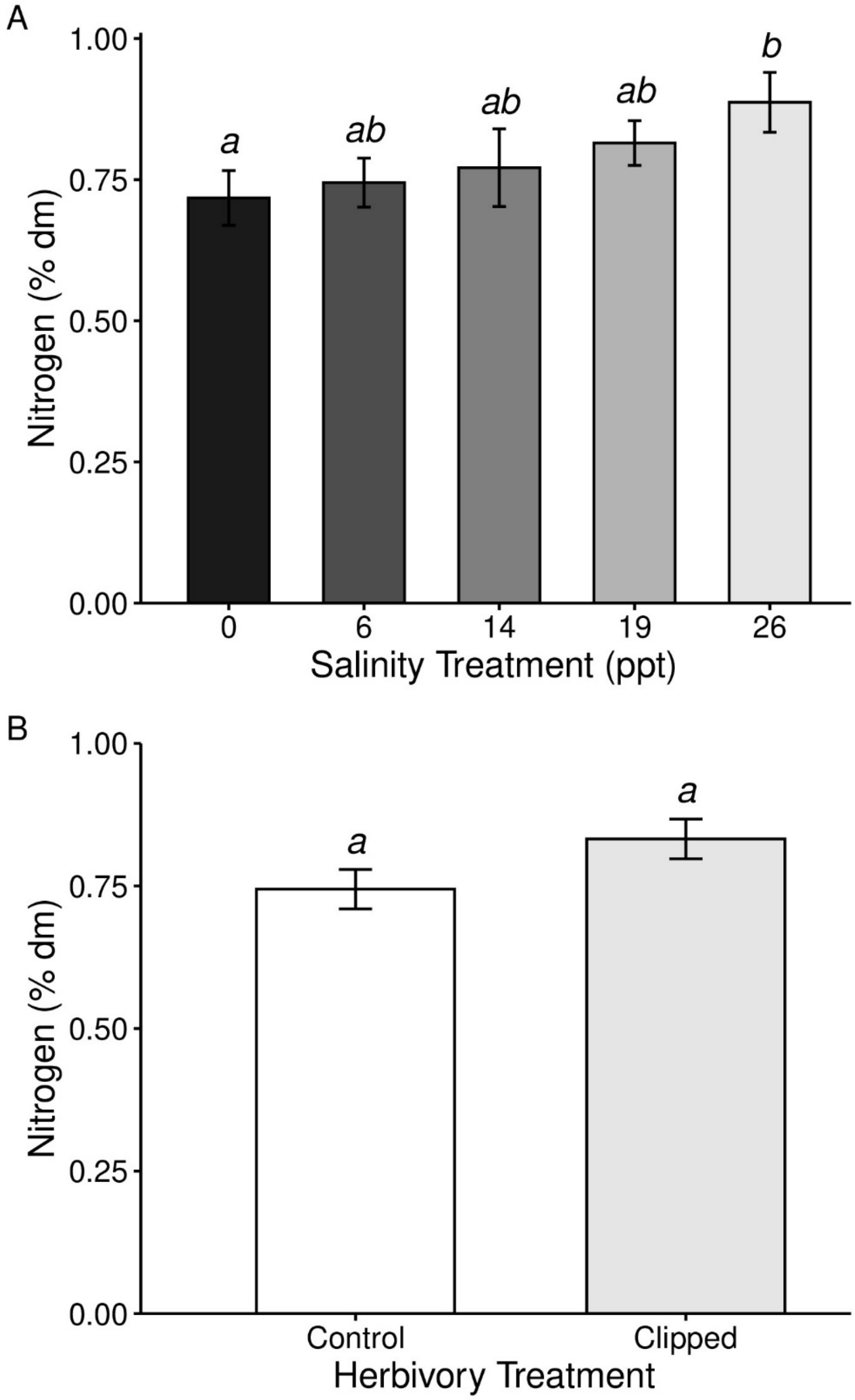


Fig. 4 Mean nitrogen content (percent dry mass) of *S. alterniflora* tissues A) subjected to one of five salinity treatments (0, 6, 14, 19, or 26 ppt) and B) by simulated herbivory treatment (control or clipped). Italicized letters above bars indicate significance between treatments. Error bars represent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

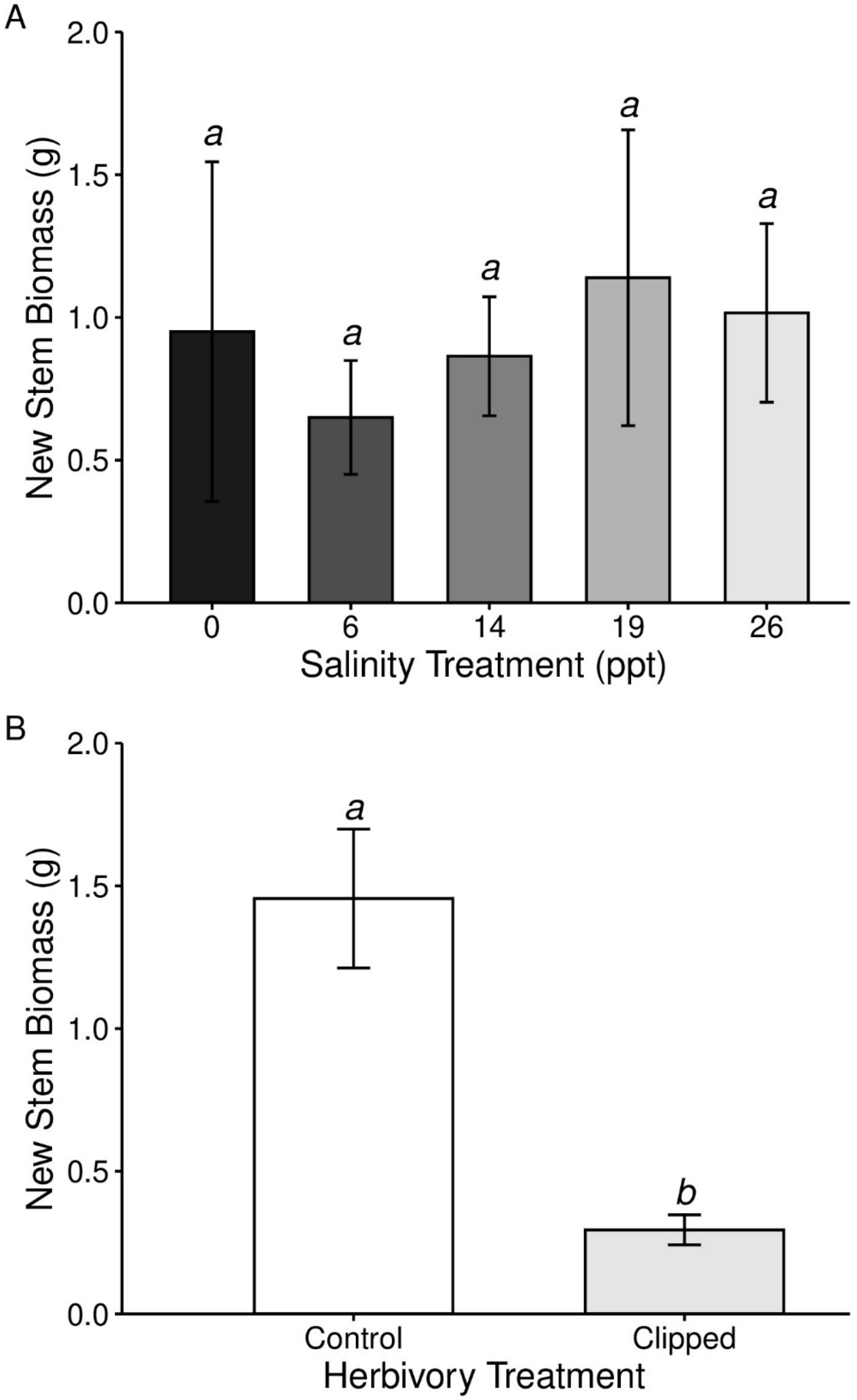


Fig. 5 Mean dry biomass (grams) of new stems produced by *S. alterniflora* A) subjected to one of five salinity treatments (0, 6, 14, 19, or 26 ppt) and B) by simulated herbivory treatment (control or clipped). Italicized letters above bars indicate significance between treatments. Error bars represent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

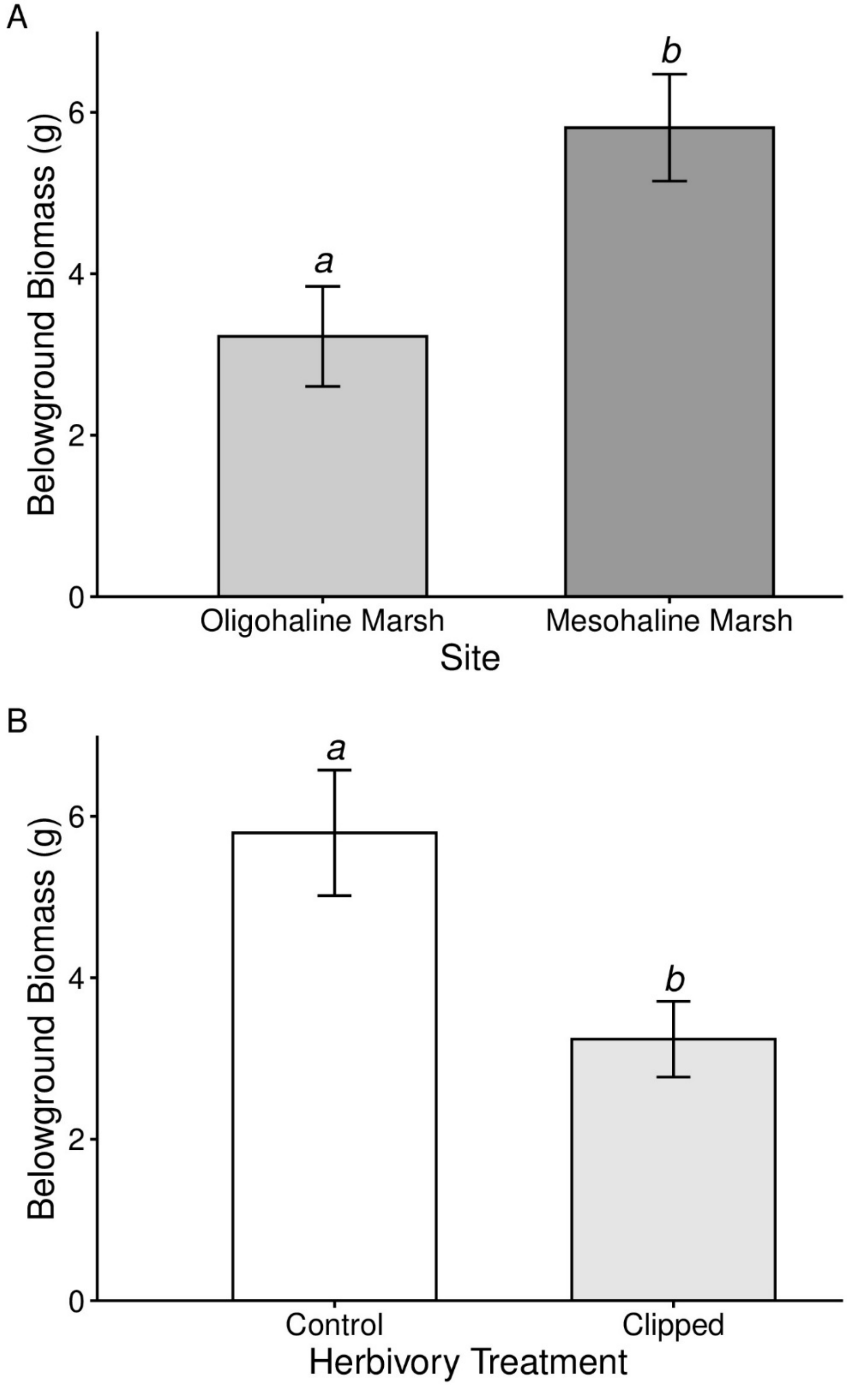
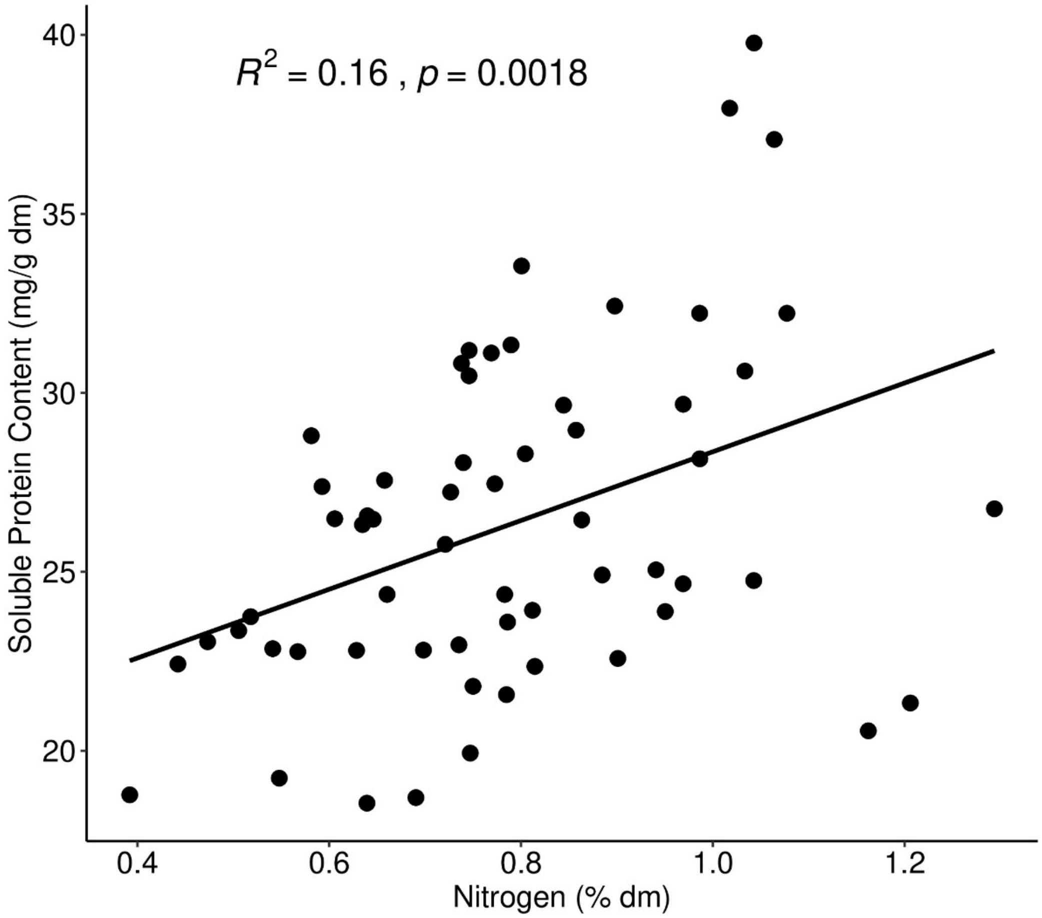
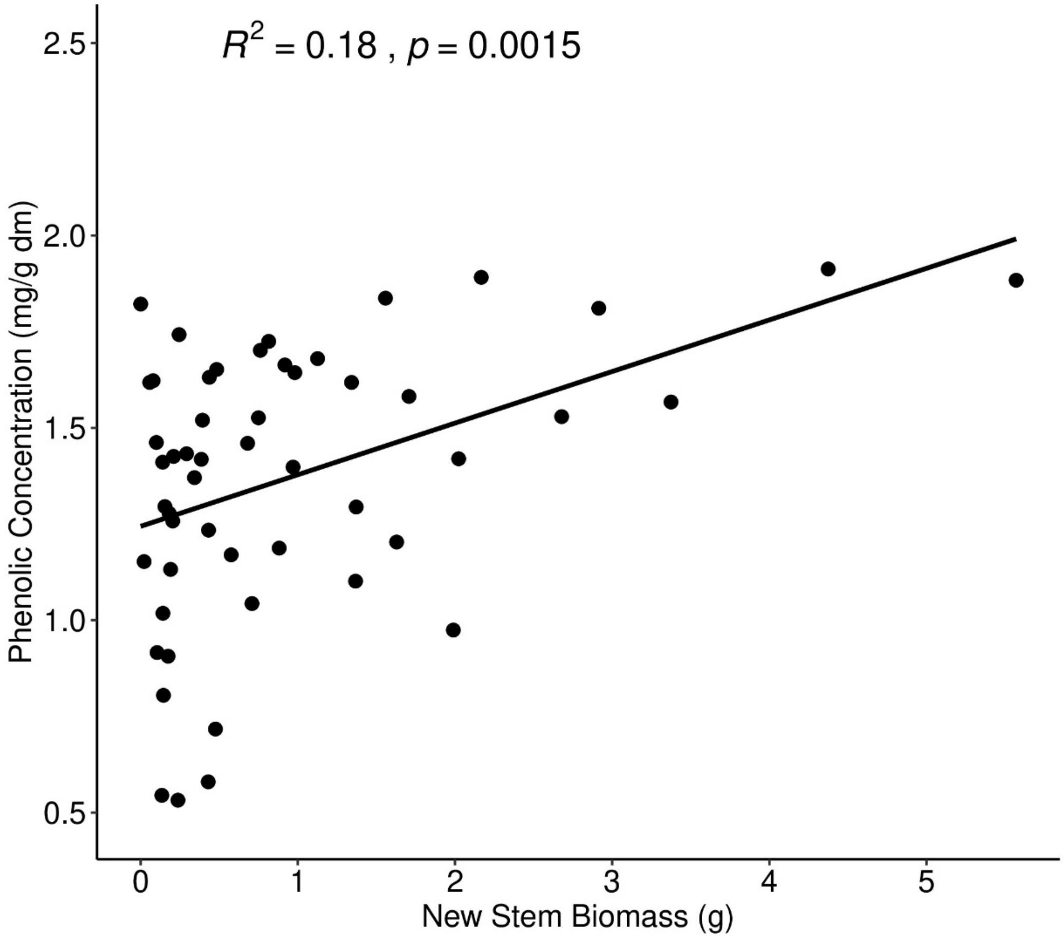


Fig. 6 Mean dry belowground biomass (grams) of *S. alterniflora* A) by collection site (oligohaline or mesohaline marsh) and B) by simulated herbivory treatment (control or clipped). Italicized letters above bars indicate significance between treatments. Error bars represent standard error.

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

CHAPTER III

Resource availability and plant age drive defense against herbivory in salt marshes

<u>This chapter is in preparation for submission as:</u>

Wittyngham, S.S., Carey, J., and Johnson, D.S. (*in preparation*). Resource availability and plant age drive defense against herbivory in salt marshes. *Journal of Ecology*.

Abstract

1. Plants can employ various defenses to prevent or deter attack from their herbivores. Based on this concept, multiple hypotheses have been generated to explain how abiotic factors control plant defense strategy selection. For example, the resource-availability hypothesis (RAH) posits that resources, such as nutrients, control plant anti-herbivore defenses. It predicts in high-resource environments, plant growth is fast, and constitutive defense is low (‘tolerance strategy’), whereas in low-resource environments, plant growth is slow, and constitutive defense is high (‘resistance strategy’). Accordingly, whether grazing induces tolerance or resistance traits is determined by resource availability. Salt marshes are threatened by eutrophication and chronic herbivory, yet we know little about how these stressors shape saltmarsh plant traits and antiherbivore defenses, which directly influence ecosystem resilience. Thus, we tested the RAH in salt marshes for the first time.

2. We manipulated resource availability via nutrient addition and herbivory via the marsh periwinkle, *Littoraria irrorata*, on the salt-marsh foundation species, *Spartina alterniflora*, in mesocosms. Because plant age can influence trait variation, we also measured traits in both original and clonally-grown new stems. We then used a feeding assay to evaluate how treatments and plant age affected subsequent *L. irrorata* consumption of *S. alterniflora*.

3. Nutrient addition promoted tolerance traits (e.g., above- and belowground biomass, new stem production, number of leaves), while decreasing constitutive resistance (e.g., tissue fiber, silica content), following the RAH. Herbivory amplified tolerance traits (e.g., belowground biomass, thicker stem diameter) and did not induce resistance traits, contrary to our expectations. Herbivory plus nutrients increased tolerance traits (e.g., belowground biomass, new stem production) and had mixed effects on resistance traits (e.g., increased nitrogen content, decreased

phenolics), partially supporting the RAH. Regardless of treatment, clonally-grown new stems had greater variation in measured traits. Despite altered traits, however, treatments and plant age did not affect *L. irrorata* consumption.

4. *Synthesis.* We found support for the RAH and our results suggest 1) nutrient availability is a primary driver of plant trait change and 2) plant age controls the magnitude of trait variation in *S. alterniflora*. Understanding how nutrient enrichment and herbivory influence the traits of foundation species such as *S. alterniflora* is critical in assessing ecosystem stability under varying levels of anthropogenic disturbance and future climate change.

Keywords: growth-rate hypothesis, plant functional traits, *Sporobolus alterniflorus*, compensatory growth

1. INTRODUCTION

Herbivores shape plant biomass, abundance, and ecosystem service provision (Silliman & Zieman 2001; Daleo et al. 2015; Freitas et al. 2016). In response to grazing from herbivores, plants can alter their morphological, chemical, and structural traits to mitigate or deter future attack. These trait alterations define the two primary defense strategies: tolerance and resistance (Mauricio et al. 1997; Burghardt & Schmitz 2015). Plants *tolerate* grazing by overproducing or reallocating biomass, altering morphology, and/or increasing photosynthetic capacity to compensate for mass lost to herbivores (Strauss & Agrawal 1999; Stowe et al. 2000; Tiffin 2000). In contrast, plants *resist* grazing by producing chemical or structural defenses which decrease plant palatability, thus shaping herbivore preference and preventing or limiting further grazing (Painter 1951; Strauss et al. 2002). Traits associated with both tolerance and resistance strategies can be either constitutive (i.e., inherent to the plant with or without herbivores present) or induced (i.e., direct result of herbivory) (Karban & Baldwin 1997; Garcia et al. 2021).

Defense against herbivory comes at a cost, as resources allocated to defense cannot be used for reproduction or new growth (Herms & Mattson 1992; Strauss et al. 2002; Leimu et al. 2006). This tradeoff between anti-herbivore defense and plant growth forms the foundation of the resource availability hypothesis (hereafter 'RAH'), which offers a potential driver of this tradeoff (Coley et al. 1985; Endara & Coley 2011). The RAH posits that in low-resource environments, plants have slow growth and high constitutive defense to protect existing tissues from herbivory (resistance strategy). In contrast, in high-resource environments, plants have low constitutive defense and fast growth to compensate for herbivore damage (tolerance strategy). Further, plant age can also influence the magnitude of trait expression and defense strategy selection in addition to resource availability (Henn & Damschen 2021). For example, in terrestrial plants, newer leaves tend to have some higher tolerance traits (e.g., chlorophyll) and resistance traits (e.g., nitrogen, protein) than older leaves, and these traits tend to be more plastic and have greater variation in younger plants (Chen & Poland 2009; Cope et al. 2020; Sola et al. 2020; Funk et al. 2021). Thus, increased presence of newer leaves, which grow quicker but are more nutritionally dense, may further shape herbivore preference.

Salt marshes are intertidal grasslands found at the land-sea interface and provide shoreline protection from storms, nutrient cycling, and habitat for valuable species (Friess et al. 2020; Whitfield 2020). Similar to other coastal ecosystems, salt marshes are threatened by a variety of stressors including chronic herbivory (Gedan et al. 2009) and nutrient runoff from land (Deegan et al. 2012). Through their direct grazing, herbivores can remove large areas of saltmarsh plants, at times converting these ecosystems into mudflats (He & Silliman 2016; Angelini et al. 2018; Williams & Johnson 2021). Under ambient conditions, salt marshes are historically nutrient limited (‘low-resource environment’; Deegan et al. 2007; Lu et al. 2019; Bowen et al. 2020). Thus, according to the RAH, we would expect saltmarsh plants to have relatively high constitutive resistance traits. However, under eutrophic conditions driven by anthropogenic nutrient loading (‘high-resource environment’), the RAH predicts saltmarsh plants would have increased tolerance traits and decreased resistance traits. This suggests that higher nutrient availability via runoff could alter plant defense strategy selection, ultimately influencing plant response to herbivory and saltmarsh resilience.

Thus, the overarching goal of this study was to test predictions from the RAH in salt marsh ecosystems and to understand how plant age may mediate trait variation in the salt marsh foundation species, *Spartina alterniflora* (hereafter ‘*Spartina*’). We hypothesized that relative to ambient conditions, 1) nutrients alone would decrease constitutive resistance and stimulate tolerance traits, 2) herbivory alone would induce resistance traits and 3) herbivory plus nutrients would induce tolerance traits. Further, we expected newer, clonally grown stems to be better defended (i.e., higher resistance and/or tolerance traits) than older stems and that the induction of resistance or tolerance traits by our treatments would influence *Spartina* palatability, affecting subsequent feeding by herbivores. To test these hypotheses, we first ran a factorial mesocosm experiment manipulating nutrient availability via fertilizer addition and herbivory from the marsh periwinkle, *Littoraria irrorata* (hereafter '*Littoraria*'). We then measured the resistance and tolerance traits of clonally-grown new stems and original stems. Lastly, we conducted a feeding assay with experimentally manipulated *Spartina* and *Littoraria* to assess how altered traits and plant age affected herbivore consumption.

2. MATERIALS & METHODS

2.1 Collection Site

In June of 2018, 300 roots and shoots of *Spartina* were collected from Cushman's Landing marsh, in Cape Charles, Virginia (37.175395°N, -75.942638°W; Fig. 1). Individual *Spartina* stems were collected at least 1 m apart to minimize collecting ramets from the same genetic clone. Following collection, plants were then immediately transported to the Virginia Institute of Marine Science (VIMS) in Gloucester Point, Virginia.

2.2 Mesocosm Set-Up & Maintenance

Within 24 hours of collection, 10 individual *Spartina* stems were planted in 11 L nursery pots containing a 90:10 potting mix to sand ratio, with a total of 28 pots planted. This stem density (10 stems per pot or 9 stems per 0.0625 m^2) was slightly less than average stem densities at this site (~13 per 0.0625 m^2). At the time of planting, stem height, diameter, and the number of leaves were recorded for each plant. A waterproof paint line was added to each stem at the sediment surface so that stem height could be tracked throughout the course of the experiment. Of the ten shoots planted, five were randomly selected and marked with a colored zip tie. These plants served as a composite sample for each replicate pot to be analyzed for plant traits at the end of the experiment. The remaining five plants were designated for feeding assays (see below). Nursery pots were suspended in 19 L buckets and made tidal mechanically (MacTavish & Cohen 2014; Wittyngham 2020). Programmable timers were used to simulate the natural diurnal tidal cycle of the Chesapeake Bay region. Reservoir buckets were filled with seawater directly from the York River (average salinity of 17-20 ppt) via a flow-through system. This water was replaced every 3 days to avoid excess algal growth and nutrient depletion.

Mesocosms were then randomly assigned one of four treatments manipulating *Littoraria* herbivory and nutrient availability: 1) herbivory only, 2) nutrients only, 3) herbivory plus nutrients, and 4) an unmanipulated control, with seven replicates per treatment. After approximately 2 weeks of growth, *Littoraria* were collected from Cushman's Landing and their height and width was measured with digital calipers (Failon et al. 2020). Twelve adult snails were added to their respective mesocosms (herbivory only; herbivory plus nutrients treatments). Although this treatment density was ~3 times the average natural density in Cushman's Landing marsh (4 snails per 0.0625 m^2; Wittyngham, *personal observation*), it was still within the range of natural densities observed. This treatment density was selected to ensure herbivory would elicit a response in *Spartina*. Each week, snails were counted and replaced as necessary to maintain these densities. In nutrient addition treatments (nutrients only, herbivory plus nutrients), two 15 mL centrifuge tubes drilled with holes and filled with ~14 grams of ammonium-nitrate fertilizer (Plantacote slow-release fertilizer, N:P:K = 14:14:14) were pushed into the sediment of their corresponding mesocosm.

At the end of the experiment (June to August, 10 weeks total), the stem height, diameter, and the number of leaves were measured for each of the ten originally planted *Spartina* stems. The number of new stems produced was also counted and recorded on a per pot basis. Any remaining fertilizer was dried and weighed, and the average nitrogen loading rate was calculated as 0.65 ± 0.012 g N m^{-2} day^{-1}. This loading rate falls between those of other enrichment studies (lower than: Langley et al. 2013; similar to: Hill et al. 2020; higher than: Johnson et al. 2016). All plant material was rinsed in an outdoor sieve (1 mm^2 mesh) to remove sediments, and belowground biomass was separated from aboveground biomass. Because it formed thick root mats that could not be disentangled, belowground biomass was compiled per pot, not per stem. Belowground biomass was placed in a 60°C drying oven for 12 days and dry masses were recorded. The remaining aboveground biomass was divided into three groups: 1) the five stems marked with zipties for plant trait analysis, 2) the five stems selected for feeding assays, and 3) new stems (i.e., not originally planted stems). Dead stems were recorded and removed when applicable. All aboveground biomass (for plant traits, feeding assays, and new stems) was rinsed to remove sediments and then was stored in a -80°C freezer.

2.3 Plant-Trait Analysis

To quantify each defense strategy, above- and belowground biomass, root:shoot ratio, carbon content, C:N ratio, and chlorophyll content (*a*, *b*, total) were considered ‘tolerance traits’, as these variables can help plants mitigate damage from herbivory (Hernán et al. 2019). Nitrogen content, total phenolics, total soluble protein, neutral detergent fiber (NDF; hereafter ‘fiber’), and biogenic silica (hereafter ‘silica’) content were considered ‘resistance traits’, as they can affect the palatability and nutritional quality of plant tissues, which may influence herbivore preference (Pennings et al. 1998; Massey et al. 2007; Hernán et al. 2019). We measured each of these traits in both original *Spartina* stems (5 stems for plant traits and 5 stems for feeding assays) and clonally grown new stems. All *Spartina* aboveground biomass was freeze-dried using a Labconco FreeZone system, and then ground to a fine powder using a mini Wiley Mill fitted with a 40-mesh sieve. All three groups (plant traits, feeding assays, and new stems) were analyzed for carbon, nitrogen, C:N ratio, chlorophyll, phenolic, protein, silica, and fiber content. Samples were run on a FlashEA CHN elemental analyzer for carbon and nitrogen analysis and compared to a standard curve for acetanilide. To measure chlorophyll content (*a*, *b*, total), 1 mL of 100% methanol was added to 10 mg of plant powder, covered in foil, and stored at 4°C for 24 hours (Warren 2008; Tran et al. 2018; Nguyen et al. 2020). Aliquots (200 µL) of the extract were transferred in duplicate to a 96-well plate with one 100% methanol blank after every ten wells. Absorbance was read on a Molecular Devices plate reader at 652 and 665 nm and then corrected to a 1 cm pathlength using the following equations (Warren 2008; Tran et al. 2018):

$$A_{652,1cm} = (A_{652,microplate} - blank)/pathlength$$

$$A_{665,1cm} = (A_{665,microplate} - blank)/pathlength$$

Following corrections, chlorophyll *a* concentrations were calculated using the equation (Wellburn 1994; Tran 2018):

$$Chl\ a\left(\frac{\mu g}{mL}\right) = 16.72(A_{665,1cm}) - 9.16(A_{652,1cm})$$

Chlorophyll *b* concentrations were calculated using the equation (Tran 2018):

$$Chl\ b\left(\frac{\mu g}{mL}\right) = -15.28\left(A_{665,1cm}\right) + 34.09(A_{652,1cm})$$

Chlorophyll *a* and *b* concentrations were then converted and reported as milligrams per gram (mg/g). Total chlorophyll concentrations are the sum of both chlorophyll *a* and *b* (Tang et al. 2018). Total phenolic concentrations were measured using a modified Folin-Ciocalteu protocol (Ainsworth & Gillespie 2007; Wittyngham et al. 2019; Wittyngham 2020) in which 2 mL of ice-cold 95% (vol/vol) methanol was added to 20 mg of plant powder and incubated at room temperature for 48 hours in the dark. Samples were then centrifuged at 13,000 rpm for 5 minutes. 200 µL of 10% (vol/vol) Folin-Ciocalteu reagent was then added to 100 µL of sample and vortexed thoroughly. 800 µL of 700 mM sodium carbonate solution was then added to each sample and samples were left to sit at room temperature for 2 hours for color development. 200 µL of sample was then transferred to a 96-well plate and absorbance was measured on a plate reader at 765 nm and compared to a gallic acid standard curve. Total soluble protein content was analyzed using a modified Bradford assay (Thermo Fisher Scientific Coomassie Plus (Bradford) Assay Kit, 2016; Wittyngham et al. 2019; Wittyngham 2020) in which 1 mL of 1 M sodium hydroxide was added to 5 mg of plant powder and incubated at 4°C for 24 hours. Samples were then centrifuged at 6,000 rpm for 10 minutes and 15 µL of each sample was mixed with 300 µL of Coomassie reagent in a 96-well plate. Absorbance was measured on a microplate reader at 595 nm and compared to an albumin standard (BSA) curve. Silica was measured using a wet chemical alkaline extraction (DeMaster 1981; Conley & Schelske 2002) in which 40 mL of 1% sodium carbonate solution was added to 30 mg of plant powder. Samples were placed in a shaker bath at 85°C and 100 rpm for 4 hours. Following digestion, 1 mL aliquots were added to 9 mL of 0.021 N hydrochloric acid and then transferred to the VIMS Analytical Laboratory for measurement of dissolved silica concentrations in aliquots using the blue-molybdate colorimetric technique (Strickland and Parsons 1972). Fiber, comprised of lignin, cellulose, and hemicellulose, was analyzed by Waters Agricultural Laboratories, Inc. in Warsaw, North Carolina, U.S.A.

2.4 Feeding Assays

We conducted a feeding assay to assess how 1) treatment-induced changes in plant traits and 2) plant age (original versus new stems) influenced *Littoraria* consumption of *Spartina*. This full factorial feeding assay measured *Littoraria* consumption of *Spartina* from each of the four mesocosm treatments (herbivory only, nutrients only, herbivory plus nutrients, and unmanipulated control) across both plant ages (original stems and new stems), with 15 replicates per treatment combination. No snail controls for each treatment combination were also included to assess potential changes in food sources in the absence of *Littoraria*. To make each food source, 25 mg of plant powder was suspended in 25 mL of 2% agar solution and poured into petri dish lids (4 mm height x 50 mm diameter), covered in parafilm, and allowed to cool at 4°C overnight (Long et al. 2011; Hughes et al. 2015). On the morning of the feeding assay, *Littoraria* were collected from Cushman's Landing and measured for height and width with digital calipers. Snails were introduced to their food sources within 5 hours of collection. One petri dish lid was placed in a 250 mL beaker with a single *Littoraria* snail and 1 mL of seawater collected from Cushman's Landing to prevent desiccation. Window screen was rubber-banded to each beaker to prevent snail escape. Snails were allowed to forage for 48 hours. At this time, petri dishes were removed from their beakers, placed on top of window screen and photographed. ImageJ software was used to count the number of squares consumed (Long et al. 2011; Hughes et al. 2015).

2.5 Statistics

All statistical analyses were conducted in R version 4.1.2 (R Core Team, 2020). Three separate multivariate regression models were used to 1) test the effects of treatment on *Spartina* morphometrics (stem height, stem diameter, number of leaves, and number of new stems), 2) test the independent and interactive effects of treatment and plant age (original stem and new stem) on *Spartina* plant traits, and 3) test the independent and interactive effects of treatment and plant age on *Littoraria* consumption. The covariate, pot number, was included in models 1 and 2 to assess whether this explained variance among responses. For the first model, morphometric data was averaged for all ten originally planted *Spartina* stems per pot. For the second model, plant trait data was averaged per pot for the five originally planted composite *Spartina* stems ('plant traits'; see methods) and clonally grown new stems and all response variables were scaled for standardization (function "scale" in R) prior to fitting the model. Assumptions of normality and homoscedasticity were met for all three models.

Due to unequal variances among our response variables, standardized effect sizes were calculated for plant morphometrics, traits, and feeding assays using Glass's delta (function "glass_delta" in R package 'effectsize'). For this calculation, the mean of the unmanipulated controls for each response variable was subtracted from the mean of each treatment group and divided by the standard deviation of the control group.

3. RESULTS

At the beginning of the mesocosm experiment, average plant height, stem diameter, and number of leaves for *Spartina* plants were 73.8 ± 14.2 cm, 9.9 ± 2.1 mm, and 9 ± 1.5 leaves, respectively, and there were no initial differences in morphometrics by pot or by treatment.

Littoraria added to mesocosms for herbivory treatments (herbivory only, herbivory plus nutrients) had an average height of 23 ± 1.7 mm and an average width of 17.5 ± 1.2 mm.

3.1 Tolerance Traits

Nutrient only treatments increased above- and belowground biomass (p=0.015 and p=0.001, respectively; Fig. 2A,B), the number of leaves per original stem (p= 0.051; Fig. 3C), and the number of new stems produced (p= <0.001; Fig. 3D). These treatments also decreased root:shoot ratio (p=0.012; Fig. 2C), and C:N ratio (p=0.029; Fig. 2E). Nutrient only treatments had no effect on carbon content (p=0.070; Fig. 2D), chlorophyll *a* (p=0.888; Fig. 2F), chlorophyll *b* (p=0.682; Fig. 2G), total chlorophyll (p=0.9604; Fig. 2H), original stem height (p=0.401; Fig. 3A), or original stem diameter (Fig. 3B).

In contrast, herbivory only treatments increased belowground biomass (p=0.029; Fig. 2B), root:shoot ratio (p=0.025; Fig. 2C), and the diameter of originally planted stems (p=0.041; Fig. 3B). Herbivory only treatments had no effect on aboveground biomass (p=0.901; Fig. 2A), carbon content (p=0.831; Fig. 2D), C:N ratio (p=0.761; Fig. 2E), chlorophyll *a* (p=0.761; Fig. 2F), chlorophyll *b* (p=0.587; Fig. 2G), total chlorophyll (p=0.667; Fig. 2H), height of originally planted stems (p=0.086; Fig. 3A), and the number of leaves per original stem (p=0.508; Fig. 3C).

Herbivory plus nutrient treatments increased belowground biomass (p=0.001; Fig. 2B), C:N ratio (p=0.028; Fig. 2E), and the number of new stems produced (p=<0.001; Fig. 3D). This treatment had no effect on aboveground biomass (p=0.087; Fig. 2A), root:shoot ratio (p=0.214; Fig. 2C), carbon content (p=0.179; Fig. 2D), chlorophyll *a* (p=0.127; Fig. 2F), chlorophyll *b* (p=0.518; Fig. 2G), total chlorophyll (p=0.207; Fig. 2H), original stem height (p=0.559; Fig.

3A), original stem diameter (p=0.124; Fig. 3B), and the number of leaves per original stem (p=0.320; Fig. 3C).

New stems had higher aboveground biomass (p=<0.001; Fig. 2A), chlorophyll *a* (p=<0.001; Fig. 2F), chlorophyll *b* (p=<0.001; Fig. 2G), and total chlorophyll (p=<0.001; Fig. 2H) than original stems. Plant age had no effect on carbon content (p=0.061; Fig. 2D), and C:N ratio (p=0.143; Fig. 2E).

3.2 Resistance Traits

Nutrient only treatments decreased fiber content (p=0.010; Fig. 4D), and silica content (p=0.018; Fig. 4E), while having no effect on nitrogen content (p=0.086; Fig. 4A), phenolic concentrations (p=0.193; Fig. 4B) or protein content (p=0.906; Fig. 4C). Herbivory only treatments had no effect on resistance traits (nitrogen content: p=0.835, Fig. 4A; phenolics: p=0.073, Fig. 4B; protein: p=0.400, Fig. 4C; fiber: p=0.080, Fig. 4D; silica: p=0.849, Fig. 4E). Herbivory plus nutrient treatments increased nitrogen content (p=0.044; Fig. 4A), decreased phenolic concentrations (p=0.013; Fig. 4B) and had no effect on protein (p=0.406; Fig. 4C), fiber (p=0.265; Fig. 4D), or silica content (p=0.836; Fig. 4E).

New stems had higher nitrogen (p=0.022; Fig. 4A) and protein content (p=<0.001; Fig. 4C) and lower silica content (p=<0.001; Fig. 4E) than original stems. Plant age had no effect on phenolic concentrations (p=0.880; Fig. 4B) or fiber content (p=0.798; Fig. 4D).

3.2 Feeding Assays

Littoraria snails used in the feeding assays had an average height of 22.9 ± 1.1 mm and an average width of 17.8 ± 1.0 mm. *Littoraria* consumption of *Spartina* was not affected by treatment (herbivory only: p=0.414; nutrients only: p=0.101; herbivory plus nutrients: p=0.691; Fig. 5) nor plant age (p=0.151; Fig. 5).

4. DISCUSSION

Coastal ecosystems, such as salt marshes, are historically nutrient-limited (Deegan et al. 2007; Lu et al. 2019; Bowen et al. 2020), with nutrient availability directly tied to anthropogenic activities (e.g., runoff from land; Billen et al. 2013). Further, these ecosystems are threatened by chronic herbivory (Gedan et al. 2009) which can alter their provision of critical services on which humans rely. Thus, the RAH provides a testable framework for understanding how eutrophication affects trait change and plant defense against herbivores, which directly influences ecosystem resilience (Lavorel 2013; Wright et al. 2016). Our results support the RAH, as nutrient addition stimulated tolerance traits while reducing resistance traits, indicating that elevated nutrient availability switched *Spartina* defense strategy from resistance to tolerance. Further, trait induction was mediated by plant age, with more pronounced defenses in new growth. A decline in resistance traits can increase plant palatability, ultimately leading to higher grazing pressure (Hernán et al. 2019) and potentially ecosystem loss. However, despite altered traits and defense strategies, our feeding assays revealed *Littoraria* consumption was similar across treatments and plant age, although these results are influenced by the removal of structural defense inherent in agar feeding assays (Long et al. 2011; Hughes et al. 2015). Overall, our results suggest that eutrophic conditions may not always increase top-down control (Silliman & Zieman 2001; Sala et al. 2008), and that a shift to a tolerance strategy in *Spartina* may increase vertical accretion via stimulated biomass production, potentially enhancing marsh resilience to sea-level rise (Graham & Mendelssohn 2014).

Based on the RAH, we expected nutrient addition alone to stimulate tolerance traits and reduce constitutive resistance traits in *Spartina*. Indeed, these treatments enhanced tolerance traits (e.g., above- and belowground biomass, new stems, number of leaves on original stems), while reducing resistance traits (e.g., fiber and silica content). This follows our hypothesis and aligns with previous nutrient enrichment studies (e.g., Johnson et al. 2016; Li et al. 2017; Crosby et al. 2021; Moore et al. 2021). Interestingly, in two other studies that evaluated the RAH in seagrasses, elevated nutrient availability decreased resistance traits yet had no effect on tolerance traits (Hernán et al. 2019; Hernán et al. 2021). The authors attributed this result to a potential limiting resource other than nutrients that hindered plant growth. Our results, however, show an almost 40% increase in new stem production in response to nutrient enrichment relative to controls, confirming that constitutive tolerance traits such as biomass production were nutrient limited in this system, a finding well-established in the salt marsh literature (Valiela et al. 1985; Deegan et al. 2012).

Contrary to our expectations, herbivory alone did not induce resistance traits. In fact, *Littoraria* grazing only influenced tolerance traits, promoting belowground biomass production and increasing original stem thickness. However, grazing alone also decreased new stem production by more than 20% in comparison to controls. Despite declines in new growth, enhanced belowground biomass and plant morphology suggests *Spartina* followed a tolerance defense strategy in response to *Littoraria* grazing even in low resource conditions, contrary to the RAH. This reallocation of biomass belowground has been recognized as a consequence of herbivory in terrestrial ecosystems (Thomas et al. 2017) and may ultimately be the result of our experimental design using *Littoraria*, an herbivore which only attacks aboveground portions of *Spartina*. There are two possible explanations for a lack of induced resistance by *Littoraria* grazing. First, *Littoraria* herbivory on *Spartina* can induce chemical defensive compounds other than phenolics (Long et al. 2011; Sieg et al. 2013; Kicklighter et al. 2018), so perhaps a resistance response was elicited that was not captured by our measured traits. Alternatively, it has been suggested that there is a tradeoff between constitutive and induced defenses, such that plants with high constitutive resistance will not invest resources in induced resistance (Kempel et al. 2011). Cushman's Landing marsh is relatively free from anthropogenic disturbance and thus may simulate a low resource environment. Accordingly, *Spartina* collected from this site may already have high constitutive resistance, thus *Littoraria* herbivory would not have induced a further resistance response.

If adhering to the RAH, we expected *Littoraria* herbivory plus nutrients to induce tolerance traits while decreasing resistance traits (Coley et al. 1985). In fact, these treatments stimulated belowground biomass and new stem production, both tolerance traits. Interestingly, these treatments had mixed effects on resistance traits, increasing nitrogen content while reducing phenolic concentrations. Our results agree with previous studies of other aquatic macrophytes which showed that compensatory growth (e.g., increased biomass) led to decreased phenolics (seagrasses: Vergés et al. 2008; mesohaline marsh plants: Rejmánková 2016). Because nutrient addition stimulated tolerance traits and reduced a resistance trait (phenolics) in the presence and absence of herbivores, we conclude that *Spartina* followed the RAH in our study. Interestingly, herbivory plus nutrient treatments acted as an intermediary, with some similar responses to both nutrients only (above- and belowground biomass, new stem production, C:N ratio) and herbivory only (belowground biomass) treatments. These results suggest nutrient addition (i.e., resource availability) exerts more control on plant traits and defense response than herbivory, further supporting the RAH.

The RAH has been primarily tested in terrestrial ecosystems between plant species (interspecific; Bryant et al. 1989; Massey et al. 2007; Endara & Coley 2011). There is a growing body of literature, however, suggesting the RAH may not apply within a species (intraspecific) due to patchiness of herbivore abundances and thus grazing pressure. This led to the development of the intraspecific RAH ($\text{RAH}_{\text{intra}}$), which predicts that high resource environments facilitate and support higher herbivore abundances and thus grazing pressure, causing plants in these areas to have greater constitutive resistance, a pattern opposite of the RAH (Hahn & Maron 2016; López-Goldar et al. 2020). Both the RAH and the $\text{RAH}_{\text{intra}}$ have been applied in grasslands (Hahn et al. 2021), pine forests (López-Goldar et al. 2020), deciduous forests (Lynn & Fridley 2019), and seagrasses (Hernán et al. 2019; Hernán et al. 2021), with mixed results. Three of these studies found their ecosystems followed the RAH (Hernán et al. 2019; Hahn et al. 2021; Hernán et al. 2021), while the other two found support for the $\text{RAH}_{\text{intra}}$ (Lynn & Fridley 2019; López-Golder et al. 2020). In our study, we found the RAH applied rather than the $\text{RAH}_{\text{intra}}$. One possible explanation for this is the artificial conditions created by our mesocosm environment. We held both grazing pressure and nutrient enrichment constant and equal among treatments. In the field, however, there is often patchiness in resources within a population, leading to differential plant palatability, herbivore preference, and thus grazing intensity (Hahn & Maron 2016; Hahn et al. 2021), and may ultimately have influenced our findings and dismissal of the $\text{RAH}_{\text{intra}}$.

In addition to herbivory and nutrient treatments, we expected plant age to affect *Spartina* traits and defense strategy. Similar to our predictions, we found new stems had enhanced tolerance (e.g., chlorophyll (*a*, *b*, total)) and resistance (e.g., nitrogen, protein) traits when compared to original stems. This suggests that, regardless of herbivore presence or nutrient availability, there is higher resource investment in clonally-grown new growth than in existing tissues. Increased chlorophyll content may be driven by palatability, as plant new growth tends to have higher nitrogen content than older leaves and stems (Tomczak& Müller 2017; Funk et al. 2021), and herbivores often forage for nitrogen-rich food sources (Mattson 1980). Thus, resources are devoted to fast new growth and high rates of photosynthesis to mitigate intense herbivory pressure. Although the influence of age on traits is understudied in salt marsh plants, these results follow similar trends to those of a seagrass species and terrestrial studies (seagrasses: Sola et al. 2020; forests: Chen & Poland 2009; coastal sage scrub: Funk et al. 2021; tallgrass prairies: Henn & Damschen 2021). Further, we saw much greater variation in measured plant traits among new stems than in original stems, supporting others' recent findings that plant age strongly determines intraspecific variation in plant traits (Cope et al. 2020; Funk et al. 2021; Henn & Damschen 2021).

Contrary to our expectations, neither treatments nor plant age affected *Littoraria*'s consumption of *Spartina*. Although altered nutritional quality and palatability can influence grazing intensity and herbivore abundance in salt marshes (Pennings et al. 1998; Long et al. 2011), our results support previous findings showing that *Littoraria* abundance and density were not impacted by *Spartina* traits or defense in the U.S. mid-Atlantic region (Kicklighter et al. 2018; Failon et al. 2020). Thus, despite differences in tolerance and resistance traits driven by resource availability, herbivory, and plant age, our findings showed that potential changes in palatability did not increase top-down grazing pressure from *Littoraria*.

Overall, our results indicate that salt marshes may be resilient against sea-level rise under moderate levels of herbivory and eutrophication. Under eutrophic conditions ('high resource environment') *Spartina* had lower resistance traits and higher tolerance traits ('tolerance strategy’). Enhanced tolerance traits can directly influence salt marsh stability and resilience to sea-level rise, as *Spartina* is a foundation species responsible for regulating vertical accretion, the process by which salt marshes keep pace with rising seas (Kirwan & Megonigal 2013). Nutrient-induced above- and belowground biomass production, in particular, may enhance sediment deposition and organic matter accumulation (Graham & Mendelssohn 2014), both of which contribute to marsh elevation. Additionally, *Littoraria* grazing further stimulated belowground biomass production and produced thicker stems, which could aid wave attenuation and promote sediment capture (Lu et al. 2019). Interestingly, when both stressors (elevated nutrients and herbivory) were combined, a treatment most closely resembling field conditions, the most beneficial outcomes for vertical accretion were produced, with increases in both belowground biomass and new stem production. Ultimately, the RAH provides a context for evaluating variation in plant tolerance and resistance traits along environmental gradients (De Battisti 2021), which is critical for understanding ecosystem stability and resilience under future climate change and anthropogenic disturbance scenarios.

Author Contributions

Serina S. Wittyngham – experimental design, execution of experiment, laboratory analyses, statistical analyses, author of first draft

Joanna Carey – laboratory analyses, draft editor

David S. Johnson – experimental design, funding, draft editor

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Data Availability Statement

All data supporting this manuscript have been archived with the Virginia Coast Reserve (VCR) Long-Term Ecological Research (LTER). This database is accessible and searchable here: https://www.vcrlter.virginia.edu/home2/?page_id=105. The citation for this data set is:

Wittyngham, S. 2022. Snail Consumption of Marsh Grasses in Mesocosms, 2018 and 2020 ver 4. Environmental Data Initiative. https://doi.org/10.6073/pasta/0b70b4ed9cd773475609eb1a97cec9bd

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Figures

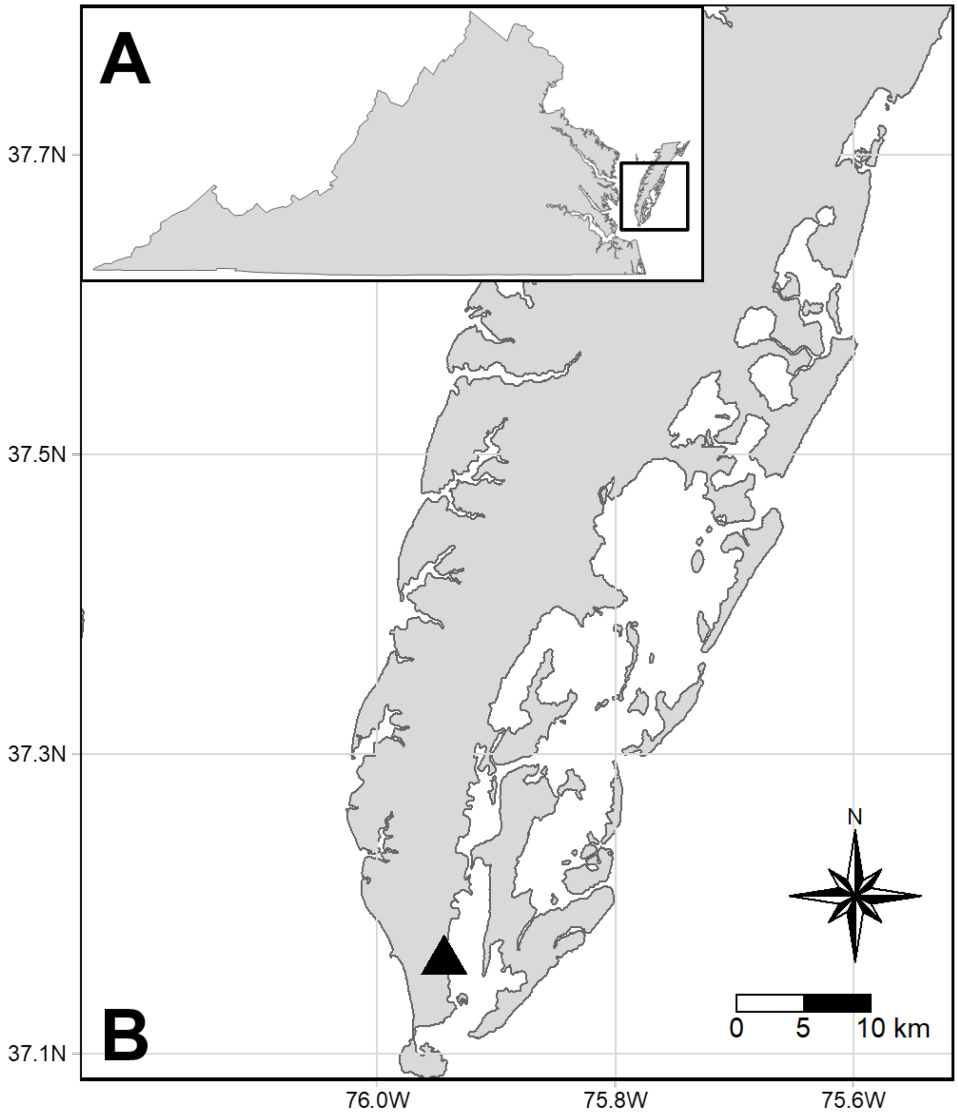

Figure 1. A) Inset map of the state of Virginia, U.S.A. Black box indicates study area shown on panel B. B) Enlarged map of the Eastern Shore of Virginia. Black triangle indicates collection site at Cushman's Landing marsh.

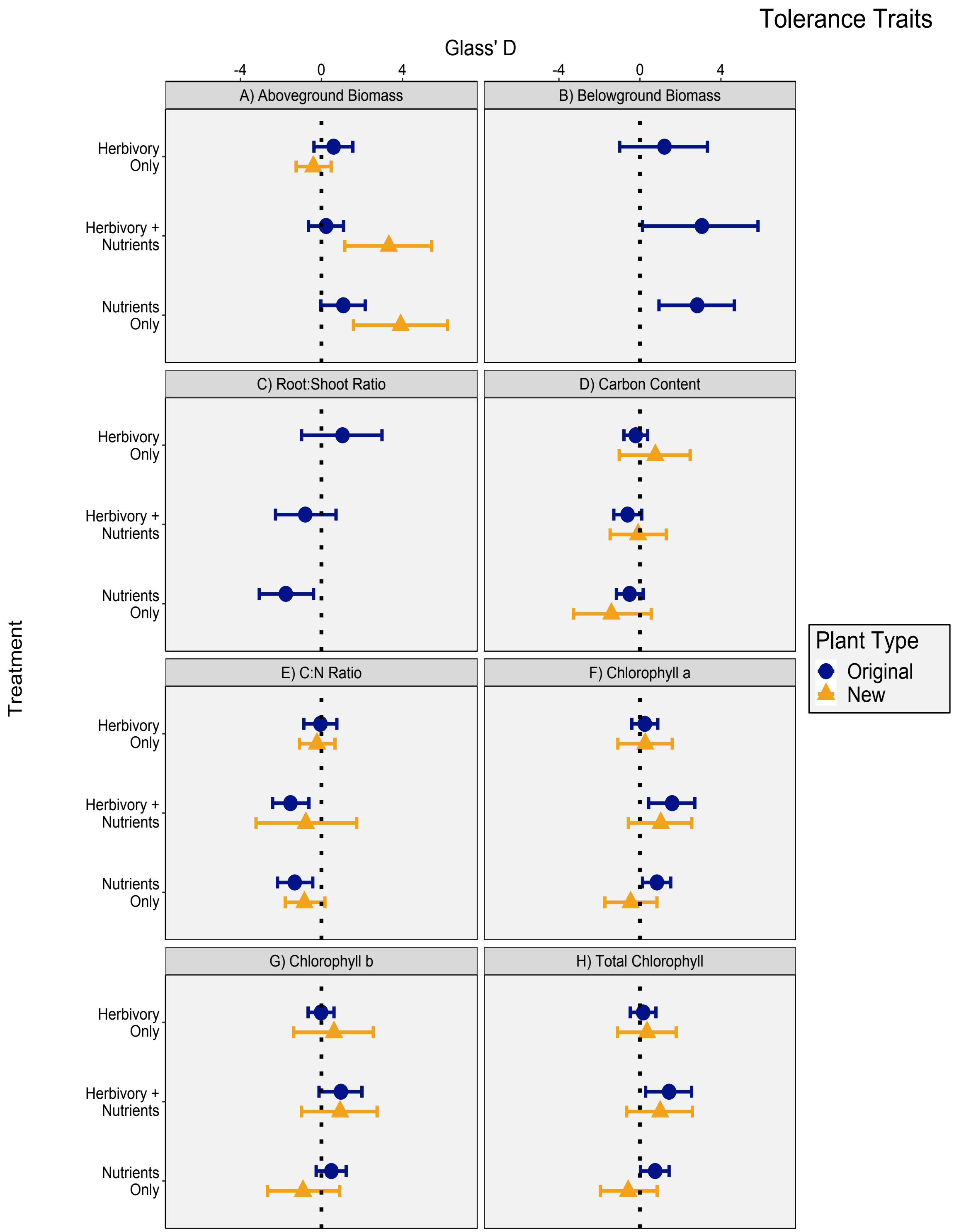

Figure 2. Standardized effect sizes (Glass's delta, 'Glass' D') for measured tolerance traits per treatment and plant age: A) aboveground biomass, B) belowground biomass, C) root:shoot ratio, D) carbon content, E) C:N ratio, F) chlorophyll *a*, G) chlorophyll *b*, and H) total chlorophyll. Note that belowground biomass and root:shoot ratio were compiled per replicate, not per stem, thus they are displayed as 'original' stems only.

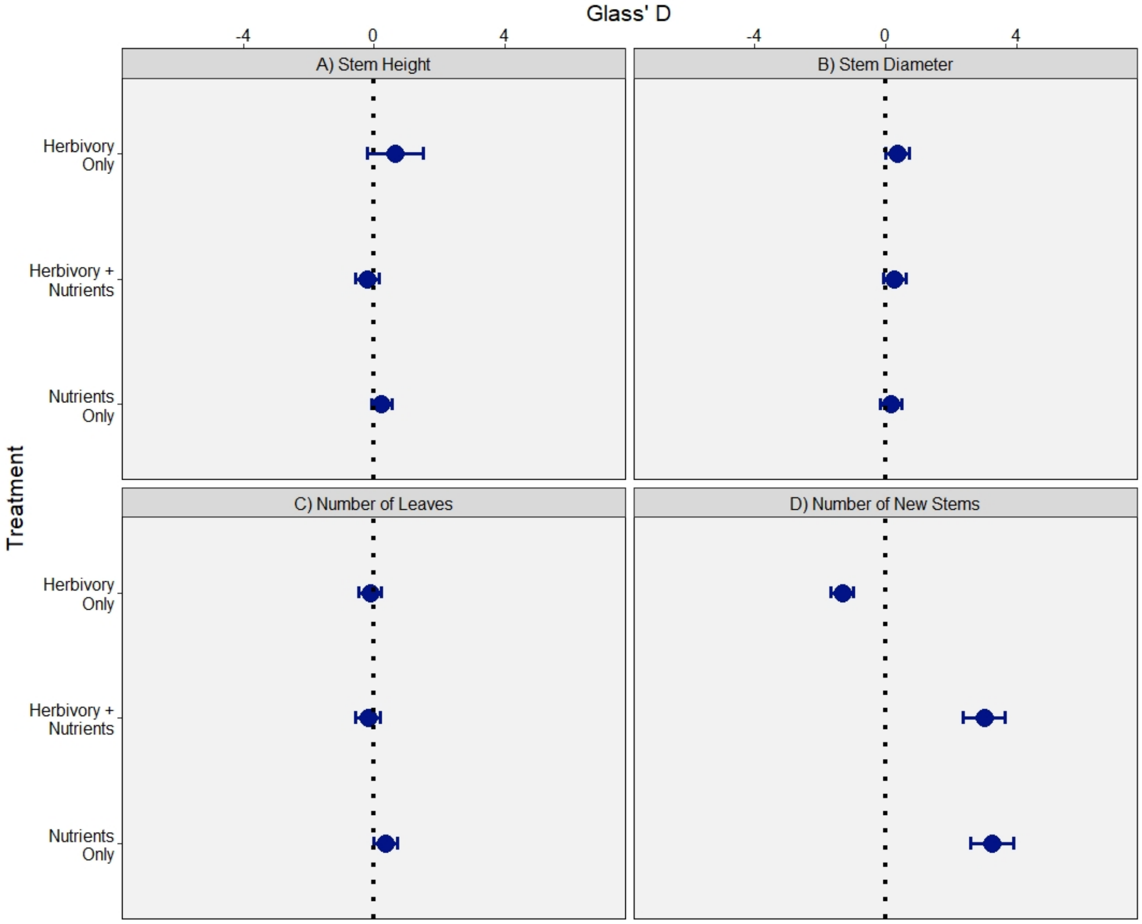

Figure 3. Standardized effect sizes (Glass's delta, 'Glass' D') for measured morphometric traits per treatment in original stems only: A) stem height, B) stem diameter, C) number of leaves, and D) number of new stems.

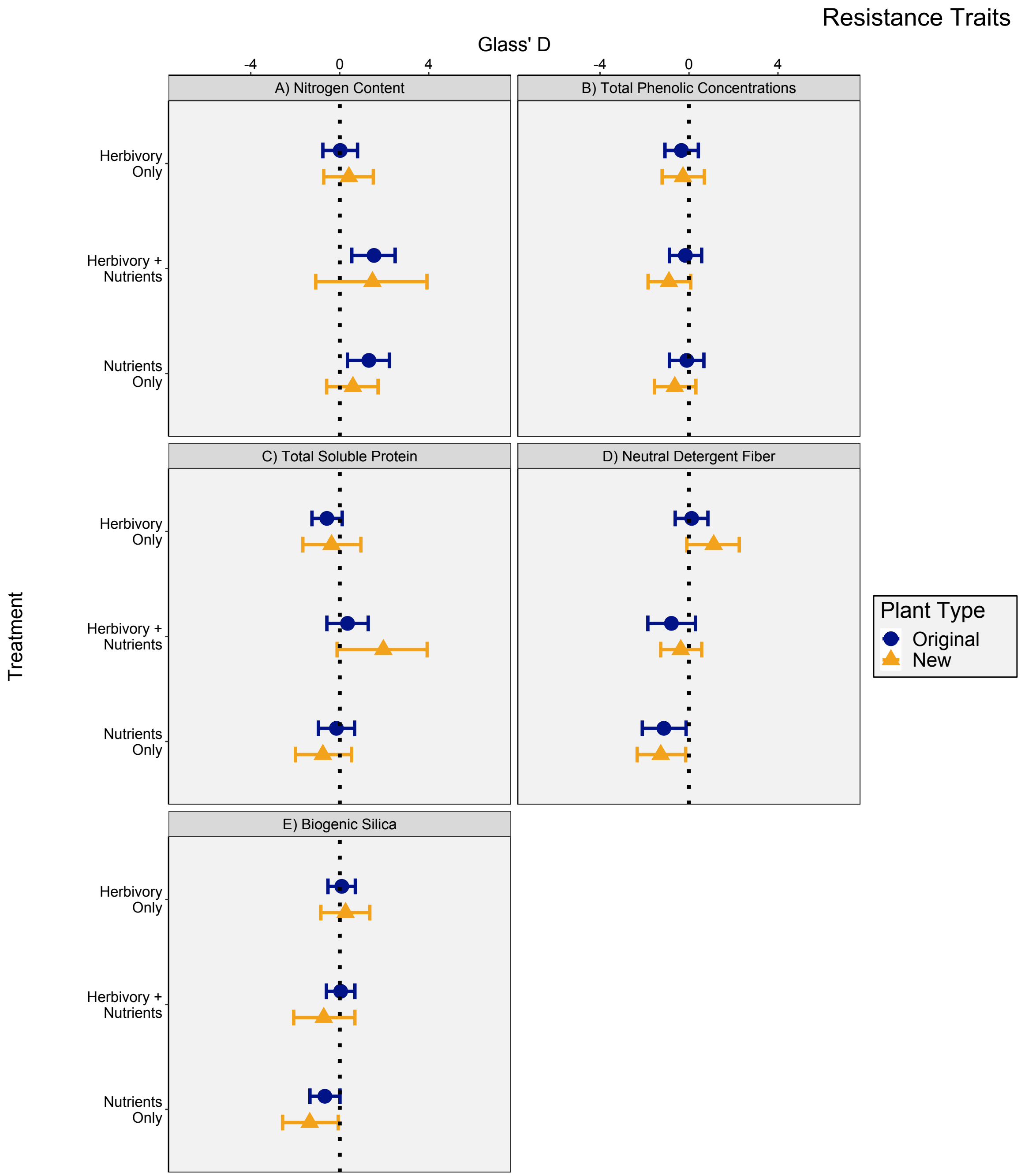

Figure 4. Standardized effect sizes (Glass's delta, 'Glass' D') for measured resistance traits per treatment and plant age: A) nitrogen content, B) total phenolic concentrations, C) total soluble protein, D) neutral detergent fiber, and E) biogenic silica.

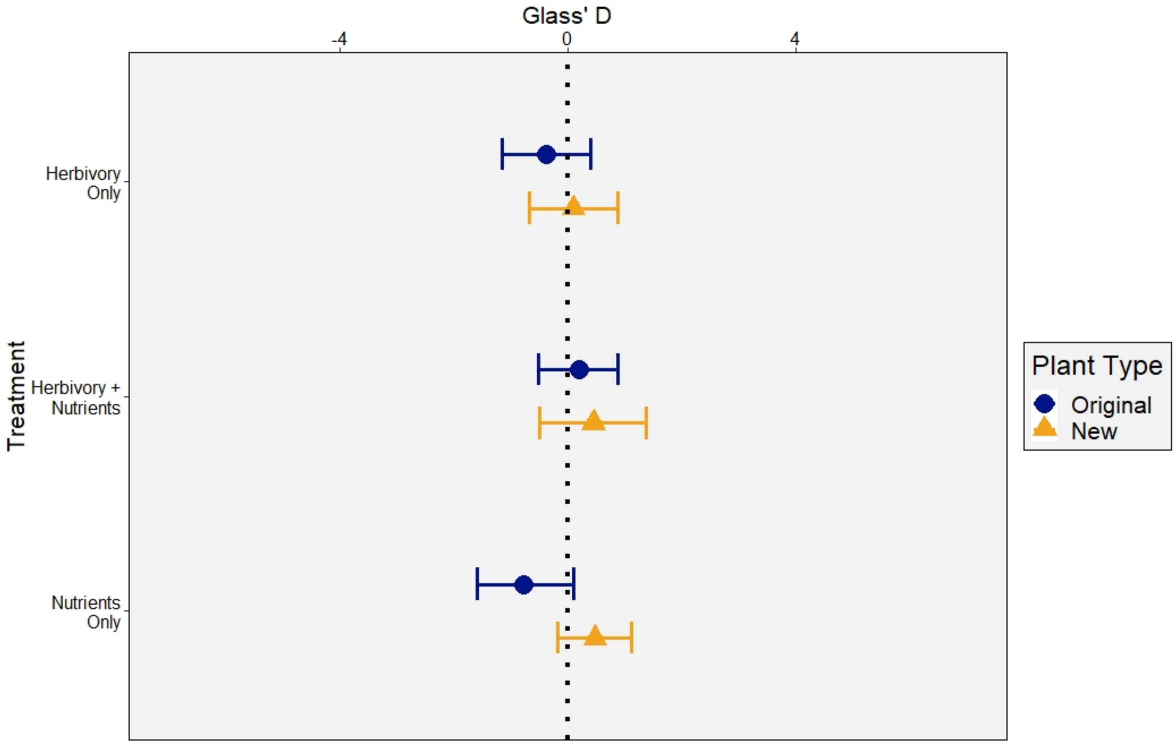

Figure 5. Standardized effect sizes (Glass's delta, 'Glass' D') for measured consumption of *Spartina* by *Littoraria* per treatment and plant age.

APPENDIX A

This appendix presents the raw means and ranges for all response variables measured from the mesocosm experiment in Chapter III. This study's primary goal was to assess the effects of increased nutrient availability and herbivory from the marsh periwinkle, *Littoraria irrorata* on *Spartina* traits. Please see the Chapter III text for additional information regarding this experiment.

Figures

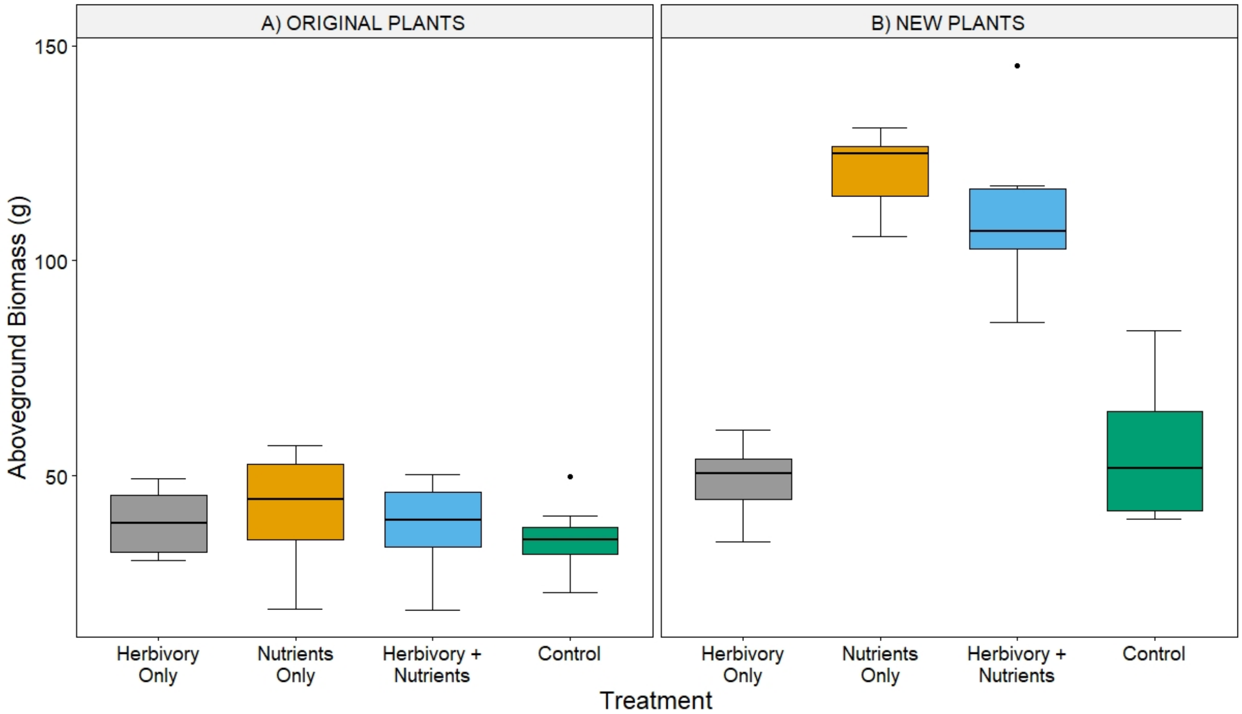

Figure A1. Average aboveground biomass in grams in A) original *Spartina* plants and B) clonally grown new *Spartina* plants by mesocosm treatment (Herbivory Only, Nutrients Only, Herbivory plus Nutrients, and Control).

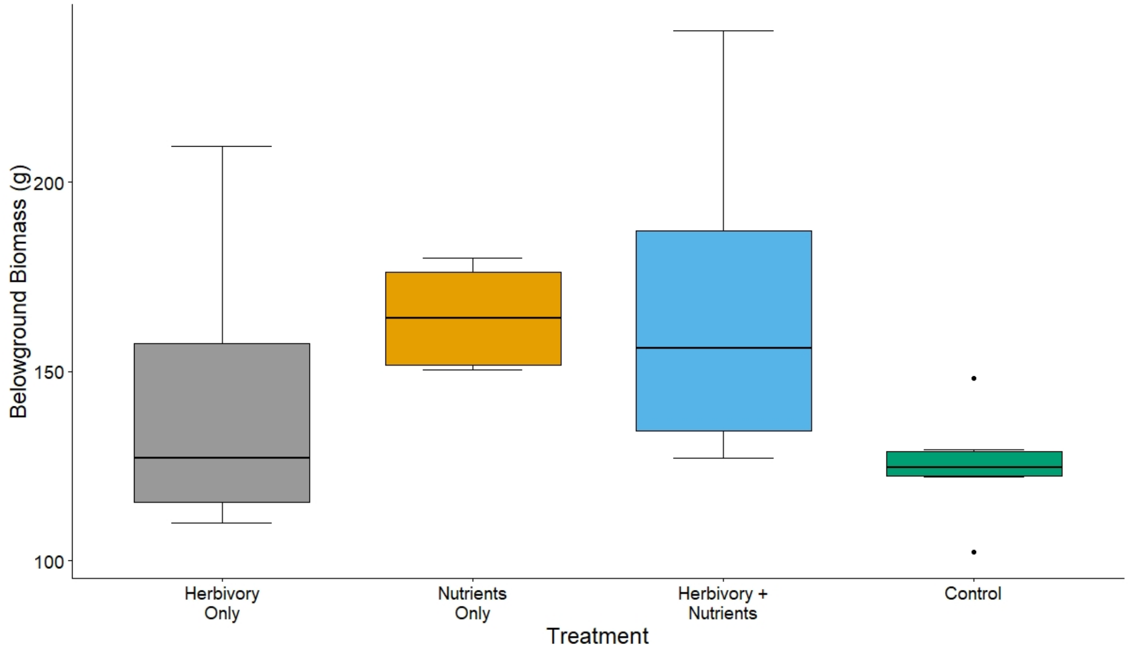

Figure A2. Average belowground biomass in grams by mesocosm treatment (Herbivory Only, Nutrients Only, Herbivory plus Nutrients, and Control).

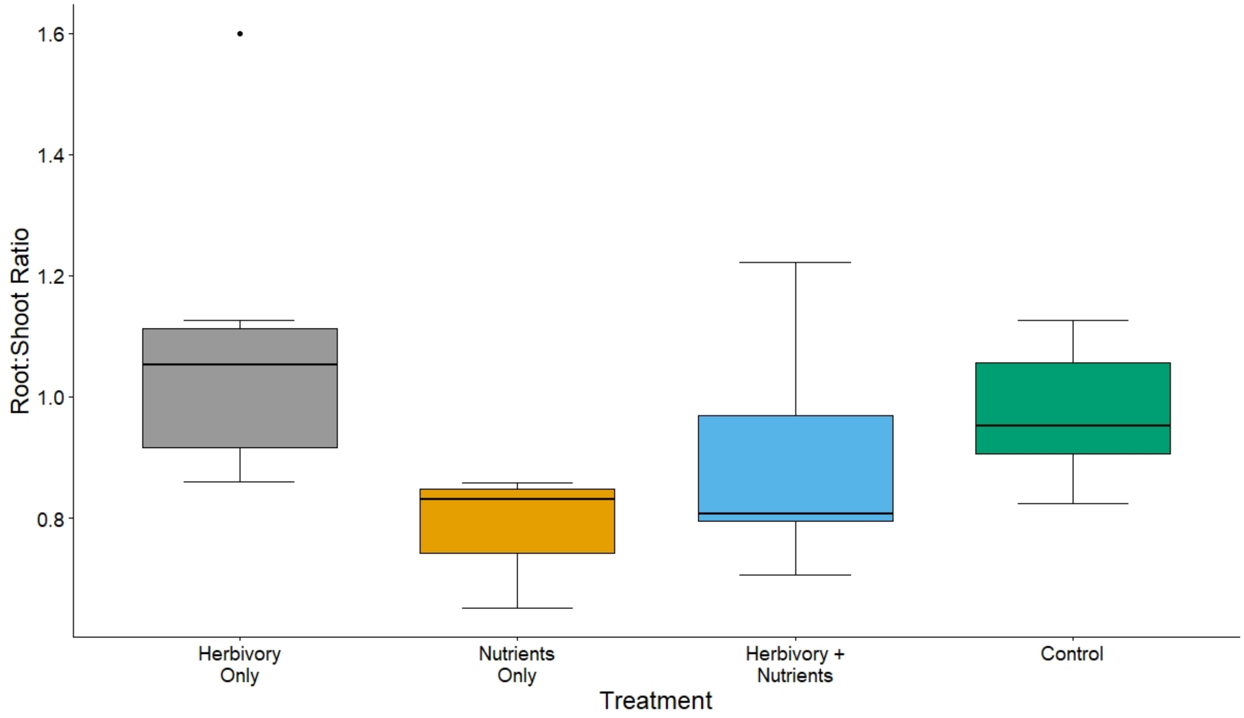

Figure A3. Average root to shoot ratio by mesocosm treatment (Herbivory Only, Nutrients Only, Herbivory plus Nutrients, and Control).

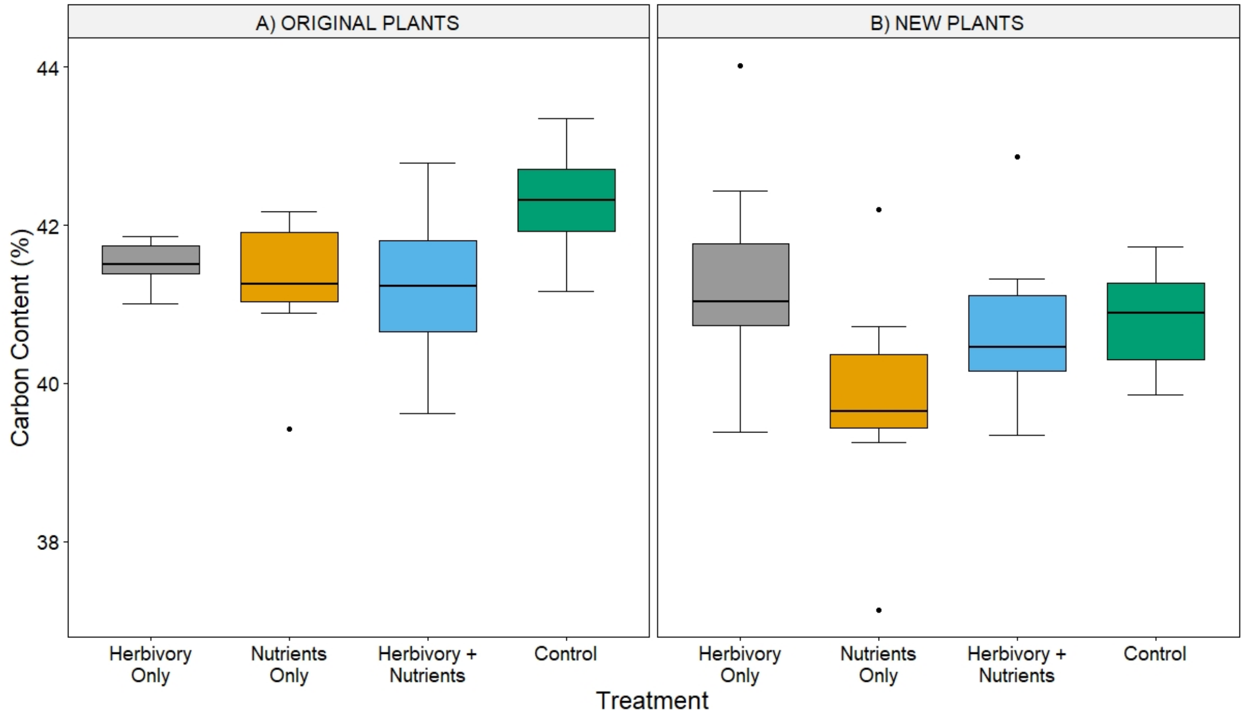

Figure A4. Average percent carbon content in A) original *Spartina* plants and B) clonally grown new *Spartina* plants by mesocosm treatment (Herbivory Only, Nutrients Only, Herbivory plus Nutrients, and Control).

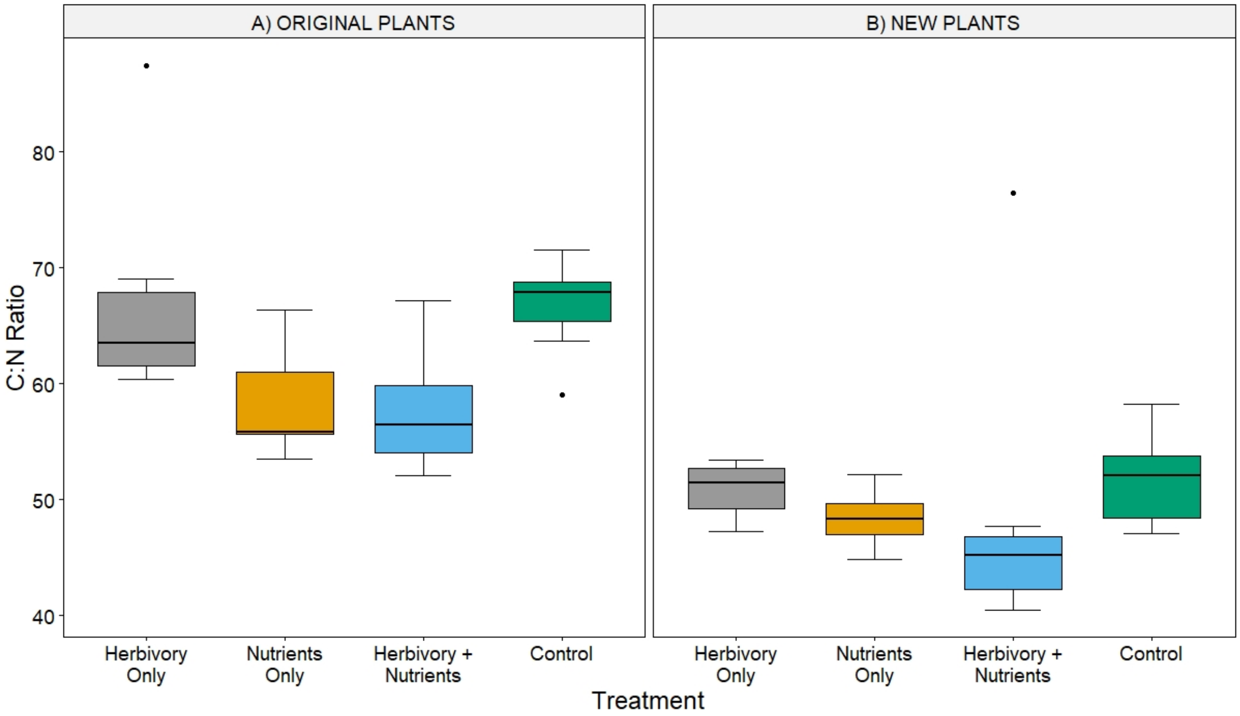

Figure A5. Average carbon to nitrogen molar ratios in A) original *Spartina* plants and B) clonally grown new *Spartina* plants by mesocosm treatment (Herbivory Only, Nutrients Only, Herbivory plus Nutrients, and Control).

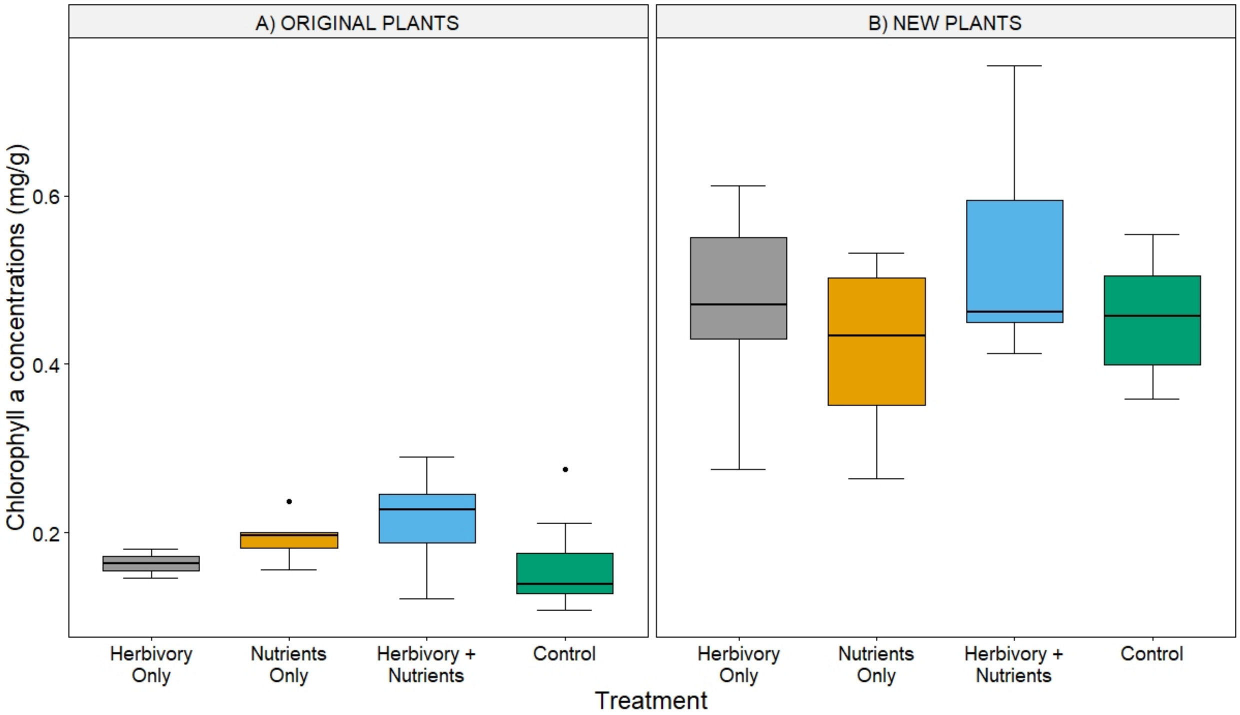

Figure A6. Average chlorophyll *a* concentration in milligrams per gram in A) original *Spartina* plants and B) clonally grown new *Spartina* plants by mesocosm treatment (Herbivory Only, Nutrients Only, Herbivory plus Nutrients, and Control).

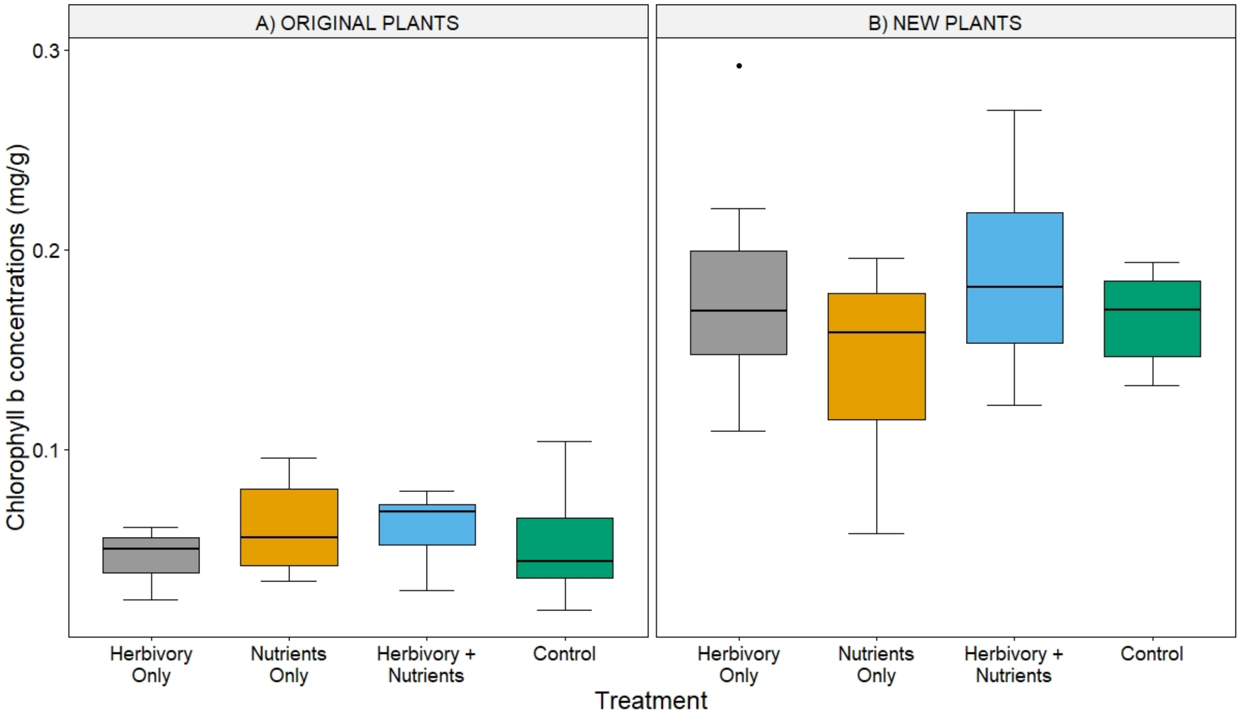

Figure A7. Average chlorophyll *b* concentration in milligrams per gram in A) original *Spartina* plants and B) clonally grown new *Spartina* plants by mesocosm treatment (Herbivory Only, Nutrients Only, Herbivory plus Nutrients, and Control).

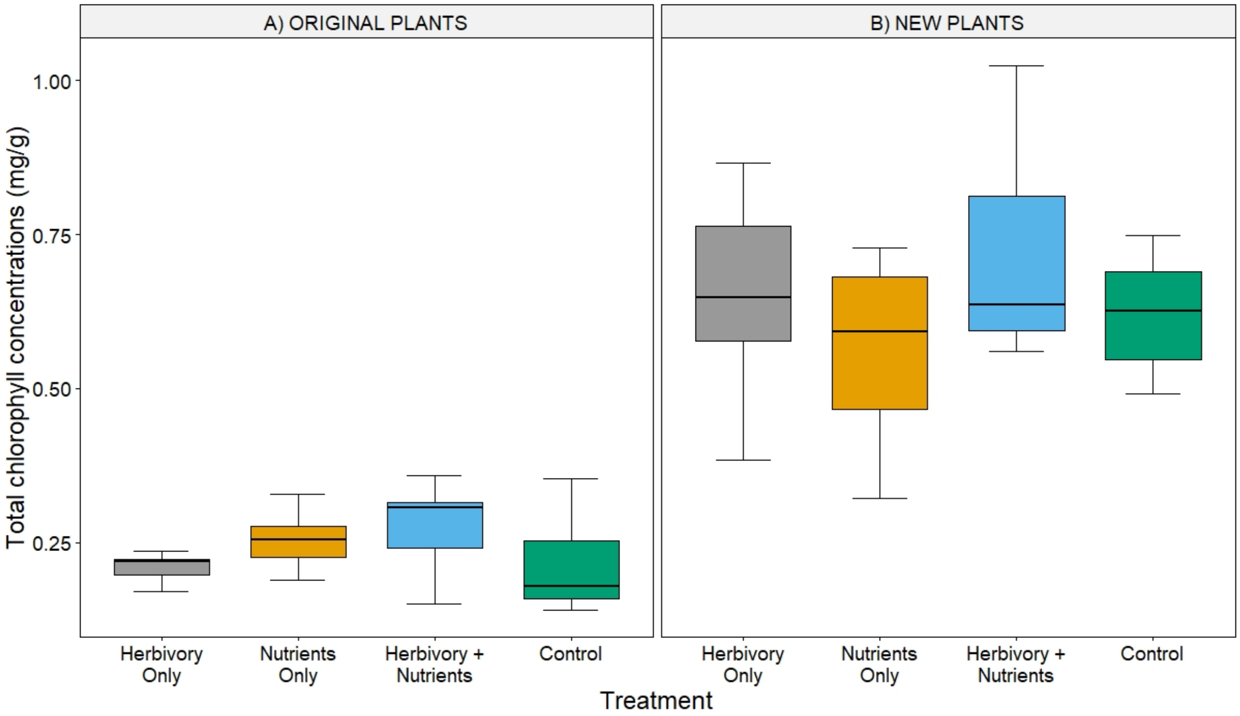

Figure A8. Average total chlorophyll concentration in milligrams per gram in A) original *Spartina* plants and B) clonally grown new *Spartina* plants by mesocosm treatment (Herbivory Only, Nutrients Only, Herbivory plus Nutrients, and Control).

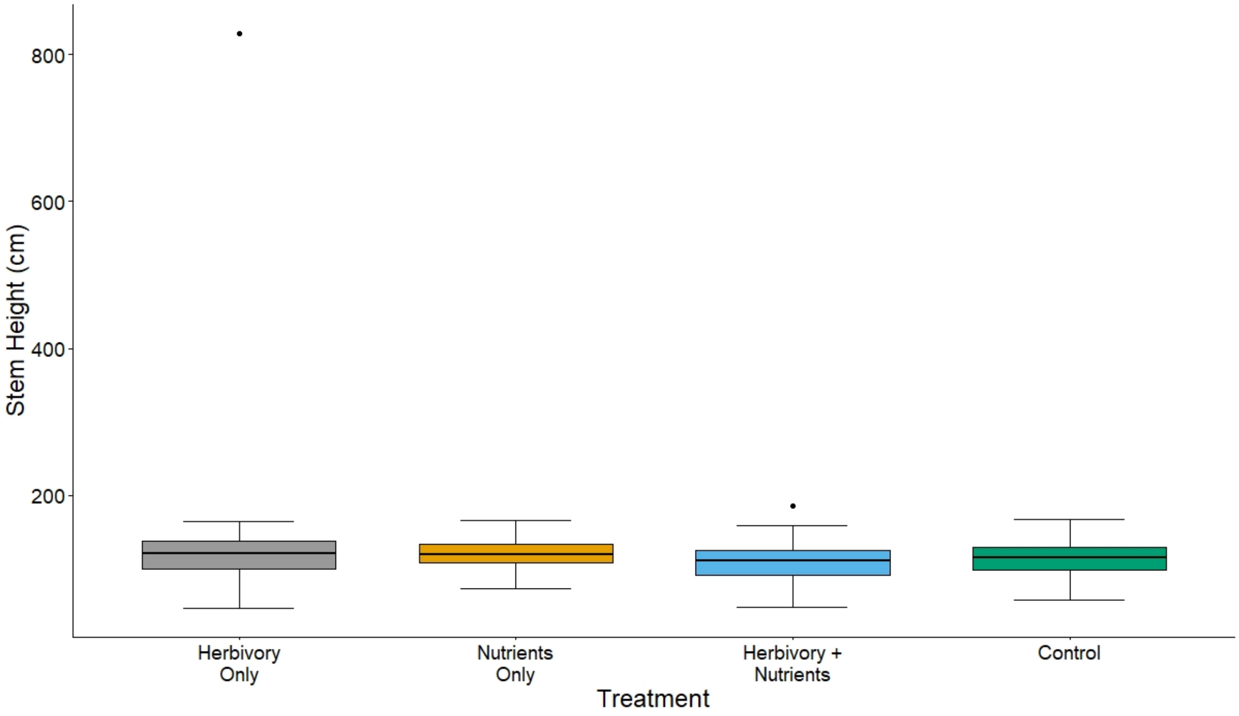

Figure A9. Average *Spartina* stem height in centimeters by mesocosm treatment (Herbivory Only, Nutrients Only, Herbivory plus Nutrients, and Control).

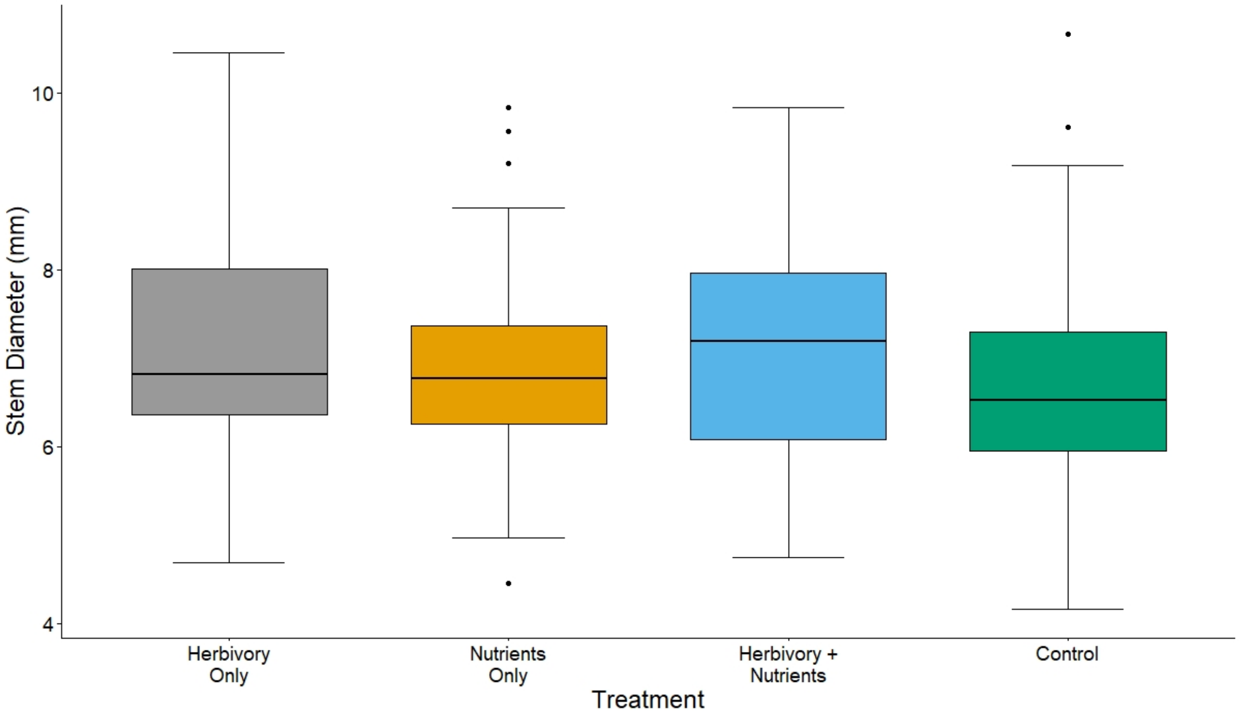

Figure A10. Average *Spartina* stem diameter in millimeters by mesocosm treatment (Herbivory Only, Nutrients Only, Herbivory plus Nutrients, and Control).

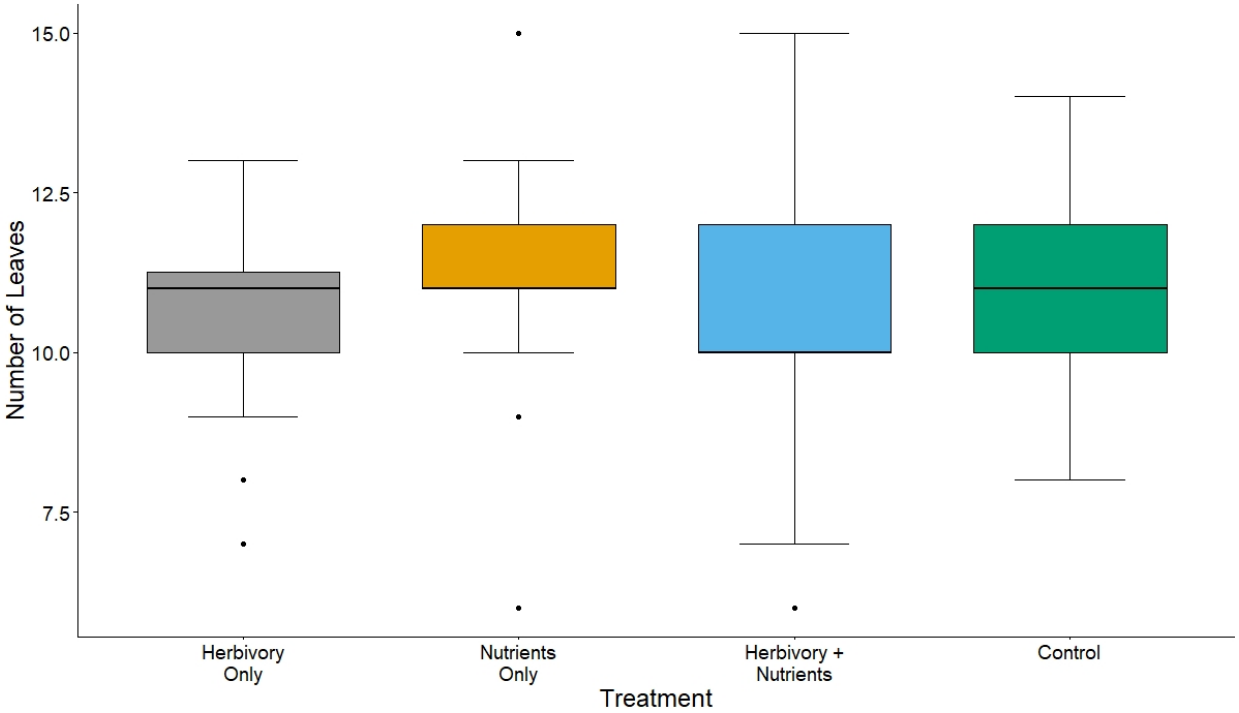

Figure A11. Average number of leaves on *Spartina* stems by mesocosm treatment (Herbivory Only, Nutrients Only, Herbivory plus Nutrients, and Control).

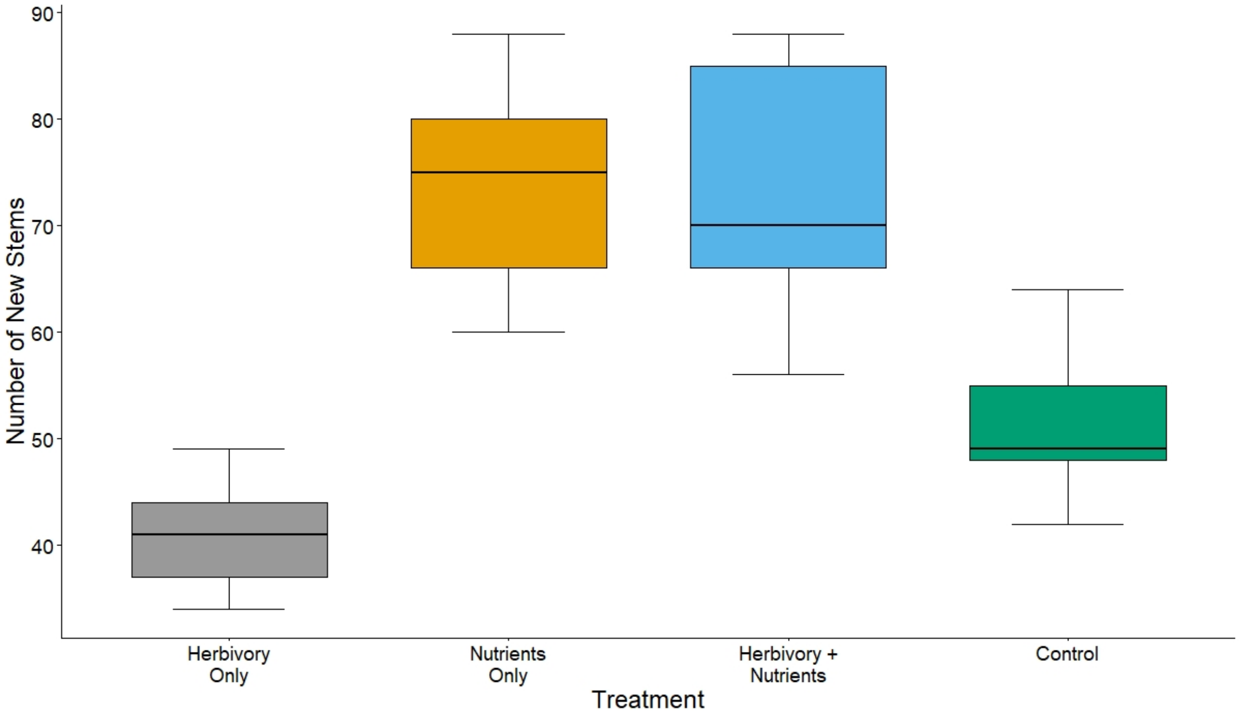

Figure A12. Average number of new stems produced by mesocosm treatment (Herbivory Only, Nutrients Only, Herbivory plus Nutrients, and Control).

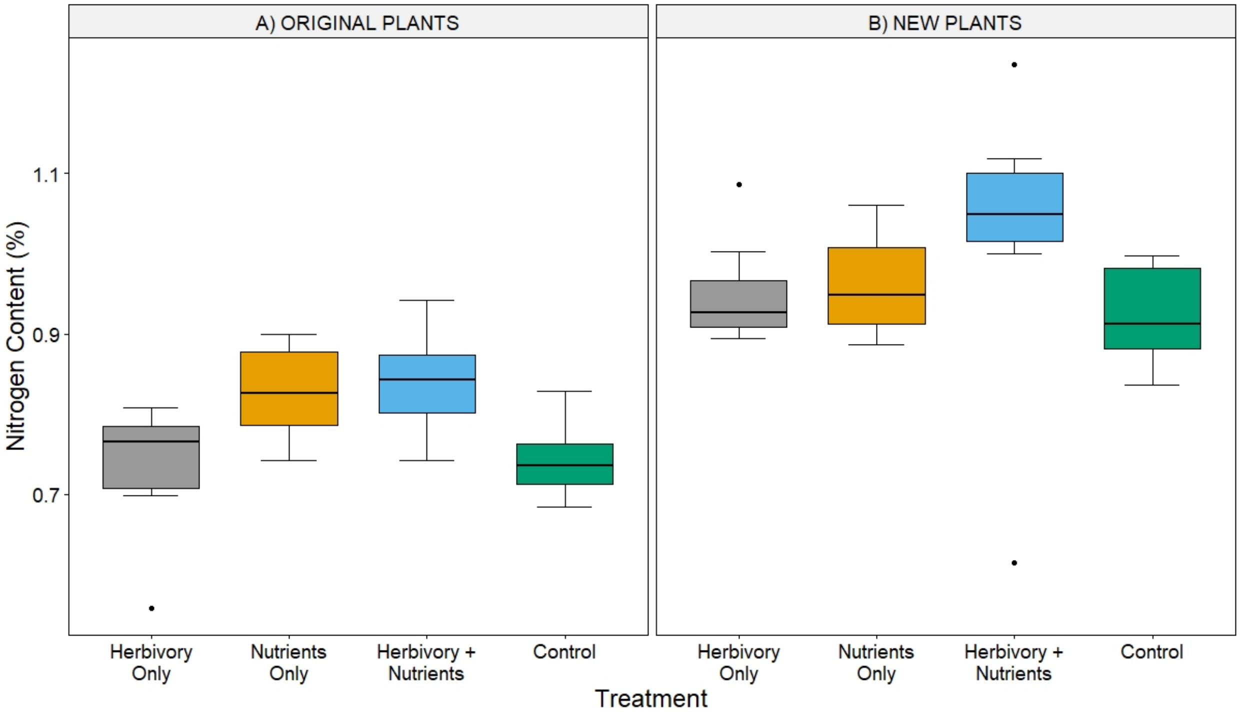

Figure A13. Average percent nitrogen content in A) original *Spartina* plants and B) clonally grown new *Spartina* plants by mesocosm treatment (Herbivory Only, Nutrients Only, Herbivory plus Nutrients, and Control).

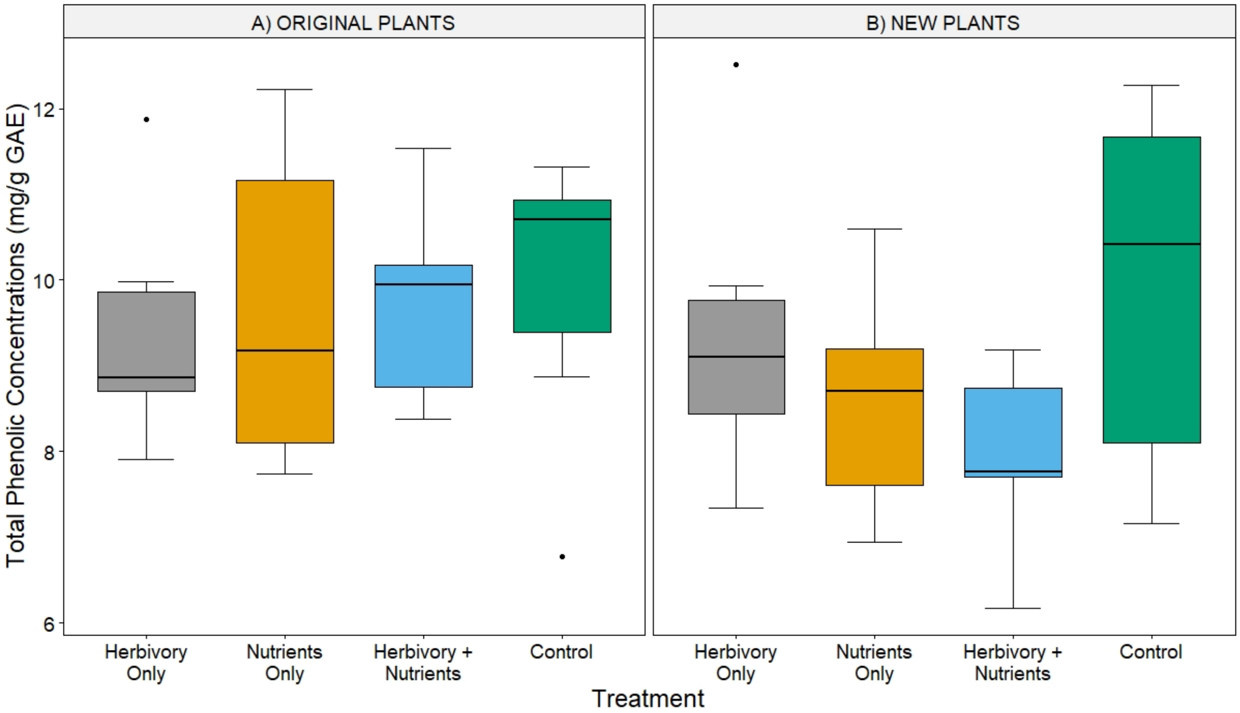

Figure A14. Average total phenolic concentrations in milligrams per gram gallic acid equivalent (GAE) in A) original *Spartina* plants and B) clonally grown new *Spartina* plants by mesocosm treatment (Herbivory Only, Nutrients Only, Herbivory plus Nutrients, and Control).

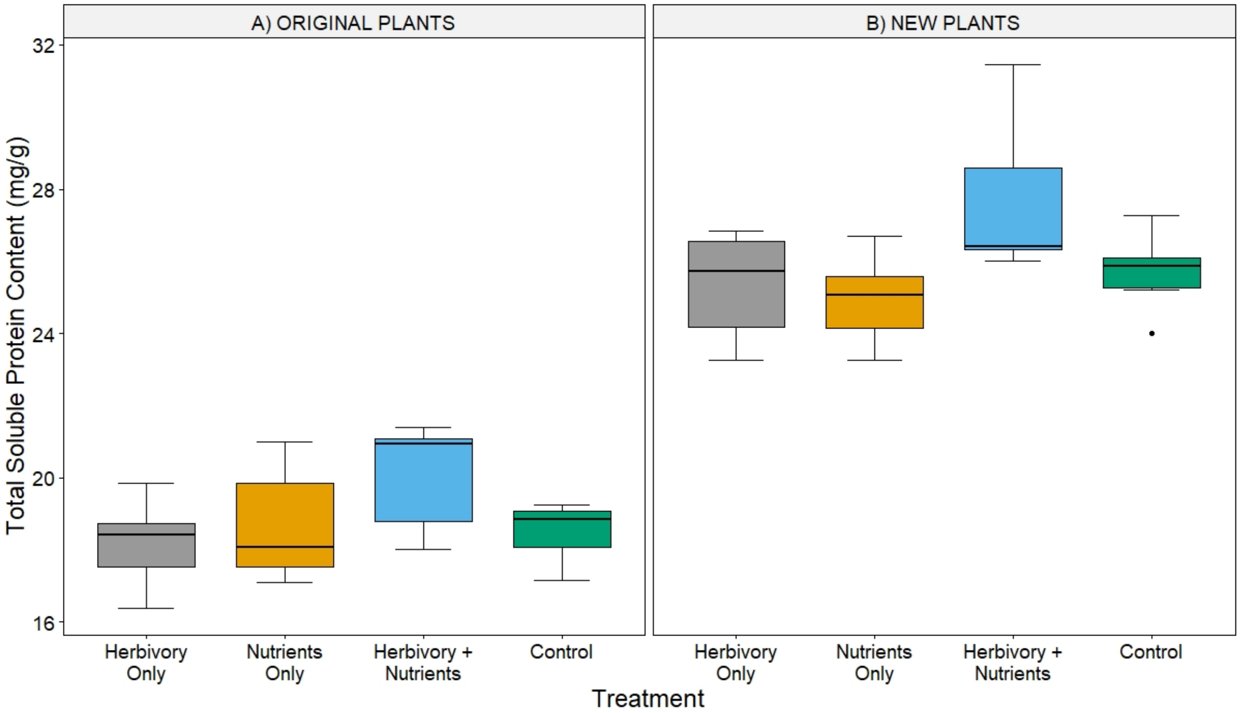

Figure A15. Average total soluble protein content in milligrams per gram in A) original *Spartina* plants and B) clonally grown new *Spartina* plants by mesocosm treatment (Herbivory Only, Nutrients Only, Herbivory plus Nutrients, and Control).

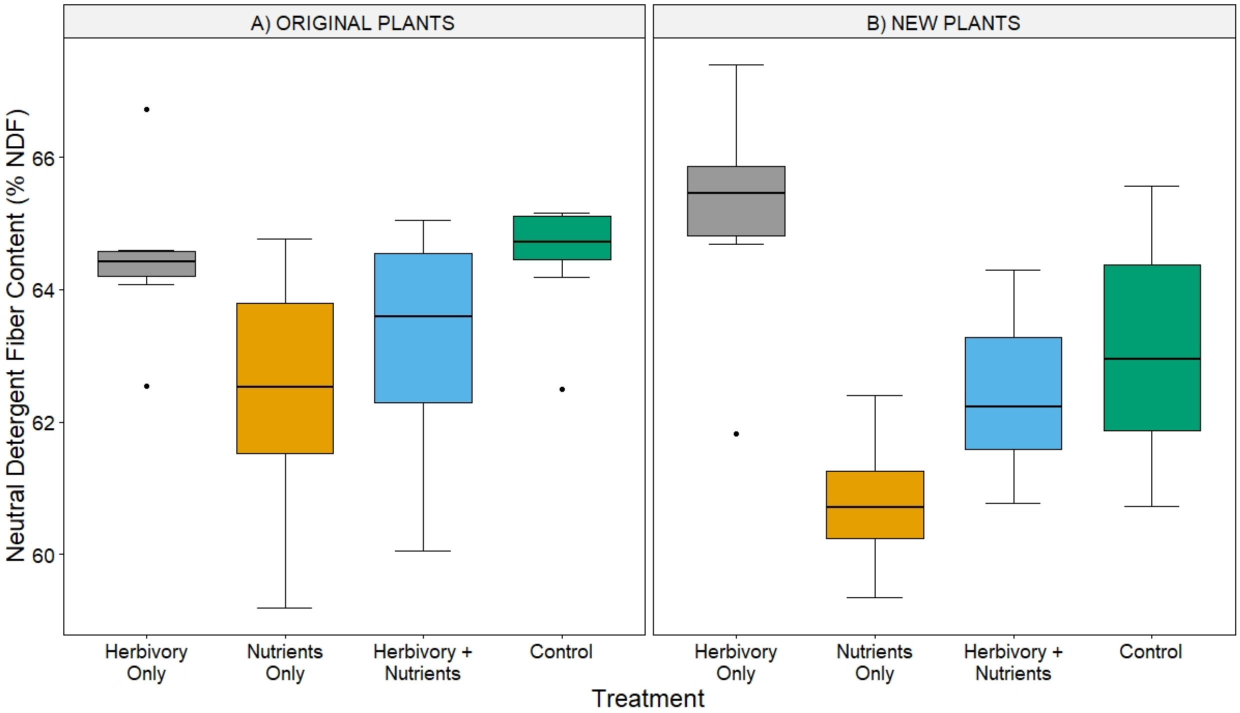

Figure A16. Average percent neutral detergent fiber (NDF) content in A) original *Spartina* plants and B) clonally grown new *Spartina* plants by mesocosm treatment (Herbivory Only, Nutrients Only, Herbivory plus Nutrients, and Control).

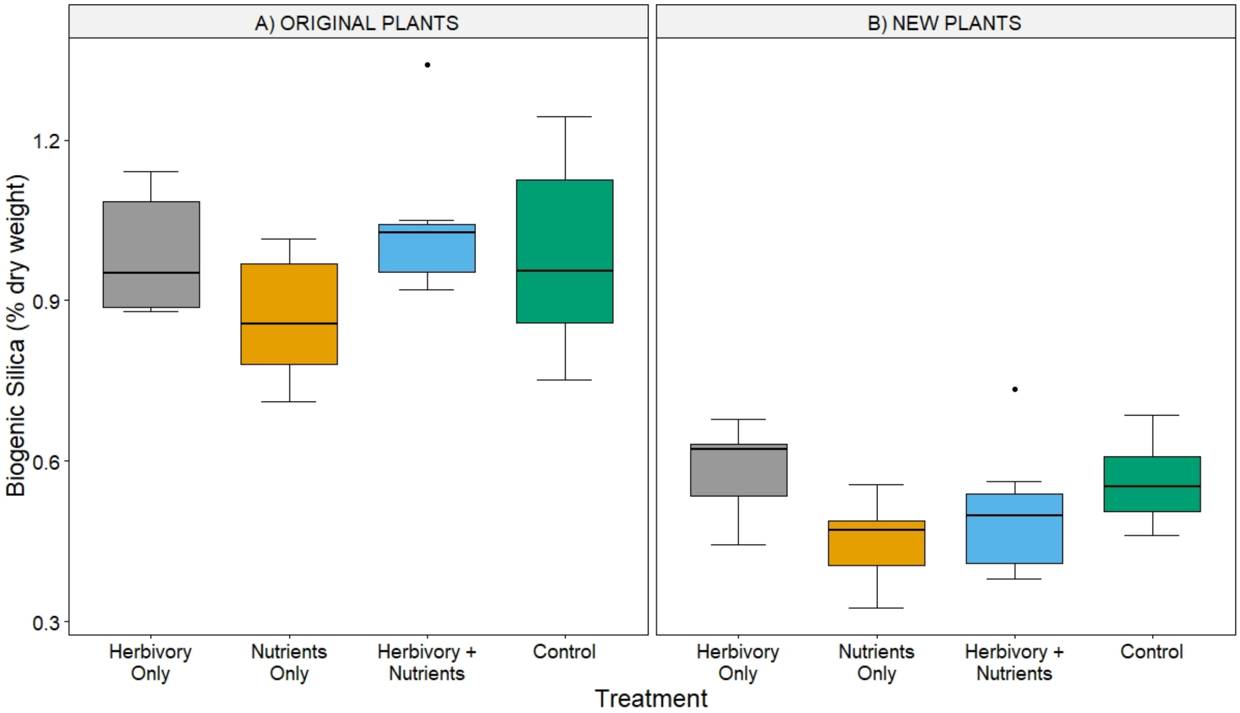

Figure A17. Average percent biogenic silica in A) original *Spartina* plants and B) clonally grown new *Spartina* plants by mesocosm treatment (Herbivory Only, Nutrients Only, Herbivory plus Nutrients, and Control).

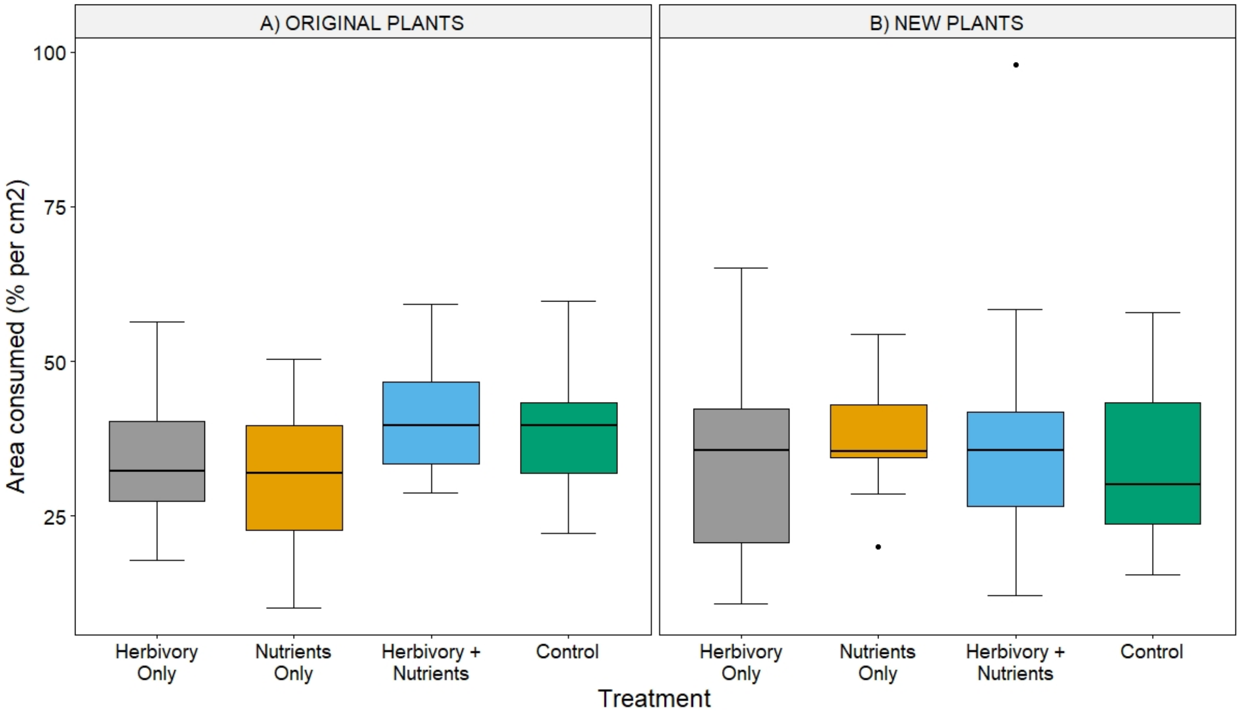

Figure A18. Average percent area consumed per square centimeter by *L. irrorata* of A) original *Spartina* plants and B) clonally grown new *Spartina* plants by mesocosm treatment (Herbivory Only, Nutrients Only, Herbivory plus Nutrients, and Control).

CHAPTER IV

Predation pressure and plant traits drive consumer fronts in salt marshes

This chapter is in preparation for submission as:

Wittyngham, S.S. and Johnson, D.S. (*in preparation*). Predation pressure and plant traits drive consumer fronts in salt marshes. *Ecology*.

Abstract

Consumer-driven disturbance remains a major influence on ecosystem functioning and resilience and, in many cases, ‘consumer fronts’ develop as highly concentrated populations of consumers move through the landscape depleting resources. Although a well-known driver of consumer fronts in terrestrial ecosystems, plant traits have been overlooked as a driver in marine ecosystems, despite their critical role in shaping herbivore preference and ecosystem stability. To test the role of plant traits in influencing herbivore movement, we focused on consumer fronts created by the purple marsh crab, *Sesarma reticulatum*, in U.S. Atlantic salt marshes. *Sesarma* consumes the foundation species, *Spartina alterniflora*, reducing primary production and causing wide-spread marsh die-off. In some cases, however, tall-form *Spartina* revegetates previously denuded areas as the *Sesarma* front advances inland towards the short-form *Spartina*-dominated high marsh. Previous work suggests that sediment characteristics, environmental conditions, and predation pressure drive this directional movement. Here, we test each of these drivers concurrently, and assess the novel hypothesis that the palatability, nutritional quality, and accessibility of *Spartina* further drives the *Sesarma* front inland. We then used a caging experiment to assess if *Sesarma* herbivory alters *Spartina* traits, presenting a pathway by which grazing induces more favorable traits, stimulating consumption, and further propagating the front inland. Predation pressure and *Spartina* traits were the only predictors of *Sesarma* front movement, with sediment characteristics and environmental conditions having no influence. Predation was almost twice as high in the tall-form *Spartina* low marsh, and *Sesarma* survival was not dependent on plant stem density. *Spartina* traits influenced the advance of the *Sesarma* front inland in three distinct ways. First, short-form *Spartina* was more palatable, nutritionally dense, and easier to access than tall-form *Spartina*. Second, *Sesarma* grazing lowered the

phenolics, biogenic silica, carbon, and chlorophyll content of short-form *Spartina*, creating positive feedback in which grazing increased short-form *Spartina* palatability and minimized its ability to mitigate damage. Third, tall-form *Spartina* was more constitutively defended than short form, as grazing only affected the phenolic and carbon content of tall-form *Spartina*. Overall, predator avoidance and poor plant quality in the tall-form *Spartina* low marsh, combined with higher forage quality in the short-form *Spartina* high marsh, resulted in the *Sesarma* front moving inland. As the front advances, *Sesarma*'s grazing and burrowing activities lower marsh elevation. Together, reduced herbivory at lower elevations allows tall-form *Spartina* to revegetate, preventing marsh loss. Our study contributes to previous studies of *Sesarma* fronts and adds a unique perspective on the role of plant traits as a bottom-up control on consumer distribution.

Introduction

Consumer fronts, concentrated populations of consumers bordering a resource, occur in ecosystems worldwide (insects in terrestrial grasslands: Lejeune et al. 2005; beetles in pine forests: Birt & Coulson 2015; urchins in kelp forests: Lauzon-Guay & Scheibling 2007; green turtles in seagrasses: Gulick et al. 2021) and can shape primary and secondary production (He & Silliman 2016; Moore et al. 2020), community assemblage (He et al. 2015), and ecosystem processes and service provision (Brisson et al. 2014; Coverdale et al. 2014; Beheshti et al. 2021). In some instances, unconstrained consumer fronts can also influence ecosystem resilience (Silliman et al. 2013; Vu & Pennings 2021), ultimately causing permanent ecosystem state change (e.g., urchin barrens resulting from overgrazing: Ling et al. 2009).

Consumer fronts typically result from resource depletion, which shape the landscape as consumers move through in search of additional resources once the current habitat becomes unfavorable (Altieri et al. 2013; Silliman et al. 2013; Vu & Pennings 2021). In addition to resource availability, consumer density and front movement can be controlled by other abiotic and biotic factors such as temperature or drought stress (Alberti et al. 2007; Daleo et al. 2015; Zhang et al. 2021) and predators (Altieri et al. 2012; Vu & Pennings 2018). Despite their ability to shape herbivore distribution and movement in terrestrial ecosystems (oak trees: Feeny 1970; poplar and dogwood trees: Dudt & Shure 1994; alder and willow trees: Ikonen et al. 2002; birch trees: Muiruri et al. 2019; agricultural crops: Godinho et al. 2020; evergreen forests: Martini et al. 2022; broadleaf dock: Ohsaki et al. 2022), plant traits remain overlooked as a potential driver of consumer fronts in vegetated marine ecosystems. Plant traits are linked to ecosystem functions such as nutrient cycling (tissue stoichiometry), productivity (aboveground biomass), and stability (belowground biomass) and can also influence herbivore preference (Pennings et al. 1998;

Massey et al. 2007), body mass (Tomczak & Müller 2017), and survival (Wetzel et al. 2016). Thus, evaluating plant traits as a potential mechanism driving consumer fronts provides critical insight into ecosystem resilience.

To assess how plant traits may influence herbivore distribution and consumer front movement, we focused on fronts created by the purple marsh crab, *Sesarma reticulatum* (hereafter '*Sesarma*') as it consumes the foundation species, *Spartina alterniflora* (hereafter '*Spartina*') in U.S. Atlantic salt marshes. These consumer fronts typically form at the heads of tidal creeks (hereafter 'creekheads') and have dramatically increased in prevalence in recent decades (Crotty et al. 2020). *Sesarma* fronts can reduce a salt marsh's ability to keep pace with sea-level rise, as their direct consumption of the above- and belowground biomass of *Spartina* can decrease sediment capture and organic matter accumulation, two key components of vertical accretion (Holdredge et al. 2009; Coverdale et al. 2013A; Schultz et al. 2016; Szura et al. 2017). Further, their burrowing activities can resuspend previously consolidated sediments and stimulate decomposition by increasing soil oxygenation, both of which contribute to higher rates of erosion (Wilson et al. 2012; Martinetto et al. 2016; Vu et al. 2017; Farron et al. 2020).

Sesarma consumer fronts can transition the marsh to a mudflat as the front moves inland (Davidson & De Rivera 2010; Bertness et al. 2014; Williams & Johnson 2021), and ultimately lead to marsh die-off and tidal creek expansion (Escapa et al. 2007; Hughes et al. 2009; Wilson et al. 2012; Vu et al. 2017; Crotty et al. 2020). However, this is not a universal response, as some salt marshes can recover from *Sesarma* fronts (Hughes et al. 2009; Pettengill et al. 2018; Wu et al. 2021). As abiotic and biotic conditions confine the *Sesarma* front to a narrow band, tall-form *Spartina* at the trailing edge can revegetate previously denuded areas as the front progresses inland towards the short-form *Spartina* high marsh (Vu & Pennings 2021; Wu et al. 2021).

Previous studies show that *Sesarma* fronts are moving inland at approximately 2 meters per year in the southeastern U.S. (Hughes et al. 2009; Crotty et al. 2020), although it is unclear if this pattern holds in the mid-Atlantic region. Ultimately, this revegetation of tall-form *Spartina*, despite the continued presence of *Sesarma* nearby, may prevent further marsh loss via increases in sediment deposition and organic matter accumulation associated with revegetation.

Sediment characteristics (Bertness et al. 2009; Bertness et al. 2014; Pettengill et al. 2018), environmental conditions (Vu & Pennings 2021), and predation pressure (Altieri et al. 2012) have all been cited as drivers of *Sesarma* consumer fronts. Sediment characteristics can influence *Sesarma*'s ability to form cavernous, connected burrows (Raposa et al. 2018) and the ease with which they can access *Spartina* roots and rhizomes, their preferred food source (Coverdale et al. 2012). Thus, intermediate sediment strength and plant densities, which allow for free movement while preventing desiccation, are primed for *Sesarma* consumer front formation (Raposa et al. 2018; Wasson et al. 2019; Crotty et al. 2020). Recently, Vu and Pennings (2021) discovered that hydrological conditions at creekheads create ideal soil temperature, dissolved oxygen, and sulfide concentrations for *Sesarma*, leading to higher burrow densities and the formation of consumer fronts in coastal Georgia. This suggests that, in some instances, environmental conditions can exceed the physiological threshold of *Sesarma*, dictating where they can survive and thrive as a colony, and ultimately confining them to creekheads. Lastly, it is well-established that predator presence can dramatically shape *Sesarma* activity (Holdredge et al. 2009; Altieri et al. 2012; Bertness & Coverdale 2013; Coverdale et al. 2013A; Coverdale et al. 2013B; Bertness et al. 2014; Brisson et al. 2014; Pettengill et al. 2018; Raposa et al. 2018), feeding location (Vu & Pennings 2018), and soil nitrogen content and plant biomass (Moore & Schmitz 2021).

Despite the extensive research outlined above, each of these studies assessed these drivers independently. We lack studies that evaluate multiple, concurrent drivers and ecosystem-level consequences of consumer fronts (Moore et al. 2020) and understanding what drives the directional movement of consumer fronts is critical for evaluating and predicting whether an ecosystem will follow a trajectory of die-off or recovery (Pettengill et al. 2018). Here, we build upon previous work by 1) assessing each of these drivers (sediment characteristics, environmental conditions, and predation pressure) simultaneously, 2) testing an additional, novel driver: *Spartina* palatability, nutritional quality, and accessibility, and 3) evaluating the impacts of *Sesarma* consumer fronts on plant traits and ecosystem resilience. We used a combination of field surveys and experiments to evaluate sediment characteristics, environmental conditions, predation pressure, and plant traits as drivers of the *Sesarma* consumer front and compared differences in these factors between tall-form *Spartina* zones (trailing edge of consumer front) and short-form *Spartina* zones (leading edge of consumer front). We hypothesized that sediment (bulk density, shear strength, organic matter content) and environmental (soil salinity and redox potential) conditions are inferior, and predation pressure is higher, in tall form zones than short form zones. We expected revegetated, tall-form *Spartina* to be more constitutively defended (chemical and/or structural), and of lower forage quality (i.e., less palatable, lower nutrient content) than short-form *Spartina*, thus driving the *Sesarma* consumer front inland. We also hypothesized that *Sesarma* grazing directly influences *Spartina* traits, presenting a positive feedback by which herbivory increases *Spartina* palatability via declines in advantageous traits (e.g., chemical or structural defense, tolerance traits), further stimulating *Sesarma* grazing and promoting front movement.

Materials & Methods

Site Description

To assess the potential drivers of *Sesarma* consumer fronts, we conducted field surveys and experiments across thirteen individual *Sesarma*-impacted creekheads along the Eastern Shore of Virginia. All impacted creekheads were tributaries of the Machipongo River, thus abiotic (e.g., salinity, tidal duration) and biotic (e.g., herbivore and plant communities) characteristics were comparable across sites.

Similar to *Sesarma* consumer fronts in Georgia and South Carolina (Hughes et al. 2009; Wilson et al. 2012; Vu & Pennings 2021), the fronts in Virginia create distinct zonation between the high marsh (>0.5518 m NAVD88), which is dominated by short-form *Spartina* and the low marsh (<0.4389 m NAVD88), which is dominated by tall-form *Spartina* (Fig. 1A). The high and low marsh are separated by a narrow band (10-20 m in width) of mudflat that has been denuded of plants by *Sesarma* grazing (Fig. 1A) and is riddled with *Sesarma* and *Minuca pugnax* (fiddler crab) burrows.

Elevation & Sediment Deposition

There are clear differences in elevation between tall and short form zones separated by *Sesarma* consumer fronts. Thus, we characterized differences in elevation and sediment deposition, a critical component of vertical accretion, between short and tall form zones. We used a Real Time Kinematic (RTK) Global Positioning System (GPS) to measure elevation along transects spanning the high to low marsh at all thirteen *Sesarma*-impacted creekheads. We averaged the elevation from all sites to generate an elevation profile (Fig. 1B).

To assess short-term sediment deposition, 3-mm diameter plastic-coated steel rods were inserted in each *Spartina* zone until they reached the sturdy root mat (n=32 per zone, 64 total; Angelini et al. 2015) in April of 2021. The distance from the sediment surface to the tip of each rod was measured in the four cardinal directions at the time of insertion and again when removed in September of 2021. The difference in distances was divided by the number of days deployed to calculate a short-term sediment deposition rate for each *Spartina* zone.

Sesarma *Burrow & Grazing Scar Counts*

To estimate *Sesarma* abundance, we counted *Sesarma* burrows by randomly tossing five 0.70 m^2 quadrats in both short- and tall-form zones at five of the impacted creekheads (n=25 quadrats per plant zone). *Sesarma* burrows are easily identifiable due to their unique, hooded surface openings (Angelini et al. 2018). At the same creekheads, fifteen 0.0625 m^2 quadrats were haphazardly thrown in both short- and tall-form *Spartina* zones (n=75 quadrats per plant zone) to count *Sesarma* grazing scars, which are distinct from other types of grazing (Holdredge et al. 2009).

Quantifying Sesarma *Front Movement*

At five creekheads, we measured the rate of *Sesarma* front movement over time. We first delineated the tall form and short form vegetation boundaries with PVC poles in July of 2020. The distance from the vegetation line to the PVC poles was then measured in six-month intervals through November of 2021. The distance from the vegetation line in November of 2020 was subtracted from the distances recorded in November of 2021 to calculate an annual rate. Negative values associated with the short form zone indicated a retreat of the vegetation edge (i.e., consumer front movement inland) and positive values associated with the tall form zone indicated revegetation as the consumer front moved away from the creekhead. The average distance in meters of retraction and revegetation from the five impacted creekheads was then calculated as consumer front movement in meters per year. At the same time as pole installation, Bushnell wildlife cameras were deployed and trained on a single striped PVC pole. These cameras took a photo at 10 am ET and 3 pm ET each day to visually follow consumer front movement over time (Fig. 1C,D).

Drivers of Sesarma *Fronts*

Sediment Characteristics

To measure soil organic matter (SOM) and bulk density, we collected 16, 30-cm deep cores via a Russian peat borer (Forestry Suppliers) per *Spartina* zone at eight impacted creekheads (n=1 per zone, per creekhead) and sectioned them into four distinct depth ranges: 0-5 cm, 5-10 cm, 10-20 cm, and 20-30 cm. SOM was calculated using standard loss on ignition techniques and bulk density was calculated as the mass of the dry sample divided by the borer volume (Wilson et al. 2012).

Predation Pressure & Environmental Conditions

We assessed predation pressure between *Spartina* zones using a series of tethering assays. Tethers consisted of 15 cm of monofilament line tied to a fiberglass rod and attached to a *Sesarma* crab with a slipknot and cyanoacrylate glue. Because plant densities, diameters, and heights are heterogeneous in both short- and tall-form *Spartina* zones, and this can potentially confound tethering results, we deployed tethered *Sesarma* across three treatments, each delineated as a 0.0625 m^2 plot: 1) all vegetation removed (i.e., bare sediment), 2) vegetation removed and 15 plastic-coated rods inserted equidistant and at equal heights to simulate uniform stem densities, and 3) control with ambient plant densities. We inserted 15 rods in rod treatments because this was the average stem density between tall- and short-form *Spartina* zones. Tethers were sunk into the sediment so that crabs were even with the sediment surface. Next to each tether we dug a 10 cm 'burrow' using a piece of capped PVC to allow crabs to retreat and to prevent desiccation. We conducted two tethering assays on successive tides, with five replicates per treatment, per *Spartina* zone (n=10 per treatment, per zone). Tethers were deployed at low tide and retrieved 24 hours later at low tide. A crab was considered consumed if pieces of the carapace remained on the tether. All other crabs were counted as missing or live (i.e., not consumed). Mortality of crabs (not via consumption), sediment redox potential, and soil salinity were measured in each *Spartina* zone to assess potential differences in environmental conditions and physiological tolerances of *Sesarma*.

Plant Traits

Experimental Design

Using a block design, we installed a series of cages and reference plots in the short- and tall-form *Spartina* zones at eight *Sesarma*-impacted creekheads. Each creekhead had one block of treatments in the short form zone and one in the tall form zone. Each block consisted of four treatments: 1) *Sesarma* addition, 2) *Sesarma* exclusion, 3) cage control, and 4) undisturbed reference. Treatment plots were 1 m^2, and plots within each block were 2.5 m apart. All blocks were placed 1.5 m from the edges of the consumer front to eliminate potential confounding effects. We used hardware cloth with 6.35 mm^2 openings to construct all cages. For *Sesarma* addition and exclusion cages, caging material was dug approximately 15 cm into the sediment to prevent crab escape or entrance. Cage controls consisted of the same hardware cloth with a 15 cm portion removed from the bottom of the cage to allow mobile organisms to move freely in and out of the plots. We dug trenches around the cage control plots similar to those made for the addition and exclusion plots to simulate comparable levels of belowground disturbance. Cages were open at the top and each side had a 10-cm wide piece of aluminum flashing attached to prevent climbing organisms from entering or exiting cages. Unmanipulated references were delineated with PVC poles marking the corners of the 1 m^2 plot and had no other disturbance. Appendix B contains additional methods regarding the caging experimental design.

At the beginning of the experiment, one open pit trap (9 cm wide x 19 cm deep) was installed in each addition and exclusion plot to help remove any mobile organisms (e.g., *Sesarma*, fiddler crabs) prior to experiment start. Capped pit traps were installed in cage control and reference plots to mimic disturbance. Pit traps were emptied every other day for two weeks. At this point, open pit traps in *Sesarma* addition plots were replaced with capped pit traps and seven adult *Sesarma* (carapace width > 15 mm) were added to each *Sesarma* addition cage. This density is ~6 times greater than average burrow density in this region (Fig. 2C), but is comparable to that used in a previous *Sesarma* addition study (Angelini et al. 2018). Although, *Sesarma* are known to have 2-3 crabs per burrow (Coverdale et al. 2012), thus burrow density is not a perfect proxy for crab density. Higher densities of crabs were also chosen to ensure grazing occurred within our cages. *Sesarma* were allowed to forage for three months, and during this time, open pit traps in the *Sesarma* exclusion cages were checked and other cage maintenance (e.g., replacing zipties) was completed every two weeks. After three months, capped pit traps in the *Sesarma* addition plots were replaced with open pit traps to remove *Sesarma* from these plots. Pit traps were checked daily for one week, and then checked every two weeks for the remainder of the experiment. Once *Sesarma* were removed, we counted and attached fluorescent mini zipties to the base of *Spartina* stems that had been clearly grazed by *Sesarma*.

Plant Collections & Trait Measurements

Two weeks following *Sesarma* removal, we collected composite samples of 3-5 *Spartina* stems from each treatment plot at two-week intervals (4 time periods total). The final plant collection occurred in September of 2021, 5 months after the experiment began (see Appendix B for additional data collected during the experimental breakdown). Grazed stems were collected from *Sesarma* addition plots and ungrazed stems were collected from all other treatment plots. The traits of *Spartina* stems collected from reference plots were used to determine ambient differences between short and tall form zones and assess whether altered traits influenced the *Sesarma* front. All collected plants were thoroughly rinsed to remove sediments and measured for stem height and width. A penetrometer (Failon et al. 2020) was used to measure tissue toughness of the first 6 leaves and were averaged per stem. All *Spartina* plants were then placed in a -80°C freezer within 3 hours of collection to await further processing.

All plants were freeze-dried using a Labconco FreeZone system, weighed for aboveground biomass, and ground to a fine powder using a mini Wiley-Mill fitted with a 40-mesh sieve. Aboveground tissues were analyzed for total phenolic concentrations, total soluble protein content, biogenic silica, carbon, nitrogen, C:N ratio, chlorophyll *a*, and chlorophyll *b* concentrations. We categorized each of these traits as follows: 1) phenolics, biogenic silica, and tissue toughness were considered 'palatability traits', because these chemical and structural traits directly influence plant palatability (Pennings et al. 1998; Massey et al. 2007), 2) soluble proteins, nitrogen content, and C:N ratio were regarded as ‘nutritional quality traits’, as herbivores often forage for plants higher in protein and nitrogen content and having low C:N (Mattson 1980), and 3) aboveground biomass, carbon content, chlorophyll concentrations, and plant morphology (stem height and diameter) were characterized as ‘tolerance traits’, because they can help plants mitigate damage from herbivores (Hernán et al. 2019). Phenolic concentrations were measured using a modified Folin-Ciocalteu method (Ainsworth & Gillespie 2007; Wittyngham et al. 2019; Wittyngham 2020) and compared to a gallic acid standard curve. Biogenic silica was measured using a wet chemical alkaline extraction (DeMaster 1981; Conley & Schelske 2002) and then transferred to the VIMS Analytical Laboratory for measurement of dissolved silica concentrations in aliquots using the blue-molybdate colorimetric technique (Strickland & Parsons 1972). Protein content was measured with a modified Bradford assay (Thermo Fisher Scientific Coomassie Plus (Bradford) Assay Kit, 2016; Wittyngham et al. 2019; Wittyngham 2020) and compared to an albumin standard (BSA) curve. Nitrogen and carbon were measured on a FlashEA CHN elemental analyzer and quantified using a standard curve based on acetanilide. C:N ratios were calculated based on these results. Chlorophyll concentrations (*a* and *b*) were measured spectrophotometrically (Warren 2008; Tran 2018; Nguyen 2020) and calculated using equations from Wellburn (1994) and Tran (2018).

Statistics

All statistical analyses were conducted in RStudio version 4.0.3 (R Core Team, 2020). For site characteristics, individual linear regression models were used to assess the effects of plant zone (tall form vs. short form) on sediment deposition and *Sesarma* burrow and grazing scar counts. Similar linear models were used to evaluate differences in potential drivers using the following predictors: 1) plant zone and depth on bulk density and loss on ignition and 2) plant zone, spatial location, and sampling period on plant traits. A generalized linear model with a negative binomial distribution was used to assess the effects of plant zone, treatment (clipped, rods, control), and environmental conditions (redox potential, soil salinity) on *Sesarma* survival in tethering assays. All response variables were tested to meet the assumptions of normality and homogeneity of variances and were square root or log transformed when required.

Results

Elevation & Sediment Deposition

There was an average drop in elevation of 10.5 ± 0.5 cm between the short form, high marsh and the tall form low marsh, with the steepest scarp occurring within the denuded band where the *Sesarma* colony resides (Fig. 1B). Tall form zones had higher sediment deposition than short form zones ($p = 0.017$; Fig. 2A).

Sesarma *Burrow & Grazing Scar Counts*

On average, short-form *Spartina* had 50% higher stem densities ($p < 0.001$; Fig. 2B) and 77% more *Sesarma* grazing scars ($p = 0.001$; Fig. 2D) than tall-form *Spartina*, despite 90% more *Sesarma* burrows in the tall-form zone ($p=0.058$; Fig. 2C). There was no effect of stem density on *Sesarma* grazing scars ($p = 0.384$).

Quantifying Sesarma *Front Movement*

The *Sesarma* consumer front moved inland at an average rate of 0.88 ± 0.12 m y^{-1} at the five creekheads evaluated in Virginia (Fig. 1C,D). At these sites, short-form *Spartina* retreated at an average rate of 1.07 m ± 0.18 m y^{-1}, whereas tall-form *Spartina* revegetated at an average rate of 0.69 ± 0.05 m y^{-1}.

Drivers of Sesarma *Fronts*

Sediment Characteristics

Soil organic matter (SOM) was greater in short form zones ($p < 0.001$; Fig. 3A), and negatively correlated with depth ($p < 0.001$; Fig. 3A). Bulk density was not affected by plant zone ($p = 0.613$; Fig. 3B) but was positively correlated with depth ($p < 0.001$; Fig. 3B).

Predation Pressure & Environmental Conditions

There was no effect of trial number on *Sesarma* survival ($p = 0.433$), thus data from both trials were pooled. Survival was higher in short form zones than in tall form zones ($p = 0.036$; Fig. 4A), regardless of treatment ($p = 0.895$; Fig. 4A). Non-consumptive mortality did not occur during our tethering assays, and despite differences between plant zones in both redox potential ($p = 0.014$; Fig.4B) and soil salinity ($p = 0.039$; Fig. 4C), neither variable affected *Sesarma* survival (redox: $p = 0.696$, salinity: $p = 0.758$).

Plant Traits

Reference Plots

Short-form *Spartina* had higher phenolic concentrations ($p < 0.001$; Fig. 5A), biogenic silica content ($p < 0.001$; Fig. 5B), and lower tissue toughness ($p < 0.001$; Fig. 5C) than tall-form *Spartina*. It also had higher protein content ($p < 0.001$; Fig. 6A), nitrogen content ($p < 0.001$; Fig. 6B), and lower C:N ($p < 0.001$; Fig. 6C). Short-form *Spartina* had higher carbon content (p=0.003; Fig. 7B) and chlorophyll concentrations (*a*: p<0.001, *b*: p<0.001; Fig. 7C,D), and tall-form *Spartina* had taller (p<0.001; Fig. 7E), and thicker stems (p<0.001; Fig. 7F).

Caged Plots

Grazing by *Sesarma* lead to lower phenolic concentrations (p<0.001; Fig. 5A) and carbon content (p<0.001; Fig. 7B) in both short- and tall-form *Spartina*, while also decreasing biogenic silica in short-form only (p<0.001; Fig. 5B). Herbivory induced increases in both chlorophyll *a* (p<0.001; Fig. 7C) and *b* (p=0.009; Fig. 7D) initially, however, concentrations decreased over time and were similar to reference levels 8 weeks after grazing (Fig. 7C,D). *Sesarma* exclusion increased biogenic silica in short-form plants (p=0.021; Fig. 5B), and decreased tissue toughness in tall-form plants (p=0.024; Fig. 5C) relative to *Sesarma* addition treatments. There were some potential caging effects, as reference treatments produced plants with lower nitrogen content (p<0.001; Fig. 6B), tissue toughness (p<0.001; Fig. 5C), and higher C:N ratios (p<0.001; Fig. 6C) than all other treatments. Interestingly, sampling period affected several plant traits in distinct ways. Peak aboveground biomass across treatments and plant type was highest 4 weeks after grazing and then declined at 6 weeks, with mixed results by week 8 (p=0.016; Fig. 7A). Similarly, stem diameter decreased at week 6 in varying magnitudes (p<0.001; Fig. 7F). Chlorophyll *a* concentration followed an opposing pattern, with stark increases at week 6, especially for tall-form *Spartina*, and then declines by week 8 (p=0.009; Fig. 7C). Both biogenic silica (p<0.001; Fig. 5B) and tissue toughness (p<0.001; Fig. 5C) sharply declined across treatments and plant type with the highest values at week 2, and the lowest values at week 8.

Discussion

Consumer fronts shape primary production and ecosystem stability as high densities of herbivores move through the landscape exhausting resources. In U.S. Atlantic salt marshes, *Sesarma* consumer fronts exert top-down control on *Spartina*, and together with their burrowing activities, they influence geomorphology, hydrology, and vertical accretion capacity (Hughes et al. 2009; Wilson et al. 2012; Crotty et al. 2020). In our study, only predation pressure and *Spartina* traits significantly influenced the *Sesarma* front, whereas sediment characteristics and environmental conditions had no effect. Predation pressure was almost twice as high in tall-form *Spartina* zones, regardless of treatment. Plant traits affected *Sesarma* fronts through three distinct mechanisms. First, short-form *Spartina* was more palatable (lower tissue toughness), nutritious (higher nitrogen and protein content), and easier to access (shorter, thinner stems) than tall-form. Second, *Sesarma* grazing enhanced short-form *Spartina* palatability (lowered phenolics and biogenic silica) and decreased its ability to mitigate damage (reduced carbon and chlorophyll content), likely promoting further herbivory. Lastly, tall-form *Spartina* was more constitutively defended (higher tissue toughness, lower C:N) than short-form regardless of treatment. Overall, we infer from our results that *Sesarma* are avoiding their intertidal predators in the low marsh and moving away from the less palatable tall-form *Spartina* towards the higher quality short-form *Spartina*. Evaluating each of these drivers and differences in elevation and accretionary processes caused by the *Sesarma* front allows us to understand how, when combined, they are facilitating tall-form *Spartina* revegetation, ultimately preventing further ecosystem loss and tidal creek expansion.

Sediment Characteristics & Environmental Conditions

Surprisingly, short-form *Spartina* zones had fewer *Sesarma* burrows, higher SOM, and lower bulk density than tall-form *Spartina* zones (Figs. 2C, 3A, 3B, respectively). Previous work indicates that *Sesarma* prefer the opposite conditions (Bertness et al. 2009; Bertness et al. 2014; Pettengill et al. 2018; Raposa et al. 2018). Thus, we conclude that sediment characteristics are not a primary driver of the *Sesarma* front in Virginia salt marshes. Similarly, we did not find evidence that *Sesarma* is avoiding unfavorable conditions in tall-form zones as proposed by other ecologists (Bertness et al. 2009; Vu & Pennings 2021). For example, *Sesarma* survived in both tall form and short form zones during our caging study (3 months) and tethering assays (each 24 hours) and we saw no signs of non-consumptive mortality, indicating that inundation duration had no negative physiological effects on *Sesarma*. Redox potential was highest in the short-form zones, while soil salinity was greater in the revegetated tall-form, yet despite these differences between zones, these environmental variables did not influence *Sesarma* survival (Fig. 4A-C). Combined, these results indicate that the abiotic environment associated with tall-form zones was within *Sesarma*'s physiological threshold, suggesting that the consumer fronts at our study sites were not driven by *Sesarma* avoiding stressful conditions in the low marsh.

Predation Pressure

We found predation pressure in the tall-form *Spartina* zone was nearly double that in the short-form *Spartina* zone (Fig. 4A), consistent with previous findings showing that predation is a strong predictor of *Sesarma* distribution (Coverdale et al. 2013B; Vu & Pennings 2018). We saw equal survival across treatments in tall-form *Spartina* (Fig. 4A), indicating that predation pressure is constant in this area and that stem densities did not influence predator access to *Sesarma*. The dominant predator of *Sesarma* in the mid-Atlantic region is most likely the blue crab, *Callinectes sapidus*, which enters the marsh at high tide, and continues to reside in self-made mud pits at low tide to avoid desiccation and to ambush fiddler crabs and *Sesarma* (Johnson 2022). Following this, average blue crab densities in the tall-form zones were ~30% greater than those in short-form zones (Johnson 2021), further supporting our conclusion that blue crab predation on *Sesarma* is higher in the tall-form low marsh. Interestingly, the highest densities of blue crabs occurred in the denuded band separating the tall and short form zones (Johnson 2021), creating a predator front which "chases" the *Sesarma* consumer front inland. Additionally, the lowest survival in short-form zones occurred in clipped plots (i.e., bare sediment; Fig. 4A), suggesting that the removal of vegetation may increase *Sesarma* vulnerability to avian predators.

Plant Traits

In general, short-form *Spartina* was more chemically (e.g., higher phenolics, Fig. 5A) and structurally defended (e.g., more biogenic silica, Fig. 5B) than tall-form *Spartina*. However, short-form *Spartina* was also more palatable (e.g., lower tissue toughness, Fig. 5C), and of higher nutritional quality (e.g., more protein, nitrogen and lower C:N, Fig. 6A-C). Moreover, short-form *Spartina* had shorter, thinner stems than tall-form *Spartina* (Fig. 7E,F), indicating it may be more accessible to *Sesarma*. *Sesarma* often feed by reaching for and pulling individual leaves to their mouthparts and shorter, weaker stems may decrease the effort required to access leaves, increasing the ease of grazing. Despite higher biogenic silica and phenolic concentrations, short-form *Spartina* may still not be defended enough to stave off herbivorous crabs or to outcompete their preference for nutrient-dense plants. For example, despite having nearly 10% higher phenolic concentrations than tall form, when compared to other marsh plants in the mid- and south-Atlantic, short-form *Spartina* is still significantly less defended and often the preferred food source for herbivores (Sieg et al. 2013: *Batis maritima*, *Borricihia fruteschens*, *Sarcocornia* sp., and *Iva frutescens*; Kicklighter et al. 2018: *Phragmites australis* and *Bolboschoenus robustus*). Additionally, elevated biogenic silica in *Spartina* leaves did not deter *Sesarma* grazing in greenhouse experiments (Bazzano & Elmer 2017), further signifying that these defenses (phenolics and silica) may not be effective against *Sesarma* herbivory in this region.

In our caging study, *Sesarma* grazing influenced *Spartina* traits in both short and tall form zones, although the magnitude of trait variation and the number of traits affected was higher in short-form *Spartina*. Generally, *Sesarma* herbivory negatively impacted short-form *Spartina*. Phenolic and biogenic silica content were lower in plots with *Sesarma* grazing relative to references and exclusion plots (Fig. 5A,B). Likewise, *Spartina* in plots with grazing had lower carbon content (Fig. 7B) and photosynthetic capacity (chlorophyll *a* and *b* as proxies: Croft et al. 2017; Fig. 7C,D). Combined, these findings suggest a reinforcing cycle in which *Sesarma* herbivory increases short-form *Spartina* palatability while reducing its ability to mitigate damage, promoting further grazing, thus driving *Sesarma* consumer front movement inland. In contrast, *Sesarma* herbivory only influenced the phenolics and carbon content of tall-form *Spartina*, with no other significant trait changes. This partially supports our hypothesis that the revegetated tall-form had lower inducible defenses than short-form *Spartina*. Lower inducible defenses are typically indicative of higher constitutive defenses (Kempel et al. 2011; Hernán et al. 2019), suggesting that tall form *Spartina* regrew with better defenses (e.g., higher tissue toughness, lower C:N) than it had previously. This is further supported when we examine induced trait changes in short-form *Spartina*, as *Sesarma* grazing lowered phenolic and biogenic silica concentrations and increased tissue toughness to levels comparable to those of tall-form *Spartina*.

Interestingly, many of the measured traits showed initial induction by *Sesarma* grazing (e.g., phenolics, tissue toughness, nitrogen content), however, once grazing pressure was removed, these traits rebounded to levels similar to reference plots (Figs. 5A, 5C, and 6B, respectively). This suggests that there was rapid induction of trait change initiated by *Sesarma* grazing and when herbivory pressure was removed, many traits recovered within 1 month, presenting plant trait plasticity as an additional mechanism facilitating marsh resilience. Further, trait-based approaches which only sample at a single time point at the end of the growing season may miss induced trait responses, thus future studies should sample at multiple time points following herbivory to capture trait variation. We also saw dramatic declines in protein content (Fig. 6A), stem height and diameter (Fig. 7E,F), and sharp increases in nitrogen (Fig. 6B), chlorophyll *a* and *b* content (Fig. 7C,D) at 6 weeks post-grazing. This coincided with flowering which is known to cause resource reallocation and to spike photosynthesis in *Spartina* (Morash et al. 2007).

Implications

In our study ecosystem, the *Sesarma* fronts are moving at an average rate of 0.88 ± 0.12 meters per year, which is over 50% slower than previous findings in Georgia (Vu & Pennings 2021) and South Carolina (Hughes et al. 2009). This could ultimately be a function of seasonality in *Spartina* production, as Virginia marshes have distinct seasons for *Spartina* growth (e.g., high production during spring and summer, senescence during fall and winter), thus *Sesarma* grazing and consumer front development is limited, whereas year-round *Spartina* growth in South Carolina and Georgia may facilitate faster *Sesarma* consumer front movement. Interestingly, we found that the rate of revegetation (0.69 ± 0.05 m y^{-1}) was slower than the rate of retreat (1.07 ± 0.18 m y^{-1}) at our study sites. This suggests that if current conditions remain constant (high predation in the low marsh; better forage quality in the high marsh), the width of the front may widen over time, with potential positive feedback to geomorphic and hydrological conditions.

In conclusion, we found that heavy predation pressure and *Spartina* traits are driving the *Sesarma* consumer front inland in our study region. In the low marsh, *Sesarma* are more vulnerable to attack from intertidal predators and their food source, tall-form *Spartina*, is better defended and thus less consumable. In contrast, in the high marsh, predation pressure is significantly lower, and short-form *Spartina* is more palatable, nutritious, and easier to access. Lastly, through their grazing, *Sesarma* actively enhanced the forage quality of short-form *Spartina*, presenting a unique mechanism which further facilitates consumer front movement inland.

At the ecosystem scale, *Sesarma* fronts create an average 10.5 ± 0.5 cm drop in elevation, and when combined with reduced herbivory in the low marsh, tall-form *Spartina* can revegetate and may be able to slow creekhead expansion. Similarly, this decline in elevation creates a positive feedback, allowing increased intertidal predator access to *Sesarma* colonies, further shaping their distribution. Revegetated tall-form zones had higher rates of sediment deposition than short-form zones, which could prevent further marsh loss and creekhead expansion. In fact, tall-form zones had an average deposition rate of 0.03 ± 0.007 mm per day in our study, which translates to 10.95 ± 2.5 mm per year of sediment accumulation. Thus, if sediment supply remains constant, this deposition rate would surpass the threshold for marsh survival, even with accelerated rates of sea-level rise in the mid-Atlantic region (Kirwan et al. 2010), presenting a pathway for marsh resilience despite chronic herbivory from *Sesarma*.

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Figures

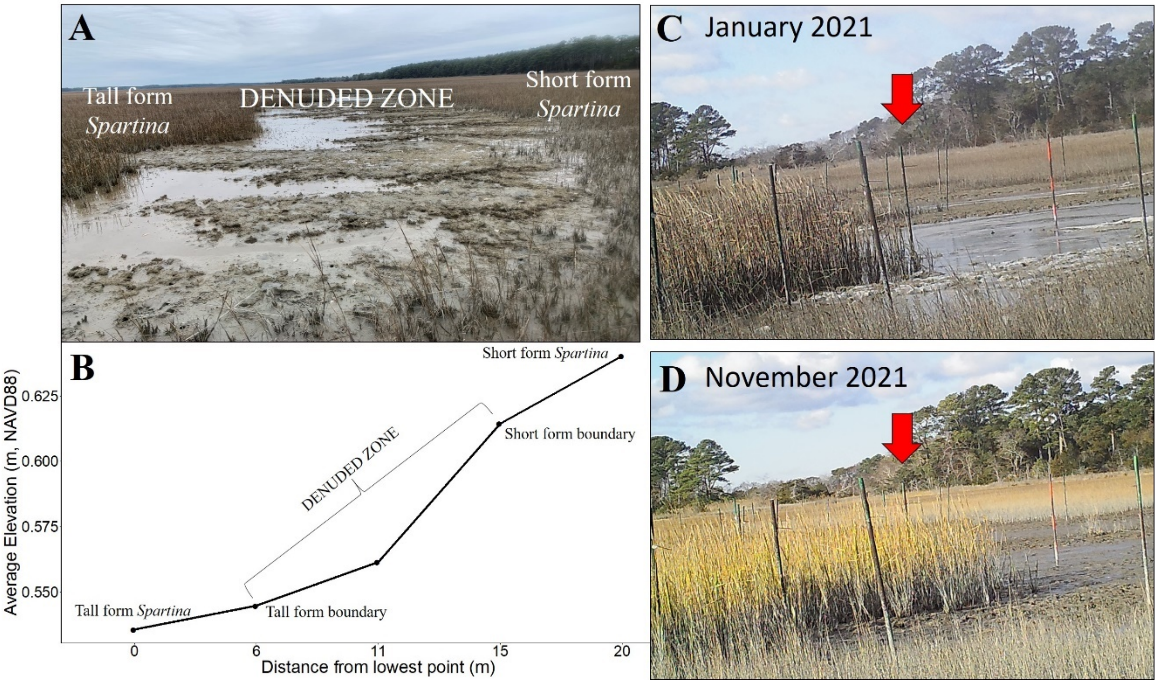

Figure 1. A) Cross-sectional photo of a *Sesarma* consumer front with zonation labels. B) Elevation profile showing average elevation in meters (NAVD88) by distance from the lowest point in meters. Labels indicate distinct zonation created by the *Sesarma* consumer front.

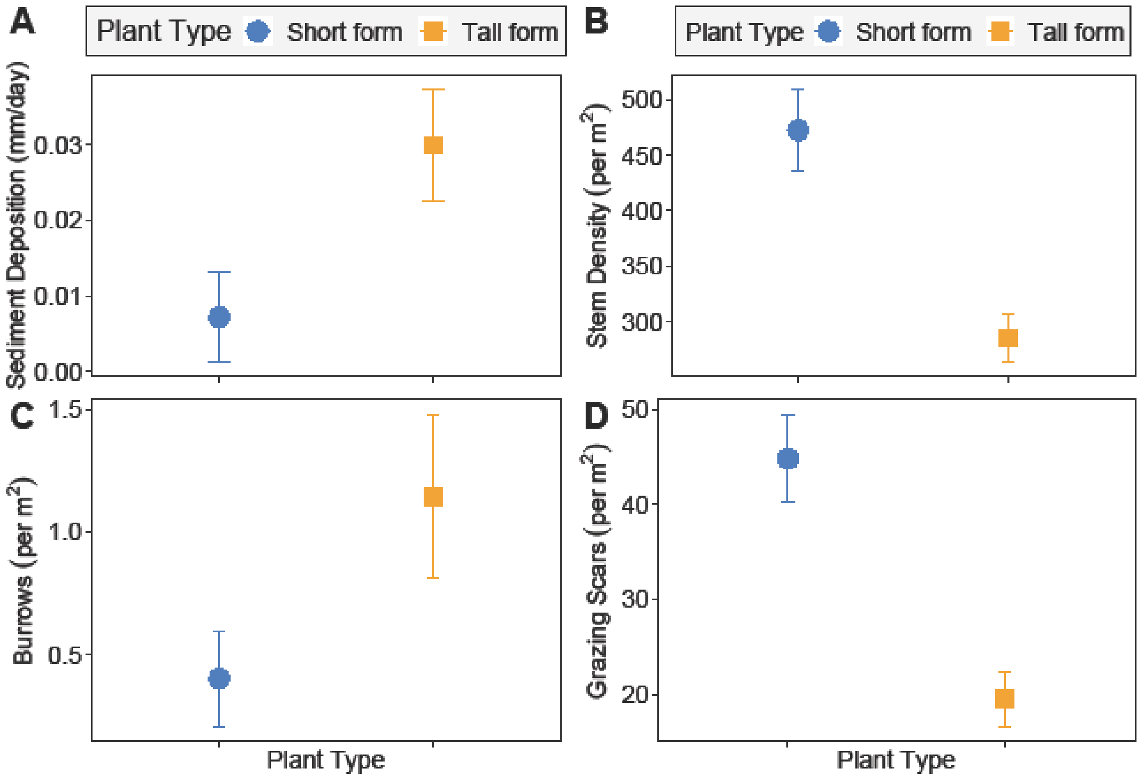

Figure 2. Average A) sediment deposition B) stem densities C) *Sesarma* burrows and D) *Sesarma* grazing scars per plant type (blue circles = short form *Spartina*, gold squares = tall form *Spartina*).

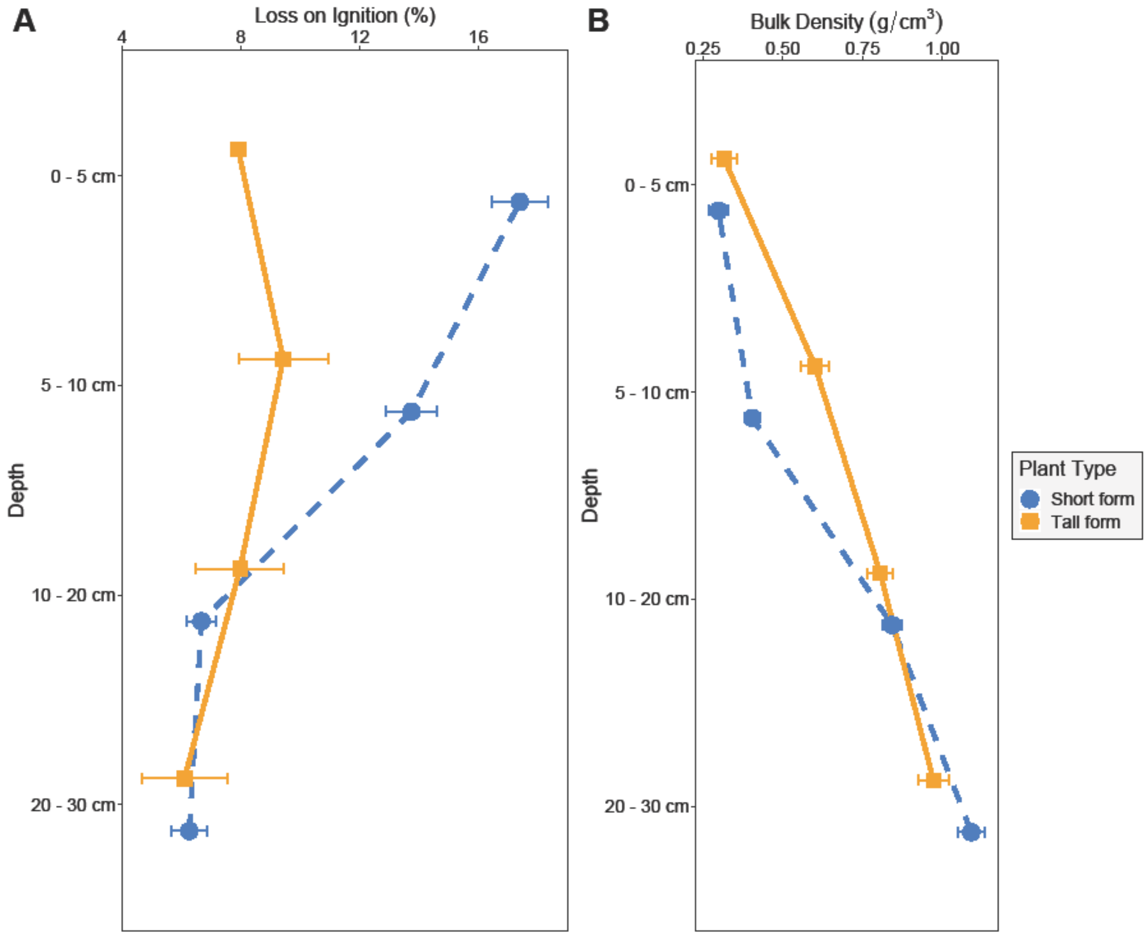

Figure 3. A) Loss on ignition ('LOI') and B) bulk density by plant type (blue circles, dashed line = short form *Spartina*; gold squares, solid line = tall form *Spartina*) by depth in centimeters.

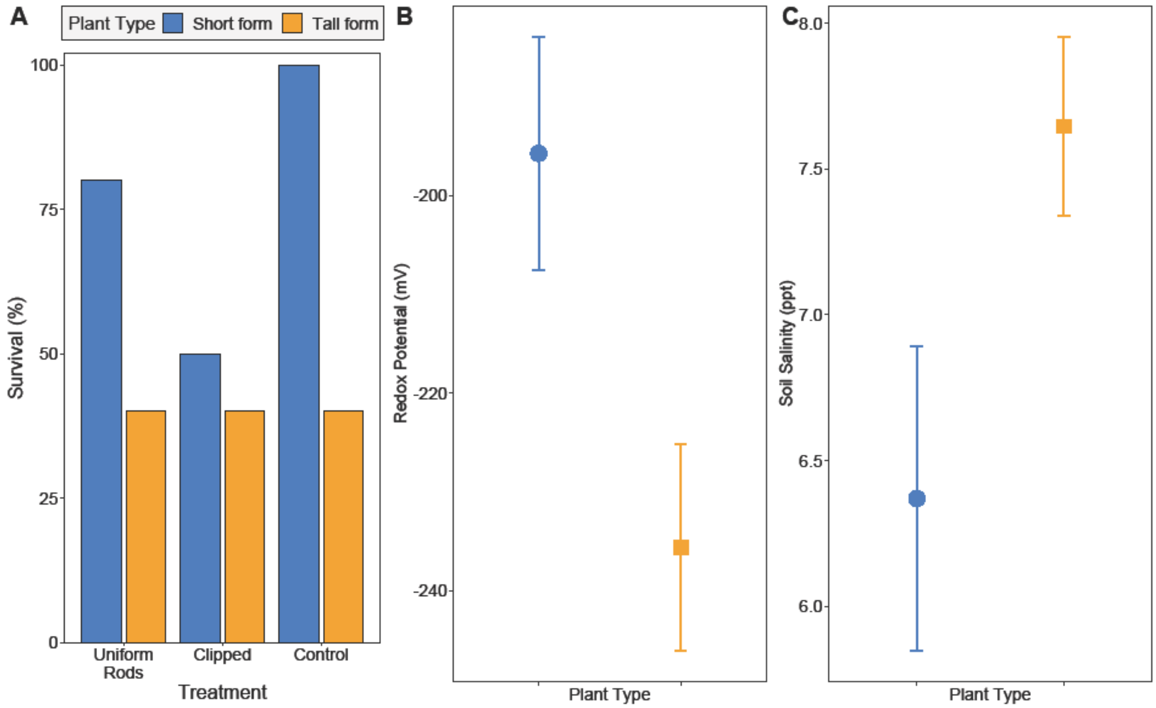

Figure 4. A) *Sesarma* percent survival across treatments (rods, clipped, control), B) redox potential and C) soil salinity by plant type (blue circles = short form *Spartina*, gold squares = tall form *Spartina*).

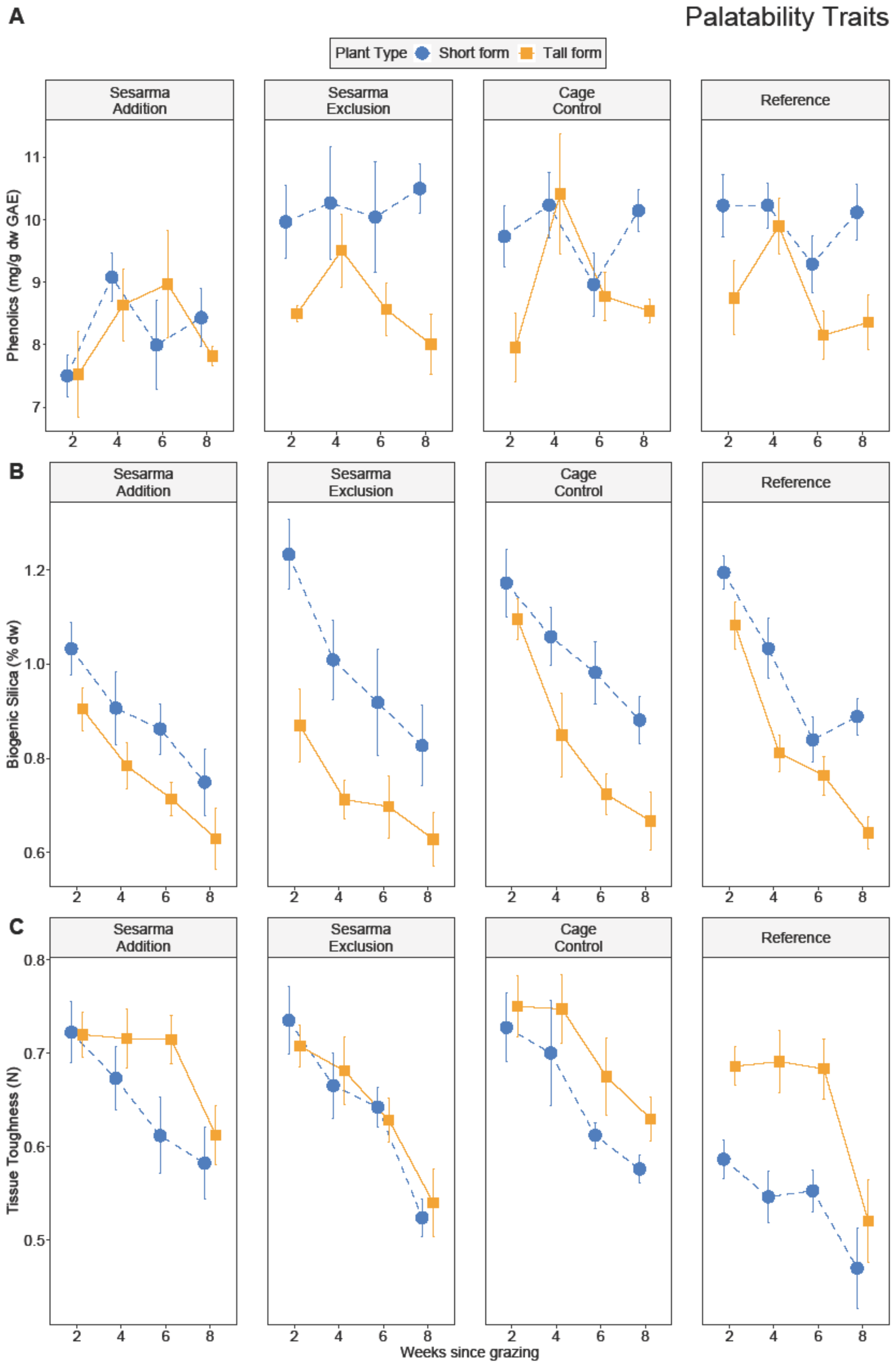
A
Palatability Traits
Plant Type ● Short form ■ Tall form
Sesarma Addition
Sesarma Exclusion
Cage Control
Reference
Phenolics (mg/g dw GAE)
11
10
9
8
7
2
4
6
8
B
Biogenic Silica (% dw)
1.2
1.0
0.8
0.6
C
Tissue Toughness (N)
0.8
0.7
0.6
0.5
Weeks since grazing

Figure 5. Average palatability traits: A) total phenolic concentrations, B) biogenic silica content, and C) tissue toughness measured at 2, 4, 6, and 8 weeks post *Sesarma* removal by plant type ((blue circles, dashed line = short form *Spartina*; gold squares, solid line = tall form *Spartina*).

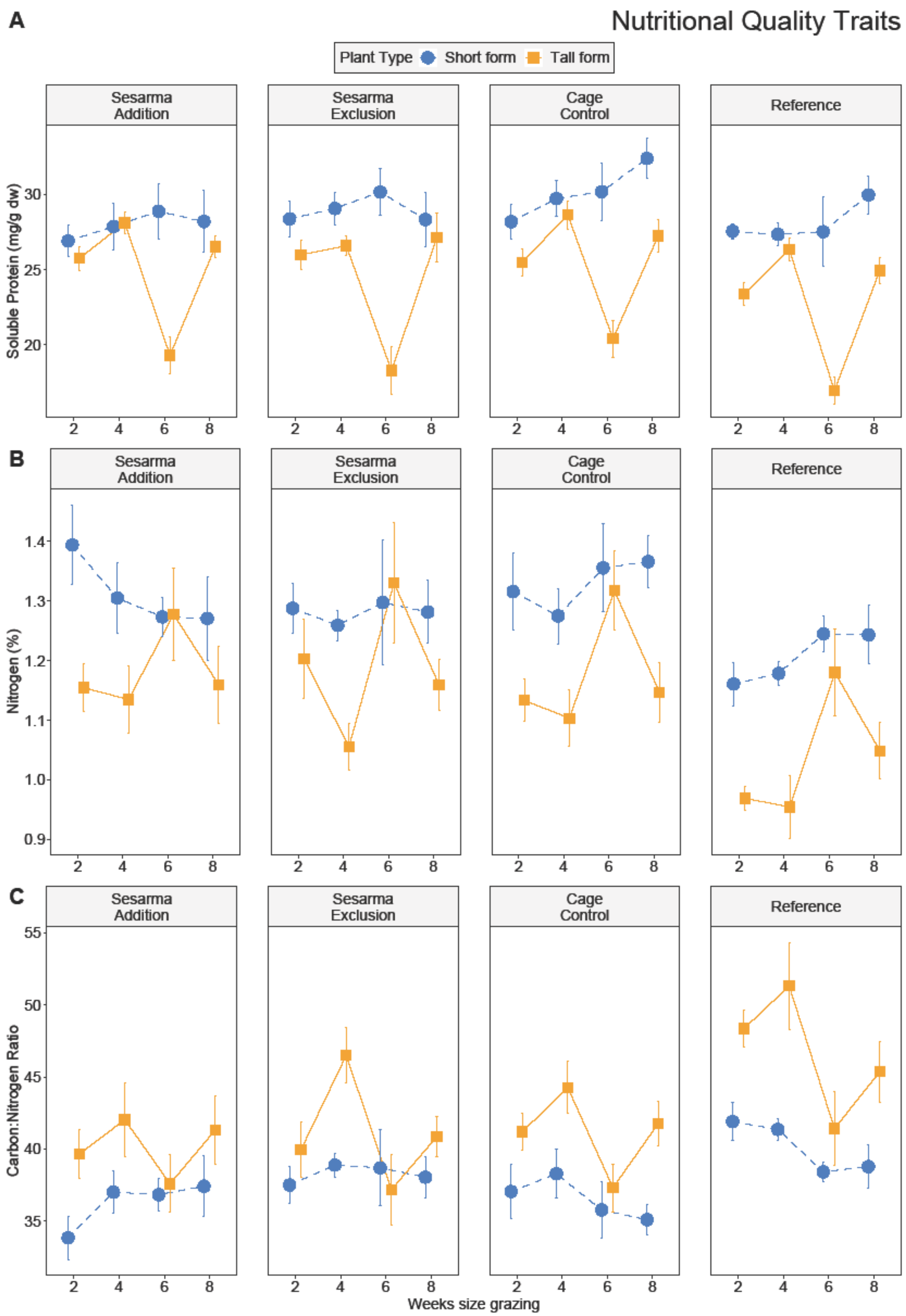
A
Nutritional Quality Traits
Plant Type ● Short form ■ Tall form
Sesarma Addition
Sesarma Exclusion
Cage Control
Reference
Soluble Protein (mg/g dw)
30
25
20
2
4
6
8
B
Nitrogen (%)
1.4
1.3
1.2
1.1
1.0
0.9
C
Carbon:Nitrogen Ratio
55
50
45
40
35
Weeks size grazing

Figure 6. Average nutritional quality traits: A) total soluble protein content, B) nitrogen content, and C) C:N ratio measured at 2, 4, 6, and 8 weeks post *Sesarma* removal by plant type ((blue circles, dashed line = short form *Spartina*; gold squares, solid line = tall form *Spartina*).

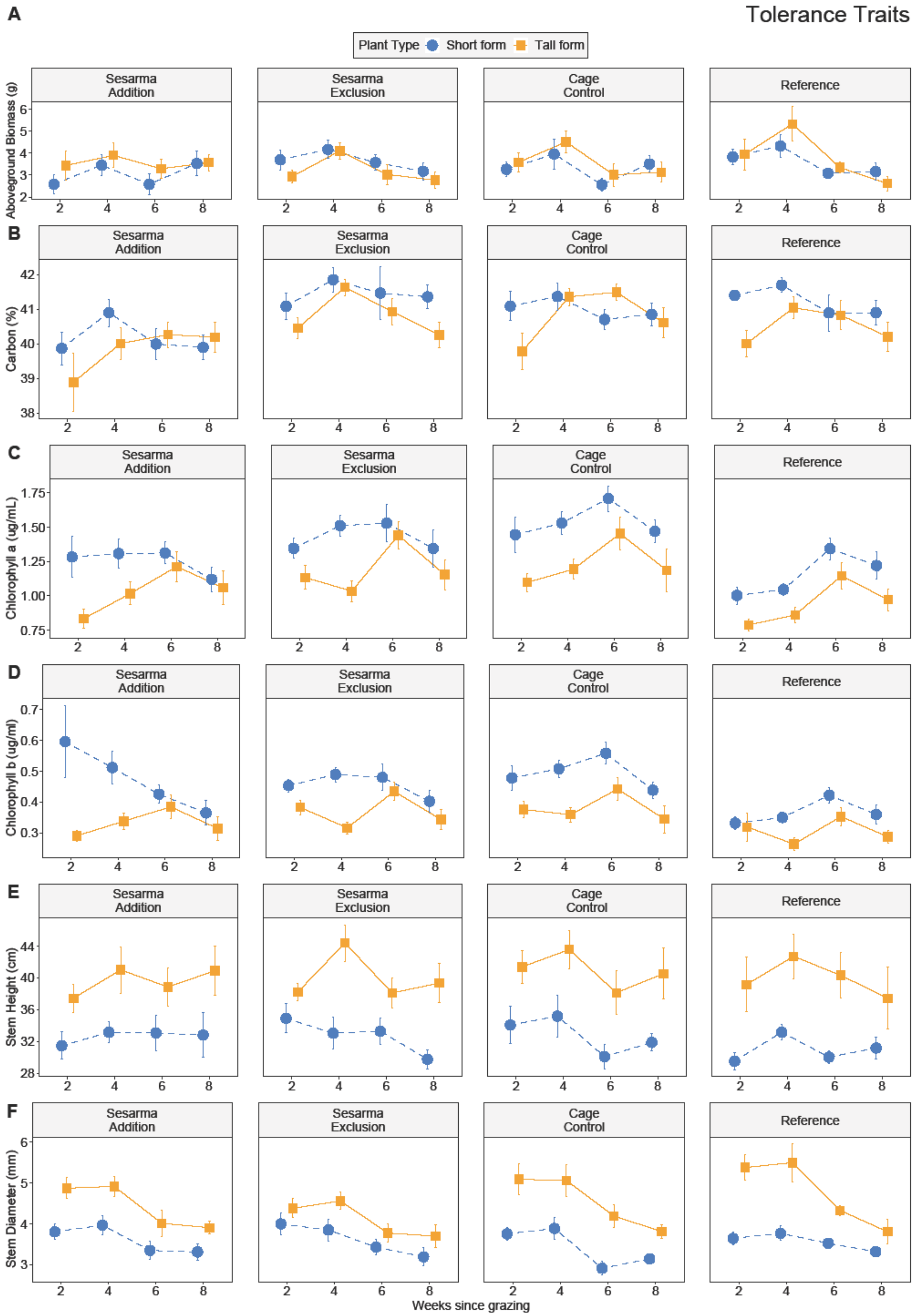
A
Tolerance Traits
Plant Type ● Short form ■ Tall form
Sesarma Addition
Sesarma Exclusion
Cage Control
Reference
Aboveground Biomass (g)
2 3 4 5 6
B
Carbon (%)
38 39 40 41 42
C
Chlorophyll a (ug/mL)
0.75 1.00 1.25 1.50 1.75
D
Chlorophyll b (ug/ml)
0.3 0.4 0.5 0.6 0.7
E
Stem Height (cm)
28 32 36 40 44
F
Stem Diameter (mm)
3 4 5 6
2 4 6 8
Weeks since grazing

Figure 7. Average tolerance traits: A) aboveground biomass, B) carbon content, and C) chlorophyll *a* concentration, D) chlorophyll *b* concentration, E) *Spartina* stem height, and F) *Spartina* stem diameter measured at 2, 4, 6, and 8 weeks post *Sesarma* removal by plant type ((blue circles, dashed line = short form *Spartina*; gold squares, solid line = tall form *Spartina*).

APPENDIX B

This appendix includes additional methods and results from the caging experiment in Chapter IV. This study's primary goal was to assess the effects of *Sesarma* grazing on *Spartina* traits. These additional data were collected to evaluate if *Sesarma* (herbivory and/or burrowing activities) had any other effects on the invertebrate community, *Spartina* above- and belowground biomass, frontal area, redox potential, benthic diatoms, cyanobacteria, and green algae, sediment bulk density, organic matter, and decomposition rates. However, due to the experimental design in which *Sesarma* were removed from plots after only 3 months, the findings for each of these variables are not ecologically relevant, thus they will not be included in the final manuscript.

Appendix B - Materials & Methods

Caging Methods

Experimental Design

At the center of each treatment plot, a 0.0625 m^2 quadrat was demarcated with wooden dowels. Because this center quadrat was most likely the least disturbed within each plot (i.e., no edge effects from cage installation), it served as a standardized collection area for final measurements detailed below.

During cage installation, heat-sealed decomposition bags made of 5-micron Nitex mesh containing dried roots and rhizomes were inserted to a depth of approximately 10 cm in each plot and marked with a pin flag. Roots and rhizomes corresponded with the *Spartina* zone they were placed in (i.e., tall-form plots received tall-form roots and rhizomes and vice versa).

Experimental Breakdown and Final Collections

The experiment ended in September of 2021, five months after it began. At each plot, the total number of fiddler burrows, *Geukensia demissa*, and *Littoraria irrorata* were counted and recorded. In the previously marked center quadrat, live and dead *Spartina* stem densities were counted and recorded, and all aboveground biomass was removed and placed in a resealable plastic bag to be dried. Frontal area (a proxy for sediment capture ability) was then calculated as the average stem diameter multiplied by average stem height and total stem density divided by area (Lu et al. 2019). A handheld oxidation-reduction/pH (ORP) meter measured redox potential (Hanna Instruments), and a benthotorch (bbe moldaenke, Germany) measured diatom, cyanobacteria, and green algae concentrations at the sediment surface. A shear vane (Humboldt Manufacturing) measured sediment shear strength at three depths: 10 cm, 20 cm, and 30 cm. We then took a 30-cm deep core and sectioned it into four depths: 0-5 cm, 5-10 cm, 10-20 cm, and 20-30 to be dried and measured for bulk density and organic matter content.

Decomposition bags were removed, and placed in clean, individual plastic bags to be dried. Two stems of the final composite sample of *Spartina* (for plant trait analysis) were randomly selected, the aboveground biomass was removed, and a 30 cm core (7.62 cm diameter aluminum corer) was taken to sample belowground biomass and to calculate root:shoot ratios. Belowground biomass cores were rinsed in outdoor sieves to remove all sediments. All biomass (above- and belowground) and decomposition bags were dried in drying ovens at 60°C until they reached a constant mass.

Appendix B - Results

The number of fiddler crab (*Minuca pugnax*) burrows did not differ between treatments (p=0.848; Fig. A1A) or plant type (p=0.645; Fig. A1A). There were more mussels in short form zones than tall form zones (p<0.001; Fig. A1B), and this did not vary by treatment (p=0.145; Fig. A1B). Lastly, there were more periwinkle snails in tall form zones than short form zones (p=0.001; Fig. A1C), except in reference plots.

Short form zones had more belowground biomass (p<0.001; Fig. A2A) and thus higher root:shoot ratios (p<0.001; Fig. A2B) than tall form zones regardless of treatment (p=0.795 and p=0.906, respectively). Caging had an effect on live stem densities in both short and tall form zones (p=0.005; Fig. A2C), with references having greater densities than all other treatments. Plant zone and treatment had no effects on frontal area (p=0.091; Fig. A2D), or the number of dead stems (p=0.179; Fig. A2E), and flowers produced (p=0.363; Fig. A2F).

Redox potential did not vary by treatment (p=0.798; Fig. A3A) or by plant zone (p=0.120; Fig. A3A). Similarly, treatment and plant zone did not affect diatom (p=0.785, p=0.530; Fig. A3B) or green algae concentrations (p=0.409, p=0.361; Fig. A3D). Cyanobacteria concentrations were comparable between treatments in tall form zones (p=0.066; Fig. A3C), however, reference plots in the short form zone had concentrations ~75% higher than all other treatments (p=0.013; Fig. A3C).

SOM was higher in short form zones (p<0.001; Fig. A4A) at depths shallower than 20 cm (p<0.001; Fig. A4A). There was no effect of treatment on SOM (p=0.231; Fig. A4A). Shear strength was also higher in short form zones (p=0.036; Fig. A4C), and in *Sesarma* exclusion plots (p=0.033; Fig. A4C). In contrast, bulk density was higher in tall form zones (p<0.001; Fig. A4B) at shallow depths (< 20 cm) and gained in compaction at depths greater than 20 cm (p<0.001; Fig. A4B). Decomposition rates were unaffected by treatment (p=0.936; Fig. A5) and plant zone (p=0.079; Fig. A5).

References

Lu, M., Herbert, E.R., Langley, J.A., Kirwan, M.L., and Megonigal, J.P. 2019. Nitrogen status regulates morphological adaptation of marsh plants to elevated CO_2. *Nature Climate Change*, doi: 10.1038/s41558-019-0582-x.

Figures

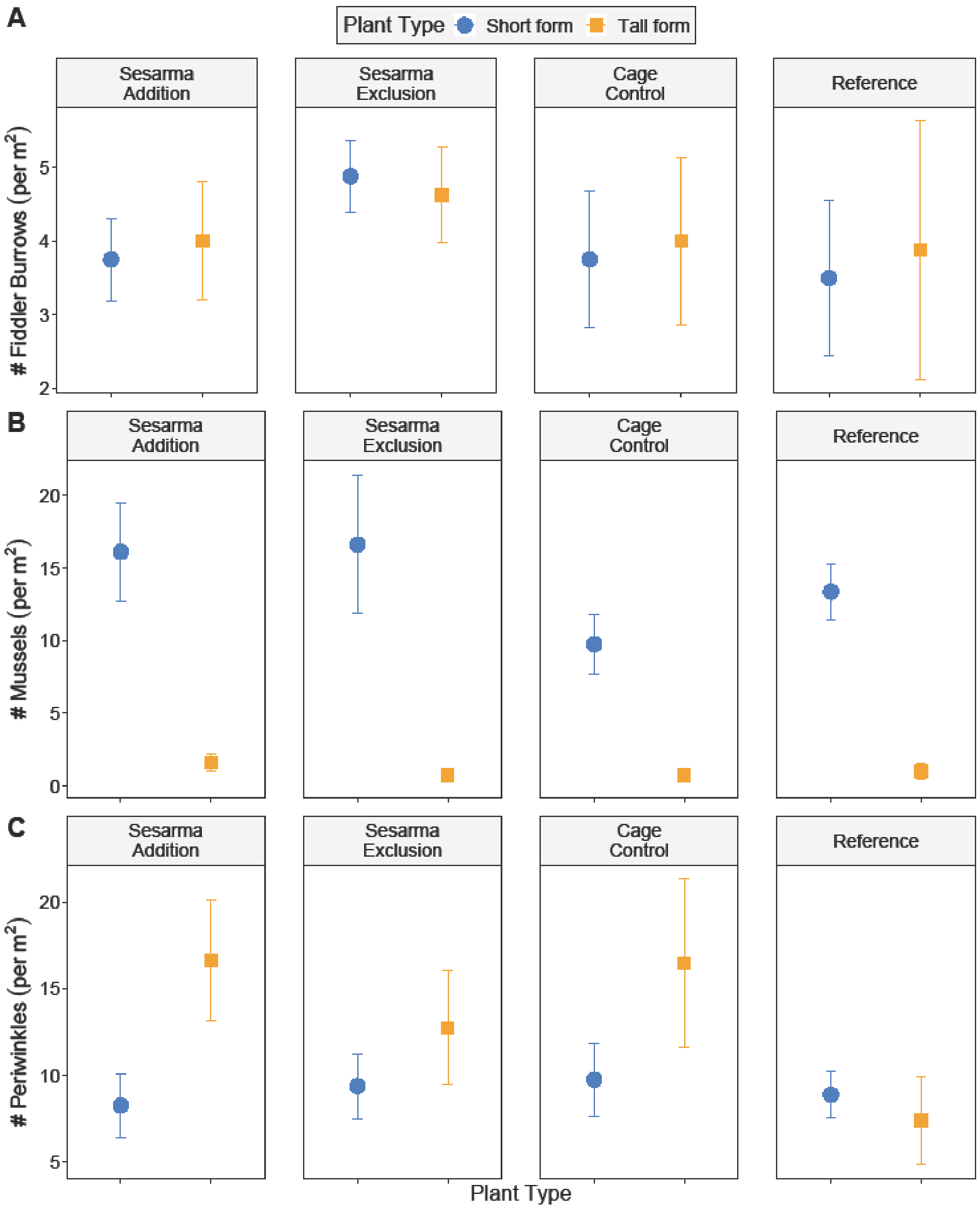

Figure B1. Average densities per meter squared of A) fiddler crab (*Minuca pugnax*) burrows, B) mussels (*Geukensia demissa*), and C) periwinkle snails (*Littoraria irrorata*) counted at the end of the caging experiment by caging treatment (*Sesarma* addition, *Sesarma* exclusion, Cage Control, and Reference) and by plant type (blue circles = short form *Spartina*; gold squares = tall form *Spartina*).

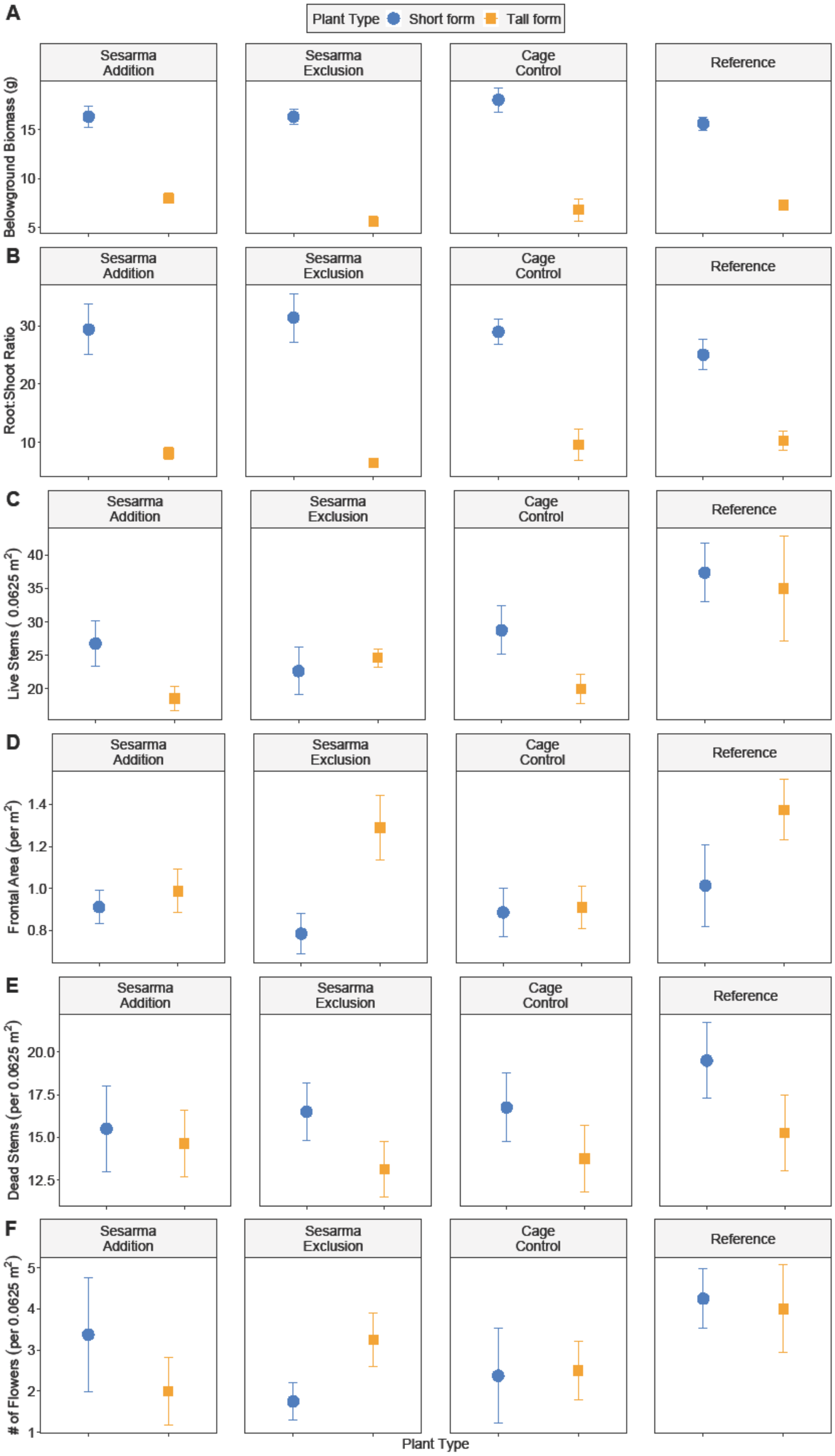
A
B
C
D
E
F
Plant Type ● Short form ■ Tall form
Sesarma Addition
Sesarma Exclusion
Cage Control
Reference
Belowground Biomass (g)
5
10
15
Root:Shoot Ratio
10
20
30
Live Stems (0.0625 m^2)
20
25
30
35
40
Frontal Area (per m^2)
0.8
1.0
1.2
1.4
Dead Stems (per 0.0625 m^2)
12.5
15.0
17.5
20.0
# of Flowers (per 0.0625 m^2)
1
2
3
4
5
Plant Type

Figure B2. Average A) belowground biomass, B) root:shoot ratios, C) live stem density, D) frontal area, E) dead stem density, and F) number of flowering stems at the end of the caging experiment by caging treatment (*Sesarma* addition, *Sesarma* exclusion, Cage Control, and Reference) and by plant type (blue circles = short form *Spartina*; gold squares = tall form *Spartina*).

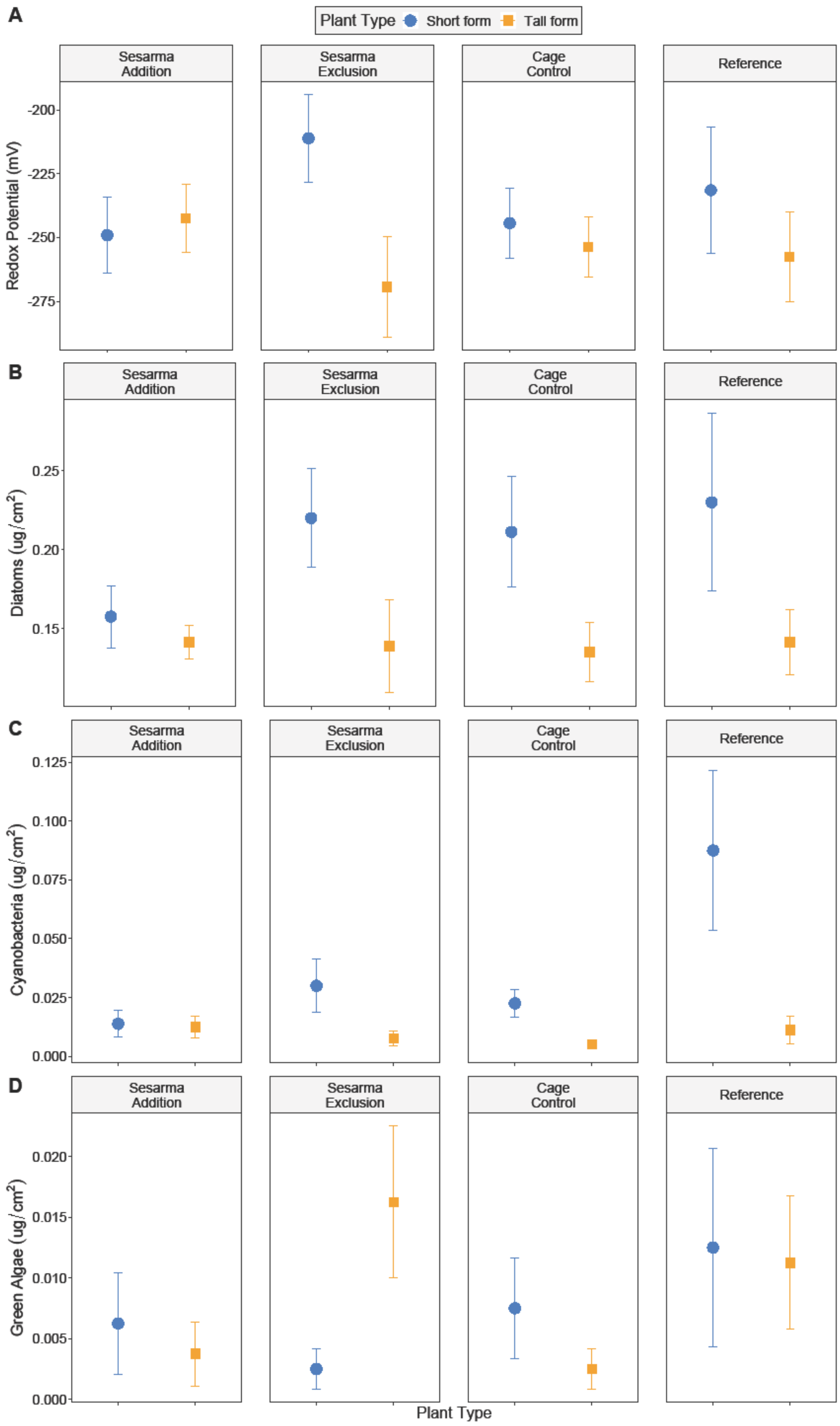
A
Plant Type ● Short form ■ Tall form
Sesarma Addition
Sesarma Exclusion
Cage Control
Reference
Redox Potential (mV)
-200
-225
-250
-275
B
Sesarma Addition
Sesarma Exclusion
Cage Control
Reference
Diatoms (ug/cm^2)
0.25
0.20
0.15
C
Sesarma Addition
Sesarma Exclusion
Cage Control
Reference
Cyanobacteria (ug/cm^2)
0.125
0.100
0.075
0.050
0.025
0.000
D
Sesarma Addition
Sesarma Exclusion
Cage Control
Reference
Green Algae (ug/cm^2)
0.020
0.015
0.010
0.005
0.000
Plant Type

Figure B3. Average A) redox potential, B) diatom concentrations, C) cyanobacteria concentrations, and D) green algae concentrations at the end of the caging experiment by caging treatment (*Sesarma* addition, *Sesarma* exclusion, Cage Control, and Reference) and by plant type (blue circles = short form *Spartina*; gold squares = tall form *Spartina*).

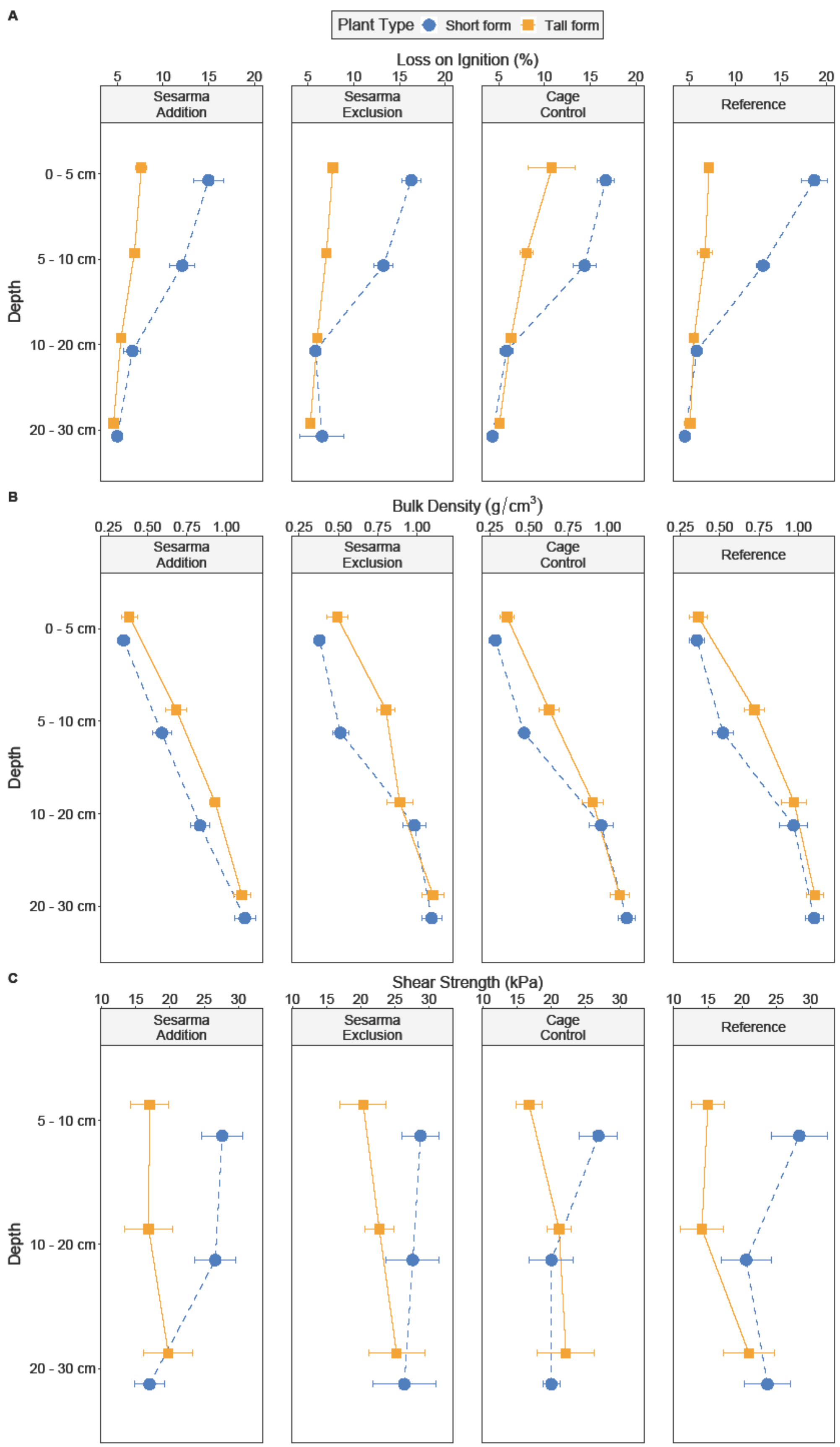
A
Plant Type ● Short form ■ Tall form
Loss on Ignition (%)
5 10 15 20
Sesarma Addition
Sesarma Exclusion
Cage Control
Reference
Depth
0 - 5 cm
5 - 10 cm
10 - 20 cm
20 - 30 cm
B
Bulk Density (g/cm3)
0.25 0.50 0.75 1.00
Sesarma Addition
Sesarma Exclusion
Cage Control
Reference
Depth
0 - 5 cm
5 - 10 cm
10 - 20 cm
20 - 30 cm
C
Shear Strength (kPa)
10 15 20 25 30
Sesarma Addition
Sesarma Exclusion
Cage Control
Reference
Depth
5 - 10 cm
10 - 20 cm
20 - 30 cm

Figure B4. Average A) loss on ignition ('LOI'), B) bulk density, and C) sediment shear strength at the end of the caging experiment by caging treatment (*Sesarma* addition, *Sesarma* exclusion, Cage Control, and Reference), by depth, and by plant type (blue circles = short form *Spartina*; gold squares = tall form *Spartina*).

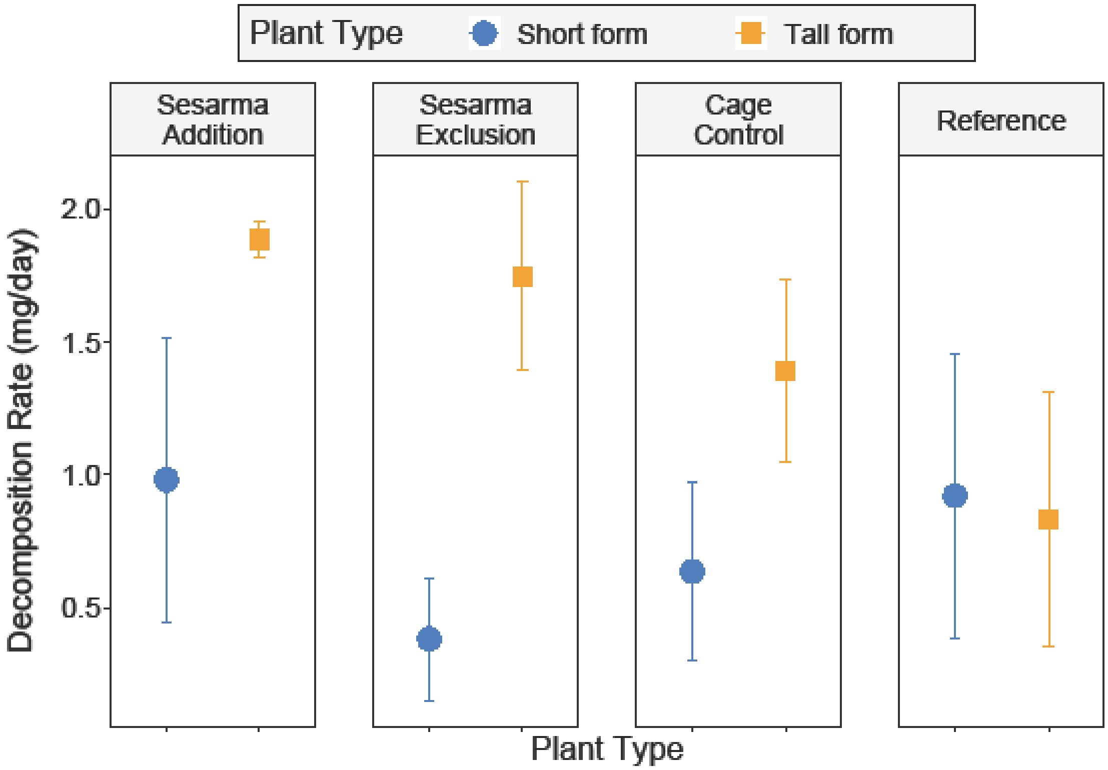

Figure B5. Average decomposition rate at the end of the caging experiment by caging treatment (*Sesarma* addition, *Sesarma* exclusion, Cage Control, and Reference) and by plant type (blue circles = short form *Spartina*; gold squares = tall form *Spartina*).

CHAPTER V

Conclusions

General Discussion

Plant defense theory and its associated hypotheses provide a foundation for evaluating biotic and abiotic controls on plant antiherbivore defenses (*reviewed in* Stamp 2003), as intense herbivory threatens plant communities across terrestrial and marine ecosystems. Understanding how plants defend themselves against their enemies is essential to predicting changes in ecosystem service provision and resilience to climate change and/or anthropogenic disturbances (Vergés et al. 2008; Hendricks et al. 2011; Di Bella et al. 2020). Yet, this suite of classic ecological theories remains understudied in coastal vegetated ecosystems, such as tidal marshes, which rely on plants as foundation species. The direct removal of plants by herbivores can shape ecosystem stability via declines in sediment deposition and organic matter accumulation, which are critical for building marsh elevation to keep pace with rising seas (Kirwan & Megonigal 2013). Hence, this dissertation first evaluated how abiotic and biotic factors (salinity and simulated herbivory via clipping: Chapter II; nutrient availability and *L. irrorata* grazing: Chapter III) associated with anthropogenic activities and climate change, shaped plant traits and defense response in the marsh foundation species, *Spartina alterniflora*. Then, it examined how *Spartina* traits and defenses influenced subsequent grazing (*L. irrorata*: Chapter III), and herbivore distribution (*S. reticulatum* consumer fronts: Chapter IV). This final chapter summarizes the primary findings of this dissertation, highlights their unique contribution to our current knowledge gaps, and offers potential future directions to continue this work.

Synthesis of Results

Climate-driven sea-level rise and changes to estuarine freshwater discharge are expected to increase the salinity of previously freshwater regions in estuaries (Neubauer et al. 2013;

Servais et al. 2020). One consequence of this is can be a conversion of diverse, freshwater marsh plant communities to monotypic stands of *Spartina* (Sutter et al. 2015). Yet, we know little about how *Spartina* traits are influenced by this increase in salinity, and how this may alter its defense against herbivores, which will most likely accompany saltwater intrusion (Geddes & Mopper 2006; Herbert et al. 2015). In general, *Spartina* collected from a brackish marsh was better defended (e.g., higher carbon content, C:N, and belowground biomass) than *Spartina* from a freshwater marsh, supporting the optimal-defense theory which posits that constitutive defense is positively correlated with the probability of herbivore attack (Rhoads 1979; Herms & Mattson 1992). Clipping with garden shears simulated a high rate of chronic herbivory which removed large quantities of aboveground biomass (i.e., clipped every other leaf to the ligule every two weeks). These treatments caused declines in new stem and belowground biomass production (tolerance traits), and increased nitrogen content (resistance trait), most likely because resources were allocated to mending remaining tissues rather than producing new growth. Salinity mediated trait variation in *Spartina*, as it was negatively correlated with tolerance traits (e.g., carbon content, C:N, and new stem biomass production). However, the magnitude of decline was determined by collection site, with salinity having a greater impact on freshwater *Spartina*, with little influence on brackish *Spartina*.

In salt marshes, nutrient enrichment can compromise marsh stability through biomass reallocation aboveground, which decreases sediment binding via reductions in belowground roots and rhizomes (Deegan et al. 2012). Plant antiherbivore defenses may also be influenced by eutrophication, as the resource-availability hypothesis (RAH) theorizes the tradeoff between plant defense strategies (resistance vs. tolerance) is driven by nutrient availability, with high nutrients facilitating a tolerance strategy, and low nutrients supporting a resistance strategy

(Coley et al. 1985; Endara & Coley 2011). Plant age can also influence trait variation and response to herbivores in terrestrial systems (e.g., Henn & Damschen 2021), yet this had never been tested in clonal plants, such as *Spartina*. I found support for the RAH, as nutrient enrichment decreased constitutive resistance traits (e.g., tissue fiber and silica content), while promoting tolerance traits (e.g., above, below, and new stem biomass production, number of leaves). Interestingly, herbivory from the marsh periwinkle, *L. irrorata*, did not influence resistance traits, but rather amplified tolerance traits (e.g., reallocated biomass belowground, produced thicker stems). When combined, herbivory and nutrient addition increased tolerance traits (e.g., belowground and new stem biomass), while having mixed effects on resistance traits (e.g., higher nitrogen, lower phenolics). Additionally, newer stems had greater trait variation and had higher tolerance traits (e.g., chloropyll *a*, *b*, total) and resistance traits (e.g., tissue nitrogen, protein content). Cumulatively, nutrient enrichment and herbivory should have enhanced *Spartina* palatability of both original and clonally grown new stems through decreased chemical resistance and elevated nitrogen content. However, *L. irrrorata* grazing did not differ between treatments or plant age.

In chapters II and III I examined how abiotic and biotic factors can influence *Spartina* traits and defense strategy. In Chapter IV, I built on these previous findings by examining how altered traits and defenses shaped herbivore distribution and movement in salt marshes. Previous work suggested that sediment characteristics (Bertness et al. 2014), abiotic conditions (Vu & Pennings 2021), and predation pressure (Altieri et al. 2012) were responsible for driving *S. reticulatum* front movement. Yet, despite their ability to shape consumer movement in terrestrial systems (e.g., Ohsaki et al. 2022), plant traits had never been examined as a potential additional driver of consumer front movement in salt marshes. Of the drivers tested, predation pressure and

Spartina palatability, nutritional quality, and accessibility were the only variables that differed between tall-form and short-form zones. Predation pressure was higher in tall-form zones, and short-form *Spartina* was more palatable (lower tissue toughness), nutritionally dense (higher nitrogen and protein), and easier to access (shorter, thinner stems) than tall-form *Spartina*. Caging results indicated that *Sesarma* grazing significantly lowered phenolics, biogenic silica, carbon, and chlorophyll content of short-form *Spartina*, while only affecting phenolic and carbon content of tall-form *Spartina*. This study was also unique in that it measured *Spartina* trait variation over time following *Sesarma* grazing and found that several *Spartina* traits rebounded to ambient levels shortly after grazing pressure was removed.

Contributions

Plant Defense Theory

I found support for each of the classic plant defense theories tested (growth-rate hypothesis, optimal-defense theory, resource-availability hypothesis). Except for clonally grown new stems (Chapter III), herbivory of any kind either increased tolerance traits *or* resistance traits (growth-rate hypothesis; Chapter II and III), supporting the hypothesis that these defenses are mutually exclusive. Results from Chapter II also supported the optimal-defense theory, as brackish *Spartina* had higher constitutive defenses than freshwater *Spartina*. Results from Chapter III confirmed that the resource-availability hypothesis can be applied to salt marshes, as nutrient enrichment promoted tolerance traits and reduced resistance traits. Although, in general, when applying the classic binary of tolerance versus resistance, I found little evidence that *Spartina* ever induced a resistance strategy in response to grazing, suggesting that either the salt marshes sampled had high enough resources to shift *Spartina* defense to a tolerance strategy (resource-availability hypothesis) or the incidence of herbivory wasn't high enough to cause *Spartina* to increase its constitutive resistance (optimal defense theory).

In some instances, the traditional dichotomy of tolerance versus resistance may not capture responses crucial to understanding plant-herbivore interactions. Historically, ecologists measured both nitrogen and protein content and classified them as resistance traits. This may have been because both nitrogen and amino acids form the foundation of some secondary metabolites responsible for antiherbivore defense (Koricheva et al. 1998), thus nitrogen and protein content may have served as a proxy. However, both nitrogen and protein content also increase the nutritional quality of plant tissues, perhaps making them more favorable to grazers, as herbivores often forage for protein- and nitrogen-rich food sources (Mattson 1980). Thus, Chapter IV re-classified the measured 'resistance traits' into two discrete categories: palatability traits and nutritional quality traits. Accordingly, chemical and structural variables (phenolics, silica, tissue toughness) were considered 'palatability traits', as they can directly influence an herbivore's ability to consume plant tissues (i.e., true resistance traits). In contrast, traits such as nitrogen, protein, and C:N were considered 'nutritional quality traits', as they can directly affect the energetic content of plant tissues. Thus, we would expect increases in palatability traits to deter herbivores, whereas increases in nutritional quality traits would stimulate herbivore consumption. This proposed re-classification provides additional insight into how plant traits can shape herbivore preference, consumption, and distribution in an ecosystem, increasing our ability to predict consumer impacts.

Lastly, in Chapter IV, I found that many traits rebounded to ambient levels within 1 month of *Sesarma* removal from cages. Ecologically, this suggests that plant trait plasticity may further facilitate salt marsh resilience. Methodologically, this challenges classic trait-based approaches, which typically only evaluate traits at a single time point at the end of an experiment.

Marsh Resilience

Overall, my results indicate that climate-driven salinity intrusion and anthropogenic eutrophication influence how *Spartina* responds to herbivory, with implications for marsh resilience to sea-level rise. As saltwater moves up estuaries, brackish marshes dominated by *Spartina* may see little to no impact, whereas increased herbivory and salinity may have compounded, negative effects on freshwater *Spartina* traits and thus marsh stability. Although increased nutrient availability, herbivory, and plant age all altered *Spartina* traits, *L. irrorata* consumption did not vary between treatments, suggesting that nutrient enrichment may not always increase top-down control on *Spartina* as previously thought (Silliman & Zieman 2001; Sala et al. 2008). In fact, eutrophication may positively benefit salt marshes in some cases by promoting tolerance traits (e.g., higher belowground and new stem biomass production) which increase vertical accretion capacity (Graham & Mendelssohn 2014), although there is a threshold beyond which nutrient loading will have deleterious effects (Deegan et al. 2012). Interestingly, clonally grown new stems had higher tolerance and resistance traits, suggesting that there is higher investment in defending new growth rather than existing stems.

My findings also suggest that grazing intensity mediates the magnitude of *Spartina* trait variation and defense response, as seen in a recent study (Hernán et al. 2021). For example, simulated herbivory via clipping removed the largest amount of *Spartina* aboveground biomass, and rather than inducing a defense response, had negative effects on both tolerance and resistance traits (Chapter II). In contrast, grazing from *L. irrorata* removed the least amount of aboveground biomass, thus *Spartina* was able to induce a defense response which varied depending on resource availability (Chapter III). Lastly, *S. reticulatum* grazing removed an intermediate amount of aboveground biomass, while also impacting belowground biomass, which caused declines in resistance and tolerance traits (Chapter IV), albeit different traits than those impacted in Chapter II. If climate change continues to create favorable conditions for herbivore reproduction and population growth (Crotty et al. 2020), then grazing activities may ultimately outpace *Spartina* defenses.

In addition to predation pressure, I discovered that *Spartina* traits indirectly facilitated marsh resilience by driving *S. reticulatum* consumer fronts inland, allowing tall-form *Spartina* to revegetate and potentially prevent or slow marsh loss. Short-form *Spartina* in the high marsh was of higher forage quality and less constitutively defended than the tall-form *Spartina*, attracting *Sesarma* inland towards the more energetically favorable food source. *Sesarma reticulatum* grazing negatively impacted short-form *Spartina* traits, presenting a unique mechanism in which herbivory increased short-form palatability (e.g., lower chemical and structural defenses) and diminished its ability to tolerate damage (e.g., lower carbon content and photosynthetic capacity), further driving the consumer front inland.

Together, in marshes suffering from intense herbivory pressure from multiple grazers, we would predict declines in *Spartina* traits with potential negative feedback to vertical accretion and thus marsh stability. However, despite consumer fronts which denude large spatial areas, marshes persist. Ultimately, patchiness in herbivore density and grazing pressure, driven by resource availability and plant forage quality and constrained by abiotic and biotic factors, may allow *Spartina* to recover and continue to vertically accrete, preventing marsh loss.

Future Directions

Each of my chapters used a different herbivore (Chapter II: simulated herbivory via clipping; Chapter III: marsh periwinkle, *Littoraria irrorata*; Chapter IV: purple marsh crab, *Sesearma reticulatum*) to elicit trait responses in *Spartina*. Further, each of these herbivores has a unique method of grazing (clipping versus scraping versus shredding) and my results indicate that they influenced *Spartina* traits differently. Future studies should test how multiple types of herbivore damage can alter plant traits to better understand how marshes respond under different grazing regimes.

Chapter IV found that plant traits induced by herbivory were plastic, and often rebounded within one month after grazing ceased. This chapter further revealed that temporal variation in plant traits is high, yet trait-based approaches rarely take this into account. Thus, factors such as seasonality of herbivory pressure and timing of flowering can further influence plant traits and should be explicitly tested.

Concluding Remarks

This dissertation builds on previous work and hypotheses critical to the development of plant trait ecology. Overall, *Spartina* is not defenseless against its herbivores. As climate change alters marsh plant communities, herbivory pressure, and abiotic conditions, we can expect *Spartina* to adapt and be resilient in many instances. I hope the information presented in this dissertation can contribute to the growing body of work evaluating marsh resilience under varying anthropogenic disturbance and future climate change scenarios.

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