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WATER COLUMN AMMONIUM CONCENTRATION AND SALINITY INFLUENCE NITROGEN UPTAKE AND GROWTH OF *SPARTINA ALTERNIFLORA*

by

RACHEL MACTAVISH

(Under the Direction of Risa A. Cohen)

ABSTRACT

Salt marsh macrophytes, such as *Spartina alterniflora,* play a critical role in uptake and transformation of inorganic nitrogen before it reaches coastal waters, thereby reducing the potential for eutrophication. Although nitrogen availability typically limits *S. alterniflora* growth, it may be possible to exceed the nitrogen uptake capacity of *S. alterniflora.* Increasing either nitrogen concentrations or salinity are key factors regulating *S. alterniflora* nitrogen uptake. Investigating the effects of nutrients and salinity on *S. alterniflora* is important given that increases in inorganic nitrogen supply to surface waters from agriculture and urbanization occur simultaneously with freshwater withdrawals that reduce flow and increase salinity. *Spartina alterniflora* nitrogen uptake in response to increasing inorganic nitrogen (ammonium, NH_4^+) (0, 10, and 100 µM), and salinity (20, 30, and 40 psu) treatments in a fully crossed factorial design were measured in greenhouse microcosms with tidal simulation in Statesboro, GA from April-October, 2013. Prior to the factorial study, a three month pilot study comparing *S. alterniflora* growth in novel tidal simulator design and salt marsh field plots revealed tidal simulation did not affect plant height, stem density, or above and belowground biomass. After 48 hours the highest water column NH_4^+ uptake occurred at the lowest salinity (20 psu) and highest ammonium concentrations (100 µM) tested. After 6 months of NH_4^+-^{15}N additions, above and belowground *S. alterniflora* plant tissue $\delta^{15}N$ increased proportionally with NH_4^+

additions and was reduced by 50% with salinity increases from 20 to 40 psu across all NH_4^+ addition levels. Furthermore, *S. alterniflora* above and belowground biomass and main shoot height was reduced with increasing salinity from 20 to 40 psu and not significantly affected increasing NH_4^+ additions. However, at high salinity (40 psu) biomass reductions were mitigated by intermediate (10 µM) NH_4^+ additions by a 50% increase over 0 and 100 µM NH_4^+ additions. Stem density and main shoot height measured weekly also reflected mitigation by intermediate (10 µM) NH_4^+ additions at elevated salinity. That *S. alterniflora* nitrogen uptake and biomass decrease with increasing water column salinity suggests alteration of coastal salinity may reduce nitrogen uptake capacities of *S. alterniflora* dominated salt marshes. Thus estuarine water column salinity should be considered when regulating inorganic nitrogen loads in aimed at conserving salt marsh nutrient retention.

INDEX WORDS: Ammonium, Greenhouse, Microcosm, Nitrogen, Retention, Salinity, Salt marsh, *Spartina alterniflora,* Tidal simulator, Uptake

WATER COLUMN AMMONIUM CONCENTRATION AND SALINITY INFLUENCE NITROGEN UPTAKE AND GROWTH OF *SPARTINA ALTERNIFLORA*

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by

Rachel MacTavish

Major Professor:	Risa A. Cohen
Committee:	Daniel F. Gleason
	Christine Hladik

Electronic Version Approved:
July 2014

DEDICATION

I would like to dedicate this thesis to my son, Luke MacTavish. You listened to all the crazy science and were happy to lend a hand in the field and greenhouse anytime I asked. The smile on your face kept me going when graduate school was challenging. We made some incredible memories during this process like fishing on Sapelo Island, GA for the "one that got away." Thanks for being the best research assistant ever! I love you.

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CHAPTER 1

INTRODUCTION

Surface water in streams and runoff deliver nutrients, primarily in the form of inorganic nitrogen (N) from inland sources to coastal areas (Reddy, 2008; Weston et al., 2009; Danielescu and MacQuarrie, 2011). Typically salt marsh plants, such as *Spartina alterniflora*, take up available N and incorporate it into new plant tissue, thereby trapping N within the marsh (Valiela and Cole, 2002; Cole et al., 2004). However, increases in agricultural fertilizer usage and urban wastes have doubled the supply of inorganic nitrogen to coastal areas in the past century (Vitousek et al., 1997). Excess inorganic N may surpass *S. alterniflora* uptake capacity resulting in higher N input to adjacent coastal waters (Drake et al., 2009; Brin et al., 2010). Surplus nutrients in coastal waters can stimulate algal growth decreasing the amount of light and oxygen necessary for marine organisms to thrive (Oswald and Golueke, 1966; Nixon 1995). In addition to altering N supply, humans restrict fresh water flow to the coast by withdrawing water and damming rivers, thereby altering the salt content in the water (salinity) at the coast (Dame et al., 2000; Flemer and Champ, 2006). Salinity can be increased further by increased temperature and reduced rainfall due to drought and other types of climate change (Scavia et al., 2006). Increasing salinity prevents N from entering plant tissue (Bradley and Morris, 1991), decreasing growth and diminishing *S. alterniflora*'s N retention ability (Haines and Dunn, 1976; Linhurst and Seneca, 1981). Therefore increasing both nitrogen and salinity concentrations in the water simultaneously has the potential to exceed the ability of *S. alterniflora* to filter N from the water at the land and sea interface, resulting in increased N export to adjacent coastal waters.

As increases in salinity and N occur due to human development of coastal areas, it is important to understand how multiple environmental stressors work together to affect N uptake and retention by salt marsh ecosystems. By addressing the effects of both nutrient additions and decreased freshwater availability experimentally, I have shown the potential for reduced efficacy of *S. alterniflora* nutrient retention in response to environmental changes faced by coastal systems globally. Information from my study may ultimately be used to inform management decisions regarding limits on N discharge into streams and rivers to reduce the risk of poor coastal water quality.

CHAPTER 2

A SIMPLE AND INEXPENSIVE TIDAL SIMULATOR FOR *Spartina alterniflora* GREENHOUSE EXPERIMENTS

Abstract

Coastal environments that experience tidal fluctuations are difficult to recreate in order to conduct controlled experiments on salt marsh plants. Greenhouse microcosms with no tidal simulation (waterlogged) are commonly used to apply treatments and measure response variables that can be redistributed by tidal ebb and flow. However, waterlogging salt marshes can reduce plant growth and associated ecosystem function. I designed a simple, inexpensive tidal simulator microcosm system that can be used with high numbers of replicates. I hypothesized that *Spartina alterniflora* growth in my tidal simulation systems would be similar to that of plants in tidally influenced plots in the field. After three months of exposure to tidal treatments, plants in the tidal simulators had similar stem density, plant height, and above and belowground biomass to plants in field plots. The tidal simulator I developed is an inexpensive and effective way to conduct studies on *Spartina alterniflora* marsh ecosystems and minimize the negative effects of waterlogging.

Introduction

Simulating tides of *Spartina alterniflora* salt marsh ecosystems in greenhouse microcosm experiments is challenging. Kitchens (1979) suggested that living tidal replicators are necessary for researchers to predict the response of tidally influenced systems to constantly increasing environmental pressures. Cord grasses in the genus *Spartina* are considered foundational species tightly linked to infauna abundance (Brusati and Grosholz, 2006) and distribution of endangered

salt marsh species such as the Light-Footed Rail clapper (Boyer and Zedler, 1999) making plant health an important factor when conducting salt marsh microcosm experiments. Plant, microbe, infauna, and water column constituent responses are often tested in greenhouse microcosm studies to investigate the effects of a wide variety of factors on salt marshes including nutrients and contaminants such as oil and heavy metals (Chambers, 1992; Millward et al., 2001, 2004; Lindgren et al., 2012). However, typical greenhouse microcosm studies are conducted by continuously submerging tidal plant roots and sediments with water at or slightly above the sediment interface for durations of up to five months (Phleger, 1971; Linthurst and Seneca, 1981; Crain et al., 2004; Craft et al., 2009; Hessini et al., 2009; Touchette et al., 2009; Merino et al., 2010; Xiao et al., 2011). Prolonged waterlogged conditions can increase anaerobic root metabolism leading to increased hydrogen sulfide concentrations and overall reductions in salt marsh plant growth (Gleason et al., 1981; Mendelssohn and McKee, 1988; Naidoo et al., 1992; Portnoy and Valiela, 1997). Waterlogging sediments also changes redox zone distribution and associated microorganism production (Hines et al., 2006). In contrast, tidal action increases oxygen in the root zone, aiding both nitrogen uptake and salt marsh plant growth (Armstrong et al., 1985), therefore simulating tidal inundation can extend the duration and external validity of salt marsh ecosystem greenhouse microcosm studies by mitigating the negative effects of waterlogging.

The ability to conduct controlled experiments is particularly useful for examining how water quality influences *Spartina alterniflora* dominated salt marshes ecosystems. In the field, tidal fluctuations often complicate the application of water column treatments. For example, field manipulations of water column isotopic tracers may result in large losses due to tidal

flooding (Tobias et al., 2001; Drake et al., 2009). Gribsholt et al. (2005) recovered only ~30% of isotopically labeled ammonium during flood tide. Therefore, greenhouse microcosms with tidal simulation and high external validity would be particularly useful in studies relying on application of water column treatments.

Greenhouse microcosm tidal simulation designs can be bound to coastal locations, expensive, or elaborately designed restricting experimental locations and number of replicates. Tidal systems that use seawater are frequently bound to coastal facilities with existing plumbing infrastructure; Boyer and Fong (2005) designed a tidal simulator using drip irrigation in 2 L pots that extracted and returned seawater to the ocean at the Redando Beach Generating Station in Redando, CA. In addition, Cohen et al. (2009) replicated tides with seawater pumped from the San Francisco Estuary into a large tank (2.3 m^2) holding several smaller pots using a flow-through system that held seawater for 6 hours and then returned to the estuary. Tidal simulators constructed away from a seawater supply can be expensive or elaborate, making replication difficult. Gleason et al. (1981) developed an effective but expensive tidal replicator that incrementally transferred water from a reservoir to the experimental unit using an expensive programmable circuit board ($200 for each replicate in 1981) that connected to a complex series of sensors to turn off water pumps after depths of 1 cm were transferred in a programmed time frame. Spaulding and Hester (2007) developed a large and elaborate tidal simulation system to examine the role of salinity and flooding level on several tidal plant species. Potted plants were placed into 108 (200 L) mesocosms connected to 4 (3000 L) reservoirs with different salinity treatments, thus recirculating one salinity treatment water to 27 smaller 200 L mesocosms. Mixing water within salinity treatments may cross-contaminate smaller mesocosms and

confound experimental results. In addition, Ulrich and Sedlak (2010) used a synthetic seawater tidal simulation system that recirculated water from 50 x 25 x 30 cm acrylic aquaria to independent 14 L reservoirs using peristaltic pumps, starting at $2300 with 2 channel ports (fishersci.com). Additional channel ports start at approximately $300 (fishersci.com), thus using peristaltic pumps to increase replication can be costly. A less expensive tidal simulator design by Brown et al. (2005) transferred synthetic seawater using aquarium pumps to push treatment water from large (240 L) staging containers to interconnected smaller (9L) microcosms and then discarded the water after one tidal cycle making it difficult to examine water-column constituents over time.

I developed an inexpensive and simple tidal simulator that could be used for application of water column treatments to *Spartina alterniflora* salt marsh microcosms. The system presented is inexpensive per unit, uses easily obtainable materials, and ensures independence of each experimental unit. I hypothesized that the tidal simulation systems adequately replicates the tidal regime experienced by *Spartina alterniflora* salt marsh ecosystems. Therefore I expected no difference in *Spartina alterniflora* growth responses between the two tidal treatments. *Spartina alterniflora* growth responses after exposure to treatments for three months provided important information about the use of *Spartina alterniflora* microcosms with tidal simulation for conducting experiments in which water column treatments are required.

Methods

I compared *Spartina alterniflora* growth in field plots to growth in microcosms with tidal simulation in an open sided greenhouse on Sapelo Island, GA (31°24'39.33"N, 81°17'8.70"W)

from June to August 2012. Sixteen marsh plugs (swards) consisting of 3-10 *S. alterniflora* ramets with surrounding sediment, rhizomes and roots were collected from a short-form monospecific stand of *S. alterniflora* on Sapleo Island near Oakland Creek (31°24'42.36"N, 81°17'7.28"W). Swards were collected ~1 m apart to ensure genetic diversity (Richards et al. 2004) and trimmed to fit buckets (20 cm depth x 28 cm diameter). Pore-water salinity was (35 ± 3 psu) for all swards at the time of collection. Half of the excavated swards (8) were placed in 18.9 L buckets that were impregnated with 12 (10 cm diameter) holes each to allow tidal flushing, swards in buckets were returned to their original location in the salt marsh. Each microcosm with tidal simulation consisted of two 18.9 L buckets, one acting as the marsh microcosm and the other as a water reservoir. Each 18.9 L microcosm received one of the remaining eight *S. alterniflora* swards in an 11.4 L nursery pot. Water was transferred between the reservoir and sward by two Tom's Aqualifter® dosing pumps (tomaquarium.com) mounted to the outer lip of each unit. Aquarium air tubing (0.65 cm diameter) secured to the side of each bucket extended from the pump to the bottom of both the experimental unit and the reservoir (Figure 2.1). Water lifting dosing pumps were connected to GE 6 Outlet Heavy Duty Outdoor Timers® set to simulate semi-diurnal tides. Every 6 hours, timers were programmed to transfer 11 L of water over 75 min from the reservoir to the microcosm and back, resulting in one fixed daily and nightly high tide. Microcosm units were established in an open sided greenhouse covered with 6 mm clear plastic sheeting to prevent rain from entering microcosms. Light transmission through the plastic sheet was reduced by ~36%, therefore plastic covers were also placed at the same height (1.5 m) above all field replicates. Initial water drawn for recirculation in the tidal simulators was from a tidal creek that connected to the field site. Tidal creek water column salinity was 31 ± 2 psu for the duration of the experiment.

Plant height and stem density were measured weekly in response to tidal treatment. Plant height was estimated as the mean of the 5 tallest shoots above 30 cm in each microcosm measured from the sediment surface to the tip of the longest leaf (Boyer and Zedler, 1998). Stem density was defined as the total number of live shoots per microcosm (Dai and Weigert, 1996). Above and belowground *S. alterniflora* biomass and soil salinity were determined only at the conclusion of the experiment due to the destructive nature of the sampling. *Spartina alterniflora* aboveground biomass was collected by clipping shoots at the surface of the sediment. Belowground biomass was separated from sediment by rinsing through a 2 mm mesh screen. Biomass samples were stored at 4°C until processing (Gallagher and Plumley, 1979); plant tissues were separated into live and dead categories according to color and turgor pressure (Darby and Turner, 2008). Briefly, aboveground tissue was considered live if it was green and turgid, and belowground tissue was considered live if it appeared white, orange, or reddish and turgid. Dead above and belowground plant tissue was identified by a yellow or brown color and flaccid texture. Following separation, plant tissue was washed free of sediment and dried at 55°C to constant weight (Darby and Turner, 2008). Live shoot density (m^{-2}) and aboveground and belowground biomass were standardized to surface area ($g \cdot m^{-2}$) were standardized to surface area (Morris and Haskin, 1990). Sediments were also dried at 55°C to constant weight and soil pastes from which soil water was expressed through filter paper onto a refractometer were used to estimate salinity (Boyer and Fong, 2005).

Data were tested for assumptions of normality using the Shapiro-Wilk *W* test and homogeneity of variances using Levene's test. Square root transformations were required only for stem density data to meet the assumptions of parametric tests. Plant height and stem density

were analyzed using repeated measures ANOVA. Above and belowground biomass, and soil salinity were analyzed at the end of the experiment using t-tests.

Results

The goal of the tidal simulator method I developed was to address the challenge of maintaining tidal regime while performing controlled greenhouse experiments on coastal plants. *Spartina alterniflora* stem density and plant height did not differ between field and tidal simulation treatments (Table 2.1). Stem density changed over time regardless of treatment, and the observed increase from June to August was consistent with *Spartina alterniflora* growth over the summer months in southeastern tidal marshes (Morris and Haskin, 1990) (Figure 2.2a). A strong trend toward a decrease in plant height over time occurred likely due to the production of new, shorter stems as main shoots in the swards senesced (Dai and Weigert, 1996), but the pattern in plant height was similar in both tidal treatments (Figure 2.2b). At the end of the experiment, no effect of tidal treatment was evident on aboveground (t-test, t_{14}=1.09, p=0.30) or belowground (t-test, t_{14}=1.24, p=0.24) biomass. Belowground biomass was 2 fold that of aboveground biomass in both tidal treatments which is within the range (0.7-14 times) for *S. alterniflora* in a Louisiana salt marsh (Darby and Turner, 2008) (Table 2.2). A strong trend toward increased soil salinity in the tidal simulator treatment relative to natural tidal inundation occurred (One-way ANOVA, $F_{1,14} = 4.5285$, $p = 0.0516$) (Table 2.1).

Discussion

The greenhouse microcosm tidal simulation system described here adequately replicated the tidal regime experienced by *Spartina alterniflora* salt marsh ecosystems. As expected, I found no difference in *S. alterniflora* plant height, stem density, or final above and belowground biomass between the described tidal simulation system and field simulator. Tidal wetland simulators also produce similar mieofauna and nutrient processing to reference field plots (Kitchens, 1979). In contrast, waterlogging salt marsh sediment can increase humic acid and hydrogen sulfides in sediments inhibiting *S. alterniflora* growth (Mendelssohn and McKee, 1988; Portnoy and Valiela, 1997). *S. alterniflora* plants in waterlogged soils exhibit lowered root porosity and decreased accumulation of osmolytes in response to salinity than drained soils (Naidoo et al., 1992), thus recreating tides is necessary to relate *S. alterniflora* plant function in greenhouse microcosms with reference field marsh plants. My comparison showed a trend toward higher soil salinity in the tidal simulator microcosms relative to reference field plots due to adding small amounts of tidal creek water to replenish nutrients. *S. alterniflora* is widely accepted as highly salt tolerant and thus the trend toward increasing salinity was not enough to reduce *S. alterniflora* growth during the three months of study (Smart and Barko, 1980).

Simple construction and potential for large numbers of independent replicates are major advantages of this tidal simulation system. Using materials found at local hardware and aquarium retailers, I constructed each tidal simulation unit for approximately $27 U.S. dollars. In addition, construction of our tidal simulation unit is quick, taking about 20 minutes to assemble each replicate. Once the materials are obtained, a researcher can have a large number of tidal simulator units set up in a few days unlike other large greenhouse tidal simulation

systems that use expensive custom tanks and can take months to set-up (Gleason et al., 1981; Spaulding and Hester, 2007). Furthermore, the tidal inundation depth can easily be changed in my simulators by adjusting the return aquarium tubing height, allowing for research questions addressing drought or sea level rise effects to be examined.

Recirculation tidal systems have the potential to become depleted of macro and micronutrients, but nutrient limitation can easily be avoided as long as the inundating water is refreshed often enough. *Spartina alterniflora* requires nitrogen, phosphorous, sulfur, magnesium, and iron (Broome et al. 1975; Smart and Barko, 1980) that can be maintained by replacing synthetic seawater containing macro and micronutrients at two week intervals (Atkinson and Bingman, 1997) or adding nutritional supplements. Continuous submersion studies typically add nutrients in the form of diluted Hoagland's solution (Touchette et al., 2009; Merino et al., 2010). I would recommend that future investigations using this tidal simulator system either completely replace inundating water at regular intervals between one (Ulrich and Sedlak, 2010) and four weeks (Smart and Barko, 1980) or add Hoagland's solution to maintain appropriate nutrient levels (Merino et al., 2010; Xiao et al., 2011). However, that the plant growth in the microcosms with tidal simulation was similar to that in the field tide treatment suggests the tidal simulation study presented is a simple and inexpensive way to enhance the findings from *Spartina alterniflora* salt marsh studies conducted in a greenhouse setting to tidally influenced plants in the field.

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Tables and figures

Table 2.1. Repeated measures analysis of variance indicating weekly growth response to tidal treatment (n=8).

	df	F	p
Live shoot density			
tidal treatment	(1,14)	0.8492	0.3724
time	(9,126)	19.7446	<0.001
tidal treatment*time	(9,126)	1.7760	0.0709
Plant height			
tidal treatment	(1,14)	0.0797	0.9096
time	(9,126)	1.8705	0.0492
tidal treatment*time	(9,126)	0.9280	0.2819

Table 2.2. Mean (± SEM) of above and belowground biomass and soil salinity after 10 weeks of exposure to tidal treatments, n=8.

	Aboveground biomass $g\ m^{-2}$	Belowground biomass $g\ m^{-2}$	Soil Salinity psu
Field plots	367.2 ± 49.2	591.9 ± 49.1	126 ± 11
Tidal simulator	437.9 ± 48.9	797.9 ± 107.3	157 ± 11

Figure 2.1. Tidal simulators consisted of one 18.9 L bucket containing a *Spartina alterniflora*

Figure 2.1. Tidal simulators consisted of one 18.9 L bucket containing a *Spartina alterniflora* sward connected to a second reservoir bucket (with a lid to prevent evaporation) by aquarium tubing attached to two water-lifting pumps. Pump 1 transferred water from the reservoir into the microcosm and pump 2 transferred the water from the microcosm back into the reservoir (A). Units were connected to an outdoor timer (3) programmed to create tidal exchange every 6 hours (B), and aquarium tubing extended from pumps to the bottom of both the microcosm and reservoir units to ensure complete transfer of water between them (C).

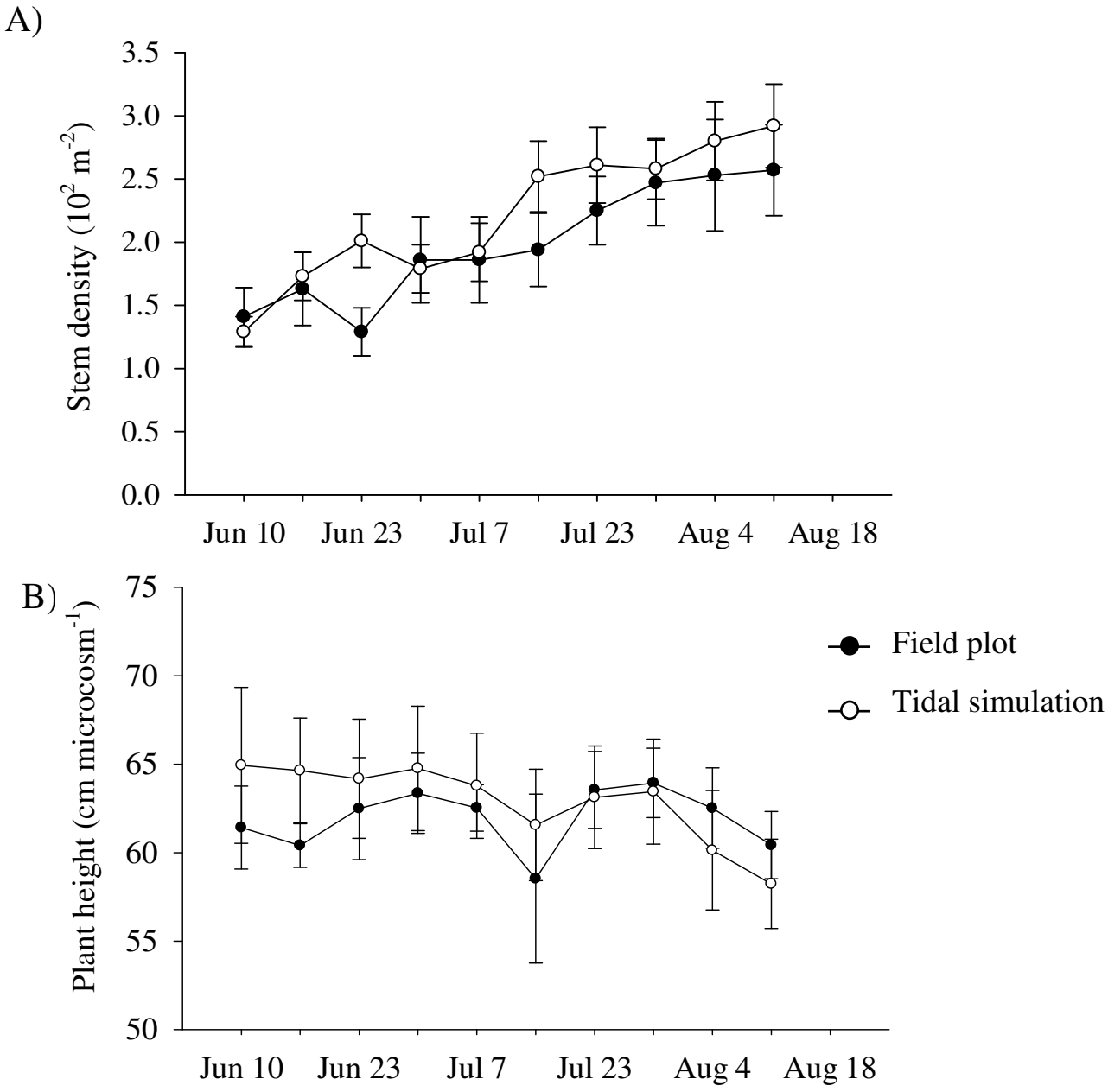

Figure 2.2. Average (A) stem density (m^{-2}) and (B) mean plant height (cm microcosm^{-1}) for *Spartina alterniflora* exposed to either tidal simulation or tidal inundation in the field from June to August. Error bars are ± one standard error of the mean (SEM) and n=8.

CHAPTER 3

WATER COLUMN AMMONIUM CONCENTRATION AND SALINITY INFLUENCE NITROGEN UPTAKE AND GROWTH OF *SPARTINA ALTERNIFLORA*

Abstract

Salt marsh macrophytes, such as *Spartina alterniflora,* play a critical role in uptake and transformation of inorganic nitrogen before it reaches coastal waters, thereby reducing the potential for eutrophication. Although nitrogen availability typically limits *S. alterniflora* growth, it may be possible to exceed the nitrogen uptake capacity of *S. alterniflora.* It is well known that altering either inorganic nitrogen availability or salinity can influence *S. alterniflora* nitrogen uptake. However, investigating the effects of both factors is essential given that changes in inorganic nitrogen supply to surface waters occur simultaneously with reductions in freshwater flow. *Spartina alterniflora* nitrogen uptake and growth responses to inorganic nitrogen (ammonium, NH_4^+) (0, 10, and 100 µM), and salinity (20, 30, and 40 psu) treatments in a fully crossed factorial design were measured using greenhouse microcosms with tidal simulation in Statesboro, GA from April-October, 2013. After 48 hours the highest water column NH_4^+ uptake occurred at the lowest salinity (20 psu) and highest ammonium concentrations (100 µM) tested. After 6 months of NH_4^+-^{15}N additions, above and belowground *S. alterniflora* plant tissue $\delta^{15}N$ increased proportionally with NH_4^+ additions and was reduced by 50% with salinity increases from 20 to 40 psu across all NH_4^+ addition levels. Furthermore, *S. alterniflora* above and belowground biomass and main shoot height was reduced with increasing salinity from 20 to 40 psu and not significantly affected increasing NH_4^+ additions. However, at high salinity (40 psu) biomass reductions were mitigated by intermediate (10 µM)

NH_4^+ additions by a 50% increase over 0 and 100 µM NH_4^+ additions. Stem density and main shoot height measured weekly also reflected mitigation by intermediate (10 µM) NH_4^+ additions at elevated salinity. That *S. alterniflora* nitrogen uptake and biomass decrease with increasing water column salinity suggests alteration of coastal salinity may reduce nitrogen uptake capacities of *S. alterniflora* dominated salt marshes. Thus estuarine water column salinity should be considered when regulating inorganic nitrogen loads in aimed at conserving salt marsh nutrient retention.

Introduction

Salt marshes provide valuable ecosystem services including carbon cycling, storm surge protection, and nutrient retention (Barbier et al., 2011; Bowen and Valiela, 2008; Cole et al., 2004; McClelland and Valiela, 1997). Salt marsh nutrient retention prevents excess nitrogen (N) and phosphorous (P) from reaching adjacent waters that could otherwise accelerate coastal eutrophication resulting in an increase in algal blooms and subsequent low dissolved oxygen conditions (Nixon, 1995; Verity, 2002.; Sousa et al., 2008; Verity, 2010). Nitrogen retention in temperate salt marshes occurs via microbial transformations and immobilization by plants such as the marsh macrophyte *Spartina alterniflora* (Weston et al., 2009), but plants appear to have the larger role. For example, the presence of *S. alterniflora* shoots increased N retention by 45% over non-vegetated sediments in salt marsh microcosms (Buresh et al., 1981). Using an N mass balance model, Anderson et al. (1997) found *S. alterniflora* N uptake rates (33 g N m^{-2} yr^{-1}) accounted for more water column N removal than microbial denitrification (0.6 to 4 g N m^{-2} yr^{-1}) in a Virginia (USA) salt marsh. Finally, a ^{15}N tracer study found *S. alterniflora* was the largest nitrogen sink with 43.6 % nitrogen uptake compared to 0.7% by denitrifying bacteria in salt

marshes of Massachusetts (USA) (Drake et al., 2009). Salt marsh plants are able to immobilize because inorganic N tends to limit growth (Valiela and Teal, 1974; Pennings et al., 2005). While *S. alterniflora* N uptake is critical to temperate salt marsh nutrient cycling, it may be possible for inorganic N availability to exceed marsh uptake capacity (Brin et al., 2010) with severe economic (Gedan et al., 2009) and ecological (Deegan et al., 2012; Scavia and Bricker, 2006) consequences.

Salt marsh nitrogen uptake capacities can be exceeded as inorganic N concentrations increase. In a greenhouse experiment Morris (1980) found *S. alterniflora* N uptake rates were increased with high water column nitrate concentration (1.18 mM) compared to rates at 135 µM nitrate. However the uptake rates did not increase enough to deplete the water column of excess nitrate (Morris, 1980). Drake et al. (2009) found that after 5 days *S. alterniflora* in nitrate-enriched salt marshes sequestered a smaller fraction (40-50% less) total nitrogen load than plants in reference marshes and the remaining nitrate was exported ebb tidewater. In a 40-year sewage sludge fertilization study, Brin et al. (2010) found ammonium export from a *Spartina alterniflora* dominated marsh to the overlying water column increased linearly with inorganic N additions. Thus even though *S. alterniflora* can increase N uptake in response to increased availability, too much N can overwhelm uptake rates resulting in export to nearby coastal waters.

In addition to excess N availability, *S. alterniflora* N uptake capacity can be influenced by salinity (Odum, 1988; Crain et al., 2007). Increasing salinity not only reduced water potential (Touchette et al., 2009), growth and sexual reproduction of *S. alterniflora* under laboratory conditions (Xiao et al., 2011), but also led to tissue damage including rolled and yellowed (chlorotic) leaves in the field (Nestler, 1977). Salinity can also reduce *S. alterniflora* ammonium uptake capacity via direct competition between sodium and ammonium ions at the root (Bradley

and Morris, 1991). Edmonds et al. (2009) demonstrated increasing salinity changes microbial respiration from methanogensis to sulfate reduction, thereby increasing hydrogen sulfide concentrations in the soil which can also reduce ammonium uptake by *S. alterniflora* (Bradley and Morris, 1990). Thus, either excess inorganic N or elevated salinity has the potential to decrease inorganic N uptake and growth of *S. alterniflora* and reduce marsh nitrogen retention capacity.

Despite the potential for simultaneous changes in salinity and nutrient concentrations, their combined effects on *S. alterniflora* N uptake have not been fully ascertained. Only a few studies to date have specifically addressed how this interaction affects plant tissue N content and growth (Haines and Dunn, 1976; Linthurst and Seneca, 1981) in *S. alterniflora* using microcosm experiments. Using hydroponic solutions with elevated ammonium, salinity, and iron levels, Haines and Dunn (1976) found an interaction between salinity and nutrients on above and belowground biomass of 11 week-old *S. alterniflora* shoots. Total plant dry weight increased 3-fold with the addition of ammonium at 5 and 20 psu salinity however, when salinity was higher (40 psu) ammonium addition only increased dry weight by 50% (Haines and Dunn, 1976). In contrast, Linthurst and Seneca (1981) examined the role of ammonium, salinity, and aeration on transplanted *S. alterniflora* growth in microcosms and found no interaction between any of the independent variables on biomass, height, or shoot density. However, adding ~2,500 µM N not only increased *S. alternifora* growth at all salinity levels (15, 30, and 45 psu) but also as salinity increased, the amount of total nitrogen in plant tissue increased, suggesting nitrogen uptake is actually enhanced at higher salinities (Linthurst and Seneca 1981). One explanation for higher salinity enhancing nitrogen uptake may be the accumulation of N based osmolytes as a response to increasing salinity aiding *S. alterniflora* salt tolerance (Cavalieri and Huang, 1979; Colmer et

al., 1996) thus additional inorganic nitrogen supply may stimulate nitrogen uptake and plant growth under high salinity conditions. Haines and Dunn (1976) and Linthurst and Seneca (1981) found conflicting effects of ammonium and salinity on *S. alterniflora* growth when roots are exposed to treatment in non-tidal greenhouse simulations. However, salt marshes receive nitrogen from the overlying water column and ground derived sources (Valiela et al., 2000), and *Spartina alterniflora* is capable of foliar inorganic nitrogen uptake (Mozder et al., 2011). To better constrain how water column ammonium and salinity influence *S. alterniflora* N uptake and growth, plant responses to both variables concurrently must be examined under tidal conditions.

Current trends of increasing inorganic N concentrations and salinity in estuaries worldwide highlight the importance of investigating the impact of both factors on *Spartina alterniflora* marsh nutrient retention (Scavia et al., 2002; Flemer and Champ, 2006). For example, using historical population data, Weston et al. (2009) found that a 142.2% increase in human population density from 1970-2000 in the Upper Ocmulgee River (GA, USA) watershed correlated with an increase in nitrate export to estuarine waters of 0.84 $\mu M\ yr^{-1}$. In addition, Verity (2002) found yearly mean nitrate concentrations doubled from 1.1 µM to 2.2 µM from 1986 to 1996 in the Skidaway River Estuary (GA, USA) due to a 64% rise in nearby coastal human populations. Human activities including dams and hydroelectric plants along rivers can influence salinity regimes by altering fresh water supply to the coast. Construction of dams along the Godavari River, India reduced river discharge by 340% between 2007 and 2009, increasing estuarine salinity by as much as 10 psu (Acharrya et al., 2012). Anthropogenic activities coupled with rising sea levels are predicted to increase nutrient delivery, change coastal salinity regimes, and in turn alter nutrient retention services provided by tidally influenced marshes along the coast. (Craft et al., 2009).

I hypothesized that altering both salinity and inorganic N concentrations affect *S. alterniflora* N uptake and growth. I predicted N uptake rates and growth would increase with increasing N concentration. Furthermore I predicted that increasing salinity would reduce N uptake and plant growth in *S. alterniflora*, thereby reducing the nutrient retention capacity of the plant. However, I expected excess availability of ammonium to alleviate the negative effects of high salinity on *S. alterniflora* N uptake and growth as the accumulation of N based osmolytes, responding to salinity increases, relieved salinity stress. I conducted a greenhouse experiment using microcosms with tidal simulation to examine ammonium uptake by and growth of *S. alterniflora* in response to simultaneous changes in water column salinity and ammonium concentration. Results from this study enhance understanding of how salinity and nutrient changes may interact to influence salt marsh nutrient retention capacity.

Methods

A microcosm study was conducted to investigate the effects of water column ammonium concentration and salinity on *Spartina alterniflora* growth and ammonium uptake from April through October 2013. At the beginning of the growing season (ca. February, 2013) marsh plugs were collected from a monospecific *S. alterniflora* stand in a low salinity marsh (20 psu) at Georgia Coastal Ecosystem LTER (31°32'12.68"N, 81°25'32.62"W) (Figure 3.1). A bulb planter (26.7 cm depth x 13.3 cm ID) was used to gently extract young shoots (10 ± 2 cm tall), their associated roots and surrounding sediment. Extracted plugs were placed into nursery pots (15.2 cm depth x 15.2 cm ID) lined with aquarium floss to prevent sediment loss. Tidal creek sediment from the collection site was placed around marsh plugs filling the nursery pots, which

were then transported to a greenhouse at Georgia Southern University, Statesboro, GA, USA (32°25'32.49"N, 81°46'48.17"W).

Each marsh plug containing a *S. alterniflora* shoot was acclimated to greenhouse conditions in water collected from the Sapleo River at GCE LTER site (19 ± 3 psu) in microcosms with tidal simulation for 3 weeks. Briefly, tidal simulators consisted of two 18.9 L buckets (36.8 cm x 29.8 cm OD) connected to Tom's Aqualifter® (tomaqarium.com) dosing pumps that transferred water from the bucket containing the potted shoot to a reservoir bucket and back again at 6 hour intervals using outdoor programmable timers (GE 6 Outlet Heavy Duty Outdoor Timers®). The result was one fixed daily and nightly high tide simulating semi-diurnal tides (Chapter 2). Shoots were then acclimated to treatment salinities (20, 30, or 40 psu) by incrementally increasing salinity concentrations by 50% of the target salinity with artificial seawater (Instant Ocean®) over the course of 2 weeks (Merino et al., 2010). For example, 20 psu water was replaced with 25 psu seawater after one week and 30 psu seawater after two weeks.

Following acclimation, microcosms within a given salinity treatment were randomly assigned to nutrient treatments in a fully crossed 3 x 3 design (n=6) to examine the effect of ammonium and salinity concentration on plant N uptake and growth. Ammonium additions (as ammonium chloride (NH_4Cl)) consisted of a no NH_4^+ addition control, 10 µM NH_4^+ addition, and 100 µM NH_4^+ addition. The maximum NH_4^+ concentration used (100 µM) represented a high level of nitrogen loading to temperate estuaries in the U.S. (Deegan et al., 2007; Drake et al., 2009; Nelson and Zavaleta, 2012). Treatment solutions of NH_4Cl were prepared using 95% $^{14}NH_4Cl$ and 5% $^{15}NH_4Cl$. This mixture yielded $^{15}NH_4Cl$ at 4 atom %, providing a ^{15}N tracer compatible with mass spectrometry sensitivity (Brandes, J., personal communication, February 8, 2012). The salinities (20, 30, or 40 psu) were prepared with deionized water and Instant

Ocean®. Salinity was monitored weekly and evaporation was compensated for by adding deionized water every other day at simulated low tide to the reservoir. Ammonium addition treatments began on April 11, 2013; water treatments were changed bi-weekly based on calculations of (Stribling, 1997) to ensure replenishment of micronutrients (e.g. sulfates).

Short-term NH_4^+ uptake experiment

A short-term ammonium (NH_4^+) uptake study was conducted for two weeks at peak biomass from 18 July to 1 August 2013, after plants had three months to acclimate to bi-weekly NH_4^+additions. I tracked water column NH_4^+concentration by taking 10 ml water samples at 0, 12, 24, and 48 hours following NH_4^+addition, which was consistent with timing of uptake measurements from Bradley and Morris (1990) that revealed NH_4^+ was depleted by *S. alterniflora* in hydroponic solutions within 30 hours. Water column NH_4^+ samples were filtered using Sartorius™ Minsart™ 0.45µm glass fiber pre-filters, and immediately frozen and shipped within one week to JBC Analytical Services at the University of Georgia for NH_4^+ determination using the phenol-hypochlorite method of Solorzano (1969). Briefly, phenol-alcohol and sodium hypochlorite solution reacts with ammonium to produce a yellow tint that can be detected with spectrophotometry to determine water column NH_4^+ concentrations.

Water column NH_4^+ uptake data was fitted to exponential models using AIC (Akaike Information Criterion) weights (Wagenmakers and Ferrell, 2004). Exponential models were compared across treatments using 85% confidence interval overlap; if treatment intervals overlapped they were not considered different (Rutting 2010; Demey et al., 2014). Confidence levels of 85% were used based on findings of Payton et al. (2000, 2003), who found 95%

confidence interval overlap corresponded with an overly conservative α=0.005, while using 85% confidence interval overlap corresponded to α=0.05 when comparing non-linear regression lines.

$^{15}NH_4^+$ tracer experiment and long-term growth

Main shoot height and stem density were measured weekly for the duration of the experiment. Main shoot height was measured from the surface of the sediment to the tip of the longest leaf (Boyer and Zedler, 1998). Stem density was determined by counting the total number of live shoots in each microcosm. Main shoot height and stem density from individual microcosms in each treatment were regressed against time to find the rate of change (slope) of each microcosm from the first treatment application (4/11/13) to peak growth (9/5/13). Peak growth occurred when shoots reached maximum height, and was consistent with *Spartina alterniflora* peak growth in previous field studies in the southeastern US (occurring between August and September, Dai and Wiegert, 1996). The slopes from the regressions for microcosms were then compared across treatments using two-way ANOVA. Only significant interactions between salinity and NH_4^+ addition are reported.

Above and belowground tissue C, N and $\delta^{15}N$ content, biomass, and spike length and weight were determined at the end of the experiment due to the destructive nature of the sampling. Aboveground plant tissue was prepared for analysis by clipping *Spartina alterniflora* shoots at the sediment surface and immediately separating into live (green and turgid) and dead (yellow or brown color and flaccid appearance) portions. Tissue was washed free of sediment and dried at 55°C to a constant weight (Darby and Turner, 2008). Belowground plant tissue was stored at 4°C until separation into live and dead portions could be performed (Darby and Turner, 2008). Live and dead belowground plant tissue was determined according to color and turgor

pressure (Darby and Turner, 2008) and dried at 55°C to constant weight. Dried *S. alterniflora* above and belowground tissue and soil samples were homogenized by grinding to a fine powder using a mortar and pestle and analyzed for $\delta^{15}N$ and C:N using an IRMS Delta V plus mass spectrometer at the Skidaway Institute of Oceanography Isotope Laboratory. Spike length was recorded at harvest from the first spikelet attachment to the tip of the spike (Xiao et al. 2009). At the conclusion of the study, spikes were removed, dried at 55°C to constant weight and seeds were removed and counted. Approximately 100 g of soil without plant material was also collected and dried at 55°C to constant weight for analysis of soil pH, salinity, C:N and $\delta^{15}N$.

Tissue and soil $\delta^{15}N$, above and belowground tissue C:N and above and belowground biomass data were tested for normality using the Shapiro-Wilk *W* test and for homogeneity of variances using Levene's test. Two-way ANOVA was used to test the effects of nutrient and salinity treatments on main shoot height, stem density, aboveground tissue C:N, spike length, and soil $\delta^{15}N$. Data transformations were performed when conditions for parametric tests were not met, or non-parametric tests were used. Log transformations were performed on aboveground tissue $\delta^{15}N$ data followed by analysis using two-way ANOVA. Differences in aboveground biomass, belowground tissue $\delta^{15}N$, and belowground C:N across salinity and nutrient treatments were analyzed using Kruskal-Wallis tests with the Scheirer-Ray-Hare extension (nonparametric analog for a two-way ANOVA).

Results

Short-term water-column NH_4^+ uptake

No differences in short-term NH_4^+ uptake occurred in any of the 0 µM NH_4^+ addition treatments (Table 3.1, Figure 3.2). In contrast, addition of NH_4^+ increased *S. alterniflora* NH_4^+

uptake rates regardless of NH_4^+ concentration (Table 3.1, Figure 3.2). However, NH_4^+ uptake rates were generally reduced with increasing salinity level. In the 10 µM NH_4^+ addition treatment, plant NH_4^+ uptake rates were lowest at 40 psu (Table 3.1). In the100 µM NH_4^+ addition treatment, NH_4^+ uptake decreased with an increase in salinity from 20 to 30 psu, but at 40 psu the NH_4^+ uptake rate was double that at 30 psu (-0.16 vs. -0.09 µM h^{-1}, respectively).

$^{15}NH_4^+$ tracer and long-term growth

Main shoot height and stem density increased over 6 months of exposure to treatments (Table 3.2, Figure 3.3, 3.4). However, the rate of increase for shoot height was influenced by salinity; heights were greater in 20 than in 40 psu across all NH_4^+ treatments (Table 3.3; Figure 3.3, 3.4). Main shoot final heights in 20 psu treatments were 33-95%, higher than at 40 psu across NH_4^+ treatments. A strong trend toward an effect of NH_4^+ concentration on rate of increase in shoot height appeared, likely due to elevated growth rate in the 10 µM NH_4^+ addition compared to growth rates in the 0 and 100 µM NH_4^+ addition treatments at 40 psu (Table 3.2, Figure 3.3 c). Salinity affected the rate of increase in stem density; at 20 psu rates were 75-220% higher than at 40 psu (Table 3.2). Rate of increase in stem density was also affected by NH_4^+, with higher stem densities in the10 µM NH_4^+ addition treatments compared to those in the 0 or 100 µM NH_4^+ addition treatments across all salinities (Figure 3.4 a-c). Final stem density was affected by salinity and NH_4^+ treatments in a similar manner to rate of increase in stem density (Table 3.2, Figure 3.4).

Spartina alterniflora aboveground tissue $\delta^{15}N$ (Two-way ANOVA, $F_{2,45} = 671.972$, $p < 0.0001$) and belowground (Sheirer-Ray-Hare, $H_{2,45} = 34.634$, $p = <0.0001$) increased in proportion to $^{15}NH_4^+$ availability (Figure 3.5). Salinity also affected aboveground tissue $\delta^{15}N$

(Two-way ANOVA, $F_{2,45} = 3.77$, $p = 0.0305$), with the highest $\delta^{15}N$ content in the 20 psu salinity treatments. In contrast, salinity had no effect on belowground tissue $\delta^{15}N$ (Sheirer-Ray-Hare, $H_{2,45} = 0.2691$, $0.50 < p < 0.90$) (Figure 3.5). The sediment did not appear to accumulate ^{15}N in the treatments receiving $^{15}NH_4^+$ addition (Table 3.4)

Tissue C:N ratio was influenced by salinity, with the lowest ratios occurring in the 40 psu treatments (aboveground: Two-way ANOVA, $F_{2,45} = 3.94$, $p = 0.025$; belowground: Sheirer-Ray-Hare, $H_{2,45} = 7.28$, $0.05 < p < 0.025$). Increasing salinity from 20 to 40 psu reduced C:N ratios by 11-22% (Figure 3.6). Tissue C:N ratios were unaffected by water column NH_4^+ concentration (aboveground: Two-way ANOVA, $F_{2,45} = 2.3681$, $p = 0.1052$; belowground: Sheirer-Ray-Hare, $H_{2,45} = 2.5714$, $0.10 < p < 0.50$). Aboveground C:N ratios were generally ~50% lower than belowground C:N ratios in all treatments. Sediment C:N ratios were similar across all treatments (Table 3.4).

Aboveground (Sheirer-Ray-Hare, $H_{2,45} = 11.5843$, $0.005 < p < 0.001$) and belowground (Two-way ANOVA, $F_{2,45} = 5.8412$, $p = 0.0056$) biomass was adversely affected by salinity (Figure 3.7). Plants grown in 40 psu had 30-60% less aboveground biomass than plants grown at 20 psu. Although NH_4^+ addition had no effect on either aboveground (Sheirer-Ray-Hare, $H_{2,45} = 5.3510$, $0.05 < p < 0.10$) or belowground (Two-way ANOVA, $F_{2,45} = 1.8661$, $p = 0.1668$) biomass, 10 μM NH_4^+ addition resulted in 50% more biomass in 40 psu than 0 or 100 μM NH_4^+ addition treatments (Figure 3.7).

Reproductive structure (spike) length was influenced by an interaction between nitrogen and salinity treatments (Two-way ANOVA, $F_{4,35} = 3.15$, $p = 0.0260$) (Figure 3.4). In general, NH_4^+ addition appeared to increase spike length while salinity decreased spike length (Figure 3.8). However, the apparent reversal of the patterns in the 30 psu salinity treatment likely led to

the observed interaction. Spike length and weight were highly correlated (Spearman's ρ, $r(102) = 0.80$, $p < 0.0001$), therefore only spike length data are presented.

Discussion

In this study, altering both inorganic nitrogen (NH_4^+) and salinity affected *Spartina alterniflora* nitrogen uptake and growth. Increased NH_4^+ availability was expected to increase *Spartina alterniflora* nitrogen uptake and growth regardless of water column salinity on time scales ranging from days to months (Morris, 1980; Bradley and Morris, 1991). Mozder et al. (2011) observed a ~10-fold increase in foliar *S. alterniflora* uptake rates across a concentration gradient ranging from no NH_4^+ addition to 100 µM NH_4^+ addition after only 1.5 hours of exposure. I found that addition of NH_4^+ increased short-term (48 h) *S. alterniflora* nitrogen uptake rates by 4-20 fold, with highest uptake rates in response to 100 µM NH_4^+ addition and that tissue $\delta^{15}N$ increased in proportion to availability over a period of six months, which supports coupling between water column and plant tissue N (Cole et al., 2002; Bannon and Roman, 2008; Bowen and Valiela, 2008). Some evidence of increased plant growth also occurred with N addition. For example, 100 µM NH_4^+ addition appeared to produce longer sexual structures (spikes) indicating *S. alterniflora* may allocate excess N to sexual reproduction. In a constructed marsh in San Diego Bay (CA, USA) the macrophyte *Salicornia biglovii* responded to 15g N m^{-2} urea fertilizations with an 800% increase in seed production and a 6 fold increase in the number of inflorescence spikes over control plots, indicating N additions increased sexual reproduction (Boyer and Zedler, 1999). In addition, water column NH_4^+concentration of 10 µM positively affected stem density, although 100 µM NH_4^+ addition did not. Furthermore, the 100 µM NH_4^+ addition did not translate to an increase in biomass after

six months of exposure to treatments. Decreased growth with increased N availability has also been reported by Deegan et al. (2012), who found *S. alterniflora* belowground biomass was greatly reduced after 6 years of exposure to ~80 µM NO_3^-. Thus, while water column NH_4^+ concentrations ≤ 100 µM (which are frequently observed for groundwater and surface water (Bowen et al., 2006) can increase short-term *S. alterniflora* nitrogen uptake, plant growth, and therefore salt marsh nitrogen retention capacity may be reduced on the longer-term.

Salinity has been suggested as a primary driver of *Spartina* sp. nutrient uptake, growth, and distribution within the salt marsh (Odum, 1988; Merino et al., 2010), therefore I expected salinity to negatively influence both *S. alterniflora* NH_4^+ uptake and growth. It was not surprising that an increase in salinity from 20 psu to 30 psu reduced short and long-term NH_4^+ uptake given tha after 30 hours Bradley and Morris (1991) found that increasing salinity from 20 to 60 psu significantly reduced NH_4^+ uptake by *S. alterniflora* through direct ionic competition in plants maintained in hydroponic solutions with altered salinity and a constant NH_4^+ concentration. I also found that a salinity increase from 20 to 40 psu reduced *S. alterniflora* tissue $\delta^{15}N$, stem density, height and above and belowground biomass. In a tidally simulated microcosm study Brown et al. (2006) found that increasing salinity from low (3-5 psu) to high (35-38 psu) reduced shoot biomass by 58% and root dry weight by 42%. White and Alber (2009) reported that *S. alterniflora* plant height along the Altamaha River (GA, USA) was reduced by 20% at sites with salinities over 14 psu. In addition, Nestler (1977) showed that increasing salinity from 20 to 30 psu across a low to high marsh gradient reduced *S. alterniflora* shoot height by 40%. Salinity also appears to be important factor in determining spike length, (Xiao et al., 2011) found that increasing salinity from 5 to 35 psu reduced spike biomass and number of spikes by 50%, suggesting salinity stress also reduces sexual reproduction. Thus

increased salinity alone has the potential to interfere with *S. alterniflora* nutrient uptake and growth.

While I generally expected that elevated salinity would reduce NH_4^+ uptake (Bradley and Morris, 1991; Brown et al., 2006), it is also possible that increased NH_4^+ availability under high salinity conditions can offset salinity stress (Cavalieri and Huang, 1979). I found that increasing NH_4^+ availability generally increased short-term NH_4^+ uptake at 20 psu, however, uptake rates for plants exposed to 40 psu were enhanced in the presence of 100 µM NH_4^+ addition. Despite the increase in uptake rate, there was not subsequent increase in biomass. *Spartina alterniflora* is an osmoconformer; as salinity increases, more N based osmolytes are required for osmoregulation (Vasquez et al., 2006). Therefore the absence of a growth response to 100 µM NH_4^+ addition at 40 psu could have been due to production of amino acid based osmolytes such as proline and glycine betaine needed for salt tolerance (Cavalieri and Huang, 1983; Colmer et al., 1996). That above and belowground C:N ratios also decreased with increasing salinity indicates the potential for decreased photosynthesis and carbon assimilation, resulting in reduced growth dilution (Rogers et al., 1999; Mateos-Naranjo, 2010). Finally, the increase in short term NH_4^+ uptake, shoot height, stem density, above and belowground biomass in the 40 psu plus 10 µM NH_4^+ addition over the 0 and 100 µM NH_4^+ addition treatments suggested that a small nutrient addition reduces the negative effects of elevated salinity. Haines and Dunn (1981) also reported at high salinity 40 psu lower ammonium additions (10 µM) increased 11 week old plant heights by 26% over 1 or 100 µM NH_4^+ additions in a greenhouse setting. Thus inorganic N in excess amounts may overwhelm *S. alterniflora* nitrogen uptake and growth potential that can be further exacerbated with concurrent increases in salinity concentrations.

My results suggest that concurrent increases in water column NH_4^+ concentration and salinity interact such that elevated salinity reduces any stimulatory effect NH_4^+ may have on *Spartina alterniflora* nitrogen uptake and growth, potentially reducing nitrogen retention capacities in *S. alterniflora* dominated salt marshes. Any beneficial effects of increased water column NH_4^+ on salinity stressed (40 psu) *S. alterniflora* were found in the 10µM NH_4^+ addition; considering N loading estimates reach as much as 100 µM (Nelson and Zavaleta, 2012; Valiela, 2000), combinations of excess N and increasing salinity can intensify coastal eutrophication. Water column salinity changes between 20 and 40 psu can occur in estuarine systems as a function of river discharge, evaporation, and residence times, particularly when compounded by high evaporation (Mikhailov and Gorin, 2012) or changes in climate and human demand for freshwater (Najjar, 2010; Flemer and Champ, 2003). For example, altered precipitation patterns may reduce winter snow pack in the Sierra Nevada Mountains (California, USA), ultimately decreasing spring river discharge and increasing salinity in the San Francisco estuary by as much as 9 psu (Knowles and Cayan, 2002). Increases in water column salinity have the potential to further exacerbate human derived exports of inorganic N to coastal areas. Howarth et al. (2002) estimated riverine N export to the North Atlantic Ocean from temperate estuaries (e.g. N. Canada, Mississipi (USA), North Sea, North and South European Coasts, and the Baltic Sea) correlated directly to an 8-fold increase in human derived N inputs per unit area of land (kg km^{-1} yr^{-1}) over the last 40 years. Management plans for regulation of coastal N loading are often developed based on information regarding water column nitrogen concentrations, riverine discharge, amount of land used, and distance water travels from source to estuary (Eichner et al., 2013) but salinity is not explicitly considered. Thus if estuarine salinity and N concentration are both elevated, nitrogen loading models have the potential to overestimate acceptable nitrogen

loading, and coastal management could benefit from considering the salinity regime in a given region to inform policy decisions.

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Tables and Figures

Table 3.1. Average short-term water column NH_4^+ uptake rates ± one Standard Error of the Mean (SEM) and 85% confidence interval overlap (n=6).

NH_4^+ (µM)**	Salinity (psu)	NH_4^+ uptake rate ($\mu M \cdot h^{-1}$)	SE	85% CI upper	85% CI lower	Significance level
0	20	-0.0452	0.0155	-0.0229	-0.0675	a
0	30	-0.0234	0.0133	-0.0042	-0.0426	a
0	40	0.0084	0.0134	0.0277	-0.0109	a
10	20	-0.1324	0.0185	-0.1058	-0.1590	b
10	30	-0.1129	0.0290	-0.0711	-0.1547	b
10	40	-0.0727	0.0189	-0.0455	-0.0999	c
100	20	-0.1672	0.0180	-0.1413	-0.1931	d
100	30	-0.0856	0.0107	-0.0702	-0.1010	e
100	40	-0.1634	0.0222	-0.1314	-0.1954	d

**0 indicates no NH_4^+ addition

Table 3.2. Two-way analysis of variance indicating weekly growth response to NH_4^+ additions and salinity (n=6).

	df	F	p
Initial shoot growth slope			
NH_4^+	(2,45)	2.5639	0.0882
Salinity	(2,45)	11.2160	<0.0001
NH_4^+ x Salinity	(8,45)	1.2286	0.3122
Initial shoot ending height (cm)			
NH_4^+	(2,45)	2.2316	0.1191
Salinity	(2,45)	13.6289	<0.0001
NH_4^+ x Salinity	(8,45)	1.0724	0.3814
Stem density slope			
NH_4^+	(2,45)	3.5837	0.0360
Salinity	(2,45)	14.9981	<0.0001
NH_4^+ x Salinity	(8,45)	0.7982	0.5327
Stem density ending			
NH_4^+	(2,45)	5.3714	0.0081
Salinity	(2,45)	15.9819	<0.0001
NH_4^+ x Salinity	(8,45)	0.7833	0.5421

Table 3.3. *Spartina alterniflora* main shoot height and stem density weekly growth. Regression slopes represent change in growth measured weekly from treatment application to peak growth (9/5/13) and ending values (end) indicate value at peak growth (9/5/13). Treatment means ± one SEM (n=6).

Treatment		Main shoot height (cm)		Stem density	
NH_4^+ (µM)	Salinity (psu)	Slope	End	Slope	End
0	20	0.533 ± 0.029	78.4 ± 3.8	0.042 ± 0.006	7 ± 2
0	30	0.522 ± 0.038	78.9 ± 4.8	0.046 ± 0.007	8 ± 3
0	40	0.275 ± 0.057	40.5 ± 7.9	0.024 ± 0.005	3 ± 3
10	20	0.507 ± 0.055	78.4 ± 8.0	0.056 ± 0.008	9 ± 3
10	30	0.489 ± 0.042	70.6 ± 5.6	0.055 ± 0.006	9 ± 2
10	40	0.412 ± 0.031	58.8 ± 4.4	0.027 ± 0.007	5 ± 2
100	20	0.437 ± 0.048	64.5 ± 9.4	0.048 ± 0.006	7 ± 2
100	30	0.416 ± 0.060	65.7 ± 8.4	0.032 ± 0.007	5 ± 3
100	40	0.306 ± 0.043	43.0 ± 7.2	0.015 ± 0.003	3 ± 1

Table 3.4. Average soil C:N ratio and $\delta^{15}N$ ± one SEM after 6 months of treatment application. No differences were detected across treatments.

Treatment		Soil	
NH_4^+ (µM)	Salinity (psu)	C:N	$\delta^{15}N$
0	20	1.03 ± 0	-23.04 ± 0.26
0	30	1.03 ± 0	-22.56 ± 0.30
0	40	1.03 ± 0	-23.44 ± 0.13
10	20	1.03 ± 0	-23.60 ± 0.22
10	30	1.03 ± 0	-23.78 ± 0.25
10	40	1.03 ± 0	-23.55 ± 0.36
100	20	1.03 ± 0	-22.88 ± 0.17
100	30	1.03 ± 0	-23.48 ± 0.40
100	40	1.03 ± 0	-23.91 ± 0.32

Figure 3.1. Map of GCE LTER site 1 adapted from GCE LTER website, http://gce-lter.marsci.uga.edu/public/gis/gcewebmap.html. Site 1 boundaries are outlined and *Spartina altneriflora* collection site is indicated by a star.

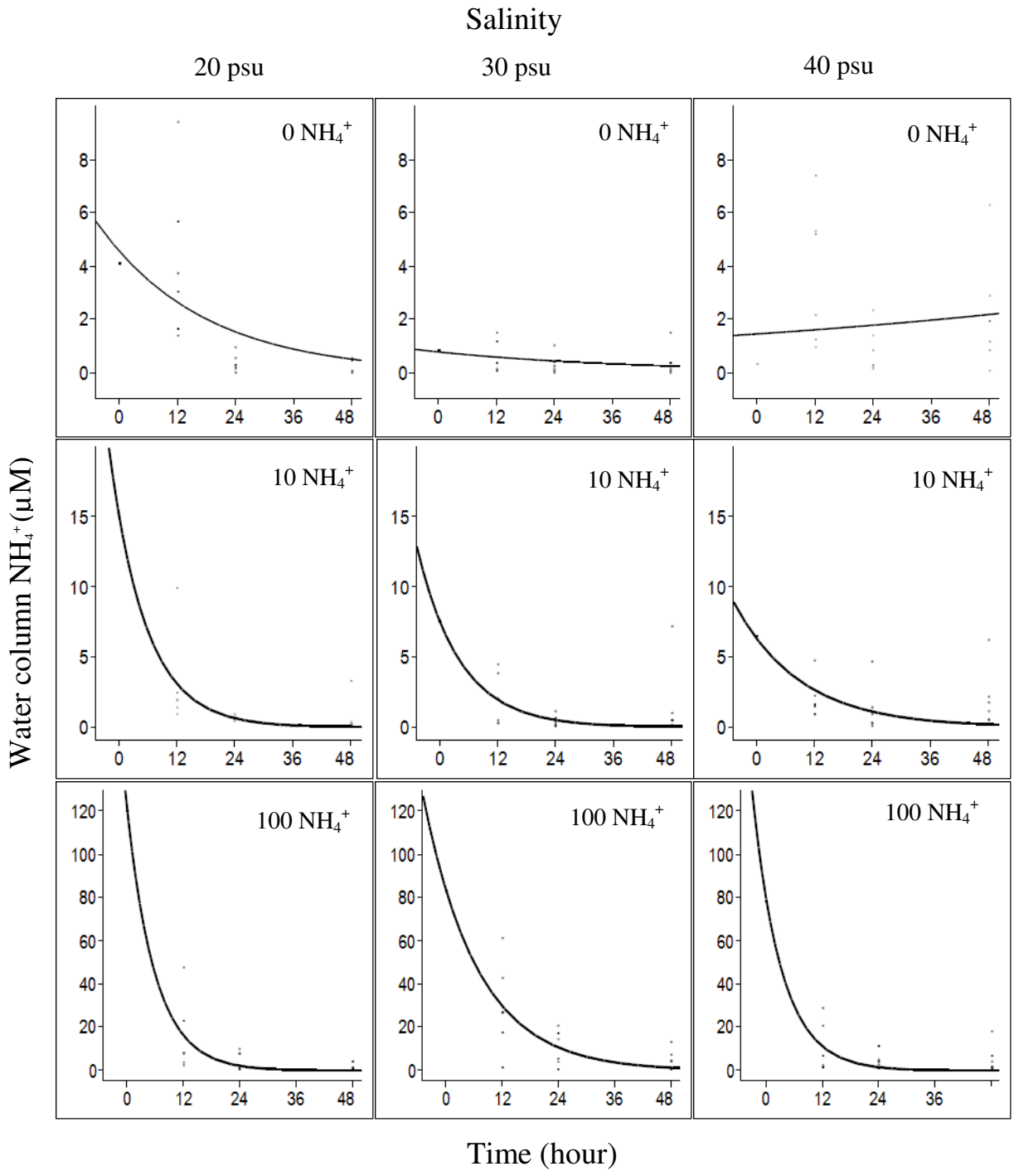


Figure 3.2. *Spartina alterniflora* short-term water column NH_4^+ uptake over 48 hours of exposure to 0, 10 or 100 µM NH_4^+ addition and 20, 30 or 40 psu salinity in August 2013 (n=6).

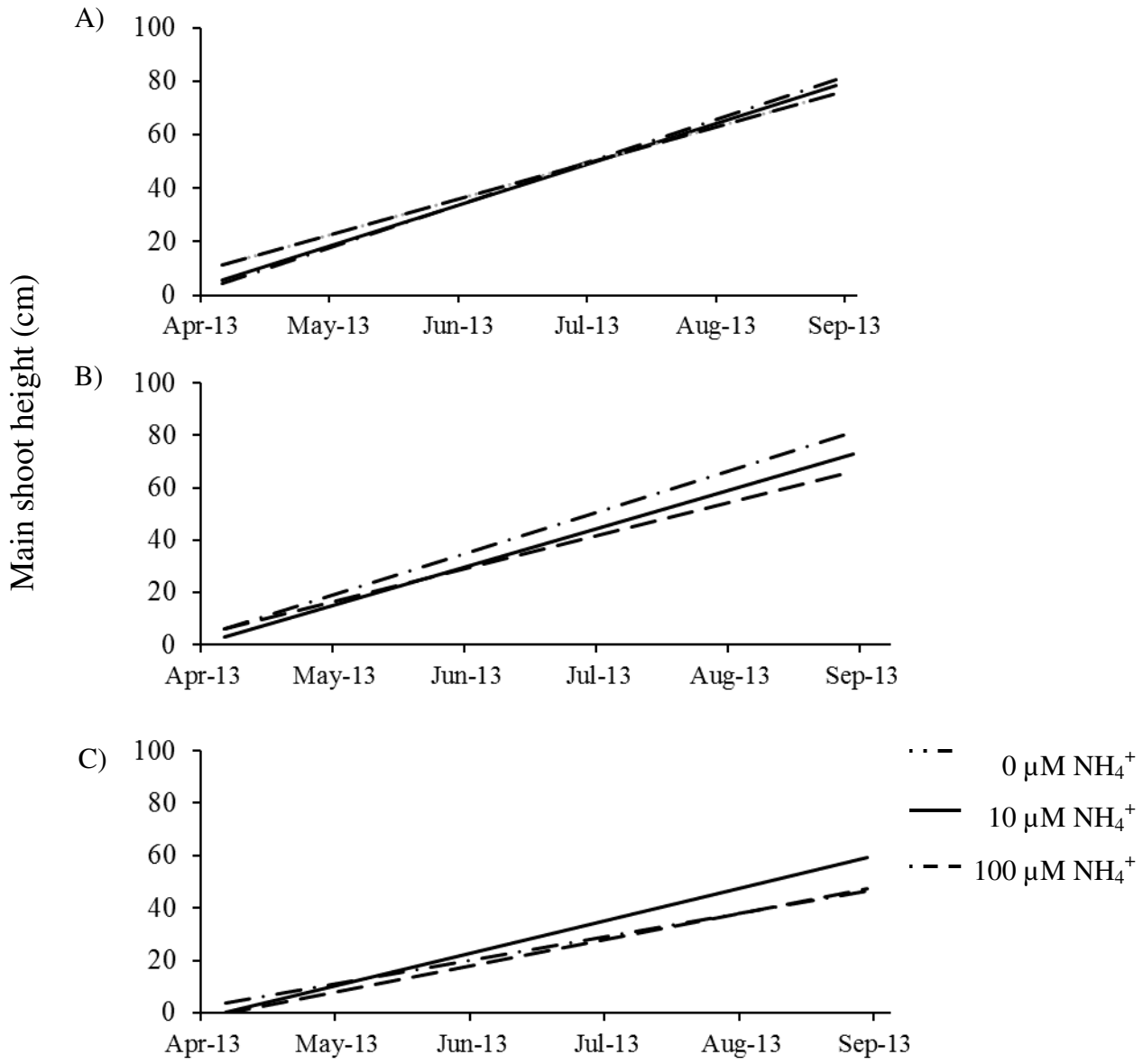


Figure 3.3. Average height of the main shoot (cm) exposed to 0, 10 or 100 µM NH_4^+ addition in salinities of a) 20, b) 30, or c) 40 psu from April to September 2013.

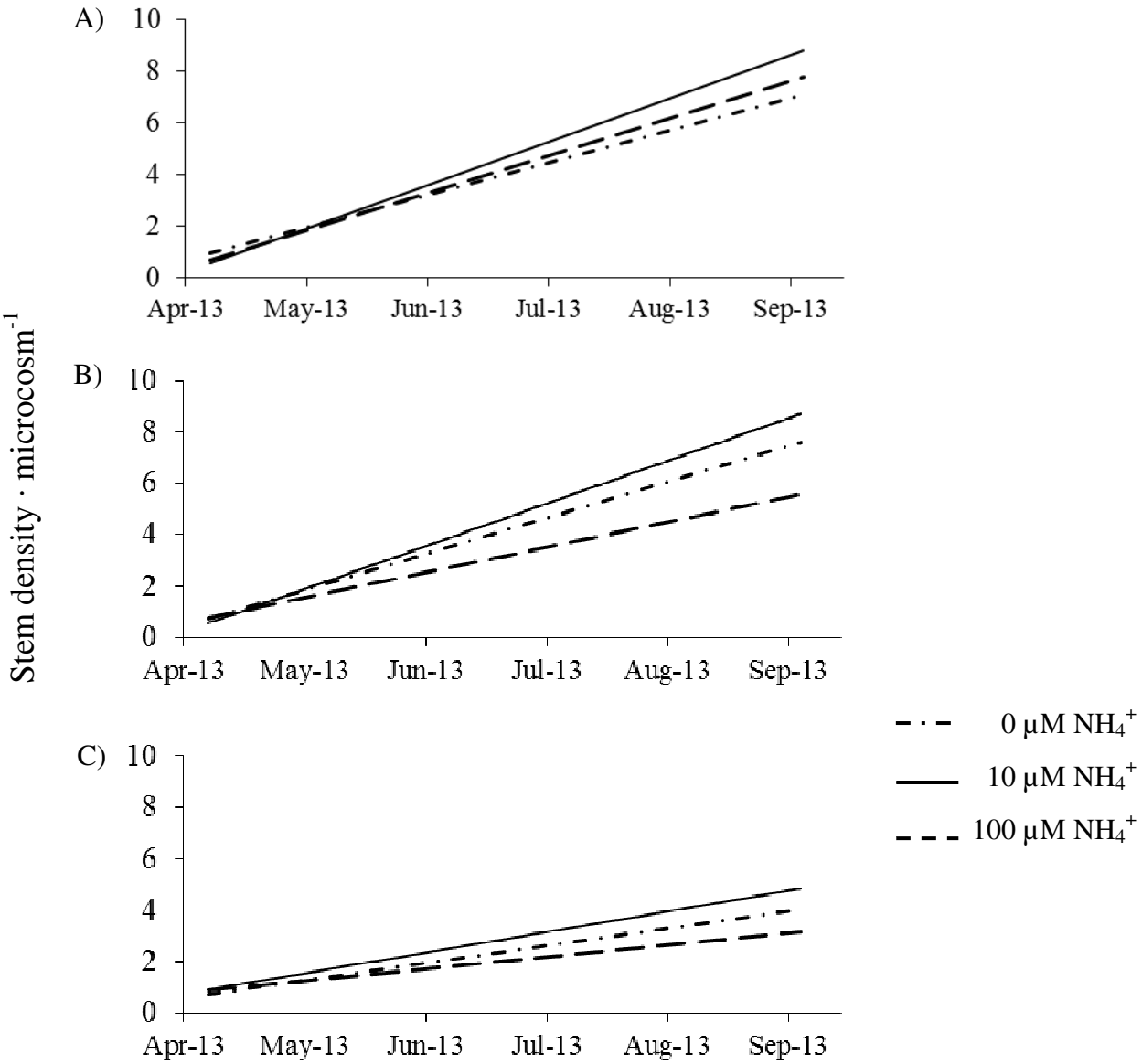


Figure 3.4. Average live stem density in microcosms exposed to 0, 10 or 100 µM NH_4^+ addition in salinities of a) 20, b) 30, or c) 40 psu from April to September 2013.

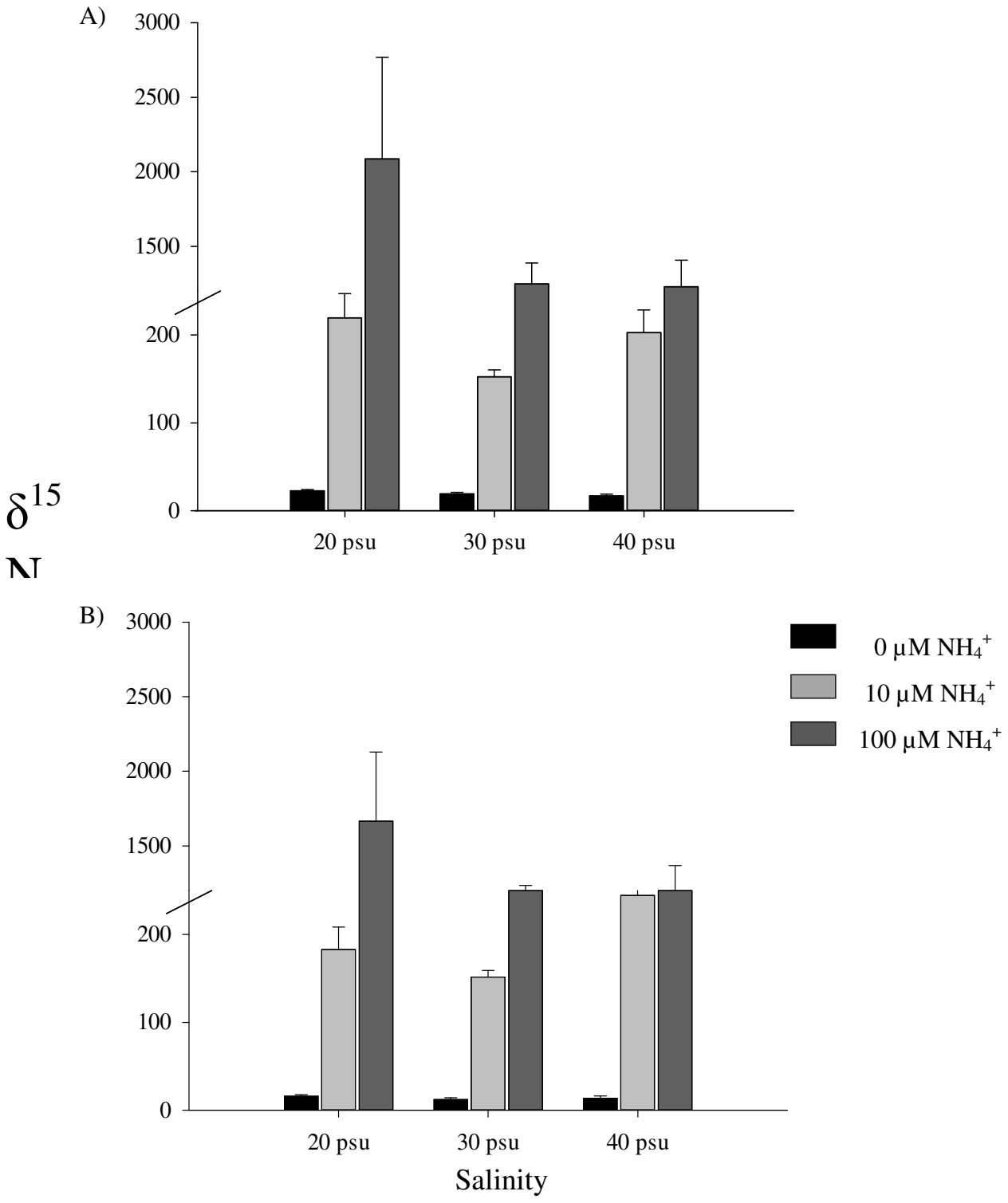
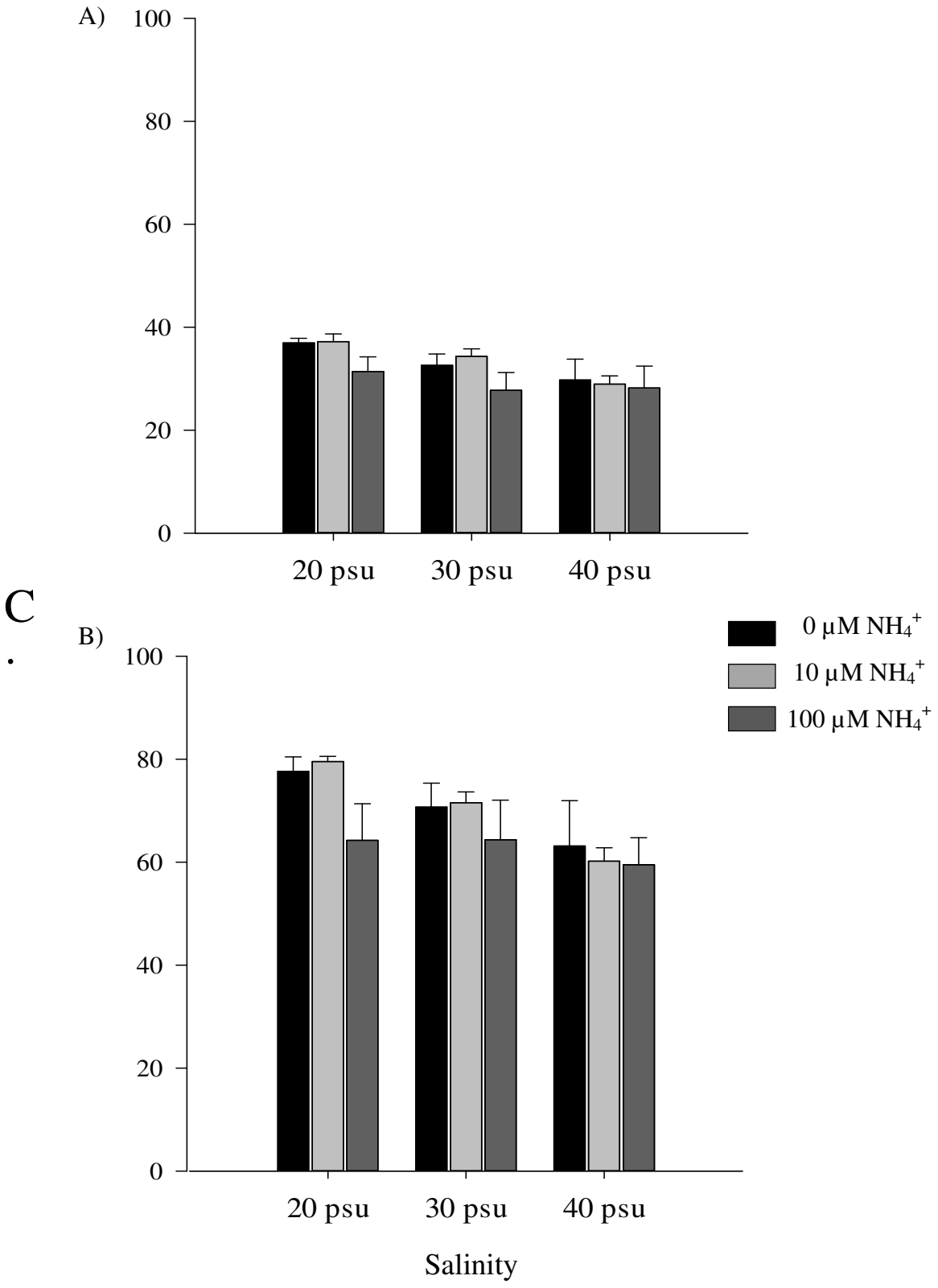

A)
3000
2500
2000
1500
200
100
0
20 psu
30 psu
40 psu
δ15N
B)
3000
2500
2000
1500
200
100
0
20 psu
30 psu
40 psu
Salinity
0 µM NH4+
10 µM NH4+
100 µM NH4+

Figure 3.5. Mean *Spartina alterniflora* tissue $\delta^{15}N$ aboveground (a-c) and belowground (d-f) after 6 months of exposure to 0, 10 or 100 µM NH_4^+ addition and 20, 30 or 40 psu salinity. Error bars represent ± one SEM (n=6).


A)
100
80
60
40
20
0
20 psu
30 psu
40 psu
B)
100
80
60
40
20
0
20 psu
30 psu
40 psu
Salinity
0 µM NH4+
10 µM NH4+
100 µM NH4+

C
.

Figure 3.6. Mean *Spartina alterniflora* tissue C:N of a) aboveground and b) belowground after 6 months of exposure to 0, 10 or 100 μM NH_4^+ addition and 20, 30 or 40 psu salinity. Error bars represent ± one SEM (n=6).

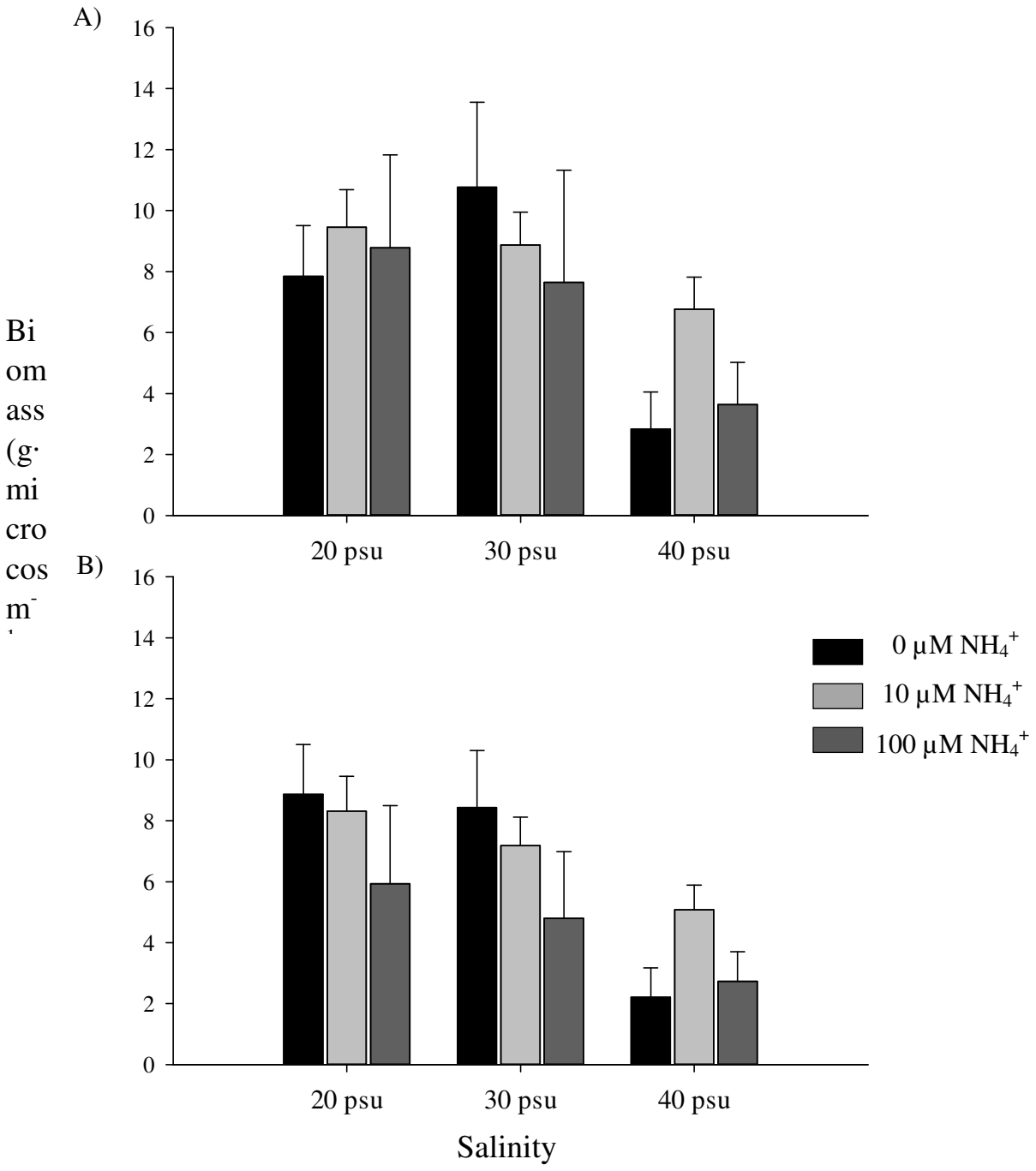


Figure 3.7. Average live *Spartina alterniflora* a) aboveground and b) belowground biomass after 6 months of exposure to 0, 10 or 100 µM NH_4^+ addition and 20, 30 or 40 psu salinity. Error bars represent ± one SEM (n=6).

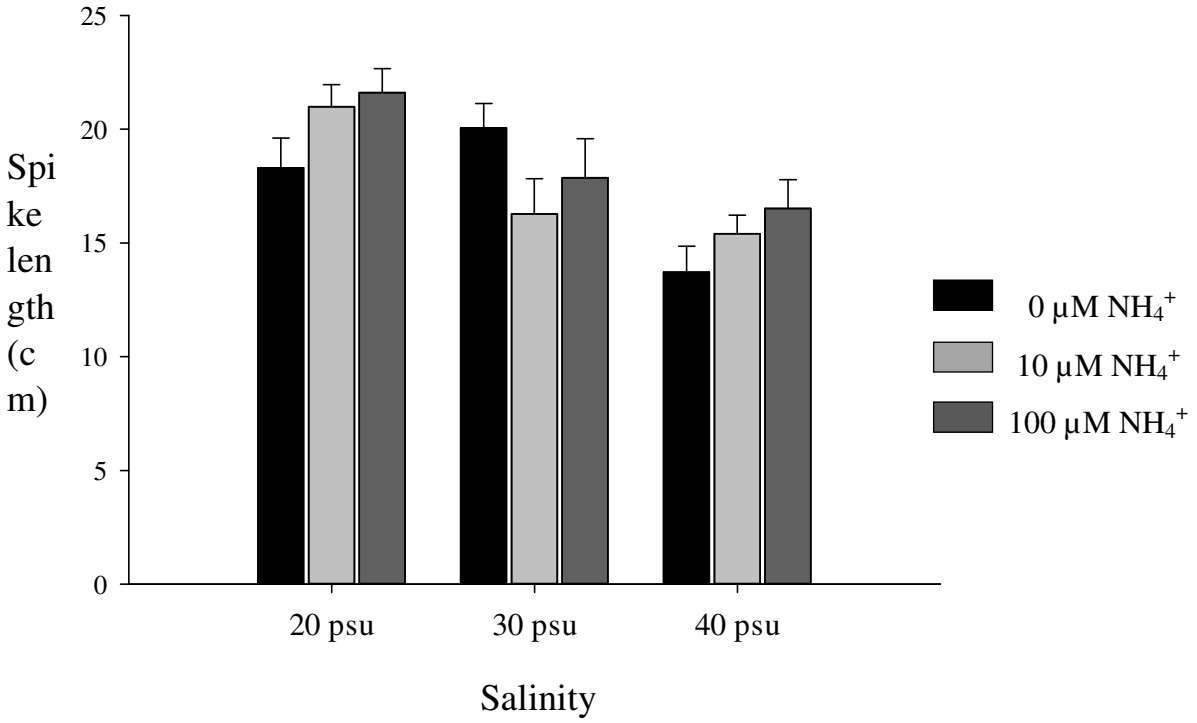


Figure 3.8. Average *Spartina alterniflora* spike length · microcosm^{-1} (cm) after 6 months of exposure to treatment combinations of 0, 10 or 100 µM NH_4^+ addition and 20, 30 or 40 psu salinity. Error bars represent ± one SEM and n=6 except for 20 psu -10 NH_4^+, 20 psu -100 NH_4^+, 40 psu -100 NH_4^+ (n=5) and 40 psu - 0 NH_4^+ (n=4).

CHAPTER 4

APPENDIX A: FIELD COLLAR TRIALS

Rationale

A pilot study was undertaken to determine whether it was possible to raise salinity *in situ* to conduct an ammonium and salinity manipulation study. I adapted methods used by Christopher Craft, from the University of Indiana, who applied elevated salinity water to the marsh using 3 m^2 Plexiglas enclosures buried ~20 cm deep, leaving ~10 cm above ground. Craft found that he could increase porewater salinity from 0 psu to ~15 psu by applying 50 gallons of seawater to the enclosures in freshwater tidal marsh (Ellen Herbert, personal communication, July 12, 2012).

Methods

I developed small-scale enclosures (collars) and applied elevated salinity water to salt marsh plots at the Skidaway Island SERF (Salt marsh Ecosystem Research Facility) site (31°58'30.46"N, 81° 1'50.86"W) from September 18, 2012 through November 25, 2012 to determine whether pore water salinity could be raised to 45 psu and 55 psu. Marsh collars were established in monospecific stands of *Spartina alterniflora* near the upland boarder where salinity was ~ 33 psu.

Marsh collars were constructed from 18.9 l buckets (36.8 cm x 29.8 cm OD) by cutting off the bucket bottom. After severing the rhizomes surrounding a marked plot, buckets were inserted in the sediment leaving ~10 cm above the sediment surface and ~20 cm below the surface, therefore enclosing an area containing roots and rhizomes. Elevated salinity treatments were prepared from Instant Ocean® and deionized water to concentrations of 100, 75, and 50 psu

(n=4) and 3.5 L of treatment solution was added weekly. In addition, control collars were installed with no added solutions (0 psu) to determine effects of the collars on plants. Treatments were applied weekly 1 hour before low tide and porewater salinity was monitored. The collars in trial 1 flooded with the next incoming tide and remained waterlogged throughout high tide. Therefore, A 4.7 mm hole was drilled at the sediment level slowing drainage of treatment solutions and tides to ~ 45 minutes after filling. I conducted three total collared marsh trials in order to achieve consistent and significant increase in porewater salinity due to application of increasing salinity solutions.

Interstitial salinity was measured weekly as response to treatment. Interstitial water samples were collected using polyvinyl chloride (PVC) 'wells' modified from Nestler, 1977. Modified wells were constructed of 35 cm lengths of PVC tubing (1.75 cm ID), 1 mm slits were placed every centimeter starting 10 cm from the top of the well. The wells were pushed into marsh sediment leaving ~5 cm of the well exposed, and then a rubber stopper was used to prevent rainfall from entering the well. Interstitial salinity was monitored weekly in the field from PVC wells with a pipette and salinity measured with a refractometer. Interstitial salinity was analyzed using rmANOVA, upon meeting normality assumptions.

Results

Interstitial salinity during the first trial period, September 18, 2012 through October 21, 2012 was significantly affected by a time*treatment interaction (Repeated measures ANOVA, $F_{3,12}=61.88$, $p<0.001$) (Figure 3A) After the first week of treatment salinity spiked in treatment collars. The elevated porewater salinity appears to stabilize with subsequent additions resulting in approximatley 60, 40, and 40 psu (100 psu, 75 psu, and 50 psu treatment additions for the

duration of the study. The 50 psu treatment appeared to produce porewater salinity similar to the 75 psu, therefore in trial 2 starting on October 21st, I dropped the 50 psu treatment and tested the effect of 75 psu and 100 psu solutions.

A second collar trial (collar trial 2) was established beginning October 21, 2012 because of confounding factors in collar trial 1. Several drainage holes were clogged in collar trial 1 after the first week of treatment so we expanded drainage holes to 6.3 mm. However, *Spartina alterniflora* ramets in the clogged plots began to brown by week 3 likely due to constant waterlogging. In order to determine if the plants were dying due to poor drainage in week 1 and 2 or due to increased salinity, all collars were moved to a new area and collar trial 2 began on October 21, 2012 and ended on November 26, 2012 with new plants (n=4). We limited the treatments to 0 psu, 75 psu, and 100 psu because post hoc analysis of collar trial 1 showed no significant difference between 50 psu and 75 psu. Collar trial 2 results (Friedman's test; $\chi^2_{3,3} = 68.4$; $p < 0.01$) show salinity treatment affected the porewater salinity (Figure 3B).

Response differences between collar trial 1 and collar trial 2 resulted in a third collar trial (collar trial 3). In collar trial 1, I found significant differences in elevated porewater salinity from the 0 psu, 75 psu, and 100 psu treatments on all dates after the initial treatment (Figure 3A). However, porewater salinity in collar trial 2 was not different due to treatment for the duration of the study (Figure 3B). From December 2, 2012 to December 19, 2012 we treated collar field plots with treatment solution twice a week see if we could strengthen the results from collar trial 2. Friedman's nonparametric test revealed significant differences due to treatment (Friedman's test; $\chi^2_{3,4} = 51.78$; $p < 0.01$), as in collar treatment 2 we were unable to clearly see differences between treatments on later dates. For example, on December 19, 2012 the 75 psu treatment (43 ± 3.8) did not appear clearly separate from the 100 psu treatment (45 ± 5.3).

Conclusions

The field collar experiment raised salinity as expected during the first trial period based on the adaptation of Christopher Crafts freshwater salinity addition experiment. During the second and third field collar trials it became difficult to detect differences in porewater salinity due to treatment. Craft was successful in slowly and evenly increasing porewater sediment in freshwater marshes by adding full strength seawater. However, due to the inconsistency of response in field trials, I would not recommend adding concentrated artificial seawater to collared sections of marsh.

My results may have been influenced by physical factors including duration of exposure to treatment and sediment porosity. Holes designed to regulate treatment water flow from the collars were easily obstructed by snails and dirt. Changes in flow rate during the second and third field collar trial could have resulted in longer treatment residence in a portion of the collars, increasing salinity from longer percolation through the soil and increased rates of evaporation (Adams, 1963). In addition, physical characteristics such as porosity of the soil can occur in small spatial areas (Bradley and Morris, 1990), thus treatments may have not absorbed into the sediment at the same rate.

References

Adams, D. 1963 Factors Influencing Vascular Plant Zonation in North Carolina Salt Marshes. *Ecology.* Vol. 44. No. 3. pp. 445-456

Bradley, P., Morris, J. 1990. Physical characteristics of salt marsh sediments: ecological implications. *Marine Ecology Progress Series.* Vol. 61. pp. 245-252

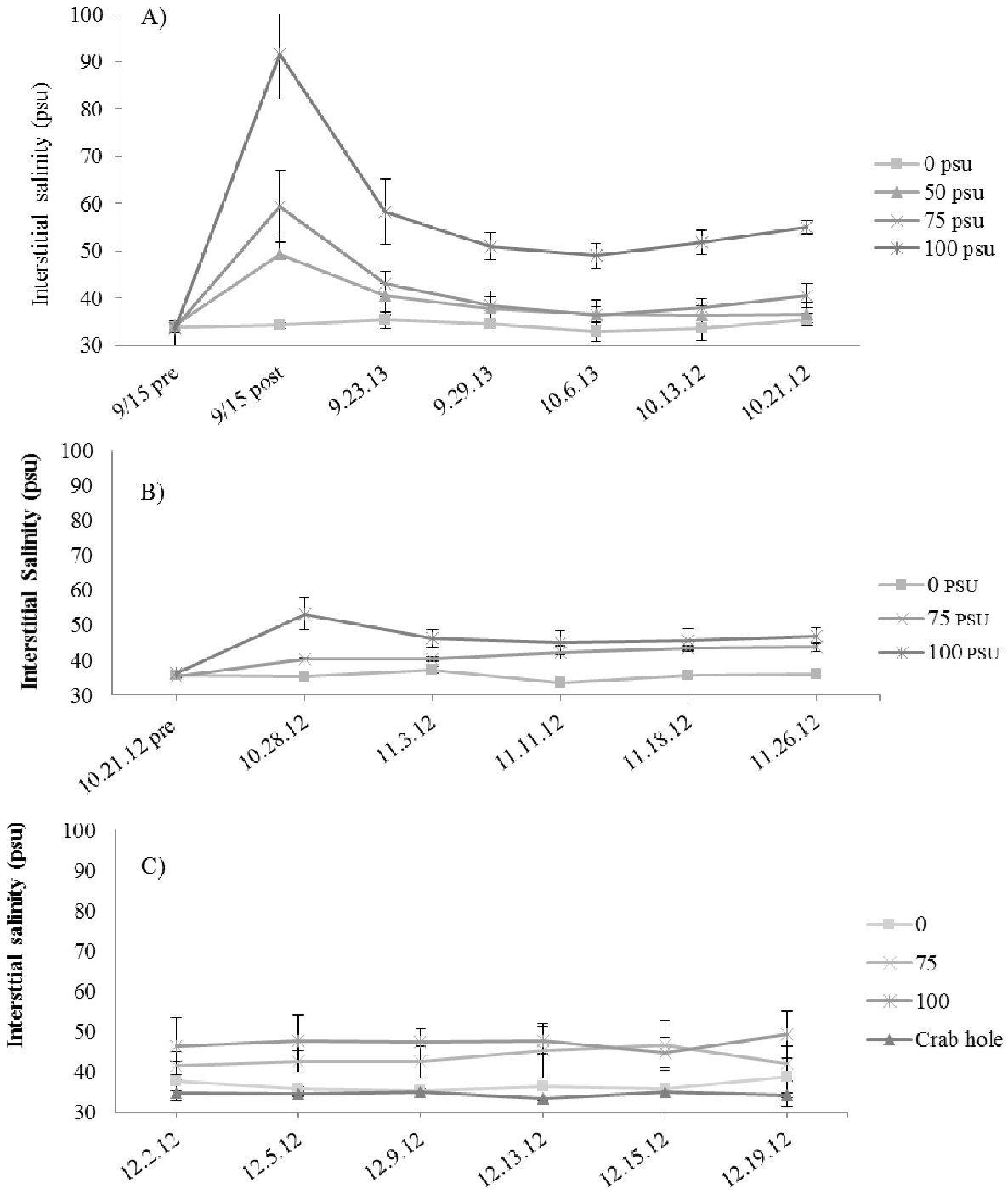


Figure 4.1 Response of interstitial salinity to increasing salinity solutions in marsh collared field plots. A) 50, 75, and 100 psu treatments from 9/15/12 to 10/21/12. B) 50 and 100 psu treatments from 10/21/12 to 11/26/12. C) 50 and 100 psu treatments applied bi-weekly from 12/2/12 to 12/19/12. Error bars represent ± one SEM (n=4).