ABSTRACT

Title of Document: A COMPETITIVE INTERACTION AND DOMINANCE EXPERIMENT BETWEEN THE VEGETATIVE MARSH SPECIES PHRAGMITES AUSTRALIS AND SPARTINA CYNOSUROIDES UNDER ELEVATED NITROGEN AND SALINITY LEVELS

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In recent decades the invasive plant Phragmites australis (common reed) has spread throughout Chesapeake Bay marshes, lowering plant community biodiversity. Excess nutrient loading and salinity intrusion due to sea-level rise make these marshes vulnerable to invasions. This study examined the interaction between Phragmites australis and the native Spartina cynosuroides (big cordgrass) to determine whether dominance of one species was detected across a range of salinity and nitrogen treatments. Aboveground biomass production of P. australis was greater than S. cynosuroides at lower salinities; however, S. cynosuroides maintained biomass production as salinity increased. Fv/Fm ratios were measured as an indirect measurement of plant tissue physiological health; only Spartina maintained the ratio at higher salinities. Nitrogen addition increased Phragmites biomass and Fv/Fm ratio at higher salinities. Results suggest salinity and nitrogen interactively affect Phragmites biomass production, and that the negative effect of increased salinity on Phragmites spread can be mitigated by nitrogen runoff.
A COMPETITIVE INTERACTION AND DOMINANCE EXPERIMENT BETWEEN THE VEGETATIVE MARSH SPECIES *PHRAGMITES AUSTRALIS* AND *SPARTINA CYNOSUROIDES* UNDER ELEVATED SALINITY AND NITROGEN LEVELS

By

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Chapter 1: Introduction

Background

Over the past century, invasive genotypes of the species *Phragmites australis* (Cav.) Trin. ex Steud. (common reed, hereafter referred to as *Phragmites*) have rapidly spread throughout coastal wetlands of North America including within the Chesapeake Bay region, due to factors such as human disturbance and increased nutrient runoff (Chambers et al. 1999). *Phragmites* is viewed as a threat to wetlands in North America including in the Chesapeake Bay because it reduces plant community species diversity wherever it establishes itself, displacing other plant species and producing tall, dense stands with extensive rhizomes (Rice et al. 2000). The global phenomenon of eutrophication from increased nitrogen inputs could continue to alter plant species composition and diversity of coastal marshes by facilitating the spread of *Phragmites* to the exclusion of other plant species (Crain 2007).

Increasing salinity due to sea-level rise may also affect the presence and spread of *Phragmites* in coastal wetlands. Increasing salinity will convert freshwater habitats to more brackish salinity regimes that will kill freshwater species and open niches for *Phragmites* to colonize (Chambers et al. 1999). However, whether this will facilitate *Phragmites* invasions is uncertain because it has not evolved salt-tolerant adaptations and therefore typically grows in lower salinity environments (such as freshwater and oligohaline wetlands), although it has been observed in brackish and
even salt marshes (Roman et al. 1984; Rice et al. 2000; Meyerson et al. 2009). As shown by my experiment, the future success of *Phragmites* in coastal marshes of the Chesapeake Bay as sea-level rise occurs may highly dependent on multiple factors, including the ability of *Phragmites* to continue to produce large amounts of biomass in increasingly saline environments, the amount of nitrogen that flows into Chesapeake Bay waters from agricultural and urban runoff, and on *Phragmites*' ability to utilize this nitrogen for biomass production and interspecies competition.

Rates of relative sea-level rise in the Chesapeake Bay are 3-6 mm/yr (NOAA 2013), and are higher than any other area along the U.S. Atlantic coast. Several studies predict that a significant portion of Chesapeake Bay coastal wetlands will be inundated, further fragmented, or eroded by future sea-level rise (Rice et al. 2000; Crain et al. 2004; Pathikonda et al. 2009). This phenomenon has the potential to shift marsh plant community structures in favor of species that are more tolerant of increased inundation and salinity that result from sea-level rise. But as sea-level rise displaces currently growing plants, niches will open that may be filled by typical colonizers such as *Phragmites*. This species is able to rapidly produce large amounts of biomass, precluding other species from establishing in the area (Chambers et al. 1999; Rice et al. 2000).

Several studies have explored the concept of niches with respect to habitat zonation, the hypothesis that a tradeoff exists between stress tolerance and competitive ability (Bertness 1991; Levine et al. 1998; Liacourt et al. 2005; Lubchenco 1980; Pennings and Callaway 1992). This hypothesis presumes that the competitive ability of a species is generally inverse to its ability to tolerate abiotic
stress (Liancourt et al. 2005). Disturbances may displace plants and provide an opportunity for new species to colonize the area, as *Phragmites* has been found to commonly do (Chambers et al. 2003). However, this may only applicable to disturbances such as nutrient addition that favor *Phragmites*, a species that tends to be found in low salinity habitats. The large amount of biomass that *Phragmites* produces is able to shade and crowd out other species in competition for sunlight and space (Rice et al. 2000) which, by this hypothesis, would make it an effective competitor but less able to tolerate physical stress. In contrast, *Spartina* is generally found in higher salinity zones due to its salt tolerance mechanisms. *Spartina*’s salt tolerance mechanisms favor its growth in higher salinity marshes where the ability to tolerate harsh physical conditions, as opposed to interspecific competition, is the determining factor for plant zonation (Pennings and Callaway 1992). These two species exemplify the tradeoff between interspecies competitive ability and the ability to inhabit stressful environments.

Nutrient input from both agricultural and urban sources is facilitating species invasions in coastal wetlands throughout the world (Zhao et al. 2009). The U.S. human population has grown in all regions of the country over the past decade (U.S. Census Bureau 2012), and nitrogen runoff from anthropogenic sources such as agriculture, sewage, and atmospheric deposition are increasingly affecting natural nitrogen cycles (Galloway 2004; Crain 2007). Greater nitrogen inputs to N-limited coastal wetlands could cause important shifts in plant community structure by promoting the invasion of plant species that rapidly establish monotypic stands and drastically reduce community species diversity (Crain 2007). Disturbance in the form
of increased nutrient availability has been shown to promote the spread of *Phragmites* (Minchinton and Bertness 2003). Its occurrence in New England marshes is closely tied to increased nitrogen (Meyerson et al. 2009). The invasive *Phragmites* has also been found to have a higher demand for N compared to the native *Phragmites* and *Spartina alterniflora* and *Spartina patens* (Meyerson et al. 2000). The nitrogen demand of the introduced *Phragmites* is approximately four times that of the native in mid-Atlantic tidal marshes, but anthropogenic N input has doubled along the North American Atlantic coast since pre-industrial times (Mozdzer and Zieman 2010).

*Phragmites* may be a more efficient competitor for other limiting resources when nutrients such as nitrogen occur in surplus (Chambers et al. 1999). This characteristic could contribute to its continued success despite increased physiological stress from higher salinity due to sea-level rise. However, it is unclear how increased salinity and continued nitrogen runoff into coastal marshes will ultimately interact to alter coastal marsh plant communities by affecting the growth of invasives such as *Phragmites*.

This question is crucial to predicting the future spread of *Phragmites* throughout Chesapeake Bay wetlands. Understanding the distribution of native and invasive plant species along gradually changing salinity gradients will increase the ability to accurately predict how coastal wetland plant communities will respond to continued salinity increase and a possible further increase in nutrient loading (Crain et al. 2004).

This study examined the biomass production and tissue health of *Phragmites* and the native species *Spartina cynosuroides* (L.) Roth (big cordgrass, hereafter referred to as *Spartina*) grown in mixture under conditions of varying salinity and nitrogen levels. *Spartina* was chosen for this study because it is a native that is
phenotypically similar to *Phragmites*. Furthermore, the two species grow together throughout the Chesapeake Bay region in oligohaline and brackish coastal marshes. Their similar structure and overlapping habitat make it likely that the two species compete for the same resources and space. Finally, *Spartina* was chosen because it has evolved salt tolerance mechanisms that may allow it to continue to produce biomass that does not show evidence of physiological stress as salinity increases due to sea-level rise.

*Spartina and Phragmites Morphologies and Life Histories*

From a management and policy perspective, *Spartina* is classified as a native species in the U.S. Atlantic and Gulf of Mexico coasts; *Phragmites* on the other hand is classified as an invasive and is therefore subject to eradication and control methods. *Spartina* is a grass native to wetlands along the U.S. Atlantic and Gulf Coasts that reproduces primarily through rhizome production, similar to *Phragmites*. It has been found in oligohaline marshes (0.5-5 ppt), but it typically occupies habitats with slightly higher salinities than the 0-5 ppt range where *Phragmites* is commonly found (Rice et al. 2000). *Spartina* is additionally described as being found in the muck of brackish coastal marshes (Godfrey and Wooten 1979) and “often in water” (Brown and Brown 1984). *Phragmites* is described as being most common in salinities less than 5 ppt, but its presence has been noted to some extent in salinities up to 18 ppt, and it has been documented in even higher salinities (Chambers et al. 1999, 2003). One study found that mortality did not occur in a greenhouse setting until 35 ppt (Hartzendorf and Rolletschek 2001). Upper limits of 45, 50 and 65 ppt have been reported for *Phragmites* in greenhouses (Hellings and Gallagher 1992). *Phragmites* is
most abundant in high marshes in the Northern Atlantic Coastal Region (Chambers et al. 1999).

*Spartina* has evolved several salinity adaptations such as the water-efficient C4 photosynthesis pathway, and specialized leaf glands that excrete salt that was absorbed in the soil porewater by its rhizomes (Maricle et al. 2007). These adaptations may enhance its competitive ability against *Phragmites* as sea-level rise creates an increasingly saline environment for both species.

All plant nomenclature and descriptions, except where specifically cited, are according to the USDA Plants database, http://plants.usda.gov, accessed 05/20/11.

**Objectives and Hypotheses**

Enhancing our understanding of how nitrogen and salinity interact to influence plant community distribution will improve our ability to anticipate the extent and location of the spread of *Phragmites* in the region as nitrogen input and sea-level rise continue. It will also increase our ability to successfully construct created and restored wetlands that incorporate a high diversity of native species with the highest chance of long-term survival, by preferentially including species that have evolved salt tolerance mechanisms and that have been shown to effectively compete against *Phragmites*, reducing the likelihood of the establishment of monoculture *Phragmites* stands throughout coastal wetlands.

The objectives of this study were to determine through biomass and fluorescence measurements the effects of salinity and nitrogen addition on the growth and physiological health of *Phragmites* and *Spartina*, and to determine whether dominance of one species over the other existed across a range of treatment
combinations. I hypothesized that *Spartina* would comprise the majority of relative biomass at high salinity levels, because of its salt tolerance adaptations. I further hypothesized that at high nitrogen levels *Phragmites* would comprise the majority of relative biomass, because of its high production of biomass in response to nitrogen.
Chapter 2: Methods

Plant Collection and Greenhouse Setup

Live rhizomes of *Spartina cynosuroides* and *Phragmites australis* were collected from the same location of the Clyde Watson Boating Area in Brandywine, Maryland (38°38’N, 76°41’W), where they were found growing together (Figure A1.1). The boating area is located on the Upper Patuxent River (Figure A1.2). The wetland is microtidal (<1 m difference between high tide and low tide) and semidiurnal (2 high and 2 low tides per day) system. It is categorized as a marsh, i.e. dominated by herbaceous plants, and is located on the Upper Patuxent River, a tributary of Chesapeake Bay in southeastern Maryland, USA. On July 27, 2011, salinity measurements of 1.7-2.2 ppt were taken during a site visit, indicating that the marsh is oligohaline. Rhizomes were transported to the University of Maryland Research Greenhouse and placed in standing fresh water until new shoots emerged. *Spartina* stems were removed from rhizomes by clipping, and the rhizomes were then cultivated standing upright in freshwater in a constructed wooden trough (2.6 m x 0.79 m, 0.41 m depth) lined with 45mm-thick Firestone Pond liners (Nashville TN). To cultivate new *Phragmites* shoots, rhizome masses of *Phragmites* were placed horizontally in a second similar trough with the stems attached, until new shoots emerged at the stem nodes (Figure A1.3). Both troughs were filled with fresh
water to a depth of 20 cm, submerging the rhizome masses approximately 80%. A photograph of cultivation in the troughs is included in Appendix 1 as Figure A1.4.

The new growth was separated into clumps of 3-4 live stems of similar total stem length and rhizome mass dimensions. Similar dimensions (rhizome mass and total stem length) of plants of both species were potted together in a 25/75 (v/v) sand/soil mixture in cylindrical plastic buckets (14.5 cm x 34 cm, radius x height). During the experiment both species were grown in mixture in all mesocosms. Sand and “Sunshine LC1 professional growing mix” soil were provided by the greenhouse facility.

Each bucket was placed on two 0.61x0.61-meter wood blocks and nested inside a rectangular plastic tank (39 cm x 32.5 cm x 47 cm, length x width x height) that contained standing water maintained at 10 cm below the soil surface of the inner bucket (Figure A1.5). Approximately 35 0.6-cm holes were drilled into the bottom of each bucket for drainage (Figure A1.6). To simulate the natural shaded stands that both species grow in, a 60.96-cm-tall 40% white shade cloth was attached with bamboo stakes to the upper rim of each inner bucket. Treatments began 14 days after planting. The water level was raised to 10 cm above the soil surface for 2 days per week and then lowered to 10 cm below the soil surface for the rest of the week, creating a 30% duration of inundation similar to that of high marsh communities (Mitsch and Gosselink 2007). To simulate tidal fluctuations in salt marshes, McKee and Rooth similarly established a flooding regime, manually raising the water level 7-10 cm at regular intervals (2008). Although flooding is a major component of sea-level rise along with salinity, differing periods of inundation were not chosen as a
treatment variable for several reasons. It is challenging to simulate realistic daily, weekly, and seasonal flooding cycles in a greenhouse without automated equipment or a significant commitment of time and labor. Additionally, for simplicity the experiment was limited to two treatment variables, salinity and nitrogen.

Within the greenhouse chamber, daytime temperature was set to 26.6° C (80° F) and nighttime temperature was set to 21.1° C (70° F). Daytime relative humidity was set to 80% and nighttime relative humidity was set to 55%. Conditions were maintained by automated controls. Figures 2.1 and 2.2 show the daily average minimum and maximum of the temperature and light intensity that were recorded in half-hour increments throughout the experiment.

**Salinity and Nitrogen Treatments**

Six salinity levels were created using dissolved Instant Ocean: 0, 4, 10, 18, 28, 40 ppt. Two nitrogen levels were also created using dissolved urea 46-0-0 fertilizer: a “Low” treatment level of 0.09 g/m²/wk nitrogen (equivalent to 30 g/m²/yr), and “High” treatment level of 0.9 g/m²/wk nitrogen (equivalent to 300 g/m²/yr). Treatments were distributed randomly in 3 blocks, totaling 36 mesocosms (Figure A1.7). Each salinity level was chosen in order to represent a scenario currently existing in or around the Chesapeake Bay, and to represent scenarios that may occur in the Clyde Watson Boating Area wetland and other oligohaline wetlands as sea level rises.

At the beginning of the experiment, salinity levels were gradually raised every other day by one treatment level until all levels were achieved, e.g. from 0 to 4 ppt, and then from 4 to 10 ppt. Salinity levels were then maintained using a weekly
application of dissolved Instant Ocean. Treatments were maintained for 14 weeks. Porewater salinity measurements were taken during the experiment to ensure that salinity levels were achieved. Dissolved nitrogen was added weekly to the inner bucket standing surface water. The “high” rate of 300 g/m²/yr is lower than the rate used in previous related field studies including Levine et al. (1998) (450 g N/m²/yr), and Pennings et al. (2002) (452 g N/m²/yr), and resulted in no obvious signs of plant burning in this study. This is a typical rate found in natural salt marshes (Mitsch and Gosselink 2007). The “low” loading rate of 30 g/m²/yr is equivalent to the average annual nitrogen loading rate in natural freshwater inland marshes (Mitsch and Gosselink 2007). Similarly, the current estimated average annual nitrogen loading rate for tidal fresh marshes is 50 g/m²/yr, and for salt marshes is 90 g/m²/yr (Mitsch and Gosselink 2007). A survey performed 1973-1980 at the Rhode River, a subestuary of the Chesapeake Bay, calculated approximately 1.065 g/m²/yr average annual total N area loading into the subestuary due to bulk precipitation (Correll and Ford 1982). A more comprehensive modeling study a decade later estimated that the total N input into the Chesapeake Bay was 20.54 g/m²/yr into the upper main stem of the bay and 29.33 g/m²/yr into the Potomac River alone (Boynton et al. 1995). These 1995 estimates represent an 8-fold increase in total N inputs from colonial times (Boesch et al. 2001). The calculations by Correll and Ford are smaller than the estimates made by Boynton et al. because of the smaller forested area of the Rhode River subestuary and because of the time difference of more than a decade between the two studies.
Vegetation and Soil Measurements

The lengths of all stems in each experimental unit (bucket) were measured and added to calculate total stem length. Total stem length per species per mesocosm was measured weekly while treatments were applied and recorded as Weekly Total Stem Length. Change in Total Stem Length was calculated as the final week’s total stem length minus the initial week’s total stem length. Weekly average stem length was calculated by averaging each week’s Weekly Total Stem Length. Weekly total stem number was calculated by adding the number of stems counted during each week’s measurements. Analyses for the week of April 5 were not performed and data was not included in any data sets, due to missing data.

The maximum chlorophyll fluorescence (Fv/Fm ratio) of leaves of both species was measured in order to indirectly estimate the physiological health of the live aboveground biomass. A Walz PAM-2100 Chlorophyll Fluorometer (Heinz Walz GmbH, Effeltrich, Germany) was used on two different dates during the experiment: Thursday, April 19, 2012 (58 days after treatments began), and Tuesday, May 14, 2012 (83 days after treatments began). Readings were taken at night to eliminate introduced variability from time of day and angle of sunlight. Three live leaves per species per mesocosm were measured. For standardization, chosen leaves were the newest growth that was wide enough to fill the 1-cm² measurement area of the instrument. Measurement of changes in Fv/Fm ratios was chosen because it is a rapid and widely used method of measuring chlorophyll fluorescence, so that it is well documented in previous literature (Maxwell and Johnson 2000).
Explanations of the theory behind the F_v/F_m ratio can be found in Maxwell and Johnson (2000) and Mateos-Naranjo et al. (2007). The basic process begins with dark adaptation of the measured leaf for at least 15 minutes, to “empty” electrons from all PSII reaction centers and reduce photochemical quenching (PQ) and non-photochemical quenching (NPQ) and fluorescence. Next, to establish a baseline fluorescence, a light source is directed onto the leaf with a light that is not of a wavelength that drives photosynthesis, to obtain the value of minimum fluorescence (F_0). Then, a light pulse of a defined wavelength is directed at the leaf, and the light energy is absorbed by the leaf’s chlorophyll molecules. Light energy can take one of three paths: the energy can enter a Photosystem II reaction center to drive photosynthesis by the creation of ATP, or be re-emitted as heat (NPQ) or light of a longer wavelength (chlorophyll fluorescence). These second two processes act to protect the plant from damage from excess light energy. An instantaneous reading of maximum fluorescence (known as F_m or F_max) is taken before NPQ occurs and when all Photosystem II reaction centers have accepted an electron but before they have time to pass on the electron to Photosystem I. The relative proportions of each of the three possible pathways provide information about the leaf’s health, since a greater proportion of fluorescence and NPQ indicate that fewer Photosystem II reaction centers are available to perform photosynthesis. At this instantaneous reading, NPQ is eliminated as a potential electron pathway. The proportion of light that is fluoresced back to the instrument indicates the proportion that has been absorbed by the reaction centers; a larger amount of fluorescence indicates that fewer photosystems are
available to accept electrons, attributable to abiotic stress. A greater proportion of fluorescence creates a smaller $F_v/F_m$ ratio in the following equation:

$$F_v/F_m = (F_m - F_0) / F_m$$

Ultimately, $F_v/F_m$ ratios measure the maximum intrinsic efficiency of photosystem 2 (PSII) (Maricle et al. 2007). A physiologically “optimal” range for most plant species has been found to be near 0.83 (Bjorkman and Demmig 1987; Johnson et al. 1993; Maxwell and Johnson 2000).

At the end of the experiment the aboveground biomass was separated by clipping all live plants at the soil surface. Belowground biomass was rinsed from the soil through a 1-mm x 2-mm filter. After harvest, all biomass was dried in an environmental chamber at 35° C and 12% humidity until the samples reached a constant mass, approximately two weeks. The biomass was then weighed to the nearest 0.1 g. Collection, transplant and treatment application methods were adapted from Crain (2004). During treatment applications, nitrate nitrogen (NO$_3$-N) and ammonium nitrogen (NH$_4$-N) were measured twice in porewater samples with a Hach Model DR 2400 portable spectrometer (Hach Company, Loveland, Colorado, USA). Additional detailed methods descriptions are included in Appendix 3.

**Data Analysis**

The arrangement of treatments was a 6×2 factorial. All data were analyzed using two-way analysis of variance (ANOVA) except weekly change in stem height results, which were analyzed using repeated measures analysis of variance.
(RMANOVA). The Tukey’s procedure was used to separate treatment means for all analyses except for weekly change in stem height results. All weekly change in stem height results, including weekly total stem height, weekly average stem height, and weekly totally number of stems, were log-transformed to meet the assumption of homogeneity of variance. Analyses were conducted using SAS 9.2 (SAS Institute, Cary, North Carolina) and SigmaPlot 10 (Systat Software, San Jose, California). An example of the SAS code is included in Appendix 2.
Figure 2.1: Greenhouse Temperature: Daily Maximum and Minimum. Daily maximums and minimums are based on continuous automated half-hour measurements.

Figure 2.2: Greenhouse Light Intensity: Daily Maximum and Minimum. Daily maximums and minimums are based on continuous automated half-hour measurements.
Chapter 3: Results

Change in Total Stem Length

For both species, as salinity increased, the effect of N addition on the change in total stem length (difference between final and initial weekly measurements of combined length of all stems within mesocosms) decreased, resulting in a significant salinity*nitrogen interaction (Figure 3.1, Table 1). At lower salinities, addition of N resulted in a significant increase in *Phragmites* change in total stem length (almost 400% increase at 0 ppt, more than 500% at 10 ppt) but there was no significant effect of N addition at 18 ppt or above in *Phragmites*. In contrast, N addition only increased *Spartina* total stem length at 0 ppt, where it increased by a similar proportion to the increase seen in *Phragmites* biomass at 10 ppt.

Figure A1.9 shows a photo example of the Block 2 mesocosms arranged from 40-0 ppt salinity with High nitrogen during the final week of treatment, just prior to harvest. Figure A1.10 shows a photo example of Block 2 mesocosms arranged from 40-0 ppt salinity with Low nitrogen.

Weekly total stem length

At 0 ppt, the final week’s *Phragmites* total stem length at high nitrogen was triple the total stem length under low nitrogen, and double the final *Spartina* total stem length at high nitrogen.

At low salinities (0 & 4 ppt) a significant difference between nitrogen treatment levels appeared in *Phragmites* weekly total stem length after 4-5 weeks of
treatment application. For Spartina, at 0 ppt this significant difference did not form until the final weeks of the experiment, and above 0 ppt no significant difference existed at any time during the experiment.

Similar to its weekly stem lengths at 0 and 4 ppt, at salinity 10 ppt a significant difference between nitrogen treatment levels appears in Phragmites after 4-5 weeks of treatment applications. This indicates that nitrogen addition still enhanced Phragmites biomass growth despite increased salinity. At 18 ppt, a significant difference in Phragmites weekly stem lengths existed between nitrogen levels and the difference in total stem lengths per nitrogen treatment level became increasingly large after 4 weeks. However, at 28 and 40 ppt, no significant difference existed at any time during treatment application.

For Spartina, as salinity increased, differences in weekly total stem length over time became minimal and growth rate appeared to level off. At 28 and 40 ppt there was no significant increase or decrease in weekly total stem length for either species between the first and final measurement weeks.

These results suggest that at lower salinities, N addition helped both species to increase stem growth, but the effect of additional N decreased as salinity increased, and sooner for Spartina; Phragmites was able to benefit from N addition to increase stem growth rate at higher salinity levels than Spartina. At the lowest salinities, Phragmites stem growth rates resembled exponential curves, highlighting its ability to utilize nitrogen to produce additional biomass over time.
**Weekly average stem length**

For both species, there was no significant difference in the weekly average stem lengths at any time at all salinity and nitrogen levels, with the exception of *Phragmites* at 18 ppt with high N addition. In this instance, average stem length actually decreased over time.

The average *Spartina* weekly average stem length was similar to *Phragmites* at lower salinities but became increasingly taller than *Phragmites* at 10, 18, and 28 ppt. At these higher salinities the negative effect of increasing salinity was possibly reducing *Phragmites* stem growth.

At all salinity levels there was much greater variation in each mesocosm between the tallest and the shortest *Spartina* stems, e.g. at 0, 18, and 28 ppt. *Spartina* continued to increase the stem length of existing individuals to very tall heights relative to *Phragmites*. But at 40 ppt salinity, there was less of a height difference between the two species.

**Weekly total stem number**

At 4, 10, and 18 ppt, the number of *Phragmites* stems increased over time at high N. *Phragmites* stem production slowed as salinity increased but addition N boosted production. At 0 ppt, a significant difference in the number of *Phragmites* stems did not occur between N levels until the final week; until then, the number of *Phragmites* stems rapidly increased significantly at both N levels, possibly because of lack of salinity stress. At 4, 10, and 18 ppt, the number of *Phragmites* stems did not
increase at low N. At these increased salinity levels, salinity stress appeared to reduce stem production.

There was little or no increase in number of *Spartina* stems over time at any salinity level, with a few exceptions, and N level never had a significant effect on the number of *Spartina* stems. At 0 and 10 ppt, there was a significant difference between number of *Spartina* stems between the first week and the final week at high N, but this was because the differences in stem number were small, e.g. an increase from 2 initial stems to 4 final stems. In general, *Spartina* simply did not produce a large number of stems in comparison to *Phragmites* during the experiment. At the highest salinities 28 & 40 ppt, there was no increase in the number of *Spartina* or *Phragmites* stems over time at either N level. Where significant differences between stem numbers occur due to N level, the number of *Phragmites* stems increased more slowly than the 4-5 weeks that were observed for *Phragmites* average stem length: it was not until the final weeks of the experiment at 0, 10 and 18 ppt, and not until 6 weeks into the experiment at 4 ppt.

At all salinity and N levels, the initial number of stems was similar between the two species (intentionally created during the experimental setup) but by the end of the experiment at 0, 4, and 10 ppt the number of *Phragmites* stems at the high N level was 30-40 times the number of *Spartina* stems at either N level. The difference between the two species was smaller as salinity increased.

In these graphs and in the graphs of weekly average stem height, it appeared that *Spartina* was allocating resources to stem length as opposed to increasing number of stems. Wheares *Phragmites* seemed to allocate resources to producing a large
number of shorter stems, *Spartina* seemed to allocate resources to producing a small number of taller stems.

**Biomass**

Similar to change in total stem length, the effect of N addition on the aboveground biomass of both *Phragmites* and *Spartina* decreased as salinity increased, but only resulted in a significant salinity*nitrogen interaction for *Phragmites* (Figure 3.5a-b, Table 1). At 0, 4 and 10 ppt, N addition increased *Phragmites* aboveground biomass by about 60-75%, although N had no significant effect above 10 ppt. Nitrogen addition increased *Spartina* aboveground biomass by nearly 300% at 0 ppt and by about 100% at 4 ppt, but above these salinities there was no significant effect.

Salinity and nitrogen addition did not significantly change the belowground biomass of *Phragmites* or *Spartina* (Figure 3.5c-d, Table 1) or the total (aboveground plus belowground) biomass of *Spartina* (Figure 3.6b, Table 1), or the total biomass of both species combined (Figure 3.6c). At high nitrogen, salinity did significantly decrease the total biomass of *Phragmites* from approximately 360 grams at 4 ppt to 135 grams at 40 ppt (p<0.05) (Figure 3.6a, Table 1). The lack of significant effects in belowground biomass data caused the majority of total biomass data to also have few significant effects.

*Phragmites* and *Spartina* produced similar proportions of relative aboveground biomass at lower salinity levels but *Spartina* produced the majority of biomass at the highest salinities (Figure 3.7, Table 1). At 40 ppt, *Spartina* produced more than 80% of the aboveground biomass with high N addition and nearly 70%
with low N addition. At 40 ppt, N addition doubled *Phragmites* relative aboveground biomass from about 15% to more than 30%.

Nitrogen addition did not significantly affect relative aboveground biomass for either species at other salinities, and did not affect relative belowground biomass for either species or in combination (Figure 3.8, Table 1).

For both species combined and for each species individually, N addition did not significantly increase the root-to-shoot ratio (Figure 3.9, Table 1).

\[ F_v/F_m \text{ Ratio} \]

In April, salinity addition had no effect on the *Phragmites* \( F_v/F_m \) ratio except at 40 ppt. At this salinity level there was a significant salinity*nitrogen interaction; the high nitrogen treatment reduced the ratio by 25% and the low nitrogen treatment reduced it to a ratio of almost 0 (p<0.05) (Figure 3.10a, Table 1). When N addition was low the *Phragmites* ratio at this highest salinity level was 20% the ratio of 0.83 cited by multiple papers as “optimal” but N addition significantly increased the ratio by a factor of 6 (p<0.05) although the increased ratio was still below the optimal range. In April, there was a significant difference in the *Spartina* \( F_v/F_m \) ratio at 4 ppt under high nitrogen compared to both high N and low N ratios at 40 ppt, but the actual change was relatively small, from 0.80 to 0.77 for both high and low N (Figure 3.10b).

In May, the highest salinity level and low N addition each decreased the *Phragmites* \( F_v/F_m \) ratio, similar to the April results (Figure 3.10c). With high N addition, the *Phragmites* ratio at 40 ppt was approximately 55% of the ratio at 18 ppt. On this date greater variation was measured at all salinities in both *Phragmites* and
Spartina ratios and there was a significant salinity*nitrogen interaction for both species (Figure 3.10c-d, Table 1). As in April, N addition did not significantly affect the Spartina Fv/Fm ratio.

The difference in variation between the measurements taken on each date could possibly partly be explained by the different day of the week on which each measurement was taken. The water level was raised and lowered during the week, and increased inundation could have increased the physiological stress that the leaves experienced, leading to greater variation and lower measured ratios. Through this explanation, more variation due to stress would be expected during a high water level on Thursday, April 19, but additional variation was actually measured on Tuesday, May 14. This indicates that the greater variation is more likely due to the negative effects of raised salinity over time.

Soil Porewater Nitrate and Ammonium

At high nitrogen, porewater nitrate increased as salinity increased because of the measured decrease in aboveground biomass production that Figures 3.5a-b demonstrate (Figure 3.11, Figure 3.13a). Both these results correlate with aboveground biomass production results – Figures 3.11-3.13 can be viewed as inverses of Figures 3.5a-b. As salinity levels increased, reduced biomass of both species caused a decrease in the amount of soil nitrate absorbed by rhizomes so that it accumulated in the soil instead. At low nitrogen, nitrate did not accumulate in the soil because only a small amount of nitrogen was being added to the soil.

Later in the experiment there was less difference among porewater nitrate concentrations measured across salinities at high nitrogen. On May 12 there was no
significant difference in porewater nitrate at any salinity or nitrate level, and on May 23 a difference only existed between the highest salinity level at the high nitrogen level, and the lowest salinity levels (0, 4, and 10 ppt). This may be because of the strictness of the Tukey-Kramer test – the ANOVA indicates that there was a significant difference as salinity increased, but it may not have been indicated by the Tukey-Kramer test because the test is the most conservative analysis method. However, when averaged across all dates the same trend seen in Figure 3.11 was also reflected in Figure 3.13a.

This increase in concentration as salinity increased was also observed for porewater ammonium from 4 ppt to 40 ppt (Figure 3.12). Decreased aboveground biomass can explain these results, as well. At high nitrogen at 0 ppt, ammonium porewater concentrations were higher than at 4 ppt possibly because in general plants preferentially absorb nitrate, as opposed to ammonium, so that there was less ammonium absorbed at this salinity level.
Table 1 ANOVA results for total stem length, biomass, and fluorescence. The Randomized Complete Block Design included three blocks with a total of 36 mesocosms. Treatments were applied using a factorial arrangement of 6 salinity levels and 2 nitrogen levels. Both species were grown in mixture in all mesocosms. **Significant effects (p<0.05) are indicated in bold.**

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<td></td>
<td></td>
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<tr>
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Table 2 ANOVA results for weekly stem measurements. Measurements were taken weekly while treatments were applied. Significant effects (p<0.05) are indicated in bold.

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<th>p</th>
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<td>S^T</td>
<td>N^T</td>
<td>S^N^T</td>
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<td></td>
<td></td>
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<td></td>
</tr>
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<td>0.9991</td>
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*10^-8

*10^-7
Figure 3.1: Change in total stem length of *Phragmites* (a) and *Spartina* (b) across salinity levels and nitrogen addition levels. Change in total stem length was calculated as the difference between final and initial weekly measurements of combined length of all stems, and stem lengths were measured weekly during treatment application. Error bars represent standard error of the least squares mean. Within each species, means with different letters are significantly different (Tukey test, p<0.05).
Figure 3.2: Weekly total stem length of Phragmites (a, c) and Spartina (b, d) across salinity levels and nitrogen addition levels. Weekly total stem length was calculated as the sum of the combined length of all stems per week. Error bars represent standard error of the arithmetic mean. All data was log-transformed to meet the assumption of homogeneity of variance.
Figure 3.2: Weekly total stem length of Phragmites (e, g) and Spartina (f, h) across salinity levels and nitrogen addition levels. Weekly total stem length was calculated as the sum of the combined length of all stems per week. Error bars represent standard error of the arithmetic mean. All data was log-transformed to meet the assumption of homogeneity of variance.
Figure 3.2: Weekly total stem length of Phragmites (i, k) and Spartina (j, l) across salinity levels and nitrogen addition levels. Weekly total stem length was calculated as the sum of the combined length of all stems per week. Error bars represent standard error of the arithmetic mean. All data was log-transformed to meet the assumption of homogeneity of variance.
Figure 3.3: Weekly average stem length of Phragmites (a, c) and Spartina (b, d) across salinity levels and nitrogen addition levels. Weekly average stem length was calculated as the average of the sum of the combined length of all stems per week. Error bars represent standard error of the arithmetic mean. All data was log-transformed to meet the assumption of homogeneity of variance.
Figure 3.3: Weekly average stem length of Phragmites (e, g) and Spartina (f, h) across salinity levels and nitrogen addition levels. Weekly average stem length was calculated as the average of the sum of the combined length of all stems per week. Error bars represent standard error of the arithmetic mean. All data was log-transformed to meet the assumption of homogeneity of variance.
Figure 3.3: Weekly average stem length of Phragmites (i, k) and Spartina (j, l) across salinity levels and nitrogen addition levels. Weekly average stem length was calculated as the average of the sum of the combined length of all stems per week. Error bars represent standard error of the arithmetic mean. All data was log-transformed to meet the assumption of homogeneity of variance.
Figure 3.4: Weekly total stem number of Phragmites (a, c) and Spartina (b, d) across salinity levels and nitrogen addition levels. Weekly total stem number was calculated as the sum of the number of stems per week. Error bars represent standard error of the arithmetic mean. All data was log-transformed to meet the assumption of homogeneity of variance.
Figure 3.4: Weekly total stem number of Phragmites (e, g) and Spartina (f, h) across salinity levels and nitrogen addition levels. Weekly total stem number was calculated as the sum of the number of stems per week. Error bars represent standard error of the arithmetic mean. All data was log-transformed to meet the assumption of homogeneity of variance.
Figure 3.4: Weekly total stem number of Phragmites (i, k) and Spartina (j, l) across salinity levels and nitrogen addition levels. Weekly total stem number was calculated as the sum of the number of stems per week. Error bars represent standard error of the arithmetic mean. All data was log-transformed to meet the assumption of homogeneity of variance.
Figure 3.5: Aboveground biomass of *Phragmites* (a) and *Spartina* (b) across salinity levels and nitrogen addition levels. Belowground biomass of *Phragmites* (c) and *Spartina* (d) across salinity levels and nitrogen addition levels. Biomass was separated, dried and weighed at the end of the experiment. Error bars represent standard error of the arithmetic mean. Within each species, means with different letters are significantly different (Tukey test, p<0.05).
Figure 3.6: Total biomass of Phragmites (a), Spartina (b), and both species together (c) across salinity levels and nitrogen addition levels. Total biomass represents the sum of aboveground and belowground biomass for a single species or for both species combined. Error bars represent standard error of the least squares mean. Within each species, means with different letters are significantly different (Tukey test, p<0.05).
Relative aboveground biomass was calculated by dividing the proportion of one species’ aboveground biomass by the total aboveground biomass of both species. Error bars represent standard error of the least squares mean. Within each species, means with different letters are significantly different (Tukey test, p<0.05).

Relative belowground biomass was calculated by dividing the proportion of one species’ belowground biomass by the total belowground biomass of both species. Error bars represent standard error of the least squares mean. Within each species, means with different letters are significantly different (Tukey test, p<0.05).
Figure 3.9: Root-to-shoot ratio of Phragmites (a), Spartina (b), and both species together (c) across salinity levels and nitrogen addition levels. Root-to-shoot ratios were calculated by dividing the ratio of belowground biomass to aboveground biomass per species and for both species combined. Error bars represent standard error of the least squares mean. Within each species, means with different letters are significantly different (Tukey test, p<0.05).
Figure 3.10: \( \text{F}_v/\text{F}_m \) ratio across salinity levels and nitrogen addition levels of *Phragmites* (a) and *Spartina* (b) measured on April 19, 2012, and of *Phragmites* (c) and *Spartina* (d) measured on May 14, 2012.

Ratios were measured using a Walz PAM-2100 Chlorophyll Fluorometer. Optimal \( \text{F}_v/\text{F}_m \) ratio range is between 0.79 and 0.85; lower ratios indicate poor physiological health of leaf tissue.

Error bars represent standard error of the least squares mean. Within each species, means with different letters are significantly different (Tukey test, \( p<0.05 \)).
Figure 3.11: Soil porewater nitrate measured on April 27, 2012 (a), May 12, 2012 (b), and May 23, 2012 (c) across salinity levels and nitrogen addition levels. Error bars represent standard error of the least squares mean. Means with different letters are significantly different (Tukey test, p<0.05).
Figure 3.12: Soil porewater ammonium measured on May 11, 2012 (a) and May 23, 2012 (b) across salinity levels and nitrogen addition levels. Error bars represent standard error of the least squares mean. Means with different letters are significantly different (Tukey test, p<0.05).
Figure 3.13: Soil porewater nitrate (a) and soil porewater ammonium (b) averaged across salinity levels and nitrogen addition levels, and averaged over all measurement dates. Error bars represent standard error of the least squares mean. Means with different letters are significantly different (Tukey test, p<0.05).
Chapter 4: Discussion

Changes in Biomass Production

Both the change in total stem length and aboveground biomass production of *Phragmites* increased more than those of *Spartina* when treated with high nitrogen addition at lower salinity levels; these results are partly explainable by the different growth strategies of the two species. Although they reach a similar mature height, *Phragmites* tends to produce dense monotypic stands. Schubauer and Hopkinson (1984) measured lower *S. cynosuroides* stem density in comparison to the similar species *S. alterniflora*, whereas *Phragmites* consistently produces densely spaced culms and high aboveground biomass that enhance its interspecific competitive ability (Farnsworth and Meyerson 1999; Meadows and Saltonstall 2007; Meyerson et al. 2009). However, even considering this explanation, *Phragmites* had proportionally higher growth rate and aboveground biomass production with high N addition when salinity was low. This supports the findings of other studies that have shown that increased nitrogen correlates highly with the presence and spread of *Phragmites* (Chambers et al. 1999, 2003; Meyerson et al. 2000, 2009; Minchinton and Bertness 2003; Mozdzer et al. 2010; Mozdzer and Megonigal 2012). As mentioned earlier, it has been hypothesized that *Phragmites* is a more efficient competitor for other limiting resources when nitrogen occurs in surplus (Chambers et al. 1999). One study compared rates of assimilation of dissolved organic nitrogen (DON) and found higher rates in introduced *Phragmites* compared to *S. alterniflora*,
citing direct assimilation of DON as a potential mechanism facilitating its expansion into temperate salt marshes. (Mozdzer et al. 2010). Another study found that the biomass of invasive *Phragmites* was stimulated by 136% with N addition, and further found that the introduced variety was more plastic than the native in its response to N addition that was equivalent to 25 g/m²/yr, by producing more total biomass (Mozdzer & Megonigal 2012). The study found that introduced Phragmites had significantly higher DON-assimilation rates and a higher affinity for dissolved inorganic nitrogen (DIN) than *S. alterniflora*.

In my study, in contrast to *Phragmites*, *Spartina* was able to maintain comparable growth rate and aboveground biomass production under increasingly saline conditions. This was presumably because of *Spartina*’s salinity adaptations. This species has evolved the ability to uptake saltwater through its rhizomes, translocate it to aboveground biomass, and excrete concentrated salt crystals through specialized salt glands in its leaves; this salt excretion has been observed in several studies in both *S. cynosuroides* and other species in the *Spartina* genus including *Spartina patens* and *Spartina alterniflora* (Morris 1995; Weis and Weis 2003; Maricle et al. 2007; Eid 2011; Subudhi and Baisakh 2011). Excretion of salt crystals from *Spartina* leaves was observed during my experiment in mesocosms that contained at least 10 ppt salinity treatments, and a greater density of salt crystals on leaves was observed at higher salinities (Figure A1.8). Although *S. cynosuroides* is typically found in oligohaline and brackish marshes, *S. alterniflora* has been documented in soils with salinities up to 103 ppt, and *S. patens* in salinities up to 52 ppt (Madrid et al. 2012). *Spartina cynosuroides* is typically documented in less saline
environments although its habitat overlaps with other *Spartina* species (Hackney and De La Cruz 1978; Stribling 1997; McHugh and Dighton 2004; White and Alber 2009). Parrondo et al. (1978) measured the greatest *S. cynosuroides* biomass production at 8 ppt and below, all other factors being equal.

*Spartina cynosuroides* additionally performs the more water-efficient C4 photosynthesis pathway, which enhances its ability to continue to produce biomass at similar rates in higher salinity environments (Maricle et al. 2007). By using additional light energy, the C4 pathway elevates internal CO₂ concentration for photosynthesis with the use of a biochemical pump, reducing loss of water via photorespiration and providing an advantage in salt marshes where physiological drought can occur due to high solute concentration (Furbank and Taylor 2005). *Phragmites* performs the C3 photosynthesis pathway and does not possess salt glands (Burke et al. 2000).

With respect to weekly average stem height and weekly number of stems, it appeared that *Spartina* was allocating resources to stem length as opposed to increasing number of stems. Wheares *Phragmites* seemed to allocate resources to producing a large number of shorter stems, *Spartina* seemed to allocate resources to producing a small number of taller stems. The average *Spartina* weekly average stem length was similar to *Phragmites* at lower salinities but became increasingly taller than *Phragmites* at 10, 18, and 28 ppt. *Spartina* continued to increase the stem length of existing individuals to very tall heights relative to *Phragmites*. This could possibly have given *Spartina* a competitive advantage over *Phragmites* because *Spartina’s* C4 photosynthesis uses more ATP and therefore requires more sunlight energy, making its taller height possibly advantageous. Taller stems are able to reach sunlight more
easily when growing in stands and are less likely to be shaded out by other species, which is a common mechanism by which *Phragmites* outcompetes other species (Rice et al. 2000). Reduced light penetration into stands of *Phragmites* lowers air and soil temperatures and in some cases delays by several weeks the “spring melt” at the soil surface of these stands (Meyerson et al. 2009).

At lower salinities, high nitrogen addition significantly increased *Phragmites* aboveground biomass production. Nitrogen addition may facilitate *Phragmites* survival by increasing its ability to outcompete other species at low salinities. Where nitrogen input from anthropogenic sources is elevated, it is possible that a shift will occur in the wetland plant community towards low diversity communities dominated by *Phragmites*. It has been found that when nutrients occur in surplus (i.e. when nitrogen is no longer the limiting factor), competition for light instead dictates competitive outcomes (Chambers et al. 1999); in environments that are high in nitrogen, the tall, dense stands that *Phragmites* produces could hold an edge over other species in the competition for light. Alternatively, the species may more directly benefit from added nitrogen in producing additional biomass, supported by evidence that higher nitrogen content has been found in the leaves of *Phragmites* growing near developed areas receiving nutrient runoff (Meyerson et al. 2009). Instead of outcompeting other species for light, increased nitrogen may simply increase *Phragmites* biomass production that would allow it to more rapidly occupy available growing space.

Although there were clear trends for aboveground biomass, there were few clear trends for belowground biomass. Nitrogen addition did not significantly
increase belowground biomass for either species or in total, although higher salinity levels did reduce total (aboveground plus belowground) biomass under the low nitrogen treatment level. Mateos-Naranjo et al. (2007) similarly found that *Spartina densiflora* aboveground biomass was reduced by increased flooding treatment but that there was no treatment effect on belowground biomass. Previous studies have found that the addition of nutrients shifts the majority of plant biomass production allocation from belowground to aboveground, and that increased nitrogen may cause slower belowground biomass growth in comparison to aboveground biomass growth (Tilman and Wedin 1991; Twolan-Strutt and Keddy 1996; Darby and Turner 2007; Zhang et al. 2007; Graham and Mendelssohn 2010). By this reasoning, the majority of growth and species competition in the current study would have occurred in aboveground measurements. At high nitrogen, biomass should have been allocated preferentially to shoots so that all high nitrogen treatment levels would have correlated with a smaller root:shoot ratio. This occurred at the highest and lowest salinity levels for total biomass of both species combined, and at the lowest salinity level for each species. However, this trend did not occur across all salinity levels. Another possible explanation is that the rhizomes may have required more time to become necrotic compared to the shoots, so that total belowground biomass death was not observable in the timespan for which this experiment ran.

**Changes in Plant Community Composition**

Similar to the total aboveground biomass and growth rate results, the relative aboveground biomass data supported the findings that *Phragmites* allocated additional nitrogen to increased biomass production and that *Spartina* allocated
resources to salinity tolerance adaptations. At 40 ppt, relative aboveground biomass was comprised mainly of *Spartina*. This proportion is explainable partly because in some higher salinity mesocosms all *Phragmites* biomass was necrotic by the end of the experiment, and because biomass was greatly reduced for both species. Additionally, where *Phragmites* continued to produce biomass at 40 ppt, *Spartina* produced similar or greater final aboveground biomass and growth rate. Although N increased *Phragmites* aboveground biomass, nitrogen did not significantly increase *Phragmites* relative aboveground biomass except at 40 ppt. This indicates that although additional N may be advantageous to *Phragmites* aboveground biomass production in high-salinity environments, this advantage appears to be limited because of the greater negative effect that higher salinity has on its biomass production. Furthermore, this suggests that the persistence of salt-tolerant species such as *Spartina* in low-salinity environments may depend heavily on continued low nitrogen input because of the possibility of displacement by *Phragmites* when N load is increased. Previous studies have found that increases in nitrogen load correlate with lowered wetland species diversity and shifts in community structure towards invasive species such as *Phragmites* (Dukes and Mooney 1999; Bart and Hartman 2000; Green and Galatowitsch 2002; Tyler et al. 2007). However, both nitrogen and salinity played roles in the current study in altering the relative biomass of the two species, and the balance of stressors (salinity) to resources (N) may be critical in determining relative species abundance in coastal wetlands as sea-level rise continues (Brose and Tielborger 1995; Crain 2007). My study indicates that even small differences in this
balance may have a significant impact on the competitive interactions among species and the plant community structure.

**Plant Tissue Measurements**

Multiple studies have linked lowered photosynthetic performance with increased abiotic stress, and have found that a sustained lowered $F_v/F_m$ ratio is indicative of plant tissue damage in response to environmental stress (Maxwell and Johnson 2000; Mateos-Naranjo et al. 2007). Reduced $F_v/F_m$ ratios have been measured in *Phragmites* that was exposed to elevated salinity (Deng et al. 2011; Zhang and Deng, 2012). In one study, an $F_v/F_m$ ratio of 0.16 was recorded for *Phragmites* exposed to 200 mM NaCl (Zhang and Deng 2012).

Maximum photosynthetic rate ($P_{\text{max}}$), a similar measurement obtained by using a chlorophyll fluorometer, was also found to decrease in *S. alterniflora* and *S. patens* as soil salinity increased; the standardized coefficient of interaction between soil salinity and $P_{\text{max}}$ was found to be -0.32 for *S. alterniflora* and -0.41 for *S. patens* (Madrid et al. 2012). However, although one study found a decrease in maximum quantum efficiency of CO$_2$ fixation (a related measurement) in *S. alterniflora* and *S. patens* under elevated salinity, $F_v/F_m$ did not also decrease, suggesting that salinity had no effect on the photosynthesis of these species by inactivation of PSII reaction centers (Maricle et al. 2007). The salinity adaptations evolved by members of the *Spartina* genus may explain these complex findings, which demonstrate that not all photosynthetic performance characteristics of plants in this genus are negatively affected by increased salinity.
It is important to note that some studies have found differences in the sensitivity of various types of stress indicators when comparing among more traditional biomass assessment methods such as growth rate and leaf expansion rates, and physiological methods such as $F_v/F_m$ ratio and adenylate energy charge ratio. In a comparison of indicators of sublethal stress due to cadmium addition, it was found that decreased leaf expansion rates and photosynthesis rates correlated with stressors earlier in the experiment than other indicators, $F_v/F_m$ ratio did not decrease until much later in the experiment, leaf spectral reflectance variables were not affected by cadmium addition, and regrowth rate after harvest depended heavily on species (Mendelssohn et al. 2001). Other studies have found that increase in proline concentration was more responsive as a short-term indicator to small changes in salinity in *S. patens* (Ewing et al. 1995) and that biomass was a more sensitive indicator as the species’ salt-tolerance decreased (1988). These findings demonstrate the need to rely on multiple indicators when attempting to characterize the effects of stressors on the health of a plant species.

In my experiment, lowered $F_v/F_m$ ratios appear to be attributable to elevated salinity levels, but may also have been lowered by possible heat stress from additional increasing greenhouse temperatures as the experiment progressed from February to May. Temperature and humidity were controlled by automated mechanics, but greenhouse mechanical setups are limited in their ability to regulate temperature during warmer months.

Figure 2.1 shows that there was more variation in the maximum temperature later in the experiment that could possibly have lowered $F_v/F_m$ ratios, but overall there
was no apparent increase in average temperature compared to earlier months. Figure 2.2 shows that there was also no apparent increase in average light intensity over the duration of the experiment.

Although *Phragmites* biomass production and growth rate were greater under many treatment combinations, only *Spartina* maintained high F$_{v}$/F$_{m}$ ratios at the highest salinity levels with low N addition. At the highest salinity level several *Phragmites* leaves became necrotic whereas *Spartina* continued to produce tissue with F$_{v}$/F$_{m}$ ratios similar to lower salinity levels. These results support the conclusion that instead of competing with other species primarily by producing large amounts of biomass at a high rate, *Spartina* channeled its resources into salinity tolerance mechanisms that maintained physiologically healthy tissue in the biomass that was produced. Where live *Phragmites* leaves existed to measure photosynthetic activity, F$_{v}$/F$_{m}$ ratio was significantly reduced, although with high N addition its F$_{v}$/F$_{m}$ ratio was higher at 40 ppt. This is further evidence that N addition can play a role in the ability of *Phragmites* to continue to produce photosynthesizing biomass under increasingly saline conditions. However, it is interesting to note that despite high N addition, the *Phragmites* ratio at 40 ppt was approximately 55% of the ratio at 18 ppt. This further suggests that N addition may have a limited positive effect on *Phragmites* physiological health. The study was concluded before rhizomes became pot-bound; it is unclear whether the species would have continued to benefit from the high nitrogen input as oceanic salinity persisted. A follow-up field study would provide additional information by allowing a longer running experiment without pot-bound belowground biomass developing and possibly altering the results.
These findings are in accordance with studies concerning habitat zonation, the hypothesis that a tradeoff exists between stress tolerance and competitive ability, and that the competitive ability of a species is inverse to its stress tolerance ability (Liancourt et al. 2005). Along stress gradients, superior competitors such as *Phragmites* have been found to dominate the least stressful regions, whereas species that are competitively inferior such as *Spartina* occupy more stressful zones where tolerance mechanisms provide a survival advantage (Lubchenco 1980; Levine et al. 1998). In my experiment, *Phragmites* $F_{v}/F_{m}$ ratios were highest when stress from salinity was lowest, whereas *Spartina* $F_{v}/F_{m}$ ratios were greater than those of *Phragmites* in the treatment levels representing the most stressful environments with the lowest resource inputs.

Previous studies have found that competitive outcomes are reversed when nutrient input is increased (Levine et al. 1998; Emery et al. 2001; Greiner La Peyre et al. 2001). However, in my study *Spartina*’s relative aboveground abundance was greatest at the highest salinity level when N addition was low. As pointed out earlier, this may be partly explainable due to the fact that all *Phragmites* biomass was necrotic at 40 ppt in several mesocosms. However, at 18 ppt and 28 ppt live *Phragmites* aboveground biomass continued to grow in all mesocosms until the conclusion of the experiment. At these salinities, additional nitrogen may have allowed it to continue to produce large amounts of biomass even as stress increased.

The limited significant effects of N addition on biomass production and $F_{v}/F_{m}$ ratios in my study demonstrates that salinity and nitrogen both contribute to quantity and physiological health of biomass in a complex and interactive way. Further study
is needed to determine to what extent each factor alone, and their interaction, influence community composition and the facilitation of invasive species spread.

**Conclusions**

Summary

The major findings of this experiment were:

- *Phragmites* had proportionally aboveground biomass production with high N addition when salinity was low.

- *Spartina* maintained comparable biomass production under increasingly saline conditions.

- *Phragmites* allocated resources to producing a larger number of shorter stems; *Spartina* allocated resources to producing a small number of taller stems.

- At higher salinities, high nitrogen addition continued to significantly increase *Phragmites* biomass production in comparison to the low nitrogen treatment level, but overall results indicate that N addition may have a limited positive effect on *Phragmites* growth and physiological health.

- Nitrogen addition did not significantly increase belowground biomass for either species or in total.

- *Spartina* relative aboveground biomass increased as salinity increased.

- At the highest salinity level several *Phragmites* leaves became necrotic whereas *Spartina* continued to produce tissue with Fv/Fm ratios similar to lower salinity levels.
• Salinity and nitrogen both contributed to quantity and quality of biomass production in a complex and interactive way.

By comparing the growth and physiological health of *Phragmites* with a species of the same family and with similar morphology and habitat, this study has demonstrated that the effects of nitrogen addition are moderated by salinity and vice versa. These results are consistent with other studies (Meyerson et al. 2000; Rice et al. 2000; Burdick and Konisky 2003; Crain 2007; Martina et al. 2010; Mozdzer et al. 2010) that have found that nitrogen addition is able to contribute to both the successful expansion of invasives such as *Phragmites* into coastal marsh areas of the Chesapeake Bay, and to the reduction of biodiversity in these habitats through the increased abundance of invasive species (Farnsworth and Meyerson 1999; Findlay et al. 2003; Havens et al. 2003; Greenwood and MacFarlane 2006; Baldwin et al. 2011). Previous studies have documented the expansion of *Phragmites* into vast areas of freshwater and, increasingly, brackish marshes (Burdick and Konisky 2003; Havens et al. 2003), and have also noted the high correlation of the expansion of *Phragmites*’ range with the development of urban areas that are able to provide large amounts of both nitrogen and disturbed spaces (Farnsworth and Meyerson 1999; Silliman and Bertness 2004).

Increased salinity from sea-level rise disturbs the salinity regime and may open niches for invasive colonizers to fill (Chambers et al. 2003). In wetlands where current plants are not eradicated by a change in salinity
regime, the primary productivity of vascular plants is reduced, and plants are forced to invest additional energy to exclude salt and sulfides (Odum et al. 1995). However, this study has shown that the salinity intrusion associated with sea-level rise may reduce the effect of excess N in promoting the expansion of *Phragmites* and other invasives, while species with adaptations to salinity may continue to produce sufficient biomass to effectively compete despite increased saltwater intrusion. In response to increased salinity, *Phragmites* may migrate upstream to lower salinity levels but development of major seaports and transportation corridors that restrict tidal water flow may prevent this (Chambers 1999). Ultimately, community scale effects will greatly depend on the amounts of nitrogen runoff from agricultural and urban sources into coastal wetlands.

Applications

This study has several applications for urban development, management of invasives, and wetland creation and restoration. As urbanization continues, low-impact development techniques such as permeable surfaces and advanced stormwater management technology must be incorporated into planning and policy to minimize the runoff of nutrients that can dramatically shift neighboring wetland plant communities. Alternative pavers that allow more groundwater infiltration would reduce the rapid movement of large volumes of runoff into streams and rivers. Further reduction in the amount of nitrogen that flows into coastal marshes of the Chesapeake Bay is possible through the incorporation of designs that catch
and filter urban runoff such as grass swales, vegetative roof systems, rain gardens, and constructed wetlands (Dietz and Clausen 2008; Bedan and Clausen 2009).

Regional organizations may more efficiently utilize their funds to control and eradicate *Phragmites* by incorporating efforts that target the reduction of nutrient runoff into surface waters, instead of exclusively funding the physical removal of *Phragmites* through common methods such as burning, mowing and pesticide application. This study demonstrated that although it is possible that *Phragmites* may outcompete other species at low salinities regardless of level of nitrogen input, nitrogen does play a role in further enhancing the interspecies competitive ability of *Phragmites*. Current invasive control methods may be simpler and provide more directly evident results, but they are time intensive and require a large staff to be effective. Typical management programs use conventional herbicide applications by either helicopter or truck sprayers, and may be combined with the burning of aboveground biomass during winter months (Meyerson et al. 2009). The U.S. Army Corps of Engineers also lists mowing and the use of tidal gates as common methods to contain stands (Saltonstall 2003). Alternatively, the availability of suitable invasion locations may be decreased by reducing the volume of runoff from developed areas and also by rerouting runoff away from coastal marshes (Burdick and Konisky 2003). Planned hydrology is a key aspect of successful wetland management programs and is critical in
promoting the growth of native species in restored marshes (Weinstein et al. 1997).

Additionally, created and restored wetlands must incorporate a diverse number of species that are able to produce a large amount of salt-tolerant biomass, in order to ensure the highest chance of long-term survival as sea-level rise and the invasion of *Phragmites* continue in the region (Chambers et al. 2003). Plants should include species such as *Spartina* that have evolved physiological adaptations to elevated salinity. The most efficient use of funds includes the planting of species that have been shown to continue producing physiologically healthy biomass under increasingly stressful conditions. It is also essential that the selected species produce low density stands in comparison to *Phragmites*, which instead creates stands that increase community vulnerability for further invasions, decrease biodiversity, and increase susceptibility to fire (Windham and Meyerson 2003). Finally, planted species must be able to produce biomass at a rate that effectively competes with the high rates of invasive species such as *Phragmites*.

Although it requires more planning to identify species that both have evolved salinity adaptations and the ability to quickly produce large amounts of biomass, this study has shown that these characteristics are important to the ability of planted wetland species to effectively compete with invasives. These findings must be incorporated into our understanding and management of coastal marshes to ensure the continued existence of diverse wetlands as environmental conditions change, and to increase the likelihood that these
wetlands continue to provide us with the essential ecosystem services that we rely on, such as flood control, wildlife habitat, erosion prevention and water filtration.
Appendices

Appendix 1: Additional Photos and Site Map

Figure A1.1: Marsh at Clyde Watson Boating Area facing away from shore. *S. cynosuroides* visible in foreground, *P. australis* stand visible in middle ground. Photograph taken October 25, 2010.
Figure A1.2: Aerial map of Clyde Watson Boating Area in relation to Washington, D.C. Boating Area indicated with arrow.
Figure A1.3: New *Phragmites* shoot emerged at an adult stem node placed horizontally in trough. After root development, the new growth was detached and used in the experiment.

Figure A1.4: Cultivation of new stems in two constructed wooden troughs (2.6 m x 0.79 m, 0.41 m depth) lined with 45mm-thick Firestone Pond liner. Pool liner has been lifted to photograph the wooden trough.
Figure A1.5: Example of mesocosm setup, with shadecloth raised for easier viewing of inner bucket. This photo was taken at the low water level.

Figure A1.6: Example of mesocosm drainage tube at bottom of outer bucket.
Figure A1.7 Mesocosm arrangement in greenhouse.

Figure A1.8: Salt excretion on *S. cynosuroides* leaves during experiment. Photograph taken April 26, 2012.
Figure A1.9: Block 2 mesocosms 40-0 ppt salinity with High nitrogen, arranged for photograph after treatments were concluded.

Figure A1.10: Block 2 mesocosms 40-0 ppt salinity with Low nitrogen, arranged for photograph after treatments were concluded.
Appendix 2: Sample SAS code

(Example: Initial - Final Change in Height)

proc mixed data=work.marthur;
class salinity fert block;
model height = salinity|fert /ddfm=satterth outp=resids;
Random block;
lsmeans salinity|fert/ adjust=tukey diff=all cl;
ods output lsmeans=lsmean1;
ods listing exclude diffs; ods output diffs=diff1;
ods output tests3=stat1;
quit;
proc plot data=resids vpercent=50;
plot resid*pred/vref=0;
quit;
data resids;
set resids;
aresid=ABS(resid);
run;
%include 'c:pdmix800.sas';
%pdmix800(diff1,lsmean1,alpha=.05,sort=yes);
proc corr spearman data=resids;
var aresid pred;
quit;
proc univariate data=resids plot normal;
var resid;
quit;
proc print data=lsmean1;
quit;
*proc print data=diff1;
quit;
proc print data=stat1;
quit;
ods graphics off;
quit;
Appendix 3: Additional Methods Description

Raising porewater salinity

Plants were potted in soil in “inner” buckets that had 4-5 holes per square centimeter drilled on the bottom with a ¼-inch drill. Each bucket was placed on 2 cut 2x2 wood planks inside a larger “outer” bucket to allow water flow under and around the inner bucket. Notches were cut in the 2x2 wood planks so that they could be fitted onto the rim of the outer bucket. Standing water was controlled by raising the level of the water in the outer bucket. The outer bucket was fitted with a drainage tube on one side near the bottom of the bucket. A removable rubber stopper was fitted in the tube.

When the water level was raised for high tide, Instant Ocean was mixed in a separate mixing bucket until the desired salinity was verified with a multimeter. The water was poured into the inner bucket and the outer bucket until the desired water level was achieved. When the water level was lowered, the wood planks were fitted onto the rim of the outer bucket. The inner bucket was placed on the planks so that space was left for water to drain through the bucket’s holes. The inner bucket’s standing water was allowed to drain into the outer bucket (if applying dissolved fertilizer, it was applied to the inner bucket’s standing water at this time to infiltrate the soil as the water drained). At the same time, the rubber stopper was removed to drain the water from the outer bucket. The planks and inner bucket were replaced inside the outer bucket.

Nitrate/ammonium standard creation

Solid NH₄Cl and solid KNO₃ were dried in an oven at 110° C for two hours. NH₄ standard concentrations were 0.0, 0.5, 1.0, 1.5, 2.0 and 2.5. NO₃ standard concentrations were 0.0, 2.5, 5.0, 7.5 and 10.0.

Solid NH₄Cl was dissolved in deionized water to create the highest concentration, 2.5 mg/L. 2.0 mg/L concentration was created by further diluting part of the 2.5 mg/L concentration in deionized water. This was repeated until all concentrations were made. Standards were tested on two instruments in the lab and compared to a regularly standardized Hach DR 5000 UV-Vis spectrophotometer borrowed from a neighboring University of Maryland laboratory.

25 mL of porewater was extracted from each mesocosm, suction filtered with a 55m paper filter, and analyzed with a Hach Model DR 2400 portable spectrometer.

Harvesting aboveground & belowground biomass

Intact plants and attached rhizomes/soil were removed from buckets. Species were separated from each other by loosening the soil by hand but keeping the belowground
biomass intact and attached to the aboveground biomass. After this, aboveground biomass were clipped from belowground biomass and soil was rinsed from belowground biomass using a 1-mm x 2-mm filter. All biomass was dried in a chamber at a temperature of 35°C (95°F) and 15% relative humidity level for one to two weeks until the samples reached a constant mass. The biomass was then weighed to the nearest 0.1 g.

**PAM measurement**

To dark-adapt the plants, readings were taken beginning at 10 PM. Ambient PAR was checked using a PAR sensor to ensure no ambient light was interfering with measurements; all PAR readings taken around the perimeter of the mesocosm arrangement were 0 µm/m²/s. A green light provided visibility. In each bucket, three leaves per species were chosen for fluorescence measurement. Leaves chosen were the newest growth (i.e. highest position on stem) that were wide enough to fill the 1-cm² area for the instrument. Live leaves were preferentially chosen. When no live leaves were present, dead leaves were measured. If no leaves were present for a species in a mesocosm, no measurements were taken. Fv:Fm ratio was recorded.
Bibliography


