

EFFECTS OF NUTRIENT ADDITIONS ON THREE COASTAL SALT MARSH
PLANTS FOUND IN SUNSET COVE, TEXAS

A Thesis

by

LESLIE ANN RULON

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of
MASTER OF MARINE RESOURCES MANAGEMENT

December 2010

Major Subject: Marine Resources Management

Effects of Nutrient Additions on Three Coastal Salt Marsh Plants Found in Sunset Cove,
Texas

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Approved by:

Chair of Committee,	Antonietta Quigg
Committee Members,	Anna Armitage
	Thomas Linton
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ABSTRACT

Effects of Nutrient Additions on Three Coastal Salt Marsh Plants Found in

Sunset Cove, Texas. (December 2010)

Leslie Ann Rulon, B.S. Texas A&M University at Galveston

Chair of Advisory Committee: Dr. Antonietta Quigg

Eutrophication, particularly due to nitrogen (N) and phosphorus (P) input, has been massively altered by anthropogenic activities. Thus it is important to understand the impact on salt marsh plants; however studies on salt marsh plants within Galveston Bay, Texas are limited. In this study, the effects of repeated nutrient additions in monospecific plots of *Spartina alterniflora*, *Batis maritima*, and *Salicornia virginica* as well as mixed plots of *B. maritima* and *S. virginica* were studied over 15 months. Results showed that nutrient loading led to an increase in height, biomass, growth rate and percent nitrogen (N) within all three species studied, but were species specific more than dose dependent. Nitrogen content in leaves had a positive correlation with P content in leaves but a negative correlation with carbon (C) content. Nutrient loading lead to a significant increase in total chlorophyll in the fertilized plots of *S. alterniflora* and *S. virginica* one month into the study. Nutrient addition to two succulent species, *B. maritima* and *S. virginica* in mixed plots did not reveal a distinct superior competitor within the 15 month study in terms of growth and nutrient use efficiencies; however using the maximum growth rates of the monospecific plots, the Monod model was used to determine which species would dominate at high nutrient loads. Based on height data *S. alterniflora*

would dominate, while *B. maritima* would dominate according to the Monod model based on biomass.

DEDICATION

To my mom, Linda Rulon and Miss Kitty:

Thank you for the love and support and for
keeping me sane when there was only one way to go-insane.

ACKNOWLEDGEMENTS

First and foremost, I am forever in debt to my committee chair, Dr. Antonietta Quigg for providing me this opportunity and commitment to see me through this process.

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Last, thanks to my family and friends for their support through this process! I could have not done it without you all!

NOMENCLATURE

C	Carbon
CSR Theory	Competitor/stress tolerator/ruderal theory
DMSO	Dimethylsulfoxide
EPA	Environmental Protection Agency
H	Hydrogen
K	Potassium
M	Mean
NOAA	National Oceanic and Atmospheric Administration
N	Nitrogen
%	Percent
P	Phosphorus
TX	Texas
TAMUG	Texas A & M University at Galveston
USEPA	United States Environmental Protection Agency
USGS	United States Geological Survey

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1. INTRODUCTION

Population growth and its associated activities continue to have a significant impact on the flux of nutrients, especially nitrogen (N) and phosphorus (P) which leads to eutrophication (Rabalís 2002) which is defined as

“a process whereby water bodies, such as lakes, estuaries, or slow-moving streams receive excess nutrients that stimulate excessive plant growth” (United States Geological Survey (USGS) 2008).

Annual worldwide inputs of anthropogenic N and P are concentrated into our rivers which have inputs of approximately 150 tetragrams (Tg) N yr⁻¹ and 22 Tg P yr⁻¹ (Galloway 2002, Howarth et al. 1995, Bennett et al. 2001).

Two major contributors of anthropogenic N and P are point and non point sources. Point sources are defined as any discrete source of pollution such as, but not limited to pipe, conduit, ditch, wastewater discharge, and sewage discharge. Non-point sources are any sources of water pollution that do not meet the legal definition of point source according to the Clean Water Act of 1987 Section 502(14) (United States Environmental Protection Agency (USEPA) 2005). Non-point source pollution is often the result of precipitation, land runoff, stream flow, ground water, hydraulic modifications, nitrogen fixation, tidal flows and atmospheric deposition. These are significantly impacted by urban and agricultural development. The Clean Water Act, which regulates point source discharges, has made significant strides in the past 25 years,

decreasing the impacts of point source pollution including in Galveston Bay, Texas (TX) (Lester and Gonzalez 2002); however, recent studies indicated that the majority of the remaining water quality impairments are due to non point source pollution (US EPA 2005).

Moderate increases in nutrients have been found to stimulate growth and production but each plant species responds in a specific manner which is both inter- and intra-specific according to Invers et al. (2004). This positive relationship has been observed between nutrient-loading rates in waterways and biomass increases, but this is non-linear due to the different pathways through which external nutrients are converted into biomass (Rabalais 2002). At high nutrient concentrations, there can be negative effects on these same plants. Elevated nutrient loads to marshes can lead to significant change in plant species composition resulting in adverse affects on the surrounding ecosystem. In addition, eutrophication can lead to increased algal biomass, which in some systems, leads to fish kills (McInnes and Quigg 2010), alters plant community composition (Quigg and Roehrborn 2008) and reduces ecosystem services (Quigg et al. 2009).

In the United States phosphorus above natural levels, 0.01 milligrams per liter (Environmental Protection Agency (EPA) 2003), contributes to eutrophication especially in freshwater systems which are commonly P limited, unlike estuarine and marine systems that are often N limited (Howarth et al. 1995) including Galveston Bay, TX (Örnólfssdóttir et al. 2004). Phosphorous is primarily found in the soil in dissolved, colloidal, or particulate forms (EPA 2003). The main sources of P are from fertilizers,

sewage runoff, mined P minerals, manures, and other non-bioavailable forms in the soil. In many situations, P can be the limiting nutrient on a seasonal basis (Sylvan et al. 2006) or can become the limiting nutrient when the excess levels of N are present, such as that which occurs in the area in the Gulf of Mexico adjacent to the outflow of the Mississippi River (Rabalais 2002).

Nitrogen below 0.3 milligrams per liter in waterways of the United States is considered to be natural levels, but with a dramatic increase it may adversely affect surrounding communities (EPA 2003). Biologically available inorganic forms of N are commonly found in forms of ammonium, nitrate (NO_3^-), and nitrite (NO_2^-); however the nitrate form is more readily available for primary producers, while ammonium is generally adsorbed by the soil (EPA 2003, Purvaja et al. 2008).

A majority of N is in the form of organic matter, including plant and animal matter. This organic matter is then broken down through microbial decomposition producing ammonium (NH_4^+) through ammonification (Purvaja et al. 2008). Assimilation of ammonium can also occur, which is the processes in which plants uptake this form of N through its roots and incorporates them into various parts of the plant, including proteins and nucleic acids. Ammonium can also remain in the sediment or become suspended in water which quickly leads to nitrification transforming ammonium into nitrite and then into nitrate, which is more readily available for plant uptake (EPA 2003; Purvaja et al. 2008). Nitrate's solubility in water is often associated with water quality issues (EPA 2003). Excess N loading, particularly to bayous which have low flushing rates, leads to excess plant productivity and ultimately a reduction in

ecosystem services such as reduced fishing and tourism. This was recently documented in the nearby Dickinson Bayou, TX (Quigg et al. 2009).

1.1 Wetlands

Since nutrients collect in streams and rivers that eventually lead to the coast, salt marshes in these areas can be severely affected by excessive nutrient loading. Salt marshes are defined by the upper coastal intertidal zone between land and brackish water dominated by halophytic plants. Such habitats are unique because of their role as ecotones between terrestrial and aquatic systems and play a positive role in the ecosystem which depends greatly on plant diversity.

Salt marshes are effective tools for reducing the impacts of eutrophication by attenuating nutrients via adsorption, deposition, filtering, and uptake (Phillips 1989). However, studies on salt marsh vegetation are limited since they are considered less sensitive and slower to respond to nutrient enrichment, making its response more difficult to quantify. The concepts that these highly productive systems are filters that lead to improvements in water quality are generally based on studies that focused on phytoplankton and submerged aquatic vegetation (McGlathery et al. 2007) as well as other marsh properties such as the soils ability to hold moisture, infiltration capacity, resistance to flow, and size and slope of the marsh (Phillips 1989).

Often, with nutrient enrichment, studies have proven that salt marshes efficiently remove nutrients through biological-uptake in vegetation, denitrification, chemical-precipitation and adsorption, as well as physical-settling and filtration processes (Hemond and Benoit 1988, Day et al. 2004). However, nutrient enrichment can lead to a

removal of nutrient limitation resulting in an increase in plant and algal biomass, which in turn leads to a reduction in light penetration into coastal water and increases the biological oxygen demand. This demand can ultimately lead to hypoxic zones which are becoming a common occurrence along the coastal areas (Parson et al. 2006).

In Galveston Bay, TX, the nutrient concentrations, although they have been decreasing since the 1970s (Lester and Gonzales 2002), are still approximately four times that of nearby TX estuaries (Örnólfssdóttir et al. 2004) and bayous (Quigg et al. 2009). Peak nitrate and phosphate levels reached in the summer often results in an increase in phytoplankton biomass (Santschi 1995, Örnólfssdóttir 2004, Quigg et al. 2009). In 2007 and 2008, nutrient addition bioassays conducted by Quigg (Personal communication 2009) found primary producers remained N limited; consistent with findings of Örnólfssdóttir et al. (2004). No nutrient addition bioassays on vascular plants have been performed in Galveston Bay, TX. This leads to the need for understanding how nutrient loads affects vascular plants in salt marshes around Galveston Bay, TX.

Today with increasing development, the United States is losing about 24,281 ha (243 km²) of wetlands each year which is equivalent to as much as a cumulative loss of 53 percent of the original wetlands in the United States (EPA 2003). This significant loss makes it essential to understand the filter capabilities of marshes and limits in order to protect our remaining wetlands as well as to restore and create new marshes. In TX, 52% of salt marshes have been lost since the early 1780's (White et al. 1993). These wetland losses have a negative impact on nutrient loads, suspended sediments, fisheries and numerous other biological and physical properties of the surrounding ecosystem.

Spartina alterniflora is a common subject for nutrient enrichment experiments, focusing on the impacts of N and P on biomass accumulation, C, N and P attenuation and when nutrient enrichment has the maximum impact (Dai and Wiegert 1996, Patrick Jr. and Delaune 1976). Dai and Wiegert (1996) found that with N addition in the short form of *S. alterniflora* a 68%/m²/year increase in biomass occurred at Sapelo Island, Georgia. Patrick and Delaune (1976) in Barataria Bay, Louisiana also found an increase in yield (15%) of biomass in *S. alterniflora* plots with N addition. They also found that although P had no direct effect on yield, it did show a significant increase in P content.

Boyer et al. (2001) studying another common salt marsh species, *Salicornia virginica*, also found that with N or N and P additions, a significant increase (2-fold) in succulent tissue biomass occurred after 14 months relative to the control. However, studies on other common species found in salt marshes are limited. This constrains our understanding of the salt marsh ecosystem as a whole, since individual species will have a unique reaction to nutrient enrichment including nutrient retention, biomass accumulation, and erosion control just to name a few.

1.2 Competition

In Rumstick Cove in Barrington, Rhode Island, *S. alterniflora*, which is the dominant species in low marsh habitats, was capable of growing in the high marsh with competitors excluded; however, when competitors were present, *S. alterniflora* was excluded in the high marsh (Bertness and Ellison 1987). This competitive interaction limits the upper elevation distribution of *S. alterniflora*, such that this particular species is competitively displaced to lower tidal elevations. Snow and Vince (1984) studying four

Alaskan salt marsh species at the Susitna Flats (*Puccinellia nutkaensis*, *Triglochin maritimum*, *Carex ramenskii*, and *Carex lyngbyei*) also found that each species was able to inhabit all zones of the marsh when competitors were excluded. These findings support the competitor/ stress tolerator/ ruderal theory (CSR theory) of competition, that species were limited to lower elevations by physical conditions, such as salinity and inundation, and by competition at higher elevations (Grime 1977).

Tilman's resource-ratio hypothesis predicts that competition occurs below ground for nutrients when nutrients are limiting and shift to above ground competition for light as productivity increases (Tilman, 1982, 1988, 1990, 1997; Wilson and Tilman, 1991, 1993). After a two-year fertilization experiment, Levine et al. (1998) found that *S. alterniflora*, which is normally restricted to the lowest elevations, was able to invade higher zones and exclude *Spartina patens* and *Juncus gerardii* in Rumstick Cove, Barrington, Rhode Island. This showed that nutrient additions changed the outcome of competition between plant species, which supports Tilman's resource-ratio hypothesis as well as the CRS theory. While Levine et al. (1998) found that with nutrient additions *S. alterniflora* was able to invade higher zones; Bertness et al. (1987) found that with excluding competitors *S. alterniflora* grew vigorously at higher zones. These studies (Levine et al. 1998; Bertness et al. 1987) suggest that nutrient plays a critical role in competition and zonation of salt marsh species.

At Nag Cove West and Nag Cove East, Rhode Island, Emery et al. (2001) performed a nutrient addition experiment at naturally occurring species borders (zonal and patch boundaries) and measured the percent cover of three species to determine the affects of nutrient additions on the competitive interactions between three species (*J.*

gerardii x *S. patens*, *J. gerardii* x *Distichlis spicata*, and *S. patens* x *S. alterniflora*).

Their findings also showed that nutrient additions could lead to substantial shifts in plant composition, or relative abundance of each species in the mixture, which could lead to substantial shifts in the zonation of plants across the tidal gradient. In this case fertilization increased the absolute biomass of the ambient marsh inferior, while decreasing the absolute biomass of the dominant (Levine et al. 1998). Therefore, it is important to consider the effect of eutrophication on the community composition in salt marshes. Although salt marshes maybe able to mitigate the results of additional nutrient loading, the consequences may include a shift in plant dynamics. This would in turn lead potentially to a shift at higher trophic levels.

1.3 Objectives and Hypothesis

The objective of this study is to gain a better understanding of the physical (height and above ground wet weight biomass) and chemical (C, N, P and total chlorophyll) effects that nutrient enrichment has on three salt marsh plants, *Batis maritima*, *S. virginica* and *S. alterniflora*. The need to understand the impact of eutrophication on critical ecosystems, i.e., salt marshes that occur in Galveston Bay, is to assist in the management and conservation of wetland ecosystems. Measurements of plant height, biomass, C, N, P and total chlorophyll will be used to determine if there is a significant difference in the response of the plants to different levels of fertilization in monospecific plots. However, it is predicted that the response to nutrient enhancement will be more species dependent than dose dependent. This entails that, with any addition of fertilizer,

the effects (physical or chemical) will be similar within the same species, but the response to the nutrient loading will be different for each species.

In addition, the aim of this study was to also examine competition between two plant species (*B. maritima* and *S. virginica*). Height and biomass will be compared to determine if fertilization has a significant effect in competition plots, i.e., those which have both species present. It is expected that nutrient additions will change the outcome of competition between the two plant species, with the subordinate species in the control able to dominate with nutrient additions. Below are the hypotheses to be tested:

Monospecific Plots:

H_0 : There is no significant difference in plant responses among fertilization levels.

H_a : There is a significant difference in plant responses among fertilization levels.

H_0 : There is no significant difference between the individual species tested and their response to different levels of fertilization.

H_a : There is a significant difference between the individual species tested and their response to different levels of fertilization.

Competition Plots:

H_0 : There is no significant difference in plant responses between levels of fertilization in competition plots.

H_a : There is a significant difference in plant responses between levels of fertilization in competition plots.

2. MATERIALS AND METHODS

2.1 Study Site

Located on the west end of Galveston Island, TX, the study area is adjacent to a new residential development, Sunset Cove (N 29°09.013', W 095°02.458') (Figure 2.1). The site, at the commencement of this study, was a relatively undisturbed native salt marsh habitat. The site was chosen because of negligible disturbance prior to the study period, the need to obtain baseline information and an ability to monitor changes in salt marsh plant abundance and/or diversity that may occur as the result of the new development on its borders. By the end of the study period, roads were completed, several houses established and large areas of Saint Augustine grass (*Stenotaphrum secundatum*) covered the developed area above the high marsh. Little to no disturbance was observed to the immediate sampling site.

A plant survey was performed prior to the beginning of this study. The vegetation of the lower and middle marsh is representative of TX Gulf coast salt marshes, mainly consisting of *S. alterniflora* (Figure 2.2a) in the lower marsh and *S. virginica* (Figure 2.2b) and *B. maritima* (Figure 2.2c) in the middle marsh. Salt marsh vegetation at this site is mainly monospecific with little mixing between the species. The high marsh was relatively undisturbed and remained characteristic of natural TX salt marshes. However, above the high marsh, the land had been maintained (mowed and cleared of debris), a road built and an aquifer constructed.

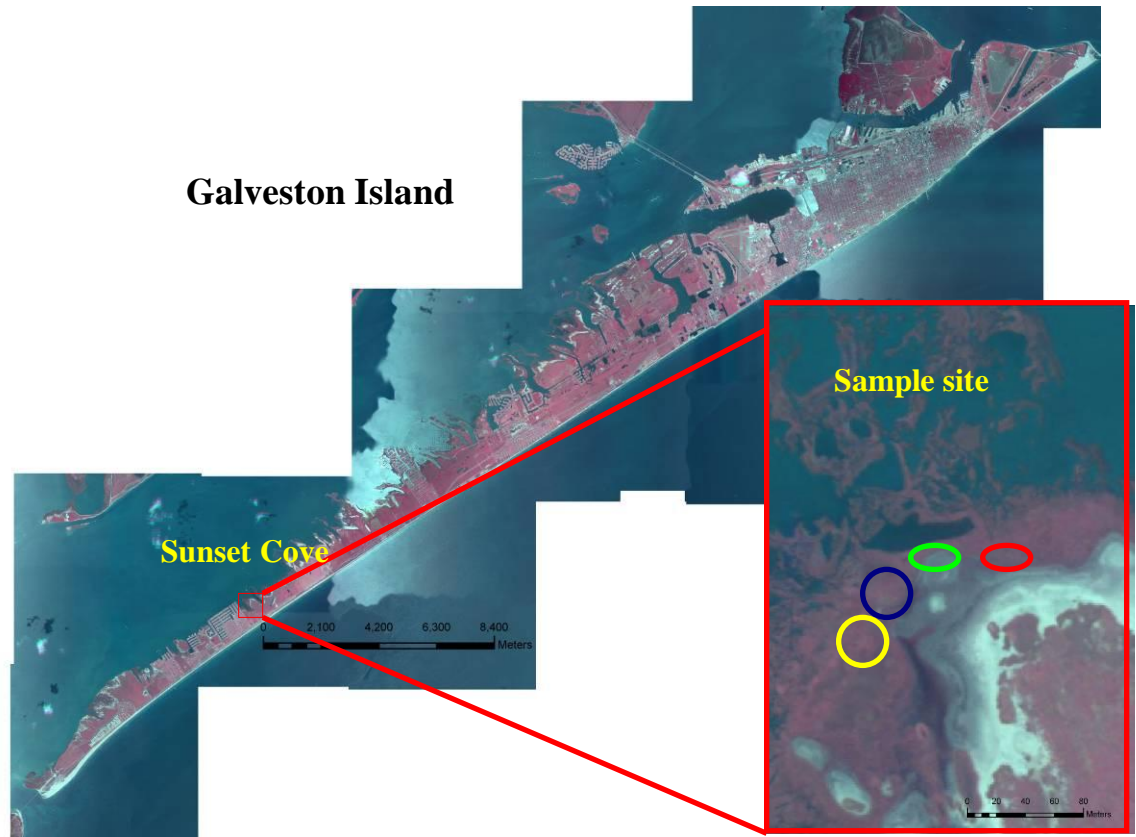


Figure 2.1. Galveston Island (left) and the sampling site at Sunset Cove (West et al.) on Galveston Island's west end. The yellow circle indicates areas of *S. alterniflora* plots, green indicates *B. maritima* plots, blue indicates *S. virginica* plots and red indicates areas of competition plots.

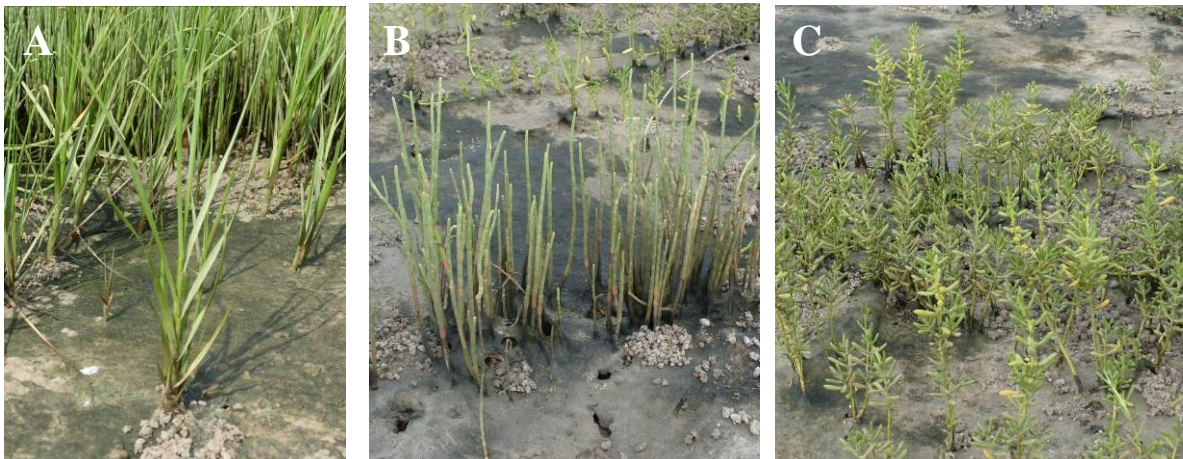


Figure 2.2. Examples of (a) *S. alterniflora* (b) *S. virginica* and (c) *B. maritima* at Sunset Cove, Galveston, Texas on 27 June 2007.

2.2 Plots and Nutrient Additions

Forty-eight permanent experimental plots (0.5 x 0.5 m) were established with a minimum 1 m buffer between them. Stakes were forced into the marsh and each plot site numbered. Plot sites were chosen randomly but fulfilled the following criteria: twelve plots of *S. virginica*, twelve plots contained *B. maritima*, twelve plots contained *S. alterniflora*, and the remaining twelve plots contained a mixture of *S. virginica* and *B. maritima* to conduct competition experiments between these two succulent species.

For each plant group (i.e. plots 1-12, 13-24, 25-36, and 37-48), the plots were subject to one of four treatments, each performed in triplicate: 0 (control), 5, 15, or 30 grams (g) of Scott's Southern Turf Builder© with 2% Iron fertilizer (26% N, 2% P, 13%K). This was applied every fourteen days by hand from June 13, 2007 to September 3, 2008. This application strategy was similar to previous fertilization studies performed by Levine et al. (1998) and Pennings et al. (2002).

2.3 Sampling Protocol

2.3.1 2007

The initial thirty-six monoculture plots were established on June 13, 2007 with measurements of the shoot length of ten randomly selected plants. Each shoot was measured as the distance from the shoot base to the shoot top (Kiehl et al. 1997). This included measuring ten randomly selected plants of the dominant species as well as up to ten randomly selected plants of each remaining species within the plot. On July 14, 2007 the remaining 12 competition plots (37-48) were established, fertilized and all measurements recorded again.

For the first two sampling periods, plots 1-36 were sampled weekly. However, due to the slow assimilation of nutrients and the limited amount of biomass to sample, the period between was increased to biweekly from June 27, 2007 to July 11, 2007. Biomass was collected from a random area (0.02m^2) within each plot. Further, as a result of the impacts observed from cutting biomass and the destruction of surrounding plants, despite great care, the period between sampling had to be increased to bimonthly until the plants began to senesce due to the onset of winter. Additional samples were taken on November 8, 2007. Plots 36-48 were sampled on the same dates as the original 1-36 plots. If sampling could not occur on the desired days due to storms or high tides, sampling was delayed until weather and tides permitted, but generally by no longer than a week.

2.3.2 2008

Sampling started after the period of senescence and growth was again evident (May). Sampling continued essentially as done in 2007, but ceased in September due to the loss of the stakes marking plots as a result of Hurricane Ike. In brief, all 48 plots were fertilized every 14 days and plant heights were measured monthly in 2008 while plants biomass was collected every other month.

2.4 Soil

Soil provides plants with nutrients and water as well as stability. The spatial distribution of resources in the soil of any system is limited, but shows variability that ranges from within the root zone (0.1 meter or less) to the landscape level over time (Ehrenfeld 2001, Levang-Brilz and Biondini 2002). A theoretical analysis conducted by Gleeson and Fry (reviewed in Levang-Brilz and Biondini 2002) suggested that grown in

patchy soil nutrient environments, plants will develop their root systems in such a way that the gains from the nutrient patches within their root zone are equilibrated. For this study it is understood that soil-plant interactions do occur, however, due to the close proximity of the plots of the same species it was assumed that the effects were consistent across all treatments. It is also understood that when comparing species at difference marsh levels, comparisons are looking at the individual species in their natural habitat. However, previous studies such as Reed and Cahoon (1992) have stated significant interactions do exist.

2.5 Biomass

Biomass was collected bimonthly from an area of 0.02m^2 within the plots. Plants were placed in bags and placed directly on ice for transportation back to the laboratory. Once back at Texas A & M University at Galveston (TAMUG), the plants were gently washed in deionized water to remove sediment, salt, detrital material and algae. Plants were separated by species and then patted dry. Each species in each sample was divided into two visually equal halves and wet weights were recorded. Half the plant material was frozen and the other half dried in an oven at 50°C until a constant dry weight was achieved. The wet weight (g/m^2) and dry weight (g/m^2) were recorded.

2.6 Carbon and Nitrogen Analysis

Dried samples from the monoculture plots were ground using a Wiley plant mill (mesh size $60\mu\text{m}$). Carbon and N were measured using a calibrated Perkin-Elmer 2400 CHN Elemental Analyzer. Plant material ($5.5\text{mg} \pm 0.5$) was prepared and run according

to Newbery et al. (1995) and Ngai and Jefferies (2004). Five blanks were run before each sample set and were reproduced as follows: C ± 30 , Hydrogen (H) ± 100 , and N ± 16 .

Acetanilide was used as the control and run within the tolerance range as follows: C 16 ± 3.5 , H 50 ± 20 , and N 6 ± 3 .

2.7 Phosphorus

Dried and ground plant samples were used for phosphorus analysis following the protocol of Fourqurean et al. (1992). Phosphorus concentrations were determined on 17 - 21mg of plant material from the monoculture plots using a UV/VIS 2100 Shimadzu Spectrophotometer. A standard (no phosphorus) was used as the blank, running between every ten samples, allowing for correction of background absorption. Standard curves were prepared each day samples were run.

2.8 Chlorophyll Extractions

Chlorophyll extractions were performed on samples of *B. maritima*, *S. alterniflora*, and *S. virginica* that had been previously frozen from the monoculture plots only. The plant samples were thawed on ice and cut to size with a 3mm cork borer in order to produce consistent weight and surface area exposed per sample. Chlorophyll was extracted from 10.0 (± 5) mg of *B. maritima* and *S. virginica*, and 1.0 (± 1) mg of *S. alterniflora* using dimethylsulfoxide (DMSO) according to the protocol of Shinano et al. (1996). Chlorophyll *a* and *b* absorbance was measured using a UV/VIS 2100 Shimadzu Spectrophotometer; concentrations were calculated using the equations of Shinano et al.

(1996) according to Equation 1. The blanks consisted of DMSO, run between every ten samples, in order to correct for background absorption.

Equation 2.1: Calculations for determining chlorophyll *a* and *b* concentrations using DMSO (Source: Shinano et al. 1996):

$$\begin{aligned}\text{Chl } a &= 14.85A_{665} - 5.14A_{648} \\ \text{Chl } b &= 25.48A_{648} - 7.36A_{665}\end{aligned}$$

2.9 Statistical Analysis

Statistical analyses were performed using SPSS 16.0 software to test the null hypothesis that there was no significant difference between fertilized and unfertilized plots of each species or competition plots. The effect of fertilization on height and total wet weight biomass of each species in monoculture was tested with an independent-sample t-test. This test was also used to determine if a difference existed between the control and 30g fertilized plot for percent N, percent P, percent N:P ratios, percent C:Total chlorophyll. Error bars in all figures are standard errors.

Competition plots again used the independent-sample t-test to determine if there was a significant difference in height or above ground biomass (wet weight) between only the control and 30g fertilized plot. To compare changed over time in each treatment paired-sample t-test were used. Correlation analyses were used to determine if any significant relationship existed between the physical parameters measured (N:P, C:N, and N:Total chlorophyll concentrations).

3. RESULTS

3.1 Environmental Factors

To compare environmental conditions between the two study years, weather data recorded between January 1, 2007 and December 31, 2008 was examined (Weather Underground, Inc. 2010). Ambient air temperatures ($^{\circ}\text{C}$) in 2007 were similar to those in 2008 ($22^{\circ}\text{C} \pm 7^{\circ}\text{C}$) (Figure 3.1). The growing season for salt marsh plants in Texas ranges from early spring to mid fall. Spring (March-May) average air temperatures were $22^{\circ}\text{C} (\pm 4^{\circ}\text{C})$ while summer (June-August) averages rose to $29^{\circ}\text{C} (\pm 1^{\circ}\text{C})$. These temperatures were not significantly different from the 30 normalcy year period (1971-2000) (National Oceanic and Atmospheric Administration (NOAA) 2009) which is not shown.

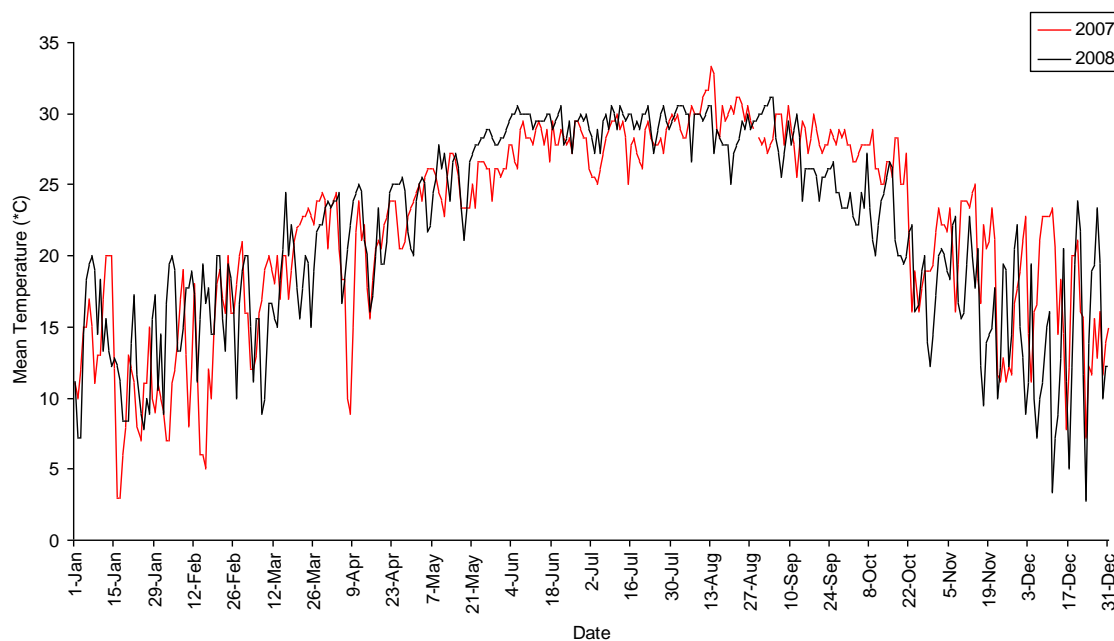


Figure 3.1. Average ambient air temperature ($^{\circ}\text{C}$) between January 1, 2007 and December 31, 2008. (Source: Weather Underground, Inc. 2010)

Precipitation (cm) was 2-times greater by the end of the first field season (September 12) in 2007 (111cm) compared with the same time frame in 2008 (53.6cm) as seen in Figure 3.2. Compared to the 30 year (1971-2000) normalcy in which average rainfall of 69.3cm per year was recorded (Weather Underground, Inc.), 2007 and 2008 had similar precipitation but this was after the experiment was concluded. Late spring and summer were wetter in 2007 than in 2008 (Figure 3.2).

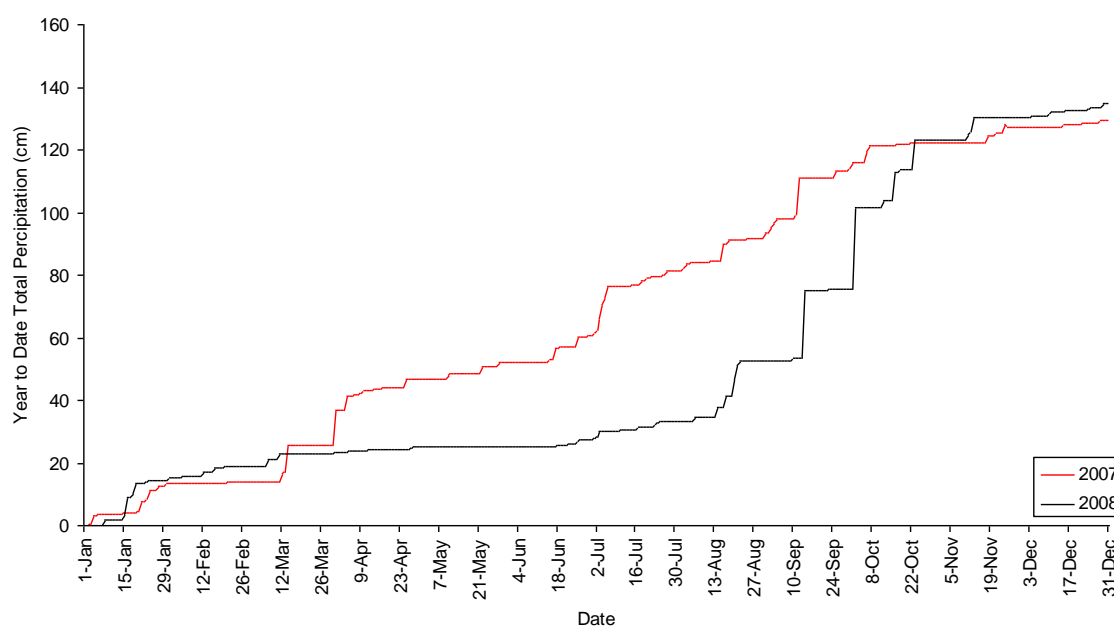


Figure 3.2. Cumulative precipitation (cm) from January 1, 2007 to December 31, 2008. (Source: Weather Underground, Inc.)

3.2 Plant Growth

3.2.1 *S. alterniflora*

S. alterniflora has an perennial growth cycle, but with a large die back during winter months; plant height and above ground biomass (wet weight) quickly increased over the growing season in the first year of the study, until the onset of winter (November 2007) (Appendix A, Table 6.1). Comparisons of plant height, using a paired-sample t-

test, in each treatment showed significant increase from June 2007 to November 2007 ($P \leq 0.02$) (Figure 3.3). The percentage increase between June 2007 and November 2007 for the control, 5, 15, and 30g treatments was 46 percent (%), 54%, 56%, and 52% increase, respectively.

An independent-sample t-test was conducted to compare each treatment to the control in November 2007 (Figure 3.3). There was a significant difference in the heights for the 5g (Mean (M)=116.22, $P=0.01$) and 15g (M=110.01, $P=0.04$), but not the 30g (M=110.75, $P=0.16$) treatment compared to the control plots (M=97.89).

In the second growing season, 2008, a significant difference was observed between all fertilized plots and the control ($P=0.02$, 0.04, and 0.04 for the 5, 15, and 30 gram treatments) according to an independent-sample t-test (Appendix A, Table 6.1). The 5, 15, and 30g treatments exceeded the control by 16, 20 and 17%, respectively in August 2008.

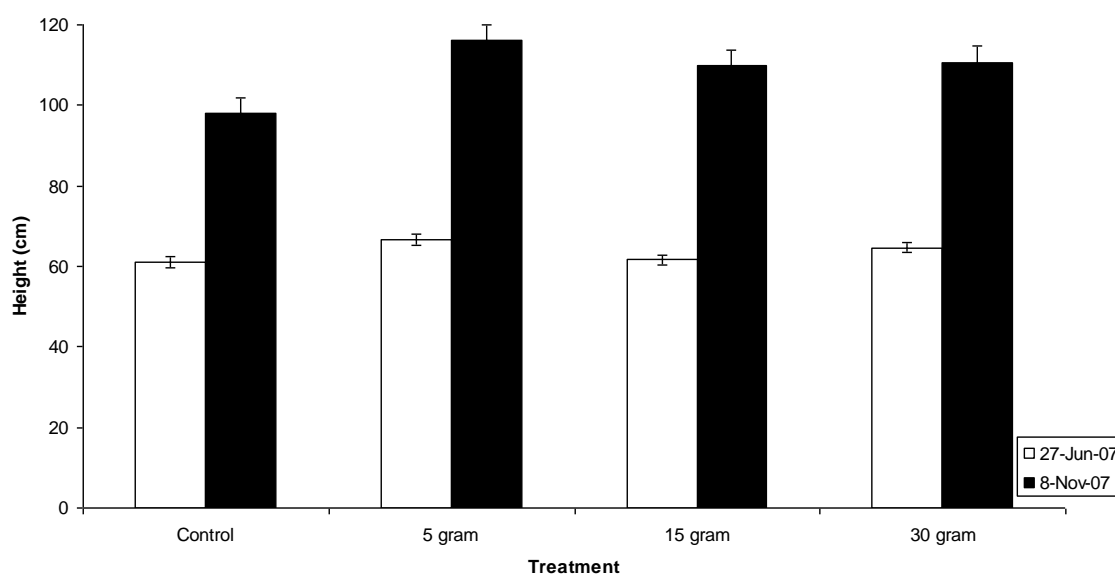


Figure 3.3. The average (\pm S.E., $n=30$) height response of *S. alterniflora* in monoculture plots at the start (27 June 07; white bars) and the end (8 November 2007; black bars) of the fertilization experiment during the 2007 sampling period.

Total wet weight biomass measurements ($\text{g}/0.02\text{m}^2$) of above ground *S. alterniflora* also showed an increase in all treatments but according to an independent-sample t-test no treatments were significantly different from the control ($P \geq 0.15$) (Figure 3.4). An increase in biomass of 88-121% in *S. alterniflora* was observed during 2007 however not significant ($P \geq 0.06$) according to a paired-sample t-test; less so (3-37%) in 2008 (Appendix A, Figure 6.1).

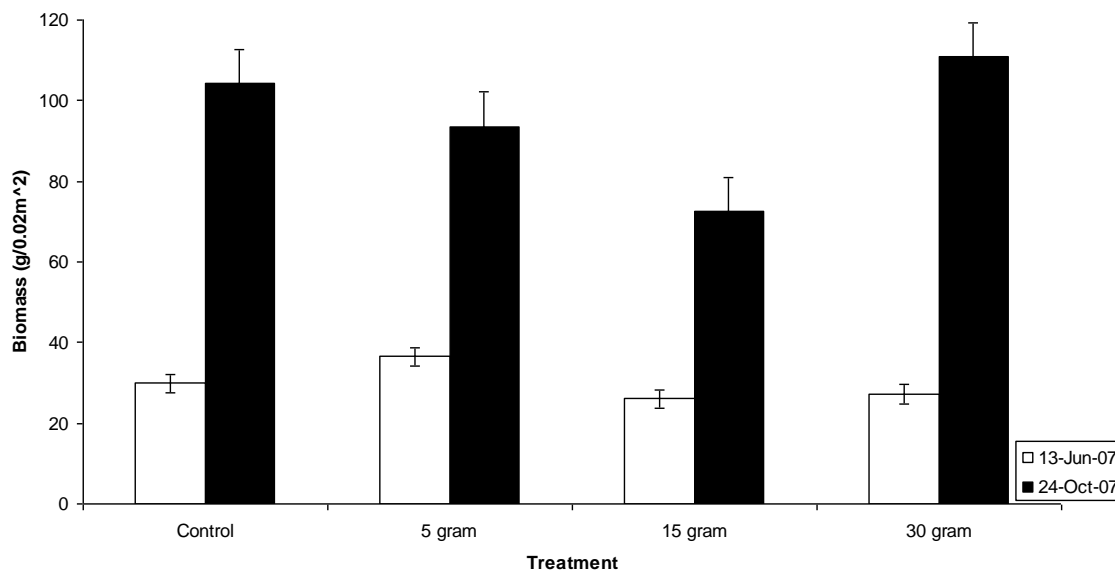


Figure 3.4. The average (\pm S.E., $n=30$) above ground wet weight biomass of *S. alterniflora* in monoculture plots at the start (13 June 07; white bars) and the end (8 November 07; black bars) of the fertilization experiment during the 2007 sampling period.

3.2.2 *B. maritima*

Changes in *B. maritima* plant heights and above ground biomass (wet weight) were recorded in 2007 and 2008. Given its perennial life cycle and prostrate, creeping stem growth form, it was necessary to calculate changes across the two year study period (Figure 3.5 and Figure 3.6). A comparison of plant heights (cm) at the beginning of the

study (June 2007) to those at the end of the study (August 2008) revealed that *B. maritima* did respond to the nutrient additions, particularly at high nutrient load in terms of above ground total wet weight biomass. A paired-sample t-test showed that there was not a significant difference for the control (June 07 M=27.13, Aug. 08 M=34.63, P=0.32) or 5 gram treatment (June 07 M=23.86, Aug. 08 M=34.39, P=0.14) but there was a significant difference in growth over the 2 field seasons for the 15g (June 07 M=25.75, Aug. 08 M=40.15, P=0.02) and the 30g treatment (June 07 M=20.76, Aug. 08 M=41.79, P=0.04) (Figure 3.5; Appendix A, Table 6.2). In the control and 5g treatment plots, the average increase in *B. maritima* plant heights was 24 and 36% whereas in the high nutrient treatments, 15g and 30g, the increase was 44% and 67% respectively.

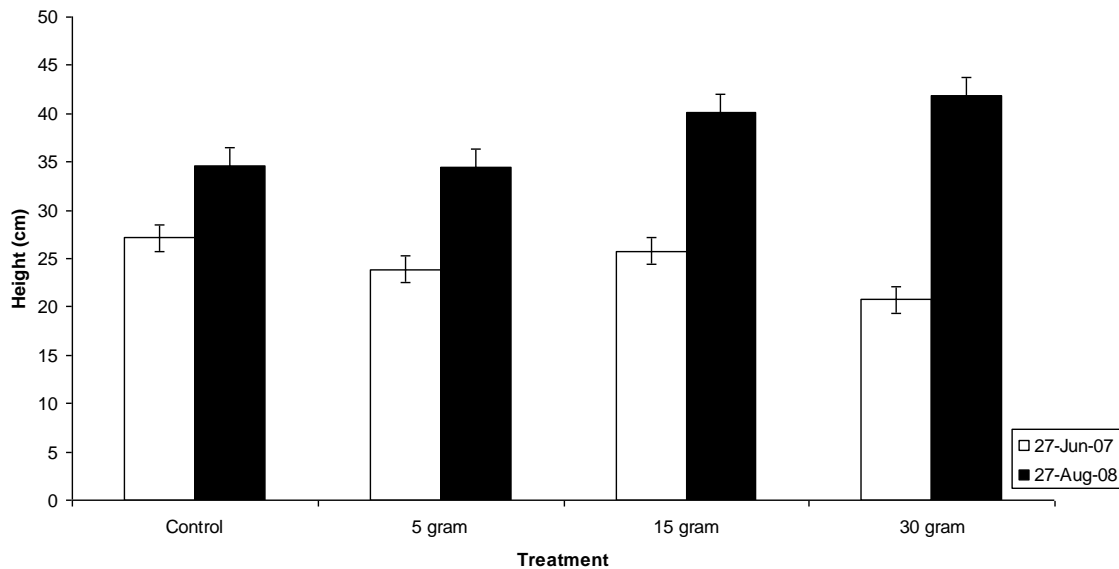


Figure 3.5. The average (\pm S.E. n=30) height of *B. maritima* in monoculture plots at the start (27 June 07; white bars) and the end (27 August 2008; black bars) of the fertilization experiment during the two year sampling period.

Total wet weight biomass ($\text{g}/0.02\text{m}^2$) of *B. maritima* did increase over the two field seasons, however, only the 5g treatment showed a significant accumulation of

biomass ($P=0.03$) according to the paired-sample t-test (Figure 3.6; Appendix A, Figure 6.2) from the initial sampling date (June 07, $M=18.85$) to the final sampling date (August 08, $M=80.51$). Comparing each treatment, 5g, 15g and 30g treatments to the control on 27 August 2008 using an independent-sample t-test showed no significant difference in total wet weight biomass ($P\geq 0.79$).

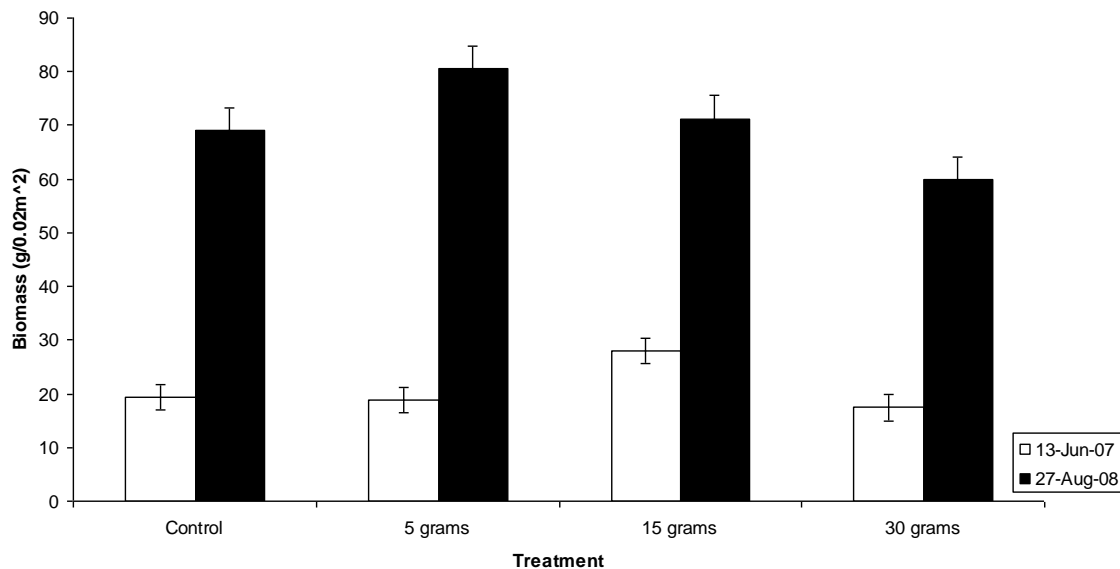


Figure 3.6. The average (\pm S.E., $n=30$) above ground wet weight biomass of *B. maritima* in monoculture plots at the start (27, June 07; white bars) and the end (27 August 2008; black bars) of the fertilization experiment during the two year sampling period.

3.2.3 *S. virginica*

In the monospecific plots of *S. virginica*, all treatments showed a general increase in height (cm) but at no time was the fertilized treatments significantly different from that of the control according to an independent-sample t-test (Figure 3.7; Appendix A, Table 6.3) due to its perennial life cycle and low spreading growth. A paired-sample t-test showed that the 15g treatment (June 07 $M=28.23$, Aug. 08 $M=43.33$, $P=0.04$) and 30g

treatment (June 07 M=27.36, Aug. 08 M=42.20, P=0.03) significantly increased over the 2 field seasons by 42% and 43% respectively.

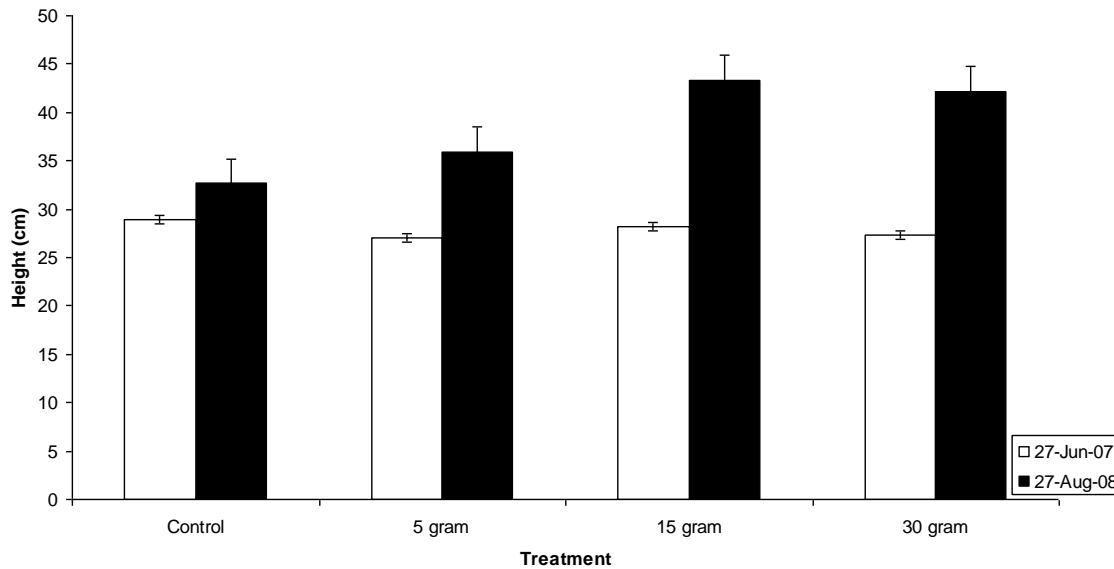


Figure 3.7. The average (\pm S.E., n=30) height of *S. virginica* in monoculture plots at the start (27 June 07; white bars) and the end (27 August 2008; black bars) of the fertilization experiment during the two year sampling period.

Biomass (total above ground wet weight in g/0.02m²) also showed similar results as that of the height data, increasing over the two sample years (Figure 3.8; Appendix A, Figure 6.3). According to a paired-sample t-test the control (June 07 M=20.01, Aug. 08 M=90.15, P=0.02), 5g (June 07 M=26.72, Aug. 08 M=104.84, P=0.04) and 30g treatment (June 07 M=22.33, Aug. 08 M=183.29, P=0.01) showed a significant increase in biomass over the 2 field seasons. According to an independent-sample t-test in August 2008, the 30g treatment was the only treatment to show a significant difference for that of the control (P=0.01) or a 58% difference. The difference between the control and 5g treatment (2%) was still much less than that of the 15g treatment (39%), however not significant.

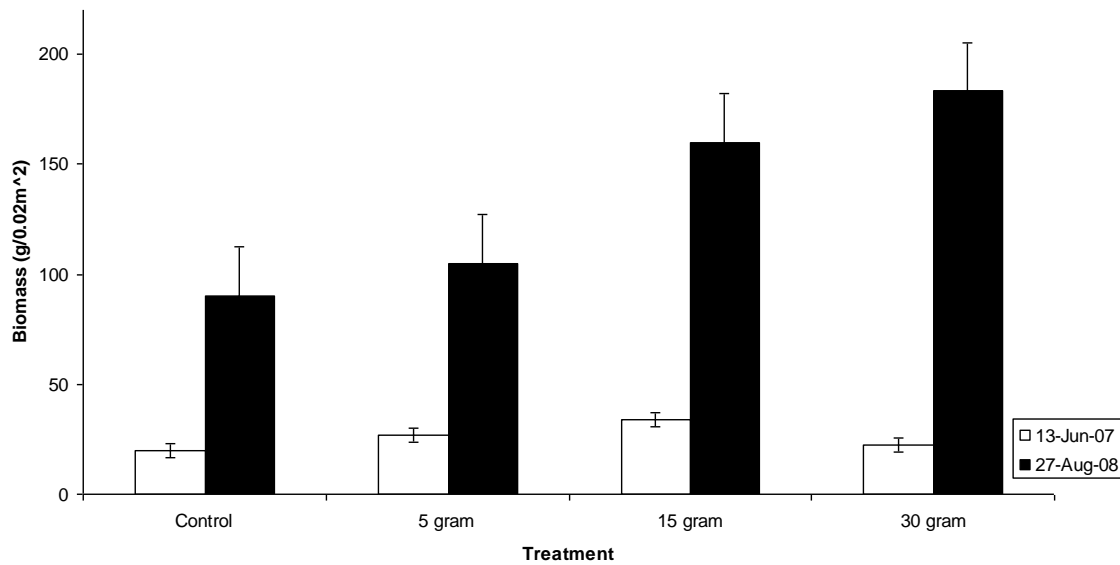


Figure 3.8. The average (\pm S.E., $n=30$) above ground wet weight biomass of *S. virginica* in monoculture plots at the start (27 June 07; white bars) and the end (27 August 2008; black bars) of the fertilization experiment during the two year sampling period.

3.2.4 Interspecies Competition

From this point on, unless specified, only the control and 30g treatments will be examined. To further simplify, the 30g treatment will be referred to as the ‘fertilized’ treatment. No below ground biomass was assessed. Within the competition plots, *B. maritima* and *S. virginica*, started with similar heights in all plots and treatments and all showed a general increase in height over the two field sampling periods, mimicking that of their monospecific counterparts (Figure 3.9; Appendix A, Figure 6.4).

B. maritima in the control treatment and fertilized treatment showed a 26% and 38% increase in height at the end of the fertilization experiment (August 2008) compared to that at the initial start (July 2007), however only the fertilized plot showed a significant increase according to a paired-sample t-test (July 07 $M=28.51$, Aug. 08 $M=42.10$, $P=0.01$).

A 24% and 37% increase in the height of *S. virginica* control (July 07 M=25.78, Aug. 08 M=32.76, $P=0.01$) and fertilized treatment (July 07 M=26.23, Aug. 08 M=38.18, $P=0.00$) also occurred over the two field sampling years, which were both significant according to a paired-sample t-test (Figure 3.9).

By the end of the second sampling season neither *S. virginica* nor *B. maritima* excluded the other species in either the control or fertilized plot nor did they show a significant difference from each other. However, due to the limited vertical growth of both species, height data is not ideal for determining if one species would exclude the other.

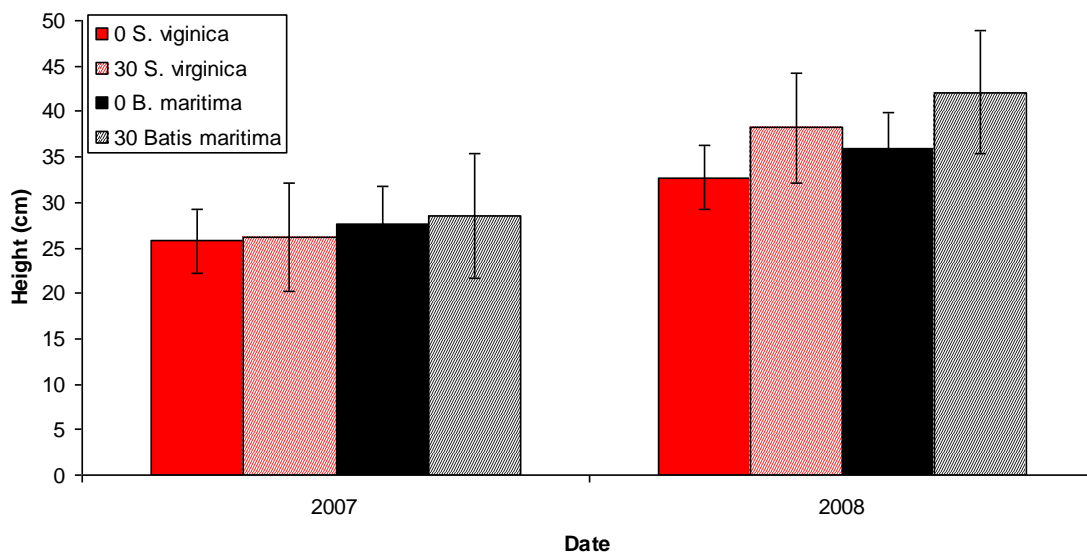


Figure 3.9. The mean (\pm S.E., $n=30$) height response of *S. virginica* and *B. maritima* to fertilization in competition plots on the initial sampling date (11 July 2007) and final sampling date (27 August 2008).

Data collected from the above ground biomass (total wet weight) within the competition plots, again mimicked that of the monospecific plots, showing a general increase in biomass over the two sampling periods (Figure 3.10; Appendix A, Figure A.5).

An increase in biomass from the initial start day (July 2007) to the final sampling date (August 2008) was observed for both treatments of *S. virginica* with increase of 86% for the control plots (July 07 M=27.31, Aug. 08 M=68.56) and 99% for the fertilized plots (July 07 M=27.18, Aug. 08 M=80.15), however only the fertilized treatment increase in biomass was significant ($P=0.01$) according to a paired-sample t-test. An independent-sample t-test showed no significant difference ($P=0.72$) between the fertilized and control plots by August 2008.

B. maritima showed a slight increase in biomass for the control (June M=32.16, Aug. 08 M=48.22, $P=0.17$) and fertilized plots (July 07 M=16.25, Aug. 08=27.07, $P=0.42$) from the initial date sampling (July 2007) to the final sampling date (August 2008), however not significant. No significant difference was observed between the fertilized plots and control plots ($P=0.41$) in August 2008.

At the end of the second field season, August 2008, the total wet biomass of *S. virginica* (M=80.15) significantly exceeded that of *B. maritima* (M=27.07) according to an independent-sample t-test ($P=0.00$) in the fertilized plots. It was observed that the biomass of *S. virginica* increased much faster than the *B. maritima* and was slightly more exaggerated in the fertilized plots than the control, suggesting that with time *S. virginica* would dominate the plots, but faster with fertilizer than without.

Monitoring the percent coverage over the 15 month sampling period did show a 25% and 35% increase in the control and fertilized plots, respectively (data not shown). Percent coverage of the control started with 54% coverage and increase to 69% coverage by the end of the experiment, still leaving 31% open ground, allowing for minimal above ground interspecific competition. *B. maritima* coverage increased by 26% while *S.*

virginica coverage increased by 24% in the control plots. The fertilized plots also started with similar percent coverage (55%) and increased to a final percent coverage of 80% by the end of the 15 month experiment. *B. maritima* coverage increased by 38% while *S. virginica* coverage increased by 37%. Again with 20% exposed area, this left room for both species to grow with minimal above ground competition.

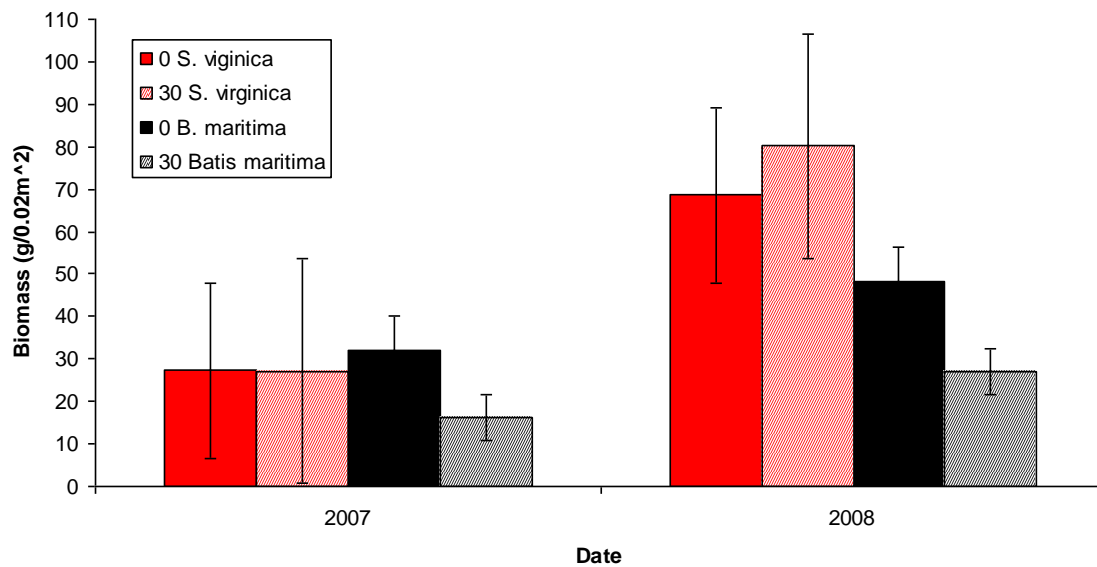


Figure 3.10. The mean (\pm S.E., $n=30$) above ground total wet weight biomass response of *S. virginica* and *B. maritima* to fertilization in competition plots on the initial sampling date (14 July 2007) and final sampling date (27 August 2008).

3.3 Nutrient Interactions

Only the control and 30g treatment samples were examined for this aspect of the study; thus, the 30g treatment samples will be referred to as ‘fertilized’ below. In all cases, C, N, and P content refers to the above ground wet weight biomass. Below ground biomass was not assessed.

3.3.1 *S. alterniflora*

The N content (% dry weight) of the above ground biomass of *S. alterniflora* did not change significantly in the control (Table 3.1; Appendix A, Figure A.6) but increased in the fertilized plants until August 2007 when the N concentration significantly different from the control (Fertilized M=1.51, Control M=1.04. P=0.04) according to an independent-sample t-test. The N in the control and fertilized plots dramatically decreased (11% and 33% respectively) from September to October.

The following year, 2008, both treatments showed a significant increase from the initial sampling date (June 2007) in the N content in June 2008 (Control P=0.01, Fertilized P=0.02) according to a paired-sample t-test. In August the N content decreased in both the control and fertilized plots, however, the fertilized plots still contained significantly more N content (P= 0.00) compared to the initial sampling date (June 2007) (Table 3.1).

Table 3.1. Total nitrogen (% dry weight) of *S. alterniflora* and its response to fertilization in monoculture plots on June 2007 and August 2008 (\pm S.E., n=30).

Treatment	27-Jun-07	11-Jul-07	8-Aug-07	5-Sep-07	24-Oct-07	10-Jul-08	27-Aug-08
0	1.0 \pm 0.00	1.2 \pm 0.1	1.0 \pm 0.1	1.0 \pm 0.1	0.9 \pm 0.1	1.4 \pm 0.1	1.3 \pm 0.1
30	0.9 \pm 0.00	1.4 \pm 0.2	1.5 \pm 0.1	1.7 \pm 0.2	1.21 \pm 0.2	1.7 \pm 0.1	1.5 \pm 0.0

S. alterniflora showed that the control and fertilized plants had similar P content (% dry weight) from June 2007, the initial sampling period, to August 2008, the end of the sampling period (Appendix A, Table A.3). The average P content of the control (0.25 \pm 0.02) and fertilized (0.24 \pm 0.01) plants at the end of the study (August 2008) was not significantly different (P=0.69) according to an independent-sample t-test. Overall it

appears that P content in both treatments followed a similar trend and that nutrient additions of P had little impact on the phosphorus content of *S. alterniflora*.

3.3.2 *B. maritima*

The N content (% dry weight) in the above ground biomass of *B. maritima* in both fertilized plots and control plots did not vary significantly from the initial sampling date (June 2007) (Table 3.2) according to a paired-sample t-test.

A general increase in N was observed in the fertilized plots (67%) and control (26%) from June 2007 to October 2007 (Table 3.2). In September, a significant difference was observed between the fertilized and control plots and maintained until June 2008 ($P \leq 0.03$). By August 2008, the N concentration in the fertilized plots was near control levels.

Table 3.2. Total nitrogen (% dry weight) of *B. maritima* and its response to fertilization in monoculture plots over the sampling period (\pm S.E., n=30).

Treatment	27-Jun-07	11-Jul-07	8-Aug-07	5-Sep-07	24-Oct-07	10-Jun-08	27-Aug-08
0	1.67 \pm 0.19	1.40 \pm 0.06	1.21 \pm 0.14	1.58 \pm 0.29	2.17 \pm 0.39	1.47 \pm 0.09	1.14 \pm 0.14
30	1.54 \pm 0.29	2.43 \pm 0.12	1.72 \pm 0.19	2.78 \pm 0.19	3.09 \pm 0.16	1.95 \pm 0.08	1.24 \pm 0.12

The P content (% dry weight) of *B. maritima*, like the N content, was variable, by did not change significantly in the control plots or fertilized plots. The average *B. maritima* P content for 2007 and 2008 in the control was 0.27 \pm 0.01 (Appendix A, Table A.4). In the fertilized plots, *B. maritima* plants P content was 0.31 \pm 0.02 for the two year sampling period. At no point during the sampling period did the fertilized plots significantly differ from the control according to an independent-sample t-test.

3.3.3 *S. virginica*

Initially, the N content of *S. virginica* in the control plots and fertilized plants was similar, with an overall average of 1.26 ± 0.07 (Table 3.3; Appendix A, Figure A.7). By the third month, August 2007, the N content of the fertilized plants ($M=1.92$) exceeded the control plots ($M=1.23$, $P=0.00$) by 44% and this was maintained through the remainder of the year according to an independent-sample t-test. In June 2008, the N contents in the control and fertilized plots were close to the initial levels measured in June 2007 and did not significantly differ from each other for the remainder of the sampling period based on paired-sample t-tests.

Table 3.3. Total nitrogen (% dry weight) of *S. virginica* and its response to fertilization in monoculture plots over the sampling period (\pm S.E., $n=30$).

Treatment	27-Jun-07	11-Jul-07	8-Aug-07	5-Sep-07	24-Oct-07	10-Jun-08	27-Aug-08
0	1.30 ± 0.03	1.41 ± 0.08	1.23 ± 0.02	1.42 ± 0.21	1.37 ± 0.39	1.17 ± 0.05	1.16 ± 0.07
30	1.23 ± 0.11	1.63 ± 0.07	1.92 ± 0.02	2.17 ± 0.10	2.01 ± 0.16	1.34 ± 0.14	1.51 ± 0.29

S. virginica showed no significant difference in the P concentration (% dry weight) between the control plots and fertilized plots over the two sampling periods (Appendix A, Table A.5).

Overall, all the plants in the fertilized plots showed an increase in the N concentration compared to the control plots while the P content remain relatively stable (Figure 3.11). Including all species N (g N/g dry weight) and P (g N/g dry weight) content, figure 3.11 shows the positive correlation between the N and P concentrations for plant growth. The correlation between N and P was much better in the fertilized plants ($R^2=0.48$) than that in the control plants ($R^2=0.28$).

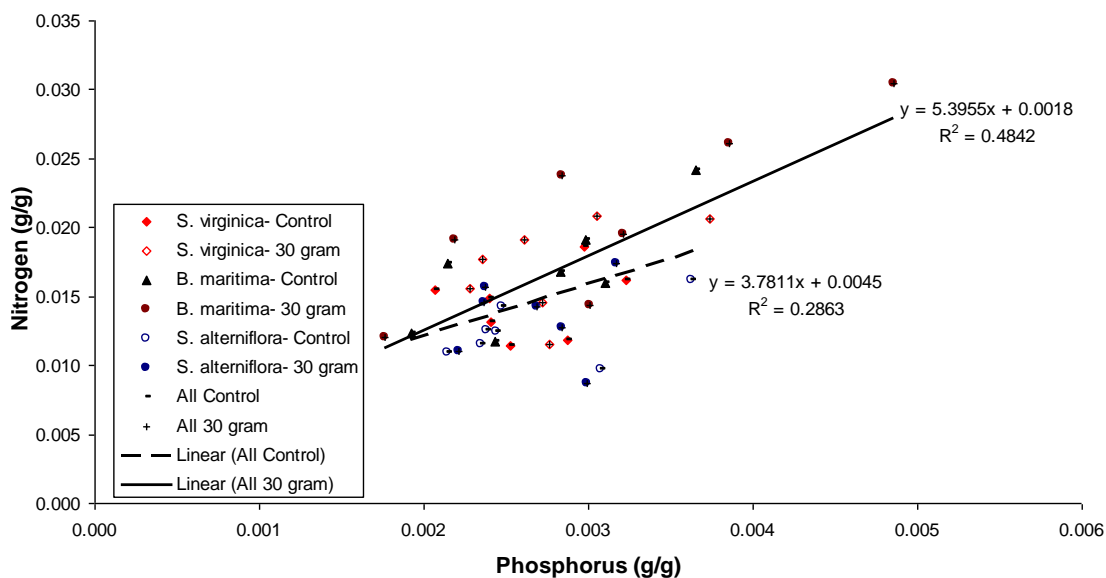


Figure 3.11. Nitrogen (g N/g dry weight) and Phosphorus (g P/g dry weight) correlation using *S. virginica*, *B. maritima* and *S. alterniflora* and nutrient impacts on the N:P uptake.

3.4 Carbon Composition

3.4.1 *S. alterniflora*

The C content (% dry weight) of *S. alterniflora*, leaves while variable, did not change significantly between the control and fertilized treatments ($P \geq 0.51$) (Table 3.4). A seasonal peak in C was observed. The control exceeded the C content of the initial sampling period in October 2007 by 8% ($P=0.02$). The fertilized plots exceeded the initial sampling period's C one month earlier, in September 2007, by 7% ($P=0.03$) and was maintained into October 2007. By June 2008, both treatments C dropped back to near control levels, however by August 2008, the fertilized plot exceeded the initial sampling date (June 07) by 6% ($P=0.03$).

Table 3.4. Total carbon (% dry weight) of *S. alterniflora* and its response to fertilization in monoculture plots over the sampling period (\pm S.E., $n=30$).

Treatment	27-Jun-07	11-Jul-07	8-Aug-07	5-Sep-07	24-Oct-07	10-Jun-08	27-Aug-08
Control	39.6 \pm 0.1	41.2 \pm 0.4	42.0 \pm 0.6	42.0 \pm 0.8	43.0 \pm 0.5	40.7 \pm 0.3	41.8 \pm 0.6
30 gram	39.6 \pm 0.0	41.4 \pm 0.2	42.3 \pm 0.6	42.4 \pm 0.5	42.9 \pm 0.5	41.5 \pm 1.0	42.2 \pm 0.4

3.4.2 *B. maritima*

Carbon (% dry weight) content in *B. maritima* showed similar seasonal trends with a significant peak for both the control (M=37.89, P=0.02) and fertilized treatment (M=32.93, P=0.03) in October 2007 relative to the starting content of 29.48 for the control and 26.96 for the fertilized plants (Table 3.5). At no point did the fertilized plots significantly differ from the control during the two sampling years (P=0.51) according to an independent-sample t-test.

In June 2008, the fertilized plot's C did fall 13% below the initial sampling date which was significant (P=0.03) according to a paired-sample t-test.

Table 3.5. Total carbon (% dry weight) of *B. maritima* and its response to fertilization in monoculture plots over the sampling period (\pm S.E., n=30).

Treatment	27-Jun-07	11-Jul-07	8-Aug-07	5-Sep-07	24-Oct-07	10-Jul-08	27-Aug-08
Control	29.5 \pm 2.0	27.6 \pm 3.6	31.1 \pm 1.1	32.0 \pm 5.1	37.9 \pm 2.2	28.5 \pm 2.1	30.1 \pm 2.6
30 gram	27.0 \pm 0.6	29.0 \pm 0.3	27.1 \pm 1.8	30.3 \pm 2.4	32.9 \pm 0.5	23.7 \pm 0.9	26.6 \pm 1.8

3.4.3 *S. virginica*

As previously seen in both *S. alterniflora* and *B. maritima* a seasonal peak in C (% dry weight) was observed for *S. virginica* (Table 3.6). Although no significant difference was observed between the control and fertilized plots over time, a difference was observed from the initial sampling period. The control exceeded the initial C content in September 2007 by 32% (June 07 M=25.59, September 07 M=35.09, P=0.01) and was maintained through the remainder of the sampling period. Only in October 2007 did the fertilized plots significantly differ from that of the initial sampling date (June 07 M=24.80, October 07 M=33.11, P=0.01) according to a paired-sample t-test.

Table 3.6. Total carbon (% dry weight) of *S. virginica* and its response to fertilization in monoculture plots over the sampling period (\pm S.E., n=30).

Treatment	27-Jun-07	11-Jul-07	8-Aug-07	5-Sep-07	24-Oct-07	10-Jun-08	27-Aug-08
Control	25.6 \pm 1.3	22.9 \pm 1.5	29.7 \pm 2.2	35.1 \pm 0.4	37.3 \pm 2.0	30.3 \pm 0.3	37.1 \pm 0.6
30 gram	24.8 \pm 3.3	24.5 \pm 0.3	31.2 \pm 1.2	33.3 \pm 1.1	33.1 \pm 2.4	28.3 \pm 0.2	38.6 \pm 1.2

All three species had similar seasonal trends with a peak in C content in October 2007 for both treatments, but the C content between the control and fertilized plots never showed a significant difference.

Although not significant, a weak negative correlation was observed between C and N content (Figure 3.12). The control showed a correlation (R^2) of 0.04 C:N (g/g) while the fertilized treatment showed a correlation (R^2) of 0.06 C:N (g/g). This suggests that with increased nutrient loading, particularly nitrogen, it can affect the carbon reserves within the plants above ground biomass.

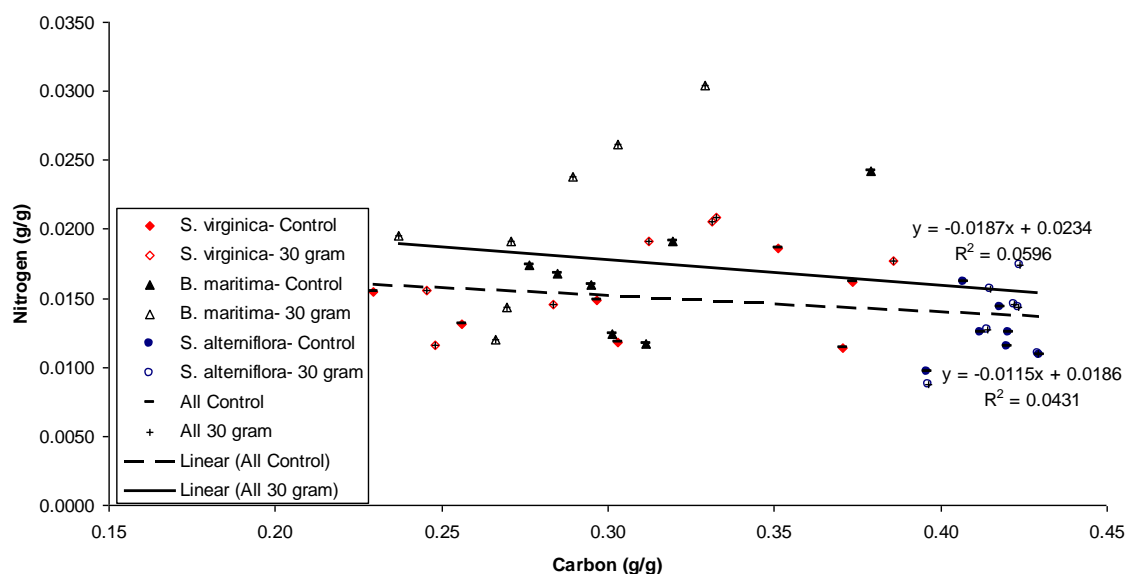


Figure 3.12. Nitrogen (g N/g dry biomass) and carbon (g C/g dry biomass) correlation using *S. virginica*, *B. maritima* and *S. alterniflora* and nutrient impacts on the C:N uptake.

3.5 Chlorophyll

S. alterniflora above ground biomass showed no significant difference in the chlorophyll a + b (total chlorophyll) concentration between the control and fertilized treatments except for July 2008 ($P=0.02$) according to an independent-sample t-test (Table 3.7). Rather, by August 2007, both the control plots ($P=0.04$) and fertilized plots ($P=0.04$) significantly decreased from the initial sampling period of June 2007 by 26 and 45% respectively. This difference was not maintained into September 2007. However, in October 2007, the control's total chlorophyll significantly dropped below the initial sampling period ($P=0.00$), but the fertilized plots did not. In the fertilized plots, by July 2008, the total chlorophyll was significantly less than the initial sampling's chlorophyll content ($P=0.04$). The total chlorophyll content of the control plots in August 2008 also showed a significant decrease ($P=0.02$) according to a paired-sample t-test.

Table 3.7. Total chlorophyll (mg/g dry weight) of *S. alterniflora* and its response to fertilization in monoculture plots over the sampling period (\pm S.E., $n=30$).

Treatment	27-Jun-07	11-Jul-07	8-Aug-07	5-Sep-07	24-Oct-07	10-Jul-08	27-Aug-08
Control	3.9 \pm 0.7	3.6 \pm 0.4	3.0 \pm 0.4	4.2 \pm 0.5	2.1 \pm 0.3	4.1 \pm 0.3	3.4 \pm 0.6
30 gram	4.5 \pm 0.4	4.3 \pm 0.4	2.9 \pm 0.6	4.3 \pm 0.3	2.8 \pm 0.7	3.2 \pm 1.0	3.2 \pm 1.2

B. maritima showed no significant variation in total chlorophyll content between treatments or from the initial sampling period according to an independent-sample t-test and paired-sample t-test (Table 3.8).

Table 3.8. Total chlorophyll (mg/g dry weight) of *B. maritima* and its response to fertilization in monoculture plots over the sampling period (\pm S.E., n=30).

Treatment	27-Jun-07	11-Jul-07	8-Aug-07	5-Sep-07	24-Oct-07	10-Jul-08	27-Aug-08
Control	4.3 \pm 0.4	4.1 \pm 0.5	4.9 \pm 1.2	6.2 \pm 1.7	5.2 \pm 0.3	4.8 \pm 1.2	4.0 \pm 0.4
30 gram	5.0 \pm 0.5	6.4 \pm 0.6	6.5 \pm 1.3	8.2 \pm 0.6	6.2 \pm 0.5	5.3 \pm 0.4	4.5 \pm 0.5

In the succulent, *S. virginica*, visual observations of fertilized plants showed a significant difference in color compared to their respective controls (Table 3.9).

Observations were confirmed spectrophotometrically by measuring an increase in the total chlorophyll content in the fertilized plants relative to the control.

S. virginica showed a significant increase in total chlorophyll for the fertilized plots one month after fertilization started ($P=0.02$) (Table 3.9). The control's (4.76 \pm 0.26mg/g) total chlorophyll in July 2007 was 43% less in the control than the fertilized treatment (6.83 \pm 0.21mg/g). The difference between the two treatments reach a peak in August 2007, with the fertilized plot (7.95 \pm 0.36 mg/g) 57% greater ($P=0.02$) in total chlorophyll than the control (5.06 \pm 0.22 mg/g). After August 2007, the chlorophyll content reduced to near control levels and did not vary significantly from the control for the remainder of the sampling period.

Table 3.9. Total chlorophyll (mg/g dry weight) of *S. virginica* and its response to fertilization in monoculture plots over the sampling period (\pm S.E., n=30).

Treatment	27-Jun-07	11-Jul-07	8-Aug-07	5-Sep-07	24-Oct-07	10-Jul-08	27-Aug-08
Control	3.9 \pm 0.1	3.6 \pm 0.2	3.0 \pm 0.2	4.2 \pm 0.3	2.1 \pm 0.2	4.1 \pm 0.2	3.4 \pm 0.1
30 gram	4.5 \pm 0.2	4.3 \pm 0.3	2.9 \pm 0.3	4.3 \pm 0.8	2.8 \pm 0.3	3.2 \pm 0.2	3.2 \pm 0.4

A positive correlation was observed between N and total chlorophyll content (Figure 3.13). The control showed a lesser correlation ($R^2=0.13$) than the fertilized treatment ($R^2=0.27$), indicating that N loading positively effects the total chlorophyll

content. The control plots correlation between chlorophyll a and b and nitrogen was 10.1 gram total chlorophyll/g N while the correlation increased to 16.2g total chlorophyll/g N in fertilized plots.

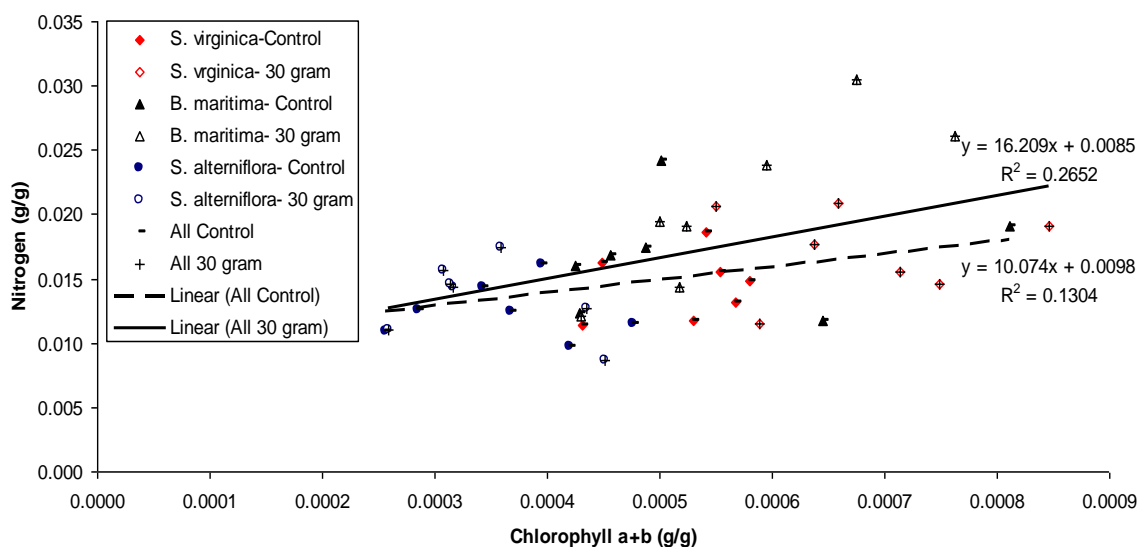


Figure 3.13. Nitrogen (g N/g dry biomass) and total chlorophyll (g chlorophyll a + b/g dry biomass) correlation using *S. virginica*, *B. maritima* and *S. alterniflora*.

4. DISCUSSION

Development along the marsh at Sunset Cove, Galveston, TX has lead to concerns of the impact it will have on the surrounding salt marsh, particularly eutrophication. This baseline study will help determine the actual impacts of increased eutrophication on this and other salt marshes. Previous studies support that nutrient additions have significant impacts on salt marsh plants, including increase in height and biomass as well as changes in N, P, C, and total chlorophyll content (Levine et al. 1998, Boyer et al. 2001, Gueswell 2004, Sicilano et al. 2008). This study in conjunction with previous studies suggest that the physical (height and biomass) and chemical (N, P, C, and total chlorophyll) properties of *S. alterniflora*, *B. maritima*, and *S. virginica* will be significantly impacted with eutrophication. The change in physical and chemical properties with prolonged eutrophication can lead to detrimental effects of the surrounding ecosystem, including functional and economic losses.

Fertilization at Sunset Cove resulted in significant changes in the physical (plant height and wet weight total biomass) and chemical (N, N:P ratios and chlorophyll) properties of *B. maritima*, *S. virginica*, and *S. alterniflora*. The hypothesis that responses to fertilization would be species dependent more so than dose dependent was confirmed, that is, *S. alterniflora* predominantly responded by getting taller in fertilized treatments within one year while *B. maritima* and *S. virginica* responded by predominantly increasing biomass in fertilized plots over a two year period.

Consistent with the literature (Edwards and Mills 2005), *S. alterniflora* had a perennial growth cycle, with a large die back each winter, seen by both decreased height and above ground wet weight biomass and then an increase in these parameters during

the spring and summer months. *B. maritima* and *S. virginica*, unlike *S. alterniflora*, have perennial life cycles with limited die off during winter months. Although height was limited by the growth form of both these species, both height and biomass increased over the two sampling years, 2007 to 2008.

The results also suggest that all physical properties, height and biomass, must be considered when comparing species due to the difference in life cycles and growth forms. That is, if only plant height was considered when comparing these three plants, the results would have been misleading. This study also shows the time frame of the study is important as *S. alterniflora* responded quickly (months) to fertilization whilst the response of *B. maritima* and *S. virginica* was more gradual (year). To detect changes in physical (height and biomass), chemical (C, N, P, and total chlorophyll) and interspecific competitive properties a year or more of sampling is required based on this study, particularly for perennial species in areas with long growing seasons (Pennings et al. 2002). Previous studies conducted by Levine et al. (1998) and Boyer et al. (2001) studying *S. alterniflora* and *S. virginica* respectively support this conclusion.

The percent cover of plants is often used in the literature when examining the response of plants to nutrient fertilization (Kiehl et al. 1997). This technique has well known problems such as variability between people and is based on estimations. As part of this experiment, percent cover was measured across both growing seasons and in all plots. However, given the data was variable and did not follow any trends, it was not included in the results of this thesis.

4.1 Monod Model

The Monod model is a common method used to describe microbial growth kinetics using maximum specific growth rate, a saturation constant, and a yield coefficient (Strigul et al. 2009). Like the Richards and Birch growth model, the Monod model is a simple model with the base of a sigmoidal growth curve (Damgaard and Weiner 2008, Kovárová-Kovar and Egli 1998), which has also been applied to explain the growth of plants (Strigul et al. 2009, reviewed in Tilman 1982). However, although the Richards and Birch growth curves can explain competitive interactions between plants (Damgaard and Weiner 2008), they lack an estimation of which species will dominate at high nutrient loads, which could be a valuable tool to predict future outcomes of eutrophication on plant communities. Predicting the future outcomes plants at high nutrient loads could then support the CRS theory by determining which species dominate at ambient conditions and which would dominate when different stresses, in this case nutrient limitation, is lessened.

The Monod model (reviewed in Tilman 1982, Strigul et al. 2009) provides a framework for comparing individual plant species in the different treatments as well as between plant species. In this study, it will be used to examine the growth response of salt marsh plants in relation to fertilizer application. The Monod model has the form shown in equation 4.1, where μ is the growth rate, S is the amount of fertilizer used in each treatment, μ_{\max} is the maximum attainable growth rate and K_s is the threshold nutrient concentration at which growth can be initiated. Growth rates can be determined based on changes in plant height, biomass or other measured factors.

Equation 4.1. Monod model equation

$$\mu = \frac{\mu_{\max} * S}{K_s + S}$$

4.1.1 Monod Model: Plant Height

Based on plant height data, *S. alterniflora* had a maximum growth rate (μ_{\max}) of 9.61cm/month which is 5-fold greater than the maximum growth rates calculated for *B. maritima* (1.73cm/month) and *S. virginica* (1.20cm/month) (Table 4.1). No published data based on plant height was found for *B. maritima* or *S. virginica*. Although, given their growth forms (stoloniferous), they were not expected to gain as much as height as *S. alterniflora* in response to nutrient additions. This increase in height of *S. alterniflora* with nutrient additions was confirmed by Pennings et al. (2002).

S. alterniflora was predicted to respond best to nutrient enrichment of the three plants examined, because it has the lowest nutrient half saturation factor (<0.01g/0.25m²/month) and the lowest nutrient affinity factor (<0.01g/0.25m²/month). This indicates, based on plant height data, that *S. alterniflora* requires the least amount of nutrients to stimulate growth. In addition, *S. alterniflora* utilizes nutrients more efficiently and has the most potential to be successful at elevated nutrient concentrations (Table 4.1). That is, only a small amount of nutrient loading is required to stimulate growth relative to the other two species examined. *B. maritima* and *S. virginica* had much higher nutrient affinity factors, 5.11g/0.25m²/month and 3.68g/0.25m²/month, respectively. These are several orders of magnitude higher indicating that the plants require substantially more nutrients to achieve maximum growth rates. Based on their nutrient affinity factors (Table 4.1), they are also less efficient at utilizing nutrients

compared to *S. alterniflora*, reducing their potential to be successful at elevated nutrient concentrations.

Table 4.1. Monod model parameters calculated using the height for the salt marsh plants in the 30 gram treatments.

Species	Max Growth Rate μ_{\max} (cm/month)	Nutrient Half-Saturation Factor (g/0.25m ² /month)	Nutrient Affinity Factor (g/0.025m ² /month)
<i>S. virginica</i>	1.20	4.41	3.68
<i>B. maritima</i>	1.73	8.84	5.11
<i>S. alterniflora</i>	9.61	<0.01	<0.01

As stated above, *S. alterniflora*, with its perennial growth cycle showed a significant increase, $54\% \pm 2$, in above ground plant heights in all treatments with fertilizer added; an increase which was similar to that seen in the control (46%), within 5 months. This is supported by the Monod model that suggests that the nutrients required to support growth at the maximum growth rate is minimal (<0.01 g/0.25m²/month) compared to *B. maritima* and *S. virginica* and with any addition of fertilizer, *S. alterniflora* is able to achieve the maximum growth rate. This is likely due to the already high nutrient load present in Galveston Bay, TX. This is supported by the relative growth rate determined from the height data that shows a plateau in height starting with the 5g treatment (Appendix B, Table B.1). The nutrient affinity factor also suggest that *S. alterniflora* can utilize the nutrients efficiently and has the highest potential to be successful at elevated nutrient concentrations compared to *B. maritima* and *S. virginica*.

The succulent species, *B. maritima* and *S. virginica* have a much lower maximum growth rate than *S. alterniflora* and the two succulents had similar results in terms of height data. This trend for plant populations to have relatively low growth rates in the

higher marsh zones, like that of *B. maritima* and *S. virginica*, than the lower marsh which is occupied by *S. alterniflora* in this case, is supported by Jefferies and Perkins (reviewed in Kiehl et al. 1997). Jefferies and Perkins stated that plant populations of high salt-marsh zones have low relative growth rates compared to the low marsh species resulting in biomass accumulation being limited with nutrient addition.

According to the nutrient half-saturation factor of the Monod model, both *B. maritima* and *S. virginica* require about 4 to 8-times the nutrient input of *S. alterniflora* to see the maximum growth rate. This is consistent with the results that both *B. maritima* and *S. virginica* at the high nutrient loads (15g and 30g) did show a significant increase in height. This was supported by Pennings et al.'s (2002) finding that N addition favors lower marsh species like *S. alterniflora* compared to higher marsh species like *B. maritima* and *S. virginica*.

Overall, it is expected that *S. alterniflora* because of its low nutrient affinity factor, nutrient half saturation factor and high growth rate should outcompete *S. virginica* and *B. maritima* overtime with high nutrient loads and progress upward into the marsh. This supports the CRS theory in which fertilization lead to the ambient marsh inferior dominating when a stressor, in this case nutrient limitation, was lessened. Levine et al. (1998) found that in general *S. alterniflora* would become the dominant species under high nutrient loads. This would result in loss of biodiversity, loss of habitat, and changes to the food web which will have detrimental effects on the surrounding community.

4.1.2 Monod Model: Plant Biomass

A more meaningful comparison may be made between species using the Monod model when examining the above ground wet weight biomass data (Table 4.2; Appendix B, Table B.2). This is especially important given that some plants grow in vertical bunch form (*S. alterniflora*) while others have a low spreading or stoloniferous growth, limiting vertical height (*B. maritima* and *S. virginica*).

Table 4.2. Monod model parameters calculated using the total above ground wet weight biomass for the salt marsh plants in the 30 gram treatments.

Species	Max Growth Rate μ_{\max} (g/0.02m ² /month)	Nutrient Half-Saturation Factor (g/0.025m ² /month)	Nutrient Affinity Factor (g/0.025m ² /month)
<i>S. virginica</i>	13.55	8.43	0.62
<i>B. maritima</i>	3.28	<0.01	<0.01
<i>S. alterniflora</i>	15.25	2.49	0.16

Supporting the height based Monod model, *S. alterniflora* had the highest maximum growth rate based on changes in biomass, accumulating up to 15.25g/0.02m²/month (Table 4.2), which resulted in biomass ranging between 2900 - 6590g/m² wet weight by October 2007. *S. alterniflora* had a high growth rate compared to other species studied. This is supported by findings of Jerries and Perkins (reviewed in Kiehl et al. 1997) who showed that plants in the lower marsh zone have high relative growth rates compared to the higher marsh zones, which relates to the limited biomass increase.

Annual above ground production estimates by Kaswadji et al. (1990) of 831 g/m² dry weight for *S. alterniflora* were similar to this study (800-850g/m²) (data not shown). In contrast, Alexander and Robinson (2006) reported much higher range of values of total

above ground biomass (250-2100g/m² wet weight). This is likely due direct human impacts on the marsh within the sampling area which had docks around the marsh (Kaswadji et al. 1990). The high spatial and large inter-annual variability also makes comparisons of biomass within marshes and between marshes problematic.

S. alterniflora is predicted to have an intermediate potential to be successful at elevated nutrient concentrations (0.16g/0.25m²/month) according to the nutrient affinity factor. This model, based on biomass, suggest that *B. maritima* should out compete *S. alterniflora* at high nutrient loads, but *S. alterniflora* will outcompete *S. virginica*. The model also suggests an intermediate nutrient half-saturation factor for *S. alterniflora* (2.49g/0.25m²/month), however, the relative growth rate determined from the total above ground wet weight biomass showed no significant difference in growth between treatments suggesting that an adequate supply of nutrients was available in the system to support the maximum growth rate and that is why, in figure 3.4, all treatments showed an increase, but that increase was not significantly different from the control.

B. maritima had the slowest maximum growth rate (3.28g/0.02m²/month) of the species sampled. This species nutrient affinity factor (<0.01g/0.25m²/month) and nutrient half-saturation factor (<0.01g/0.25m²/month) was the lowest compared to *S. virginica* and *S. alterniflora* , suggesting that *B. maritima* will be the most successful at high nutrient loading and requires only a small amount of nutrients to achieve maximum growth rate. This is supported by the monospecific plots of *B. maritima* and relative growth rates (Figure 3.6; Appendix B, Table 7.2) that showed no significant change with the addition of fertilizer. This again supports that nutrients are readily available to support maximum growth rate for both *S. alterniflora* and *B. maritima*.

S. virginica showed an intermediate growth rate of $13.55\text{g}/0.02\text{m}^2/\text{month}$ with a nutrient half-saturation factor ($8.43\text{g}/0.25\text{m}^2/\text{month}$) suggesting it needs a high nutrient load to achieve this maximum growth rate. This is supported by the relative growth rate determined from total above ground biomass which shows a plateauing effect between the 15g and 30g treatment and is confirmed by figure 3.8 which showed that the 30g treatment exceeded the control plots' biomass by the end of the sampling period (August 2008). The data also suggest that *S. virginica* is less efficient at utilizing nutrients at high nutrient loads compared to *B. maritima* and *S. alterniflora* and would be the least successful at elevated nutrient concentration.

In this case, it is unclear which measurement, height or biomass, is best to use with the Monod model since all species showed an interaction with nutrient loading. *S. alterniflora* increased in height which could result in shading of other species, while *S. virginica* and *B. maritima* increased in biomass, which could spread eventually outcompeting for space. Additional studies, including competition studies, for a longer period of time are needed to confirm which measurement is best. However, it is hypothesized that height or a combination of biomass and height measurements would give the most accurate estimate of the nutrient saturation factor. This is supported by Levine et al. (1998) and Pennings et al. (2002) which found that *S. alterniflora* did become the dominate species under nutrient-enhanced conditions, which is predicted by the Monod model based on height.

4.2 Competition Plots

Zonation of species is attributed to competitive displacement and interspecific variations in physiological tolerance (Levine et al. 1998). The CSR theory states that there are two main external environmental factors, stress and disturbance, that affects the performance of plants in their natural environment (Grime 1977). Tilman's resource-ratio hypothesis also predicts when the stress for competing for below ground nutrients is relieved, then competition shifts aboveground for light and space (Tilman 1982, 1988, 1990, 1997, Wilson and Tilman 1991, 1993).

In this experiment, the stress-factor that was manipulated was the nutrient load. With increasing nutrient loading competition should shift from below ground interspecific competition to above ground competition which would be evident by increased height and above ground biomass. The Monod model, because of the calculated nutrient affinity factor can help to predict the outcome of which species, in this case *B. maritima* and *S. virginica*, will out compete the other at high nutrient loading in mixed plots.

The Monod model, based on height, *S. virginica* with the lower nutrient affinity factor ($3.68\text{g}/0.25\text{m}^2$) should outcompete *B. maritima* at higher nutrient loads (i.e. 30g treatment) while at lower nutrient loading *B. maritima* would dominate. However, based on height data (Figure 3.9) no significant difference and definitive conclusion could be made on which species would outcompete the other. This is consistent with these plants having a stoloniferous growth form. Hence, they are less likely to invest in new growth growing taller.

The Monod model based on biomass data, however, showed the opposite trend, with *B. maritima* having the lower nutrient affinity factor ($<0.01\text{g}/0.25\text{m}^2$) suggesting that this species should outcompete *S. virginica* at high nutrient loading. However, biomass data in the competition plots (Figure 3.10) showed a trend of *S. virginica* in both the control and fertilized plots increasing faster than *B. maritima* which suggests that *S. virginica* would dominate in all plots given enough time.

The Monod model although a useful tool in determining maximum growth rate, nutrient affinity factors, and nutrient saturation factors, lacked the combination of measurements (height and biomass) to best determine which species would dominate at high nutrient loading. These Models in this case were also based on monospecific plots with no interspecific competition and does not take into account the time that nutrient additions started.

Levine et al. (1998) found that fertilization intensifies the competitive advantage of early emerging species over late emerging species by increasing growth rates and escalating the degree of asymmetric competition. This means that in competition plots the addition of limiting nutrients can stimulate the species that's spatial or temporal pattern of nutrient uptake corresponds best to the timing of fertilization. This supports why *S. virginica* was able to increase height to match *B. maritima* and to exceed the biomass of *B. maritima*, since it appears that *B. maritima* growth occurred mainly before the start of fertilization (maximum growth achieved in June). *S. virginica* growth occurred later in the year, starting in July and the maximum overall growth was achieved by September.

Competitive displacement might also take several years if current year growth is based on the belowground reserves or because of the larger size of plants and longer growing seasons (Pennings et al. 2002). In addition, this experiment did not take into account belowground competition since the plots were lost due to Hurricane Ike before below ground biomass could be collected. When nutrient limitation occurs species will allocate growth to the root systems, but with adequate nutrients the competition can be redirected aboveground for light and space.

With the large influx of nutrients, particularly N, into salt marshes they are particularly vulnerable because they are naturally N-limited (Emery et al. 2001). With the results from this study, it is predicted that eutrophication over an extended period of time will exclude species that have a high nutrient affinity factor. The Monod model based on height measurements it would suggest *S. alterniflora* would dominate which is consistent with Levine et al. (1998) and Pennings et al. (2002) findings. However, the Monod model based on biomass measurements suggest that *B. maritima* will dominate under eutrophic conditions, however, no studies were found that could confirmed or disproved this model. This shift in species composition would cause a dramatic change to the salt-marsh community. Although nutrients can lead to significant changes in salt marshes, other stressors-flooding and salinity and disturbances-wrack burial and herbivory play an important role in species competition, preventing the complete dominance by one species.

4.3 Role of Nutrients

Seasonal patterns of nitrogen in the above ground biomass for all three species were pronounced. The N content increased from spring and peaked in fall. During the winter months, N content decreased to the initial levels of the previous year, likely due to translocations of nutrients to the roots for storage (Güsewell 2004, Hopkins and Schubauer 1984). The seasonal pattern of N accumulation during the growing season of above ground biomass was comparable to observations of Hopkins and Schubauer (1984).

Fertilizer additions increased the N content significantly in all three species studied and similar trends have been reported in other salt marsh species by Boyer et al. (2001) and Hopkins and Schubauer (1984) studying *S. virginica* and *S. alterniflora*, respectively. These studies indicate that salt marsh species play a major role in transfer of nutrients from the sediments to the marsh surface through uptake and subsequent leaching and detritus production. This increase in leaching of nutrients and increased detritus production can lead to noxious and toxic algal blooms, increased turbidity, leading to loss of submerged aquatic vegetation, reduction in oxygen, disruption of ecosystem functions, loss of habitat, and loss of biodiversity (Rabalais 2002; Quigg et al. 2009).

This significant increase of N along with increases in plant height and biomass suggest plants responded due to N limitation at Sunset Cove, TX. This is comparable to other studies, particularly in the Gulf States, that have shown salt marshes are N limited (Rabalais 2002, Siciliano et al. 2008, Bertness et al. 2002), and susceptible to eutrophication. The limited change over time and between the control and fertilize plots

suggest that P was not a limiting nutrient within this system (Boyer et al. 2001). This is consistent with other studies that have found that P is readily available and not the limiting nutrient in salt marshes (Bertness et al. 2002, Rabalais 2002).

The general increase in N:P ratios in the fertilized plots of all species is correlated with an increase in relative growth rate (Güsewell 2004, Zhang et al. 2008). This is likely a consequence of faster plant growth at higher N concentrations, leading to a greater demand for other nutrients, in this case, P. The biomass N:P ratios reflects the relative availability of N and P but is not exact because of homeostatic regulation of plants, leaching, translocation and loss of detritus material. Boyer et al. (2001) suggested that N additions in *S. virginica* without P addition could lead to P limitation of primary production. The shift in nutrient stoichiometry can lead to alternations in plant community composition, when it occurs over longer periods than this study.

The weak negative correlation between C and N content (Figure 3.12) showed that N additions interfered with carbon concentrations. Invers et al. (2004) found that N assimilation interfered with carbon metabolism since assimilation required energy. In addition C skeletons were diverted from carbohydrate metabolism. This C storage is essential for survival under stressful conditions, such as overwintering and extended periods of flooding (Invers et al. 2004). If C reserves are decreased, then the health of the species is compromised and could lead to the partial or complete die off under stressful conditions, leading to the loss of biodiversity. A large die off of plants would also release a large amount of nutrients and detritus material into the surrounding community.

This reduction of C reserves, although weak, could have played a significant role in the cause for the complete die off of *S. virginica* at the 30g treatment while the other

treatments remained visually unaffected during the extend flooding of the plots due to Hurricane Ike (data not shown).

4.4 Chlorophyll

Nutrient additions in two species studied, *S. alterniflora* and *S. virginica*, did have a significant impact in total chlorophyll content. This is likely due to the increase in N and P being readily available for use. Nitrogen is a key component in chlorophyll, while P is necessary for photosynthesis. By reducing the stress of nutrient limitations the plants in the fertilized plots were able to dedicate the nutrients to other processes such as photosynthesis. The increase in total chlorophyll was consistent with past research that found a relationship in which an increase in nitrogen lead to an increase in photosynthesis (Gusewell 2004), which was also supported by the observed increase in N:total chlorophyll correlation in fertilized plots.

As in the fertilized *S. virginica* plots of this study, Sicilian et al (2008) found that the fertilized plots of *S. virginica* were visually brighter green than the dull olive green of the unfertilized plots. In this experiment and Siciliano et al. (2008), observations were spectrally confirmed by measuring the chlorophyll content which showed an increase in chlorophyll content in the fertilized plots compared to the control plots. Pennings et al. (2002) also saw a marginal increase in photosynthetic rates in *S. alterniflora* of fertilized plants compared to the control using gas exchange measurements.

It is likely that *S. virginica* and *S. alterniflora* saw a significant increase in total chlorophyll and *B. maritima* did not because of the sampling method based on plant growth forms. All samples taken came from sections of the plant with a width of 3mm.

S. virginica chlorophyll samples were taken approximately 2cm from the top where the width reached 3mm and *S. alterniflora* was sampled on the second leaf from the top, leading to sampling of younger, more immature leaves, while *B. maritima* was sampled on the first leaf from the top that allowed for a plug from a 3mm cork borer, which could have led to more mature leaves which have lower chlorophyll content (Biber 2008).

Often concentration of chlorophyll a is used as an indicator of eutrophication; however, as seen in this study, chlorophyll concentrations are variable over time and between species. If the community composition is known, since this salt marsh is nitrogen limited and nitrogen is correlated with chlorophyll a and chlorophyll b, hyperspectral data could be used to monitor changes in some communities at specific times of the year in conjunction with other sampling methods (Siciliano et al. 2008).

By monitoring chlorophyll concentrations within this salt marsh, increased concentrations could signal a large flux of nutrient loading, which would lead to increases in biomass and ultimately a change in community composition, reducing plant species richness (Bertness, et al. 2002).

5. CONCLUSION

The objective of this study was to provide a more comprehensive understanding of the relationship between nutrient additions (eutrophication) and its impact on common salt marsh species, specifically the physical (height and above ground biomass) and chemical (C, N, P, and total chlorophyll) components of plant responses as well as interspecific competition. The data collected support the hypothesis that nutrient additions would have an impact on the physical and chemical features of the three species studied, *B. maritima*, *S. alterniflora*, and *S. virginica*. This included that nutrient enhancement was more species dependent than dose dependent, with each species responding differently to nutrient enhancement. Nutrient loading did show a general increase in height, biomass, growth rate, and percent nitrogen. Phosphorus and C content did show weak correlations to nitrogen and chlorophyll content was affected in fertilized plots of *S. alterniflora* and *S. virginica*. However, data did not support the hypothesis that nutrient additions would favor the species that are the competitive inferior under ambient conditions. In this case, no definitive conclusion could be made of which species was inferior under ambient conditions.

As Sunset Cove, TX develops it is expected that the impact of eutrophication on the salt marsh will increase. This will result in increased biomass of all species present. With increasing biomass, competition will change from belowground competition for nutrients to aboveground for light and space. With high nutrient loads, the biodiversity of plants can decrease with lower salt marsh species outcompeting higher salt marsh species. This leads to loss of habitat and food for other species found in the salt marsh. With increased biomass, nutrient leaching and loss of detritus material into the

surrounding water will also increase, leading to increase in algal blooms, turbidity, loss of submerged aquatic vegetation and reduced oxygen levels. This alters the community and reduces the ecosystem services.

This study is important in the understanding of eutrophication by using a simple model (Monod model) to quantify the observed changes in height and biomass over time. By using the nutrient affinity factor of the Monod model, this can help to predict which species will outcompete at high nutrients loads, which can lead to predicting future community structures at high nutrient loads. Also, by measuring changes in chemical properties (N, P, C and total chlorophyll) of the salt marsh vegetation that has a slower response time, it could be used as a bioindicator of temporal nutrient dynamics, if a baseline, such as in this study is established.

REFERENCES

- Alexander, C., and M. Robinson. 2005. Quantifying the ecological significance of marsh shading: the impact of private recreational docks in coastal Georgia. http://www.skio.usg.edu/publications/downloads/pdfs/technical/dockshading_on_biomass2006.pdf. Accessed Sept. 9, 2010.
- Bennett, E. M., S. R. Carpenter, and N. F. Caraco. 2001. Human impact on erodible phosphorus and eutrophication: A global perspective. *BioScience* 51:227-234.
- Bertness, M. D., and A. M. Ellison. 1987. Determinants of pattern in a New England salt marsh plant community. *Ecological Monographs* 57:129-147.
- Bertness, M. D., P. J. Ewanchuk, and B. R. Silliman. 2002. Anthropogenic modification of New England salt marsh landscapes. *Proceedings of the National Academy of Sciences* 99:1395-1398.
- Biber, P. D. 2008. Determining salinity-tolerance of giant salvinia using chlorophyll fluorescence. *Gulf and Caribbean Research* 21:31-36.
- Boyer, K. E., P. Fong, R. R. Vance, and R. F. Ambrose. 2001. *Salicornia virginica* in a southern California salt marsh: Seasonal patterns and a nutrient-enrichment experiment. *Wetlands* 21:315-326.
- Dai, T., and R. G. Wiegert. 1996. Ramet population dynamics and net aerial primary productivity of *Spartina alterniflora*. *Ecology* 77:276-288.
- Damgaard, C. and J. Weiner. 2008. Modeling the growth of individuals in crowded plant populations. *Journal of Plant Ecology* 1:111-116.
- Day, J. W., J. Ko, D. Sabins, R. Bean, G. Berthelot, C. Brantley, L. Cardoch, W. Conner, J. N. Day, A. J. Englande, S. Feagley, E. Hyfield, R. Lane, J. Lindsey, J. Mistich, E. Reyes, and R. Twilley. 2004. The use of wetlands in the Mississippi Delta for wastewater assimilation: A review. *Ocean and Coastal management* 47:671-691.
- Edwards, K. and K. Mills. 2005. Aboveground and belowground productivity of *Spartina alterniflora* (smooth cordgrass) in natural and created Louisiana salt marsh. *Estuaries* 28:252-265.
- Ehrenfeld, J. G. 2001. Plant-Soil interactions. *Encyclopedia of Biodiversity* 4:689-709.
- Emery, N. C., P. J. Ewanchuk, and M. D. Bertness. 2001. Competition and salt-marsh plant zonation: Stress tolerators may be dominant competitors. *Ecology* 82:2471-2485.

- Environmental Protection Agency. 2003. Wetlands Overview. Office of Water. EPA 843-F-04-011a. <http://www.epa.gov/owow/wetlands/pdf/overview.pdf>. Accessed Nov. 6, 2008.
- Fourqurean, J. W., J. C. Zieman, and G. V. N. Powell. 1992. Phosphorus limitation of primary production in Florida Bay: Evidence from C:N:P ratios of the dominant seagrass *Thalassia testudinum*. *Limnology and Oceanography* 37:162-171.
- Galloway, J. N. and E. B. Cowling. 2002. Reactive nitrogen and the world: 200 years of change. *A Journal of the Human Environment* 31: 64-71.
- Grime, J. P. 1977. Evidence for the existence of three primary strategies in plants and its relevance to ecological and evolutionary theory. *American Naturalist* 111:1169-1194.
- Güeswell, S. 2004. N:P ratios in terrestrial plants: Variation and functional significance. *New Phytologist* 164:243-266.
- Hemond, H. F. and J. Benoit. 1988. Moist-soil management of playa lakes for migration and wintering ducks. *Wildlife Society Bulletin* 21:288-298.
- Hopkinson, C. S. and J. P. Schubauer. 1984. Static and dynamic aspects of nitrogen cycling in the salt marsh graminoid *Spartina alterniflora*. *Ecology* 65(3):961-969.
- Howarth, R. W., H. Jensen, R. Marino, and H. Postma. 1995. Transport to and processing of P in near-shore and oceanic waters. p. 54. *In* H. Tiessen (ed.) *Phosphorus in the Global Environment: Transfers, Cycles and Management*. SCOPE 54, John Wiley and Sons. Chichester, UK. 323-356.
- Howarth, R. W., H. Jensen, R. Marino, and H. Postma. 1995. Transport to and processing of P in near-shore and oceanic waters. *Phosphorus in the Global Environment: Transfers, Cycles and Management*. Tiessen, H., Eds. Chichester, UK., SCOPE pp. 54
- Invers, O., G. P. Kraemer, M. Pérez, and J. Romero. 2004. Effects of nitrogen addition on nitrogen metabolism and carbon reserves in the temperate seagrass *Posidonia oceanica*. *Journal of Experimental Marine Biology Ecology* 303:97-114.
- Kaswadji, R. F., J. G. Gosselink, and R. E. Turner. 1990. Estimation of primary production using five different methods in a *Spartina alterniflora* salt marsh. *Wetlands Ecology and Management* 1:57-64.
- Kiehl, K., P. Esselink, and J. P. Bakker. 1997. Nutrient limitation and plant species composition in temperate salt marshes. *Oecologia* 111:325-330.

- Kovárová-Kovar, K. and T. Egli. 1998. Growth kinetics of suspended microbial cells: From single-substrate-controlled growth to mixed-substrate kinetics. *Microbiology and Molecular Biology Reviews* 62:646-666.
- Lester, L.J and L. Gonzales (Eds). 2002. The State of the Bay: A characterization of the Galveston Bay Ecosystem. Galveston Bay Estuary Program Publication GBEP T-7. Webster, Texas. <http://gbic.tamug.edu/sobdoc/sob2/sob2page.html>. Accessed Sept. 9, 2010.
- Levang-Brilz, N. and M. E. Biondini. 2002. Growth rate, root development and nutrient uptake of 55 plant species from the Great Plains Grasslands, USA. *Plant Ecology* 165:117-114.
- Levine, J.M., J. S. Brewer, and M. D. Bertness. 1998. Nutrients, competition and plant zonation in a New England salt marsh. *Journal of Ecology* 86:285-292.
- McGlathery, K. J., K. Sundback, and I. C. Anderson. 2007. Eutrophication in shallow coastal bays and lagoons: The role of plants in the coastal filter. *Marine Ecology Progress Series* 348:1-18.
- McInnes, A. and A. Quigg. 2010. Near-annual fish kills in small embayments: Casual versus causal factors. *Journal of Coastal Research*. 26: 957-966.
- National Oceanic and Atmospheric Administration. 2009. National Weather Service Weather Forecast Office. Galveston: Extremes, Normals and Annual Summaries. <http://www.nws.noaa.gov/>. Accessed January 21, 2009.
- Newbery, R. M., J. Wolfenden, T. A. Mansfield and A. F. Harriosn. 1995. Nitrogen, phosphorus and potassium uptake and demand in *Agrostis capillaris*: The influence of elevated CO₂ and nutrient supply. *New Phytologist* 130:565-574.
- Ngai, J. T., and R. L. Jefferies. 2004. Nutrient limitation of plant growth and forage quality in arctic coastal marshes. *Journal of Ecology* 92:1001-1010.
- Örnólfssdóttir, E. B., S. E. Lumsden, and J. L. Pinckney. 2004. Nutrient pulsing as a regulator of phytoplankton abundance and community composition in Galveston Bay, Texas. *Journal of Experimental Marine Biology Ecology* 303:197-220.
- Parson, M. L., Q. Dortch, R. E. Turner, and N. R. Rabalais. 2006. Reconstructing the development of eutrophication in Louisiana salt marshes. *Limnology and Oceanography* 51:534-544.

- Patrick Jr., W. H. and R. D. Delaune. 1976. Nitrogen and phosphorus utilization by *Spartina alterniflora* in a salt marsh in Barataria Bay, Louisiana. *Estuarine and Coastal Marine Science* 4:59-64.
- Pennings, S. C., L. E. Stanton, and J. S. Brewer. 2002. Nutrient effects on the composition of salt marsh plant communities along the southern Atlantic and Gulf Coasts of the United States. *Estuaries* 25:1164-1173.
- Phillips, J. D. 1989. An evaluation of the factors determining the effectiveness of water quality buffer zones. *Journal of Hydrology* 107:133-145.
- Purvaja, R., R. Ramesh,, A. K. Ray, and T. Rixen. 2008. Nitrogen cycling: A review of the processes, transformations and fluxes in coastal ecosystems. *Current Science* 94:1419-1438.
- Quigg A., L. Broach, W. Denton, and R. Miranda. 2009. Water quality in the Dickinson Bayou watershed (Texas, Gulf of Mexico) and health issues. *Marine Pollution Bulletin* 58:896-904.
- Quigg, A. and L. Roehrborn. 2008. Spatial and temporal distributions of planktonic diatoms in a subtropical bayou, along the upper Texas coast. *Texas Journal of Science* 60:281-298.
- Rabalais, N. N. 2002. Nitrogen in Aquatic Ecosystems. *A Journal of the Human Environment* 32:102-112.
- Reed, D. J. and D. R. Cahoon. 1992. The relationship between marsh surface topography, hydroperiod, and growth of *Spartina alterniflora* in a deteriorating Louisiana salt marsh. *Journal of Coastal Research* 8:77-87.
- Santschi, P. H. 1995. Seasonality in nutrient concentrations in Galveston Bay. *Marine Environmental Research* 40:337-362.
- Shinano, T., T. T. Lei, T. Kawamukai, M. T. Inoue, T. Koike, and T. Tandano. 1996. Dimethylsulfoxide method for the extraction of chlorophylls *a* and *b* from the leaves of wheat, field bean, dwarf bamboo, and oak. *Photosynthetica* 32:409-415.
- Siciliano, D., K. Wasson, D. C. Potts, and R. C. Olsen. 2008. Evaluating hyperspectral imaging of wetland vegetation as a tool for detecting estuarine nutrient enrichment. *Remote Sensing of Environment* 112:4020-4033.
- Snow, A. A., and S. W. Vince. 1984. Plant zonation in an Alaskan salt marsh: II. An experimental study of the role of edaphic conditions. *Journal of Ecology* 72:669-684.

- Strigul, N., H. Dette, and V. B. Melas. 2009. A practical guide for optimal designs of experiments in the Monod model. *Environmental Modeling and Software* 24:1019-1026.
- Sylvan, J. B., Q. Dortch, D. M. Nelson, A. F. Maier Brown, W. Morrison, and J. W. Ammerman. 2006. Phosphorus limits phytoplankton growth on the Louisiana shelf during the period of hypoxia formation. *Environmental Science and Technology* 40:7548-7533.
- Tilman, D. 1982. Resource competition and community structure. Princeton University Press, Princeton, New Jersey, USA. pp. 269.
- Tilman, D. 1988. Plant strategies and the dynamics and structure of plant communities. Princeton University Press, Princeton, New Jersey, USA. pp. 376.
- Tilman, D. 1990. Mechanisms of plant competition for nutrients: the elements of a predictive theory of competition. Pages 117-141 *in* J. B. Grace and D. Tilman, editors. Perspectives on plant competition. Academic Press, New York, New York, USA. pp. 484.
- Tilman, D. 1997. Mechanisms of plant competition. Pages 239-361 *in* M. J. Crawley, editor. Plant ecology. Second edition. Blackwell Science, Oxford, UK. pp. 736.
- United States Environmental Protection Agency. 2005. Natural management measures to protect and restore wetlands and riparian areas for the abatement of nonpoint source pollution. EPA-841-B-05-003. Office of water. Washington, District of Columbia.
- United States Geological Survey. 2008. Toxic Substances Hydrology Program: eutrophication. <http://toxics.usgs.gov/definitions/eutrophication.html>. Accessed September 30, 2009.
- Weather Underground, Inc. 2010. History for Galveston, TX. <http://www.wunderground.com/history/>. Accessed September 25, 2009.
- White, W. A., T. A. Tremblay, E. G. J. Wermund, and L. R. Handley. 1993. Trends and status of wetland and aquatic habitats in the Galveston Bay System, Texas, GBNEP-31. The Galveston Bay National Estuary Program, Galveston, Texas, USA.
- Wilson, S. D. and D. Tilman. 1991. Components of plant competition along an experimental gradient of nitrogen availability. *Ecology* 72:1050-1065.
- Wilson, S. D. and D. Tilman. 1993. Plant competition and resource availability in response to disturbance and fertilization. *Ecology* 74:599-611.

Zhang, Z., Z. Rengel, and K. Meney. 2008. Interactive effects of nitrogen and phosphorus loadings on nutrient removal from simulated wastewater using *Schoenoplectus validus* in wetland microcosms. *Chemosphere* 72:1823-1828.

APPENDIX A

Table A.1. The mean (\pm S.E., $n=30$) height response of *S. alterniflora* to fertilization in monoculture plots over the sampling period.

Treatment	27-Jun-07	14-Jul-07	8-Aug-07	9-Sep-07	8-Nov-07	29-May-08	1-Jul-08	27-Aug-08
0	61.1 \pm 1.2	60.6 \pm 1.5	76.9 \pm 0.4	93.9 \pm 4.9	97.9 \pm 1.6	66.7 \pm 1.9	70.2 \pm 1.6	60.9 \pm 1.3
5	66.6 \pm 2.4	67.1 \pm 2.6	85.9 \pm 2.5	100.7 \pm 1.8	116.2 \pm 0.8	73.5 \pm 1.7	77.3 \pm 1.8	71.3 \pm 0.7
15	61.6 \pm 1.7	62.7 \pm 1.6	83.2 \pm 2.5	96.2 \pm 4.2	110.0 \pm 1.5	72.1 \pm 0.8	77.1 \pm 1.4	74.2 \pm 2.0
30	64.7 \pm 1.2	65.2 \pm 1.8	89.6 \pm 1.8	105.4 \pm 2.4	110.7 \pm 3.5	77.4 \pm 2.0	79.7 \pm 1.5	71.9 \pm 1.7

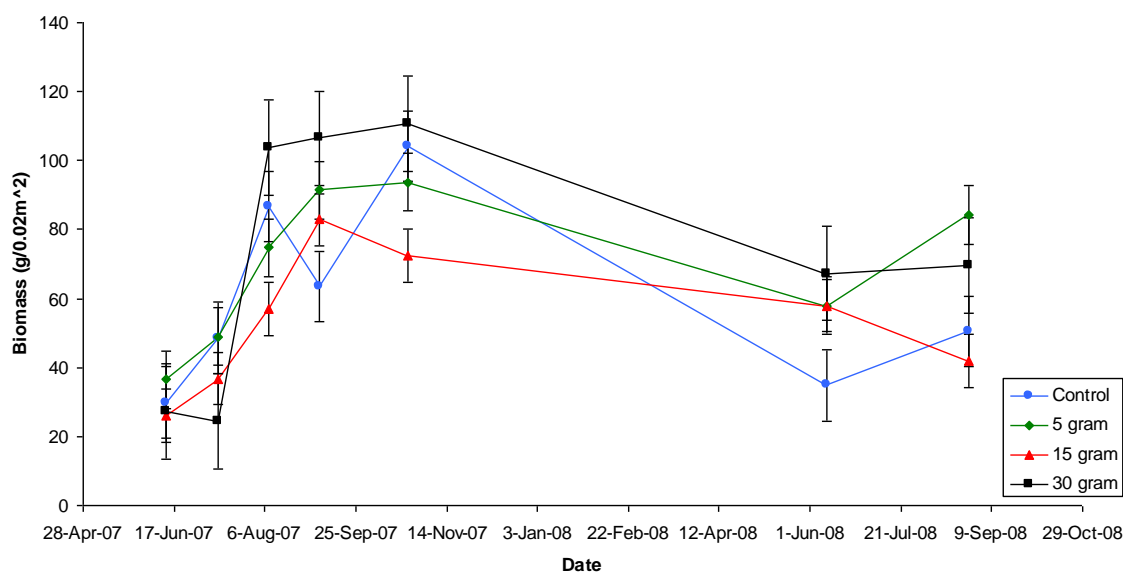


Figure A.1. The mean (\pm S.E., $n=30$) biomass response of *S. alterniflora* to fertilization in monoculture plots over the sampling period.

Table A.2. The mean (\pm S.E., $n=30$) height response of *B. maritima* to fertilization in monoculture plots over the sampling period.

Treatment	27-Jun-07	14-Jul-07	8-Aug-07	9-Sep-07	8-Nov-07	29-May-08	1-Jul-08	27-Aug-08
0	27.1 \pm 3.5	28.8 \pm 4.8	27.0 \pm 4.2	24.9 \pm 3.9	24.2 \pm 4.6	29.2 \pm 3.3	33.1 \pm 4.6	34.6 \pm 6.8
5	23.9 \pm 3.2	24.2 \pm 2.2	20.4 \pm 1.7	17.8 \pm 1.5	19.0 \pm 1.0	28.0 \pm 1.0	38.8 \pm 6.6	34.4 \pm 5.8
15	25.8 \pm 2.1	24.7 \pm 1.8	22.6 \pm 1.7	22.8 \pm 2.1	25.8 \pm 2.9	34.3 \pm 1.7	37.6 \pm 2.4	40.2 \pm 3.0
30	20.8 \pm 1.8	20.8 \pm 1.8	19.2 \pm 1.7	18.4 \pm 1.4	21.5 \pm 1.4	31.3 \pm 2.8	41.7 \pm 4.6	41.8 \pm 4.2

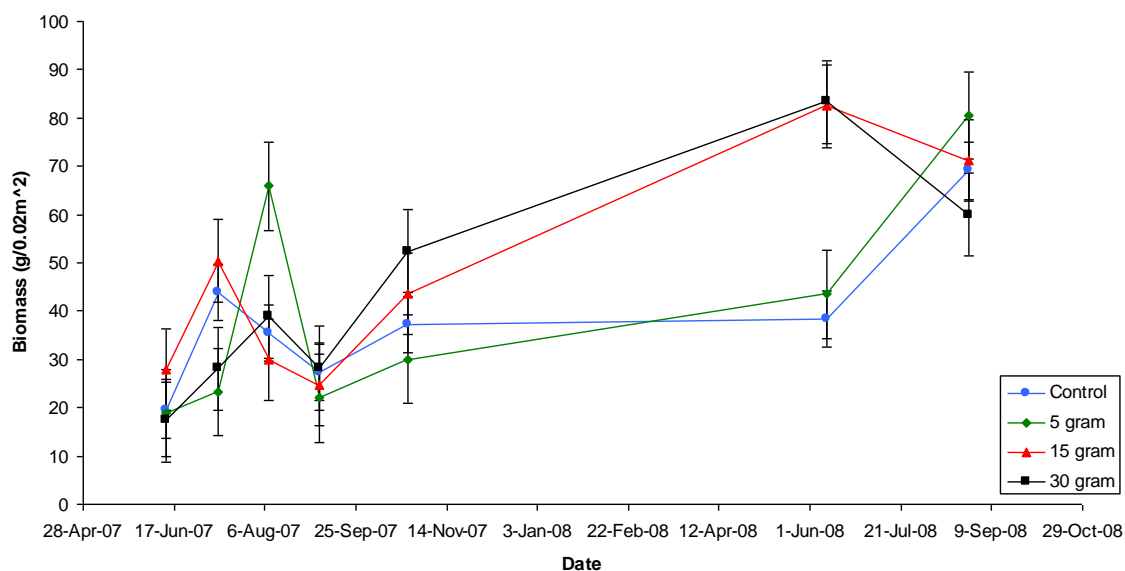


Figure A.2. The mean (\pm S.E., $n=30$) biomass response of *B. maritima* to fertilization in monoculture plots over the sampling period.

Table A.3. The mean (\pm S.E., $n=30$) height response of *S. virginica* to fertilization in monoculture plots over the sampling period.

Treatment	27-Jun-07	14-Jul-07	8-Aug-07	9-Sep-07	8-Nov-07	29-May-08	1-Jul-08	27-Aug-08
0	28.9 \pm 0.5	27.4 \pm 0.2	30.6 \pm 1.0	29.2 \pm 0.2	31.3 \pm 1.1	33.6 \pm 1.7	33.6 \pm 0.8	32.7 \pm 1.0
5	27.0 \pm 0.8	28.3 \pm 0.6	30.8 \pm 0.9	31.0 \pm 1.3	29.4 \pm 0.3	35.8 \pm 2.2	38.4 \pm 2.6	36.0 \pm 2.0
15	28.2 \pm 0.5	27.4 \pm 0.3	30.2 \pm 0.3	27.5 \pm 0.7	31.1 \pm 0.7	32.5 \pm 2.8	42.3 \pm 2.5	43.3 \pm 2.1
30	27.4 \pm 0.6	28.6 \pm 0.7	30.2 \pm 0.8	29.8 \pm 0.6	31.6 \pm 1.1	37.1 \pm 1.0	41.7 \pm 1.8	42.2 \pm 1.9

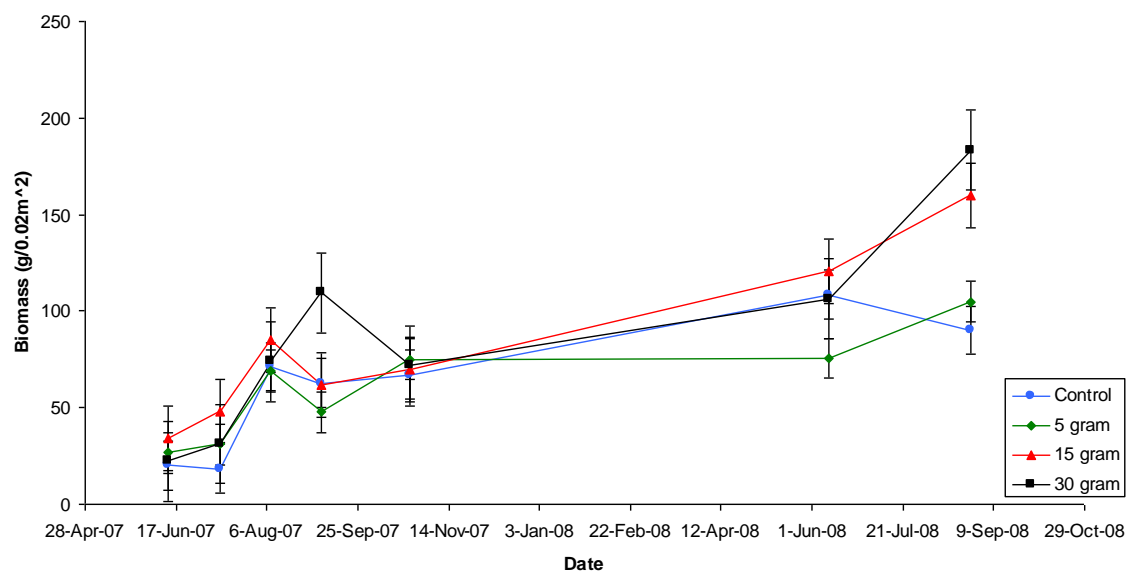


Figure A.3. The mean (\pm S.E., $n=30$) biomass response of *S. virginica* to fertilization in monoculture plots over the sampling period.

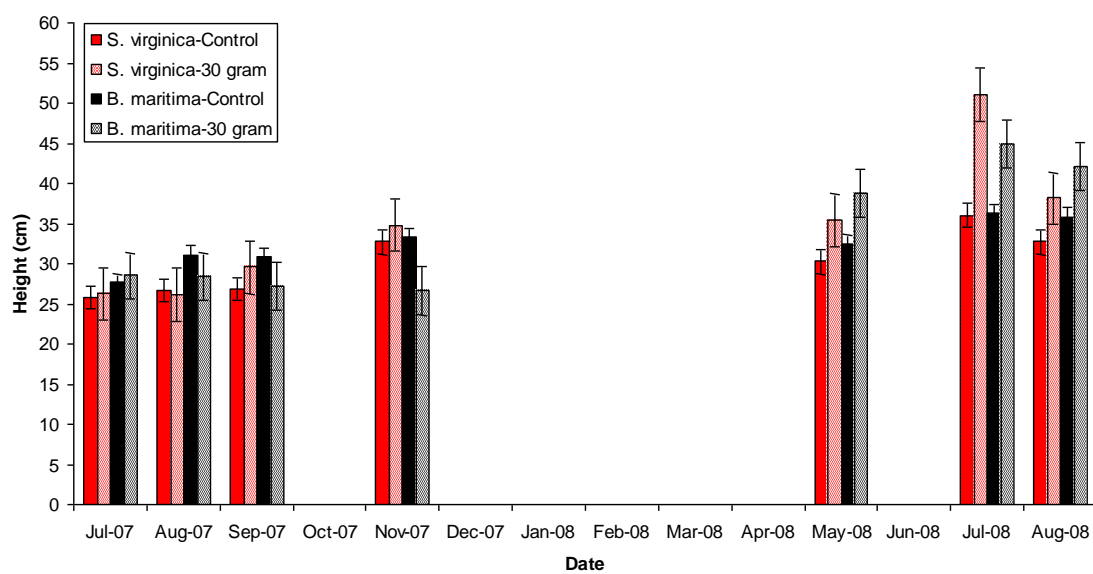


Figure A.4. The mean (\pm S.E., $n=30$) height response of *S. virginica* and *B. maritima* to fertilization in competition plots over the sampling period.

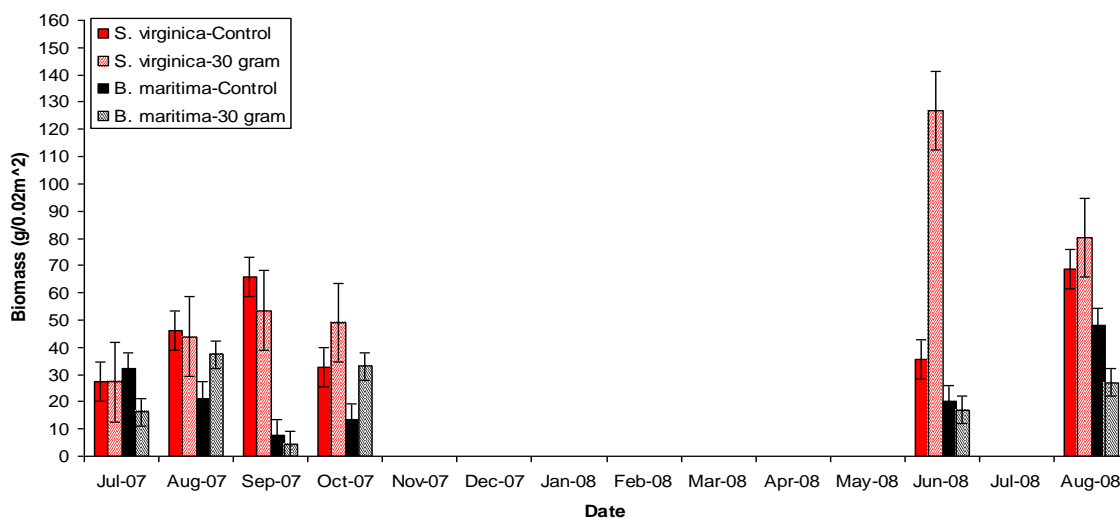


Figure A.5. The mean (\pm S.E., $n=30$) above ground biomass response of *S. virginica* and *B. maritima* to fertilization in competition plots over the sampling period.

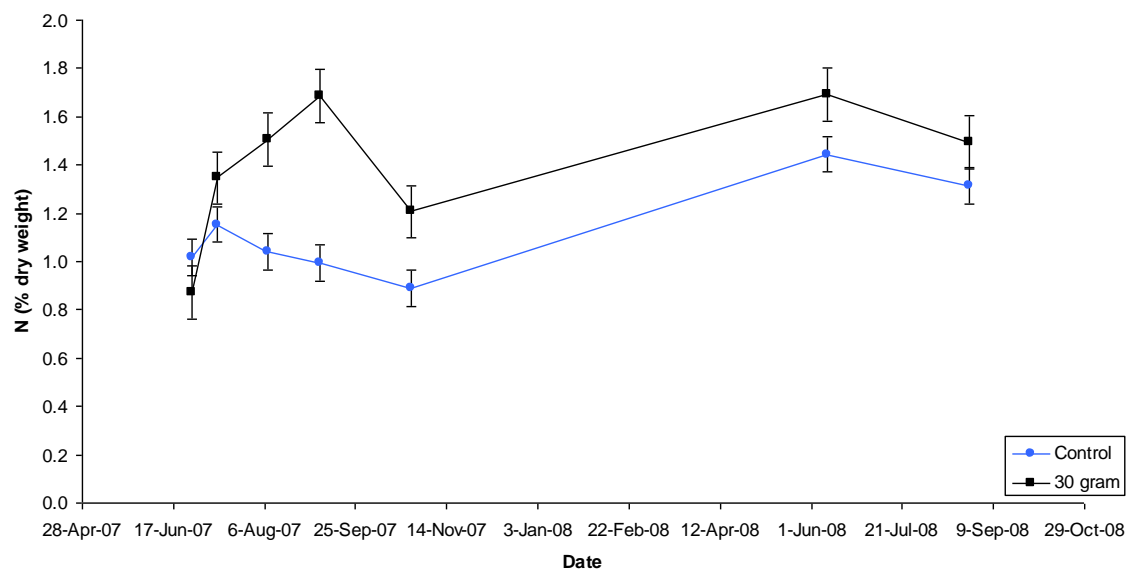


Figure A.6. Total nitrogen (% dry weight) of *S. alterniflora* and its response to fertilization in monoculture plots over the sampling period (\pm S.E., $n=30$).

Table A.4. Total phosphorus (% dry weight) of *S. alterniflora* and its response to fertilization in monoculture plots over the sampling period (\pm S.E., $n=30$).

Treatment	27-Jun-07	11-Jul-07	8-Aug-07	5-Sep-07	24-Oct-07	10-Jun-08	27-Aug-08
0	0.31 \pm 0.03	0.24 \pm 0.01	0.24 \pm 0.02	0.23 \pm 0.01	0.21 \pm 0.02	0.36 \pm 0.03	0.25 \pm 0.02
30	0.30 \pm 0.02	0.28 \pm 0.04	0.27 \pm 0.02	0.32 \pm 0.04	0.22 \pm 0.04	0.24 \pm 0.02	0.24 \pm 0.01

Table A.5. Total phosphorus (% dry weight) of *B. maritima* and its response to fertilization in monoculture plots over the sampling period (\pm S.E., $n=30$).

Treatment	27-Jun-07	11-Jul-07	8-Aug-07	5-Sep-07	24-Oct-07	10-Jun-08	27-Aug-08
0	0.31 \pm 0.01	0.21 \pm 0.00	0.24 \pm 0.01	0.30 \pm 0.01	0.37 \pm 0.02	0.28 \pm 0.00	0.19 \pm 0.01
30	0.30 \pm 0.01	0.28 \pm 0.01	0.22 \pm 0.02	0.39 \pm 0.02	0.49 \pm 0.02	0.32 \pm 0.01	0.18 \pm 0.00

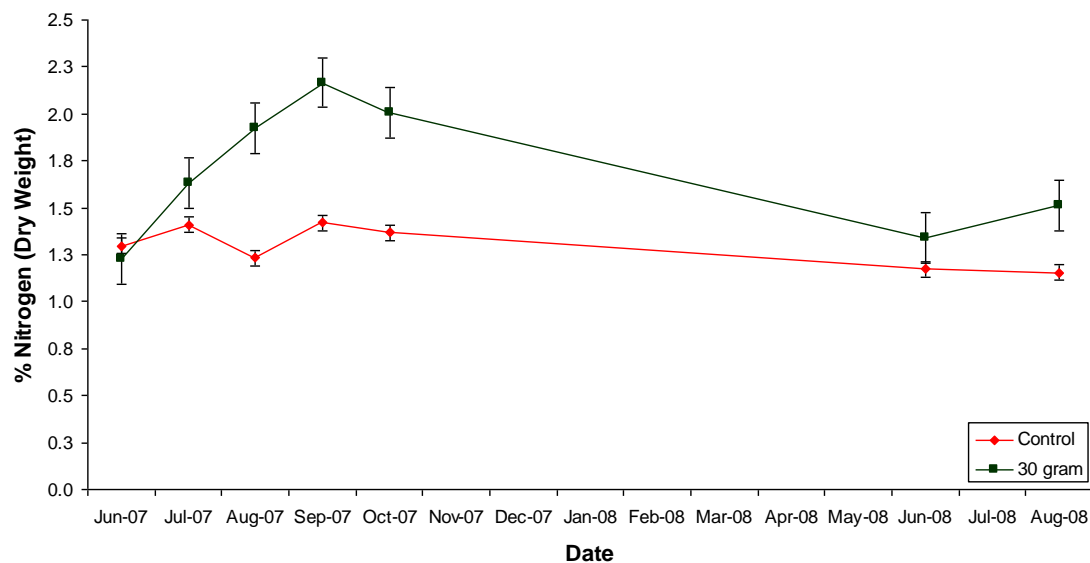


Figure A.7. Total nitrogen (% dry weight) of *S. virginica* and its response to fertilization in monoculture plots over the sampling period (\pm S.E., $n=30$).

Table A.6. Total phosphorus (% dry weight) of *S. virginica* and its response to fertilization in monoculture plots over the sampling period (\pm S.E., $n=30$).

Treatment	27-Jun-07	11-Jul-07	8-Aug-07	5-Sep-07	24-Oct-07	10-Jun-08	27-Aug-08
0	0.24 \pm 0.03	0.21 \pm 0.01	0.24 \pm 0.00	0.30 \pm 0.03	0.32 \pm 0.02	0.29 \pm 0.01	0.25 \pm 0.01
30	0.28 \pm 0.01	0.23 \pm 0.01	0.26 \pm 0.02	0.31 \pm 0.02	0.37 \pm 0.02	0.27 \pm 0.01	0.24 \pm 0.02

APPENDIX B

Table B.1. Mean (\pm S.E., n=30) relative growth rate determined from height data as a function of nutrient additions.

Treatment	<i>S. virginica</i> (cm/month)	<i>B. maritima</i> (cm/month)	<i>S. alterniflora</i> (cm/month)
0	0.25 \pm 0.11	0.50 \pm 0.32	7.36 \pm 0.30
5	0.60 \pm 0.24	0.70 \pm 0.29	9.93 \pm 0.66
15	1.01 \pm 0.20	0.96 \pm 0.13	9.68 \pm 0.19
30	0.99 \pm 0.18	1.40 \pm 0.29	9.21 \pm 1.24

Table B.2. Mean (\pm S.E., n=30) relative growth rate determined from total above ground biomass data as a function of nutrient additions.

Treatment	<i>S. virginica</i> (g/0.02m ² /month)	<i>B. maritima</i> (g/0.02m ² /month)	<i>S. alterniflora</i> (g/0.02m ² /month)
0	4.68 \pm 0.72	3.31 \pm 2.09	14.87 \pm 3.02
5	5.21 \pm 1.00	4.11 \pm 0.78	11.44 \pm 2.68
15	8.40 \pm 1.38	32.89 \pm 1.38	9.26 \pm 5.95
30	10.73 \pm 0.78	2.84 \pm 0.78	16.72 \pm 5.73

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