NUTRIENT ENRICHMENT EFFECTS WITHIN THE MARSH-MANGROVE ECOTONE: IMPLICATIONS FOR MANGROVE ENCROACHMENT

A Dissertation

by

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ABSTRACT

Over the past two centuries, woody vegetation has expanded globally in coverage, often encroaching into grasslands, including coastal habitats. Mangroves are tropical trees which are susceptible to freezing temperatures. Therefore, the frequency and severity of freezing events are often invoked as the main control in mangrove growth and distribution within the sub-tropical marsh-mangrove ecotone. However, as freezing events have occurred less frequently, mangrove coverage has increased within these regions, often encroaching into salt marsh habitats. Other factors may also influence plant composition within this ecotone by either perpetuating mangrove encroachment, or conversely, favoring salt marsh vegetation and the subsequent suppression of mangrove establishment and growth. Salt marsh and mangrove systems are exposed to anthropogenic nutrient enrichment which may be an additive factor to temperature-driven vegetation changes. Many studies have documented positive nutrient responses within both marsh and mangrove monotypic stands, but few studies have examined nutrient dynamics in mixed stands. In order to assess the impact of nutrient addition on herbaceous and woody plant composition within a marsh-mangrove ecotone, I fertilized *in situ* mixed stands of *Spartina alterniflora* (smooth cordgrass) and *Avicennia germinans* (black mangrove), two dominant species within the Northern Gulf of Mexico, for four growing seasons (2010 – 2013). Overall, I hypothesized that nutrient enrichment would augment growth more so in *S. alterniflora* than in *A. germinans*, facilitating salt marsh vegetation dominance and conversely slowing mangrove encroachment.

Contrary to what I expected, my results suggest that nutrient enrichment promoted *A. germinans* growth, which could subsequently lead to accelerated mangrove stand expansion and subsequent displacement of *S. alterniflora*. Nearly all *A. germinans* plant metrics were significantly elevated between control and fertilized plots, indicating that nutrient enrichment facilitated growth in *A. germinans*. Conversely, *S. alterniflora* plant metrics had very little response to nutrient enrichment. Collectively, these data

indicate that *A. germinans* growth was facilitated by nutrient enrichment, whereas fertilization responses were diminished with *S. alterniflora* metrics. More specifically, mangrove height distribution significantly increased within fertilized plots, augmenting its ability to displace neighboring marsh plants. These results suggest, contrary to previous studies, *A. germinans* growth may benefit more from nutrient enrichment than *S. alterniflora* in the marsh-mangrove ecotone. Although nutrient enrichment is not the main factor driving mangrove encroachment, fertilization may be perpetuating the increase in mangrove coverage and accelerating marsh displacement. Therefore, nutrient enrichment can be considered a positive feedback for mangrove stand expansion, as it can further propel climate-driven woody encroachment within the marsh-mangrove ecotone. It is important to identify and understand factors serving as feedbacks in order to better predict how ecosystem components will be influenced in various global change scenarios. Areas within the marsh-mangrove ecotone that have higher potential for anthropogenic nutrient enrichment could be more susceptible to mangrove encroachment and management strategies may need to be prioritized, because a shift from marsh to mangrove vegetation could have large implications for a variety of ecosystem services.

DEDICATION

I would like to dedicate this dissertation to my parents, Sarah Finnerty and Joe and Susan Weaver, because their love and unwavering faith and encouragement have always pushed me to accomplish and excel in all that I have set out to do.

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Finally I would like thank a great number of colleagues, friends, and family members who have offered me assistance, guidance, and encouragement throughout the various stages of my journey and without whom I could not have been as successful.

TABLE OF CONTENTS

Page

LIST OF FIGURES

Page

LIST OF TABLES

Page

Page

CHAPTER I
INTRODUCTION

Grasslands are ecosystems dominated by herbaceous vegetation (mainly graminoid species) and shrublands are dominated by woody vegetation (generally less than 2 m in height) (Giri et al. 2005). Grasslands and shrublands generally exist in areas exposed to different environmental properties that set ecosystem boundaries (Cheplick 1998, Woodward et al. 2004). Between these boundaries are ecotones: transitional areas where the dominant vegetation and environmental properties attributed to different ecosystems intermix (Neilson 1993, Risser 1995).

Grassland-shrubland ecotones occur from arid to mesic regions and are composed of interspersed, various sized patches of herbaceous and woody vegetation (Cabral et al. 2003, Briggs et al. 2005, Maestre et al. 2009, Van Auken 2009). The physiological attributes of these plant types respond to abiotic properties differently, therefore, environmental factors can drive community composition in favor of either herbaceous or woody vegetation (Scholes and Archer 1997, D'Odorico et al. 2012). Ecotones are maintained because environmental conditions fluctuate, thereby alternating herbaceous and woody plant composition, creating a landscape of shifting vegetation types and patch sizes (Archer et al. 1995, D'Odorico et al. 2012). For example, grassland-shrubland ecotone composition can be mediated by fire frequency (Scholes and Archer 1997). Fires can cause woody plant dieback, allowing fast-growing herbaceous species to fill in gaps within the landscape, reduce woody seedling establishment and survivability, and become the dominant vegetative growth form (Reich et al. 2001, Coll et al. 2004). Conversely, in the absence of fire, woody plant growth will persist, canopy sizes will increase and limit light to grass species, and woody plant stands will expand (Van Auken 2009, Eldridge et al. 2011).

Grassland-shrubland ecotones do not evenly shift between herbaceous and woody plant dominance. Over the past two centuries, woody vegetation has expanded globally in biomass and coverage, often encroaching into grasslands (Archer et al. 1995,

Briggs et al. 2005, Saintilan and Rogers 2015). Global climate changes, such as increased temperature or elevated CO_2 levels, favor woody vegetation types and can be important drivers of the vegetation shift (Archer et al. 1995, Saintilan and Rogers 2015). However, other local effects such as grazing can also influence the ecotone. Grazing reduces vegetative fire fuel, therefore increased grazing pressure can lower fire frequency, which increases woody plant growth, and maintains woody encroachment (Van Auken 2000, Briggs et al. 2005, Van Auken 2009, D'Odorico et al. 2012).

Most literature on grassland-shrubland ecotones focuses on woody encroachment in terrestrial systems (Cabral et al. 2003, Maestre et al. 2009, Van Auken 2009, Ward et al. 2014). Although not as well documented, woody encroachment is also occurring along many coastlines within marsh-mangrove ecotones (Saintilan and Rogers 2015). Salt marshes, dominated by herbaceous halophytes, are found worldwide with the highest occurrence in temperate climatic zones (Adam 2002); mangroves are woody plants that dominate the tropics (Alongi 2002). In the subtropics, these coastal vegetation types often co-exist, forming a marsh-mangrove ecotone (Friess et al. 2012). Over the last 50 years, mangrove stands within these ecotones have proliferated worldwide, encroaching into salt marshes, mainly attributed to a reduced frequency of severe freezing events (Cavanaugh et al. 2014, Saintilan et al. 2014, Armitage et al. 2015).

Mangroves, like other tropical vegetation, are susceptible to freezing temperatures which can lead to mangrove dieback and death, facilitating salt marsh dominance (Markley et al. 1982, Stevens et al. 2006). Therefore, the frequency, duration, and severity of freezing events are often invoked as the main controls of herbaceous and woody plant composition within the marsh-mangrove ecotone (Stevens et al. 2006, Osland et al. 2013, Cavanaugh et al. 2014). Similar to terrestrial systems, other factors may also influence this ecotone by either perpetuating mangrove encroachment, or conversely, favoring salt marsh vegetation and the subsequent suppression of mangrove establishment and growth.

Anthropogenic nutrients can enter coastal waters through groundwater inflows, runoff, and wastewater discharge, potentially impacting salt marsh and mangrove

systems (Vitousek 1997, Boesch 2002). Many studies have documented positive nutrient responses within both marsh (Pennings et al. 2002, Darby and Turner 2008a) and mangrove (Lovelock et al. 2004, Feller et al. 2007) monotypic stands. However, few studies have examined how mixed stands of salt marsh and mangroves respond to nutrient enrichment. Marsh vegetation has reduced mangrove seedling growth and survivability, and some marsh species may outcompete mangroves for nutrients (Patterson et al. 1993, McKee and Rooth 2008). These previous studies suggest that nutrient addition within this coastal ecotone may favor marsh vegetation dominance, subsequently slowing mangrove encroachment. However, these studies are limited in scope as previous work has focused on mangrove seedlings, been conducted within mesocosms, only compared between adjacent monospecific stands, and/or observed for no more than one growing season. Therefore, studies within mature, *in situ*, mixed stands are needed to better understand the influence of nutrient enrichment on vegetation dynamics within the marsh-mangrove ecotone. Freezing temperatures are likely the primary control of coastal woody encroachment, but nutrient enrichment within the marsh-mangrove ecotone may also influence this vegetation shift.

The aim of the present study was to assess the impact of nutrient addition on herbaceous and woody plant composition within a marsh-mangrove ecotone. I investigated nutrient responses by fertilizing mature stands of naturally co-occurring marsh and mangrove vegetation. I quantified nutrient responses in an assortment of marsh and mangrove above- and belowground plant components over multiple growing seasons. This research focused on the Texas (USA) coast in the Northern Gulf of Mexico marsh-mangrove ecotone. Here, the dominant graminoid marsh species is *Spartina alterniflora* (smooth cordgrass) and the dominant mangrove is *Avicennia germinans* (black mangrove).

Specifically, I sought to answer the following questions:

1) How will *S. alterniflora* and *A. germinans* respond to prolonged nutrient enrichment within mixed stands?

I hypothesized that after a sustained enrichment period, *S. alterniflora* would respond positively to nutrient addition and *A. germinans* would have a limited nutrient response.

2) Will *S. alterniflora* and *A. germinans* allocate biomass differently in enriched conditions?

I hypothesized that *S. alterniflora* would allocate more biomass to aboveground material in response to fertilization and *A. germinans* biomass allocation would not differ between treatments.

3) Will nutrient enrichment reduce *S. alterniflora* displacement and slow *A. germinans* encroachment over multiple growing seasons?

I hypothesized that *S. alterniflora* displacement and *A. germinans* encroachment would lessen through time within fertilized plots, as *S. alterniflora* would respond more strongly to fertilization, augmenting its growth and potential to inhibit *A. germinans* growth.

CHAPTER II

NUTRIENT ENRICHMENT EFFECTS ON CO-OCCURRING *SPARTINA ALTERNIFLORA* AND *AVICENNIA GERMINANS*: IMPLICATIONS FOR MANGROVE STAND EXPANSION

2.1 Introduction

Marsh-mangrove ecotones exist in subtropical regions worldwide (Zhang et al. 2012, Cavanaugh et al. 2014, Saintilan et al. 2014). In these areas, mangroves are interspersed with salt marsh vegetation and can range from small patches to extensive, nearly continuous stands. Mangroves generally have relatively tall, wide canopies that shade and outcompete herbaceous salt marsh species (Kangas and Lugo 1990, Alongi 2002). Marsh and mangrove coexistence in these ecotones is maintained by a variety of mechanisms. Severe freeze events are often invoked as a control of mangrove cover (Cavanaugh et al. 2014), though other factors such as fire can also contribute (Smith et al. 2013). Global changes, particularly rising temperatures, are predicted to transform these coastal systems by causing changes in species composition and dominance, subsequently altering related ecosystem services (Costanza et al. 1997, Adam 2002, Scavia et al. 2002, Alongi 2015). In particular, a reduction in the duration and severity of freezing events is widely linked to an increase in mangrove cover within marsh-mangrove ecotones (Osland et al. 2013, Cavanaugh et al. 2014).

The marsh-mangrove ecotone in the Northern Gulf of Mexico (NGoM) is comprised of *Avicennia germinans* (black mangrove) and several marsh grass and forb species. Periodic freezing events in this region have caused mangrove diebacks, thereby maintaining salt marsh dominance (Markley et al. 1982, Sherrod and McMillan 1985, Stevens et al. 2006). However, there have been no lethal freezes since the 1980s, and *A. germinans* stands within the NGoM have been expanding, often encroaching into areas previously dominated by smooth cordgrass, *Spartina alterniflora* (Sherrod and McMillan 1985, Stevens et al. 2006, Perry and Mendelssohn 2009, Armitage et al.

2015). This warming trend, and associated mangrove expansion, is likely to continue in Texas and Louisiana, as a 2 – 4 °C increase in mean annual minimum temperature by 2100 is predicted to convert these coastlines into mangrove dominated habitat (Osland et al. 2013).

The vegetation composition within the marsh-mangrove ecotone is likely to be influenced by an interaction of temperature and other factors such as anthropogenic nutrient input (Saintilan et al. 2014). Many field studies have documented that marsh (Valiela et al. 1978, Levine et al. 1998, Pennings et al. 2002, e.g., Darby and Turner 2008a) and mangrove (Onuf et al. 1977, Lovelock et al. 2004, e.g., Feller et al. 2007, Naidoo 2009) production and morphometrics, such as plant height and density, respond positively to nutrient enrichment in monotypic habitats. In one of the few studies that examined nutrient effects on mixed stands of marsh and mangrove vegetation, *S. alterniflora* responded positively to nitrogen enrichment when grown in monoculture and when mixed with *A. germinans* (McKee and Rooth 2008). Comparatively, *A. germinans* growth parameters only responded to nitrogen enrichment when grown in monoculture. These findings suggest that *S. alterniflora* has a stronger response to nutrient enrichment and its presence may also influence the nutrient response in *A. germinans*. However, there are few comparative field studies of nutrient effects of *in situ* co-occurring *S. alterniflora* and *A. germinans*, and those studies only focus on mangrove seedling metrics (e.g., Simpson et al. 2013). Furthermore, fertilization responses of both *S. alterniflora* and *A. germinans* have only been quantified for 18 months (e.g., McKee and Rooth 2008). It is unclear if after a longer enrichment period *S. alterniflora* will continue to be the only species that responds positively to nutrient resources.

Understanding the influence of nutrient enrichment on species coexistence in the marsh-mangrove ecotone is particularly important in areas of high nutrient loading. Since the 1950s, the NGoM has experienced a regional increase in precipitation by 20 – 30 % (Ning et al. 2003). The increase in precipitation is predicted to continue, leading to as much as a 34 % rise in total runoff by the end of the century along the United States Atlantic and NGoM coastlines, potentially increasing anthropogenic nutrient input

(Scavia et al. 2002, Ning et al. 2003). Therefore, the NGoM coastline is an ideal setting for investigating the influence of nutrient enrichment on the mangrove-marsh ecotone. To determine extended fertilization responses within naturally co-occurring populations, I fertilized *in situ* mixed stands of *S. alterniflora* and *A. germinans*, two dominant species within the NGoM marsh-mangrove ecotone, for 28 months (three growing seasons). I hypothesized that *S. alterniflora* would respond more strongly to nutrient addition.

2.2 Methods

2.2.1 Site description

I established an *in situ* nutrient enrichment experiment in Port Aransas, Texas, USA (27.9°N, 97.1°W) within the NGoM marsh-mangrove ecotone (Figure 2.1a). *Avicennia germinans* was first recorded in this area in the 1930s with a steady increase in coverage until several lethal freezing events in the 1980s (Sherrod and McMillan 1981, 1985, Montagna et al. 2011). Mangrove coverage, particularly in the last twenty years, has increased and is surpassing the aerial coverage reported in 1979, making Port Aransas one of the mangrove expansion "hot spots" along the Texas coast (Montagna et al. 2011, Armitage et al. 2015). At the study site, the low marsh had areas dominated by *S. alterniflora* and areas with dense *A. germinans*, which were shrub-like and typically less than 1.5 m in height. The low marsh also had areas where *S. alterniflora* and *A. germinans* co-occurred; here *A. germinans* were shorter and not as dense. The mid marsh was dominated by succulent marsh plants such as *Batis maritima* and *Salicornia* spp. Large sand flats (up to 100 m wide) separated the mid marsh from the high marsh. This study focused on the low marsh elevation contour, where *S. alterniflora* was intermixed with smaller *A. germinans* (Figure 2.1 b). Abiotic soil characteristics in this area along similarly defined elevation contours have been described by Guo et al. (2013).

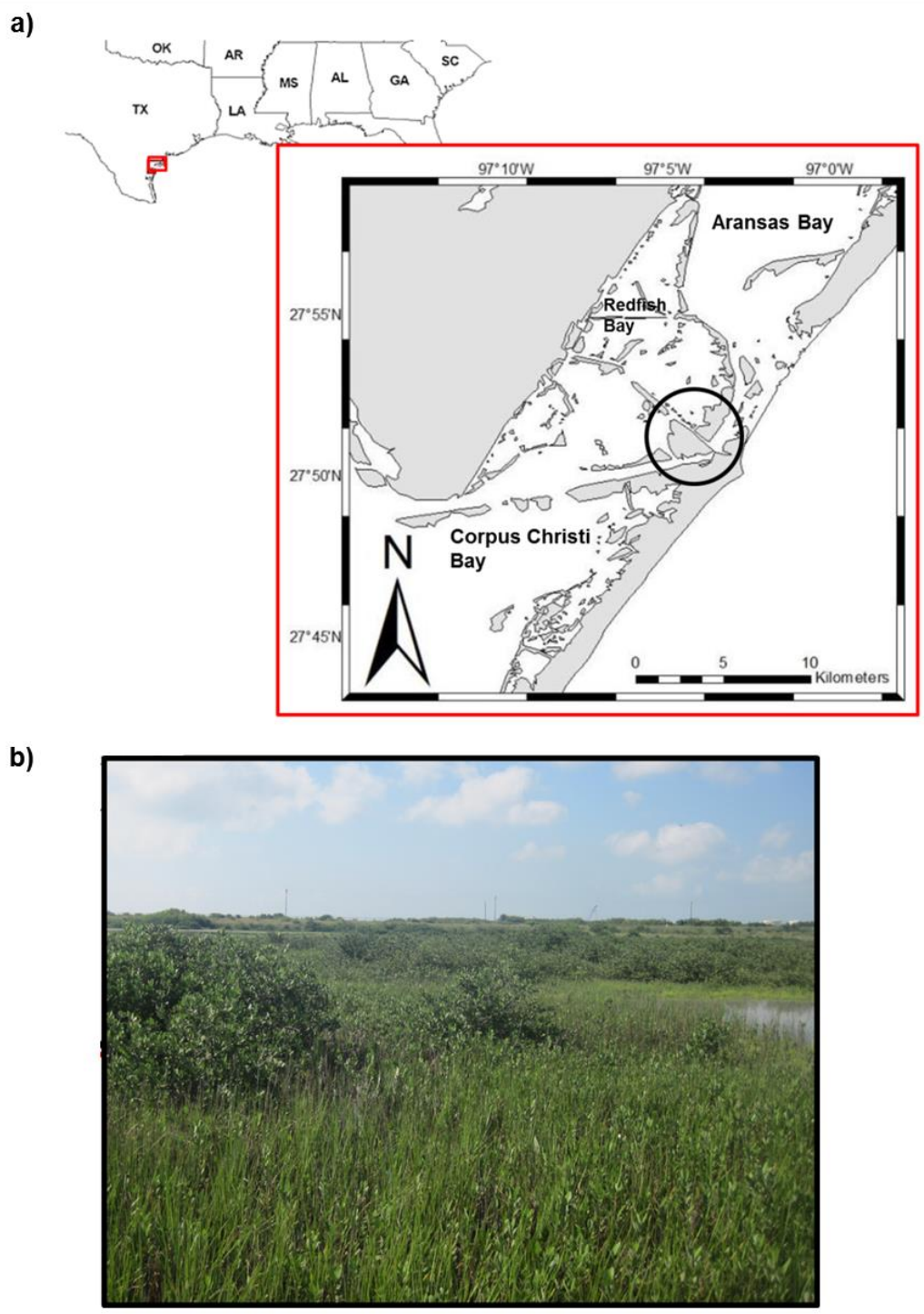

Figure 2.1 Study site in Port Aransas, TX, USA (**a**) where plots were established in co-occurring *Spartina alterniflora* (smooth cordgrass) and *Avicennia germinans* (black mangrove) stands (**b**).

2.2.2 Sampling design

The study was initiated in May 2010 along the low marsh tidal elevation contour where *S. alterniflora* and *A. germinans* co-occurred. At the time of deployment, *A. germinans* (including seedlings and small shrubs) were roughly evenly mixed with *S. alterniflora*; few *A. germinans* exceeded 50 cm in height. To control for the spatial heterogeneity of the site (e.g., variation in edaphic characteristics), plots were arranged in a randomized block design. A two-way mixed permutational analysis of variance model (treatment as a fixed factor and block as a random factor) was used to determine that there were no significant differences in density of either species between plots prior to treatment application. Each of the eleven blocks consisted of two 4 m^2 plots (no closer than 4 m) which were randomly assigned a nutrient treatment: control or fertilized. Fertilized treatment plots were fertilized with Osmocote® Outdoor & Indoor Smart-Release® Plant Food (NPK 19-6-12) at a loading rate of 0.342 g N m^{-2} day^{-2} and 0.108 g P m^{-2} day^{-1}. Loading rates were based on previous enrichment experiments in NGoM salt marshes (Darby and Turner 2008a, Slocum and Mendelssohn 2008). Osmocote® is a slow release formula and it was re-applied quarterly by broadcasting pellets onto the sediment surface. Pellet retention within plots was high due to low tidal volume exchange in this area.

2.2.3 Data collection and analyses

Plots were sampled in September 2012 after 28 months (three growing seasons) of continued enrichment. Total live *S. alterniflora* (stems m^{-2}) and *A. germinans* (trunks m^{-2}) densities were recorded within each plot. Stem and trunk densities were quantified for the entire 4 m^2 plot when logistically feasible; plots with higher stem densities were subsampled with a 30 cm x 30 cm quadrat. All density data were reported as total stems or trunks m^{-2}. Maximum height (cm) for each of the target species was recorded by measuring the tallest *S. alterniflora* and *A. germinans* individual within each plot. A SPAD-502 portable meter with leaf clip (Konica Minolta Corporation, USA), which measures 650 and 940 nm light transmission, was used on a penapical leaf from ten

S. alterniflora and *A. germinans* individuals within each plot. The SPAD-502 meter is an established method for inferring leaf chlorophyll *a* content (e.g., Markwell et al. 1995, Uddling et al. 2007). Live, mature leaves from a penapical position (n = 20 from each species) were clipped and maintained on ice for transport to the lab.

In the lab, leaves were rinsed to remove any adhered sediments, photographed, and dried to constant mass at 60 °C in a drying oven. Leaf area (cm^2) was calculated using the image processing program ImageJ (Rasband 1997). All dried leaves from each species per plot were ground with a Thomas Wiley® Mini-Mill and sieved through a 60 mesh (0.25 mm) screen. Total carbon and nitrogen content were quantified using a Costech ECS 4010 Elemental Analyzer; analytical variability ranged 2 – 5 %, as determined by running National Institute of Standards and Technology standard reference material (SRM 1941-b). Total phosphorus content was determined via a dry-oxidation, acid hydrolysis extraction followed by a colorimetric analysis on a Shimadzu UV-1800 Spectrophotometer (Fourqurean et al. 1992).

In order to determine *S. alterniflora* and *A. germinans* nutrient responses, dependent variables (tissue nutrient content, SPAD, maximum height, density, and leaf surface area) were analyzed for each species separately using two-way mixed permutational analysis of variance models (permANOVA; Anderson 2001). PermANOVAs were used because they are robust but do not require assumptions of data normality (Anderson 2001, Anderson et al. 2008). Nutrient treatment (two levels: control and fertilized) was treated as a fixed factor and block (11 levels) as a random factor; the block interaction term was excluded from the model because there was no replication within blocks, typical of randomized block experimental designs. After 28 months of enrichment, *S. alterniflora* was rare or absent from some plots. Therefore, some plots were excluded from *S. alterniflora* analyses except for density analyses; if *S. alterniflora* was not present, density was entered as zero. Data resemblance matrices were formed based on Euclidean distances, except for density data which were square root transformed and Bray Curtis similarity resemblance matrices were used (Anderson et al. 2008). Permutational *p* values were obtained from 9999 unique permutations of the data.

All data were analyzed using PERMANOVA+ version 1.0.5 in PRIMER 6 version 6.1.15 (PRIMER-E Ltd., Plymouth Marine Laboratory, UK; Anderson et al. 2008).

2.3 Results

Spartina alterniflora leaf nutrient contents did not vary between nutrient treatments whereas *A. germinans* leaf nutrient contents were significantly different in fertilized plots (Figure 2.2; Table 2.1). *Spartina alterniflora* leaves did not differ in live leaf total nutrient contents or nutrient ratios between control and fertilized plots (Figure 2.2 a-c; Figure 2.3 a-c; Table 2.1). Total carbon and phosphorus content of fertilized *A. germinans* leaves (47.6% and 0.16% , respectively) were moderately higher than leaves from control plots (46.5% and 0.14%, respectively; Figure 2.2 d, f). The largest nutrient response occurred in *A. germinans* total leaf nitrogen content, which was 1.8% in control plots and was significantly higher (2.3%) in the fertilized treatment (Figure 2.2 e). *Avicennia germinans* leaf C:N, C:P, and N:P molar ratios were significantly different between treatments (Figure 2.3; Table 2.1). Corresponding with the elevated leaf nitrogen values in fertilized leaves, *A. germinans* leaf C:N and N:P changed the most with nutrient treatment (Figure 2.3 d, f).

Other *S. alterniflora* and *A. germinans* leaf metrics had varied treatment responses. *Spartina alterniflora* SPAD measurements did not differ between treatments (Figure 2.4 a; Table 2.2). *Avicennia germinans* SPAD measurements were 13.4% higher in fertilized leaves compared to control leaves (Figure 2.4 c; Table 2.2). Leaf surface area was larger in both species in nutrient addition plots. *Spartina alterniflora* leaves were 67% larger with nutrient enrichment (Figure 2.4 b; Table 2.2) and *Avicennia germinans* leaves were 30% larger (Figure 2.4 d; Table 2.2).

A positive nutrient effect occurred in *S. alterniflora* and *A. germinans* maximum height, though *A. germinans* had a larger response (Figure 2.5 a, c). Fertilized *S. alterniflora* plants were 13% taller than control plots (Figure 2.5 a) and fertilized *A. germinans* were 34% taller than the control counterparts (Figure 2.5 c). Control

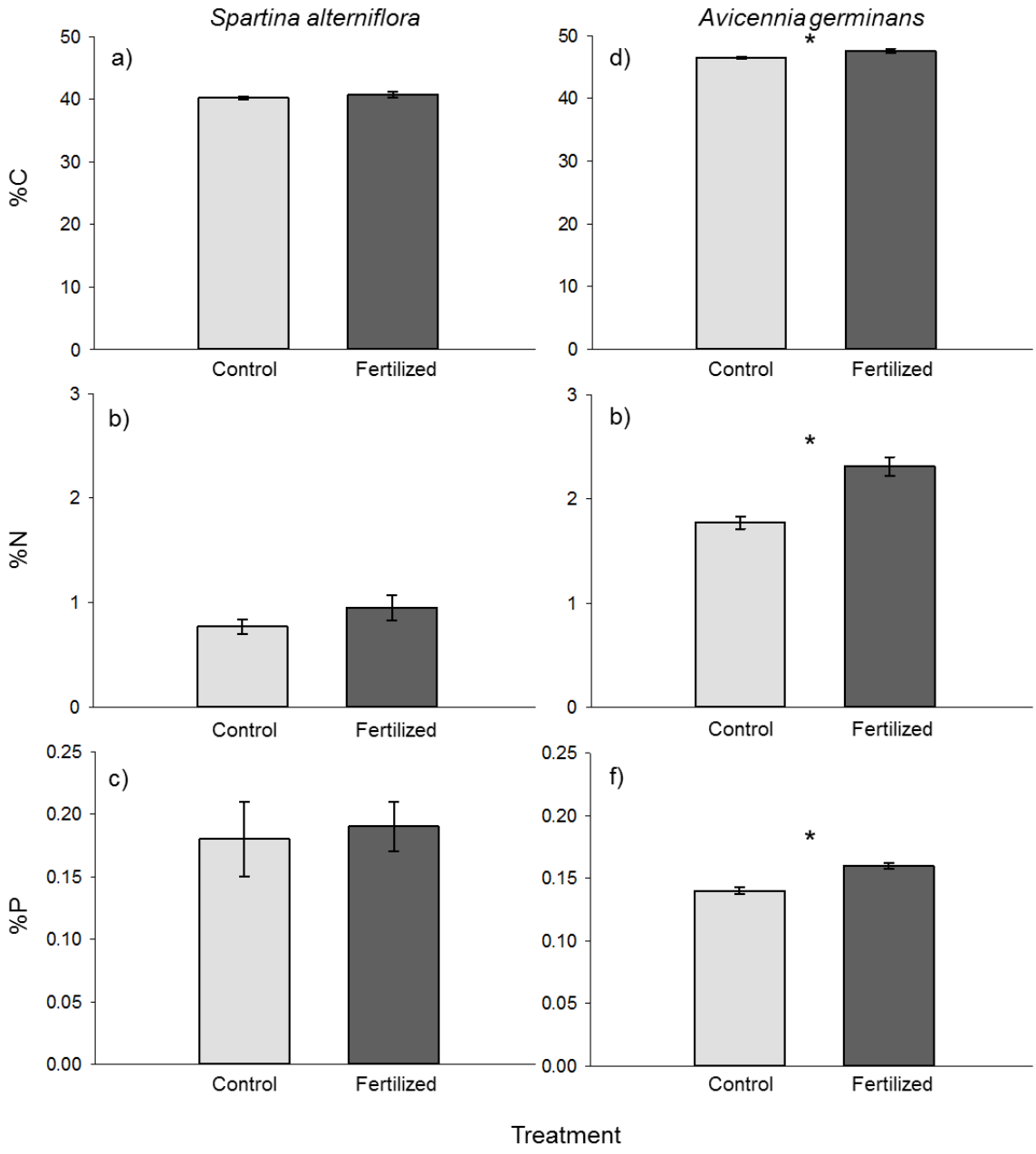

Figure 2.2 Total percent carbon (% C), nitrogen (% N), and phosphorus (% P) of live *Spartina alterniflora* (smooth cordgrass; **a-c**) and *Avicennia germinans* (black mangrove; **d-f**) leaves in control and fertilized treatment plots. Data are mean values ± standard error; n = 9 (*S. alterniflora*) and 11 (*A. germinans*). * Indicates significance at perm $p < 0.05$; see Table 2.1 for statistical analyses.

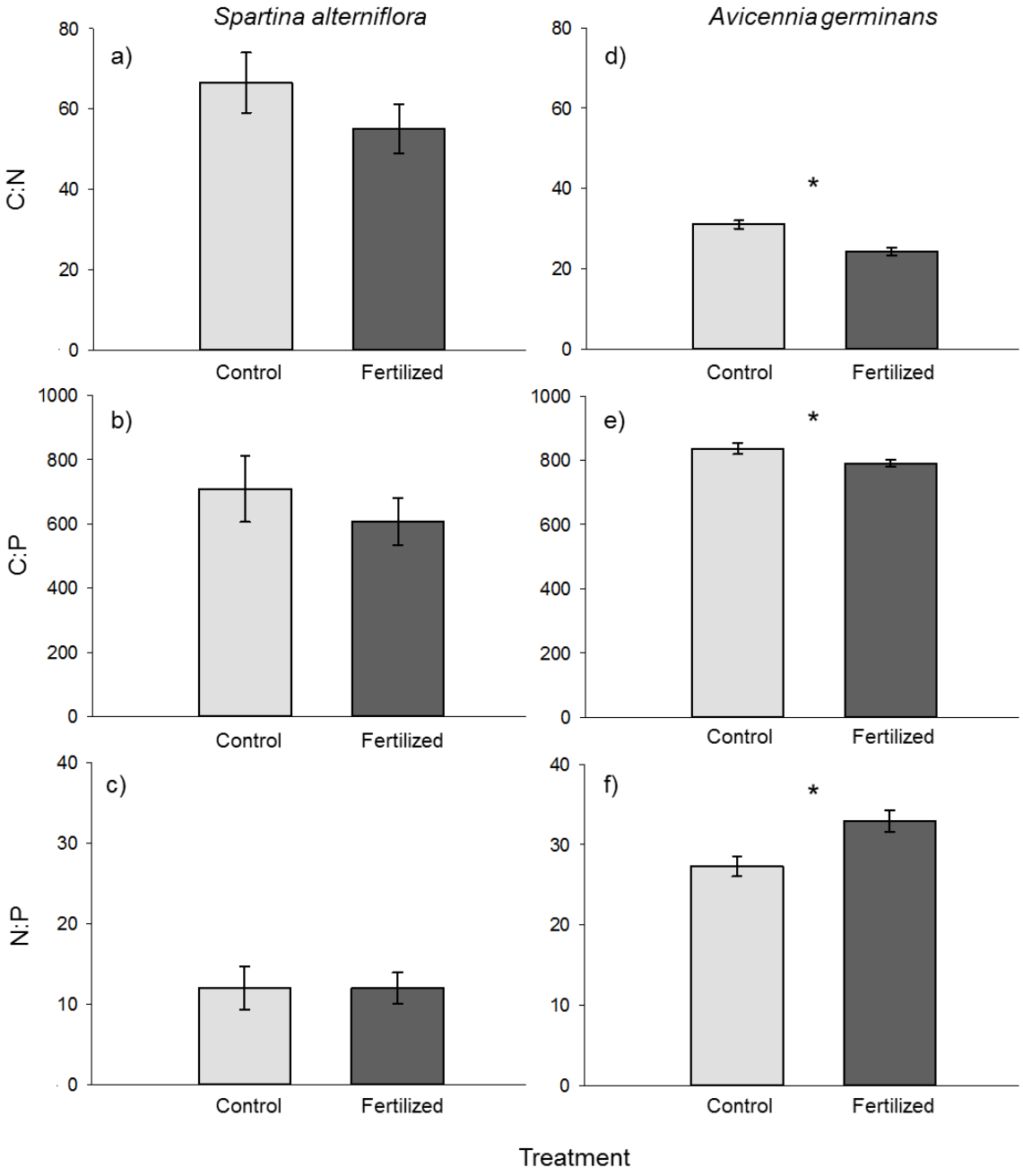

Figure 2.3 Molar carbon to nitrogen (C:N), carbon to phosphorus (C:P), and nitrogen to phosphorus (N:P) ratios of live *Spartina alterniflora* (smooth cordgrass; **a-c**) and *Avicennia germinans* (black mangrove; **d-f**) leaves in control and fertilized treatment plots. Data are mean values ± standard error; n = 9 (*S. alterniflora*) and 11 (*A. germinans*). * Indicates significance at perm $p < 0.05$; see Table 2.1 for statistical analyses.

Table 2.1 Results from separate permANOVAs to determine differences in *Spartina alterniflora* (smooth cordgrass; top portion) and *Avicennia germinans* (black mangrove; bottom portion) live leaf total carbon (% C), nitrogen (% N), phosphorus (% P), carbon to nitrogen (C:N), carbon to phosphorus (C:P), and nitrogen to phosphorus (N:P) between control and fertilized treatment plots. A two-way mixed permANOVA model was utilized: treatment (2 levels: control and fertilized) x block (11 levels). Perm *p* values obtained from 9999 unique permutations of the data. * Indicates significance at perm $p < 0.05$.

		***Spartina alterniflora* (smooth cordgrass)**											
		% C		% N		% P		C:N		C:P		N:P	
	df	Pseudo F	Perm *p*	Pseudo F	Perm *p*	Pseudo F	Perm *p*	Pseudo F	Perm *p*	Pseudo F	Perm *p*	Pseudo F	Perm *p*
Treatment	1	1.07	0.35	1.90	0.24	0.81	0.43	1.79	0.24	0.39	0.56	4.23	0.10
Block	10	3.89	0.07	3.19	0.12	2.41	0.23	1.82	0.27	6.22	0.04*	13.93	<0.01*
Residual	5												
Total	16												
		***Avicennia germinans* (black mangrove)**											
		% C		% N		% P		C:N		C:P		N:P	
	df	Pseudo F	Perm *p*	Pseudo F	Perm *p*	Pseudo F	Perm *p*	Pseudo F	Perm *p*	Pseudo F	Perm *p*	Pseudo F	Perm *p*
Treatment	1	21.77	<0.01*	35.80	<0.01*	11.68	<0.01*	27.884	<0.01*	6.88	0.03*	21.90	<0.01*
Block	10	4.48	<0.01*	2.12	0.129	1.00	0.51	1.573	0.24	1.68	0.22	0.03	0.03
Residual	10												
Total	21												

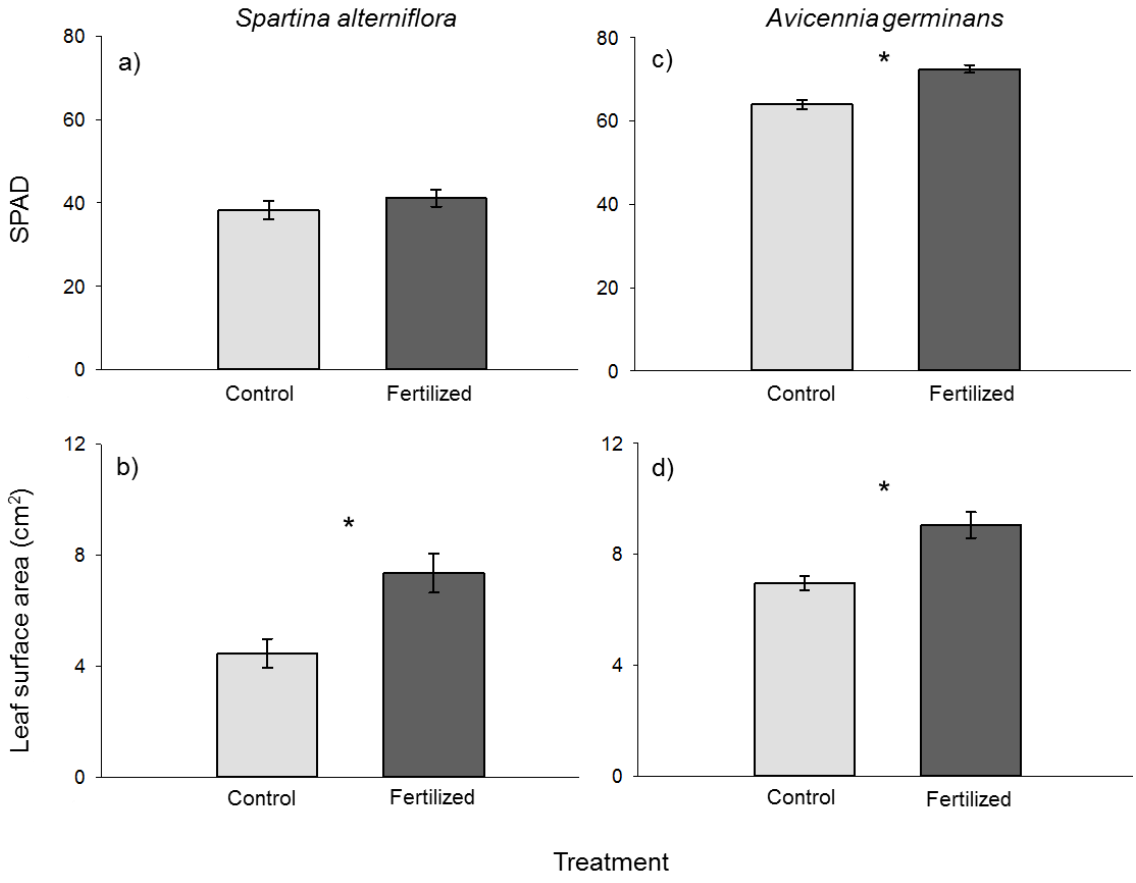

Figure 2.4 Surface area (cm^2) and SPAD readings for live *Spartina alterniflora* (smooth cordgrass; **a-b**) and *Avicennia germinans* (black mangrove; **c-d**) leaves in control and fertilized treatment plots. Data are mean values ± standard error; n = 9 (*S. alterniflora*) and 11 (*A. germinans*). * Indicates significance at perm $p < 0.05$; see Table 2.2 for statistical analyses.

S. alterniflora and *A. germinans* were nearly the same height (approx. 70 cm tall; Figure 2.5 a, c). However, in fertilized plots, *A. germinans* (94 cm) was 18 cm taller than *S. alterniflora* (76 cm; Figure 2.5 a, c). Total live *S. alterniflora* stem and *A. germinans* trunk densities were not significantly different between treatments (Figure 2.5 b, d; Table 2.2).

Table 2.2 Results from separate permANOVAs to determine differences in *Spartina alterniflora* (smooth cordgrass; top portion) and *Avicennia germinans* (black mangrove; bottom portion) live leaf SPAD, leaf surface area (cm^2), total live density (stems or trunks per m^{-2}), and maximum height (cm) between control and fertilized treatment plots. A two-way mixed permANOVA model was utilized: treatment (2 levels: control and fertilized) x block (11 levels). Perm *p* values obtained from 9999 unique permutations of the data. * Indicates significance at perm $p < 0.05$.

***Spartina alterniflora* (smooth cordgrass)**

		Leaf surface area (cm^2)		SPAD		Maximum height (cm)				Total live density (# m^{-2})	
	df	Pseudo F	Perm *p*	Pseudo F	Perm *p*	Pseudo F	Perm *p*		df	Pseudo F	Perm *p*
Treatment	1	9.83	0.02*	0.97	0.37	13.64	0.02*	Treatment	1	0.17	0.87
Block	10	1.01	0.54	1.23	0.45	9.30	0.01*	Block	10	1.75	0.14
Residual	5							Residual	10		
Total	16							Total	21		

***Avicennia germinans* (black mangrove)**

		Leaf surface area (cm^2)		SPAD		Maximum height (cm)				Total live density (# m^{-2})	
	df	Pseudo F	Perm *p*	Pseudo F	Perm *p*	Pseudo F	Perm *p*		df	Pseudo F	Perm *p*
Treatment	1	22.88	<0.01*	46.82	<0.01*	12.02	<0.01*	Treatment	1	1.44	0.26
Block	10	2.01	0.140	1.62	0.23	0.86	0.63	Block	10	1.82	0.14
Residual	10							Residual	10		
Total	21							Total	21		

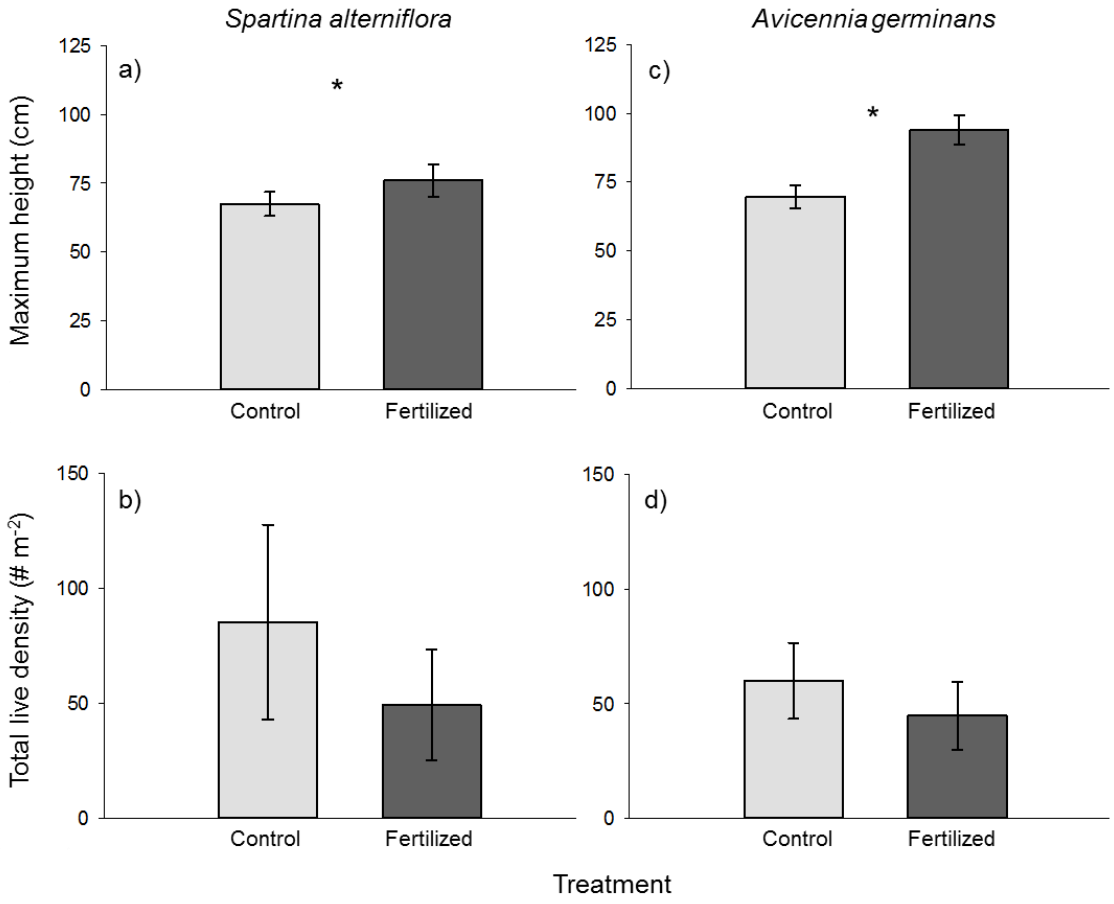

Figure 2.5 Maximum height (cm) and total live stem and trunk density (# m^{-2}) of *Spartina alterniflora* (smooth cordgrass; **a-b**) and *Avicennia germinans* (black mangrove; **c-d**) in control and fertilized treatment plots. Data are mean values ± standard error. Sample size for maximum height was n = 9 (*S. alterniflora*) and n = 11 (*A. germinans*); n = 11 for total live stem and trunk densities. * Indicates significance at perm $p < 0.05$; see Table 2.2 for statistical analyses.

2.4 Discussion

As the first study to assess both *S. alterniflora* and *A. germinans* enrichment responses within *in situ* co-occurring stands, I found that after 28 months of continued enrichment, more *A. germinans* metrics responded to nutrient addition than *S. alterniflora* metrics. These findings are contradictory to what I expected, in that I

hypothesized that nutrient addition would favor *S. alterniflora*. These findings indicate that *in situ A. germinans* respond positively to fertilization and may potentially grow faster and displace more marsh vegetation in areas of the marsh-mangrove ecotone that are prone to high levels of nutrient input.

Avicennia germinans total leaf carbon, nitrogen, and phosphorus contents were significantly higher with nutrient addition, but *S. alterniflora* leaf nutrient values were not, indicating that *A. germinans* was storing the added nutrients in leaf tissue and *S. alterniflora* was not. Leaf nitrogen content is positively correlated to SPAD (chlorophyll *a* content index) values (e.g., Bullock and Anderson 1998); SPAD measurements in *A. germinans* were also higher in fertilized plots. The lack of treatment response in *S. alterniflora* leaves was surprising, as field and mesocosm studies have shown *S. alterniflora* in the NGoM increases leaf nutrient contents when fertilized (Patrick and Delaune 1976, e.g., Buresh et al. 1980). Furthermore, in my mixed plot field study, *S. alterniflora* and *A. germinans* responses directly contrasts those from mesocosm studies where *A. germinans* leaf nutrient contents did not significantly vary with fertilization when grown with *S. alterniflora* (McKee and Rooth 2008). The novel results yielded by a longer study period and field setting clearly highlight the importance of using field tests to assess effects of nutrient enrichment in coastal environments.

Plant responses to fertilization are generally linked to the plant's limiting nutrient. Based on the significant treatment effect on leaf nitrogen, C:N, and N:P values, the *A. germinans* at the study site were likely responding to nitrogen input. Leaf C:P also significantly changed with nutrient treatment, but the relative differences between control and fertilized values were much greater in leaf C:N than leaf C:P, indicating that the relative magnitude of phosphorus uptake was smaller than nitrogen uptake. Although the leaf nutrient content does not reveal the entire nutrient economy of the plant, tissue nutrient ratios can be an informative tool (Güsewell et al. 2003). Additionally, these data match *A. germinans* nutrient limitation studies (e.g., Feller et al. 2003), suggesting that the mangroves in this study were likely nitrogen limited. Along the US Atlantic and NGoM coasts, *S. alterniflora* is also generally considered to be nitrogen limited (e.g.,

Valiela et al. 1978, Buresh et al. 1980). In this study, *S. alterniflora* had comparable total nitrogen and N:P values to other enrichment studies (Buresh et al. 1980, Darby and Turner 2008a). Therefore, it is likely that *S. alterniflora* is also nitrogen limited within this region, but the lack of fertilization response in *S. alterniflora* leaf tissue nutrient contents within the *A. germinans* mixed plots indicates that another factor (e.g., light) may be the primary limiting factor.

Although *A. germinans* may have stored more nutrients in leaf tissue than *S. alterniflora*, both species exhibited some morphometric responses to nutrient addition. In particular, both species had larger leaf surface areas and taller maximum canopy heights in fertilized treatments. In the case of *S. alterniflora*, where leaf surface area increased but there was no concurrent elevation in leaf nutrient contents, the larger leaf areas may have been a plant strategy to dilute leaf nutrient content in order to minimize increased herbivory in enriched conditions (Dai and Wiegert 1997). Maximum height was also positively influenced by nutrient enrichment in both species, consistent with other *S. alterniflora* (Valiela et al. 1978, Buresh et al. 1980) and *A. germinans* (e.g., Feller et al. 2007, McKee and Rooth 2008) enrichment studies in monocultures. In mixed mesocosms, both species also increased in height with nitrogen enrichment, but the *S. alterniflora* height difference between low and high nitrogen treatments was double the height difference in *A. germinans* between treatments (McKee and Rooth 2008). Within this study, *A. germinans* height had a larger nutrient treatment response, as the relative increase in height was nearly three times larger than the increase in *S. alterniflora* height.

Live *S. alterniflora* stem and *A. germinans* trunk densities were similar between nutrient treatments. This finding is novel, particularly in the context of previous field studies, which have primarily focused on monocultures. *Spartina alterniflora* stem density often increases in field enrichment studies (e.g., Darby and Turner 2008a). Previous field enrichment studies on *A. germinans* monocultures have focused on individual tree metrics and have not tracked changes in trunk density within stands (e.g., Feller et al. 2007, McKee and Rooth 2008). Most previous nutrient enrichment work on

mixed *S. alterniflora* and *A. germinans* assemblages has been in mesocosms (McKee and Rooth 2008); in that study, *S. alterniflora* total shoot number increased with fertilization. *Avicennia germinans* density was not measured in those mesocosms, most likely because there was no source of mangrove recruits into the mesocosms. However, in my study, the experiment contained trees of various sizes and maturity, typical of a natural mangrove stand. Total stem density of *S. alterniflora* is generally lower in stands when *A. germinans* is present, which is primarily linked to increased competition for space and light (Kangas and Lugo 1990). Mangroves have taller, wider canopies that shade *S. alterniflora* and other marsh species, typically reducing marsh plant density (Kangas and Lugo 1990). Although there were no changes in total trunk or stem densities for either species with nutrient treatment in this study, *A. germinans* maximum height was substantially taller than *S. alterniflora* in fertilized plots. Therefore, it is likely that, given more time, the taller, wider *A. germinans* canopy in fertilized plots would cause a decrease in *S. alterniflora* stem density.

Contrary to my prediction, *A. germinans* had a stronger growth and morphometric response to added nutrients than *S. alterniflora*. Other field studies have demonstrated that *S. alterniflora* becomes the dominant marsh plant in nutrient enriched conditions when mixed with other graminoid and subshrub species (Levine et al. 1998, Pennings et al. 2002). In mesocosm and transplant experiments, *S. alterniflora* suppresses *A. germinans* seedling growth and survivability (Patterson et al. 1993, McKee and Rooth 2008, Guo et al. 2013), possibly due to a higher nutrient uptake rate in *S. alterniflora* (McKee and Rooth 2008, Perry and Mendelssohn 2009). The novel results from my study may be linked to the longer fertilization period, and the focus on a full suite of *A. germinans* age classes (seedlings to small mature trees), not just on seedling metrics. Suppression of *A. germinans* growth by *S. alterniflora* is strongest at the mangrove seedling stage, but competition from neighboring marsh plants is alleviated in taller (ca. 60 cm) mangroves (Guo et al. 2013). Therefore, the nutrient enrichment effects that were detected in this study are a better representative of potential assemblage responses within real-world ecosystems.

In urbanized and agriculturally developed watersheds, excess nutrients can enter coastal ecosystems via runoff and wastewater discharges, contributing to coastal ecosystem alteration and degradation (Smith et al. 1999). The influence of nutrient enrichment on mangrove stand expansion within the marsh-mangrove ecotone has previously focused on large scale indirect effects (Cavanaugh et al. 2014) or smaller direct influences on mangrove seedlings (e.g., Patterson et al. 1993). Therefore, the current understanding of nutrient enrichment impacts on changes in species composition within this ecotone is lacking, particularly for the NGoM. The Texas and Louisiana coastlines are likely to continue experiencing increases in the size of mangrove stands, as only a 2 – 4 °C increase in mean annual minimum temperature could lead to widespread mangrove-dominance in coastal wetlands (Osland et al. 2013). In this favorable temperature scenario, these results suggest that nutrient enrichment may further accelerate the growth of *A. germinans* stands in Texas. As agricultural and developmental runoff continues to impact watersheds in this region (Castro et al. 2003) and increase the growth rate of mangrove stands, subsequent salt marsh displacement is likely to continue.

Although this study focused on *A. germinans* stands within *S. alterniflora* marshes in the NGoM, the increase in mangrove stand size within marsh-mangrove ecotones is a worldwide occurrence, with reports in North and South America, Africa, and Australia (Saintilan et al. 2014). A shift from salt marsh to mangrove dominated habitat may alter ecosystem functions such as nutrient cycling and the maintenance of water quality (Saintilan et al. 2014). Marsh and mangrove areas provide habitat for a variety of commercially and recreationally important species, yet few faunal species overlap between these vegetation types (Sheridan 1997, Bloomfield and Gillanders 2005). Therefore, it is vital to understand the implications of this shift and how nutrient enrichment may facilitate mangrove expansion.

In conclusion, these data suggest that nutrient enrichment may augment *A. germinans* growth, thereby facilitating faster mangrove encroachment and subsequent exclusion of *S. alterniflora.* The temperature-driven shift in dominance from *S.*

alterniflora to *A. germinans* along the NGoM is already occurring (Perry and Mendelssohn 2009, Guo et al. 2013, Saintilan et al. 2014), and a multitude of factors, such as nutrient enrichment via runoff may be influencing mangrove stand expansion. Regions along the NGoM, particularly the Texas and Louisiana coasts, are likely to shift towards mangrove dominance following small increases in winter temperatures (Osland et al. 2013). The NGoM region is also susceptible to anthropogenic nutrient enrichment and shifts in land use could increase the amount of nutrients entering coastal systems, subsequently affecting the rate of mangrove stand expansion. These data provide additional information for understanding how nutrient enrichment may facilitate *A. germinans* stand expansion within the marsh-mangrove ecotone, which will aid restoration and management decisions in the context of future climate change.

CHAPTER III
FERTILIZATION INCREASES WOODY NOT HERBACEOUS PLANT BIOMASS WITHIN THE MARSH-MANGROVE ECOTONE

3.1 Introduction

Grasslands and shrublands are separate ecosystems and at their boundaries, a mix of herbaceous and woody vegetation creates an ecotone (Risser 1995, Cheplick 1998, Woodward et al. 2004). Grassland-shrubland ecotones occur around the globe from arid to mesic regions, where woody vegetation intersperses with herbaceous vegetation in small to near continuous stands (Cabral et al. 2003, Briggs et al. 2005, Maestre et al. 2009, Van Auken 2009). Most research in grassland-shrubland ecotones focuses on terrestrial systems, but herbaceous and woody vegetation also intermix in intertidal zones along coastlines. The marsh-mangrove ecotone is the transitional area between temperate salt marshes, dominated by herbaceous halophytes, and tropical woody mangrove systems (Saintilan et al. 2014).

Grassland-shrubland ecotones are composed of herbaceous and woody plants in stands of various sizes. The composition of these ecotones is regulated by the environmental conditions of the ecotone (Archer et al. 1995, Scholes and Archer 1997), since herbaceous and woody plants respond differently to abiotic factors (Scholes and Archer 1997). Therefore, multiple interacting environmental factors (e.g., atmospheric CO_2 levels and precipitation), at varied degrees of severity and frequency, drive the vegetation composition of the ecotone (Archer et al. 1995, D'Odorico et al. 2012). For example, within grassland-shrubland ecotones, high fire frequency can favor fast-growing herbaceous vegetation, and fire suppression can lead to an increase in woody vegetation coverage (Archer et al. 1995, Van Auken 2009). Other local factors such as grazing pressure, which reduces vegetative fire fuel, interact with fire frequency to regulate herbaceous and woody plant composition (Van Auken 2000, Briggs et al. 2005).

Freezing temperatures are often invoked as a control of mangrove cover within the marsh-mangrove ecotone (Sherrod and McMillan 1985, Cavanaugh et al. 2014). Mangroves dominate tropical coastal systems, and similar to other tropical vegetation, are sensitive to freezing temperatures which can cause reduced growth and death (Markley et al. 1982, Stevens et al. 2006). Marsh-mangrove ecotones are a mix of mangroves and salt marshes, and generally are in the subtropics, where mangrove height and population size are limited by freezing events (Saintilan et al. 2014). A reduction in freezing event frequency and severity leads to an increase in mangrove coverage (Osland et al. 2013, Cavanaugh et al. 2014). Therefore, freezing conditions are a main driving factor that regulates herbaceous or woody plant coverage within the marsh-mangrove ecotone.

In combination with regional climatic conditions, vegetation composition is also influenced by local environmental conditions. Of particular relevance in coastal ecosystems is anthropogenic nutrient enrichment, a global management issue. Nutrients added to salt marsh and mangrove vegetation typically increase aboveground biomass, height, and productivity (Lovelock et al. 2004, Feller et al. 2007, Darby and Turner 2008a, Fox et al. 2012). However, these studies generally focus on monotypic stands of vegetation, and few studies have examined nutrient enrichment effects within mixed stands. In some mesocosm and transplant studies with mangrove seedlings, marsh species may appear to be better competitors for nutrient resources because of reduced mangrove growth and survivability (Patterson et al. 1993, McKee and Rooth 2008). However, the effects of nutrient enrichment on plant species composition within mixed-species ecotones have not been examined in the field.

Biomass allocation (i.e., belowground biomass:aboveground biomass ratios; herein BLW:ABV) can be an informative measure to compare marsh and mangrove vegetation responses to nutrient enrichment. As reviewed by Poorter et al. (2012), high soil nutrient conditions increase plant aboveground biomass, at the expense of belowground biomass. This pattern of reduced BLW:ABV in nutrient enriched conditions has been documented within monospecific stands of marsh (Darby and

Turner 2008a, Deegan et al. 2012) and mangrove (Feller et al. 2007, Naidoo 2009) vegetation. When marsh and mangrove vegetation were mixed within mesocosms, total BLW:ABV (vegetation types were not separately calculated) increased with fertilization, (McKee and Rooth 2008). To date, there has been no comparable experiment within *in situ* marsh and mangrove mixed vegetation stands.

In order to better understand how anthropogenic nutrient enrichment may influence the marsh-mangrove ecotone, I investigated how fertilization changed coastal herbaceous and woody plant above- and belowground biomass allocation. I fertilized naturally co-occurring stands within the Northern Gulf of Mexico marsh-mangrove ecotone, where the dominant salt marsh grass and mangrove species are *Spartina alterniflora* (smooth cordgrass) and *Avicennia germinans* (black mangrove). Because it is unclear if nutrient enrichment will favor herbaceous or woody vegetation when in mixed stands, I propose the following possible outcomes and interpretations:

1) *Fertilized herbaceous BLW:ABV will be lower than the control with no difference in woody biomass.* This outcome would indicate that *S. alterniflora* increases its allocation to aboveground biomass in response to increased nutrient resources but *A. germinans* does not.
2) *Fertilized woody BLW:ABV will be lower than the control, with no difference in herbaceous biomass.* This outcome would indicate that *A. germinans* increases its allocation to aboveground biomass in response to increased nutrient resources but *S. alterniflora* does not.
3) *Both fertilized herbaceous and woody BLW:ABV will be lower than controls.* This outcome would indicate that both *S. alterniflora* and *A. germinans* increase their allocation to aboveground biomass in response to increased nutrient resources.
4) *Neither fertilized herbaceous nor woody BLW:ABV are different than controls.* This outcome would indicate that neither *S. alterniflora* nor *A. germinans* change biomass allocation in response to nutrient addition.

3.2 Methods

3.2.1 Study location and experimental design

In Port Aransas, TX (USA), black mangroves have been co-occurring with salt marshes since at least the 1930s (Sherrod and McMillan 1981, Montagna et al. 2011). The study plots were in an area (27.9°N, 97.1°W; Figure 3.1) where marsh and

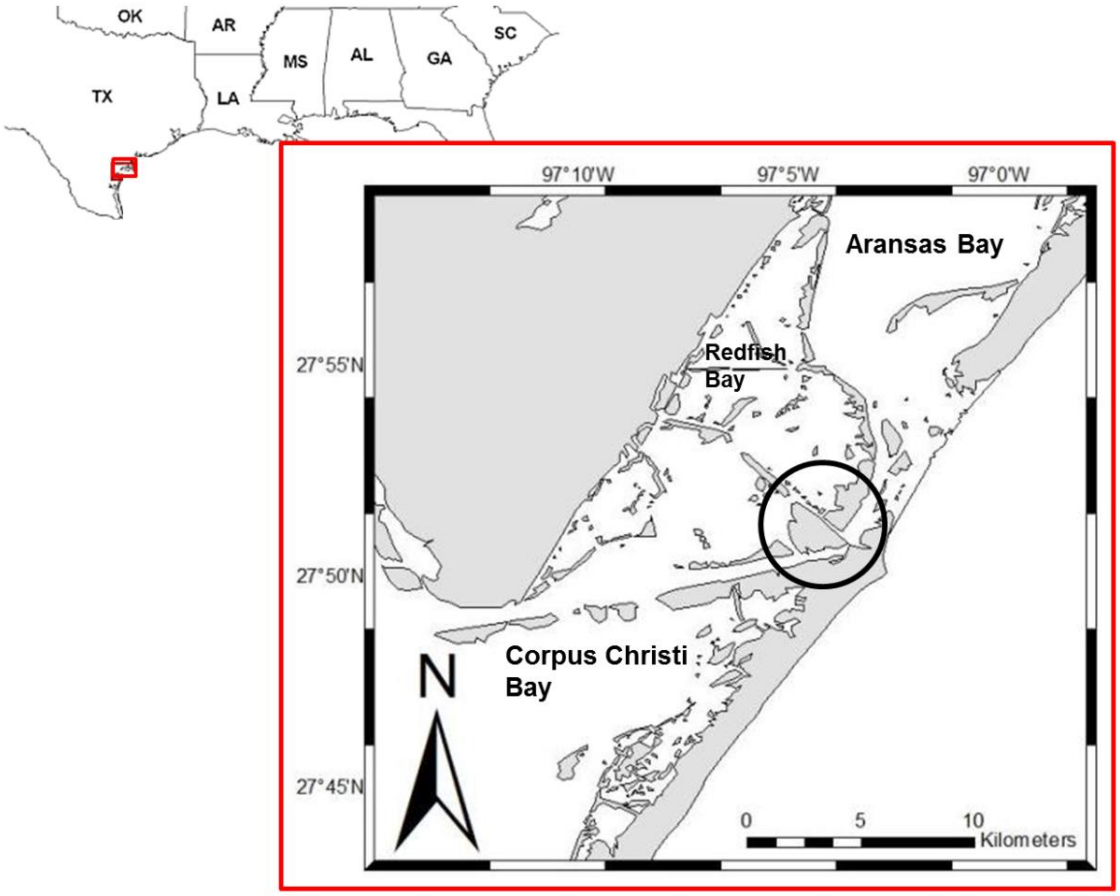

Figure 3.1 Location of study plots within co-occurring *Spartina alterniflora* (smooth cordgrass) and *Avicennia germinans* (black mangrove) stands in Port Aransas, TX, USA.

mangrove species occurred in mixed stands. The dominant salt marsh species was *S. alterniflora*, but other species such as *Batis maritima* (saltwort) and *Salicornia depressa* (Virginia glasswort) were also present in some plots. *Avicennia germinans* was the only mangrove species; all individuals had a shrub-like morphology and were rarely more than 1.5 m tall.

Plots were demarcated in May 2010 at the beginning of the *S. alterniflora* growing season (Kirby and Gosselink 1976) in areas where *S. alterniflora* and *A. germinans* coverage were intermixed. I employed a randomized block design to account for landscape heterogeneity. Each of six blocks contained two 4 m^2 plots, one of each treatment (control and fertilized); blocks were separated by at least 4 m. Plots were fertilized with slow-release fertilizer (Osmocote® Outdoor & Indoor Smart-Release® Plant Food NPK 19-6-12) that was broadcasted on the sediment surface at a loading rate of 0.342 g N m^{-2} day^{-2} and 0.108 g P m^{-2} day^{-1}, based on other enrichment experiments (Darby and Turner 2008a, Slocum and Mendelssohn 2008). Fertilizer was applied 3 – 4 times a year between May 2010 and October 2013 to ensure continuous fertilization.

3.2.2 Sample collection and analyses

Plots were sampled in October 2013 after four growing seasons of continuous fertilization. Above- and belowground samples were collected in October, as this is the peak of the *S. alterniflora* growing season (Kirby and Gosselink 1976).

Within each control and fertilized plot, *S. alterniflora* and *A. germinans* patches were selected for above- and belowground biomass collection. Aboveground biomass within a 10 cm x 10 cm quadrat was clipped of all vegetation to the sediment surface. The corresponding belowground biomass was collected with a 10 cm diameter core to a depth of 20 cm to capture the majority of live root material (Darby and Turner 2008a, Comeaux et al. 2012). Aboveground patches contained the highest density of live monospecific (or as close as possible) *S. alterniflora* and *A. germinans* within each plot that fit within the confines of the aboveground quadrat. Because of the aboveground

quadrat size, the largest shrubs within the plot could not be collected; height of sampled mangroves did not exceed 80 cm.

In the laboratory, aboveground biomass was washed with distilled water to remove adhered sediment. All clipped vegetation was identified, enumerated, and height measured. *Spartina alterniflora* shoots were divided between live and dead and *A. germinans* was divided by leaf and wood material. Aboveground tissue was dried at 60 °C to determine biomass. Leaves (3 – 5 of the newest, fully grown) from *S. alterniflora* and *A. germinans* aboveground biomass samples were used for carbon, nitrogen, and phosphorus analyses.

Cores were sectioned at 5 cm depth intervals and were washed through a 250 μm sieve to capture most root material. I did not separate belowground biomass between live and dead material as this can be highly subjective. Roots were not divided by species because this was also not always easily discernable, particularly for small roots. However, cores were removed from areas selected based on the aboveground presence of the target species (*S. alterniflora* or *A. germinans*) which was always more than 80 % of the total aboveground biomass. Therefore, the roots collected within each core type should primarily be roots associated with the target species of that sample. Roots from each core section were dried to constant mass at 60 °C, weighed, and used for carbon, nitrogen, and phosphorus analyses.

Dried leaves and roots were ground using a Thomas Wiley® Mini-Mill and passed through a 250 μm sieve. Total carbon and nitrogen contents were quantified using a Costech ECS 4010 Elemental Analyzer. The detection limit for this analyzer was 0.001 %; samples below this detection limit were recorded as 0.001 %. Total phosphorus content was determined via a dry-oxidation, acid hydrolysis extraction followed by a colorimetric analysis on a Shimadzu UV-1800 Spectrophotometer (Fourqurean et al. 1992).

All data were analyzed using PERMANOVA+ version 1.0.5 in PRIMER 6 version 6.1.15 (PRIMER-E Ltd., Plymouth Marine Laboratory, UK; Anderson et al. 2008). Total above- and belowground biomass and BLW:ABV values were analyzed

with a three-way permutational analysis of variance (permANOVA; treatment x target species x block). Total above- and belowground biomass includes all plant material collected above and below the sediment surface, respectively. Belowground biomass and root nutrient contents were analyzed in separate three-way permANOVAs (treatment x target species x block) for each core section (0-5 cm, 5-10 cm, 10-15 cm, and 15-20 cm). For both *S. alterniflora* and *A. germinans*, average height, leaf nutrient concentrations, and aboveground biomass for each component (*S. alterniflora* live and dead shoots; *A. germinans* leaves, wood, and pneumatophores) were analyzed for treatment effects within separate two-way permANOVAs (treatment x block). All data were fourth root transformed and resemblance matrices for biomass data were calculated using Bray Curtis resemblance and the remaining data (e.g., heights and nutrient contents) were based on Euclidean distances.

3.3 Results

Nutrient addition significantly increased above- and belowground biomass in *A. germinans* samples, but did not significantly affect *S. alterniflora* biomass (Figure 3.2). Overall, *A. germinans* had significantly more aboveground biomass than *S. alterniflora* (Table 3.1). Fertilized *A. germinans* biomass was nearly 2.5 times more than in control plots, and more than 10 times higher than either *S. alterniflora* treatment (Figure 3.2 a). Control aboveground biomass of marsh samples was 65 % live and 16 % dead *S. alterniflora* shoots and within fertilized samples, aboveground biomass was 60 % and 26 % live and dead *S. alterniflora* shoots, respectively (Figure 3.3). *Avicennia germinans* shrubs were 84 % of the control and 89 % of fertilized aboveground biomass. *Avicennia germinans* pneumatophores comprised 13 % and 11 % of the control and fertilized aboveground biomass (Figure 3.3). Total belowground biomass did not significantly vary between *S. alterniflora* treatments, but *A. germinans* had significantly more root biomass within fertilized samples compared to *A. germinans* controls or to fertilized or control *S. alterniflora* (Figure 3.2 b , Table 3.1). BLW:ABV ratios were significantly different between vegetation types, but did not significantly vary between treatments

(Table 3.1). *Spartina alterniflora* samples had higher BLW:ABV within control (2.39 ± 0.77) and fertilized (1.10 ± 0.49) than *A. germinans* control (0.57 ± 0.13) and fertilized (0.29 ± 0.04) samples.

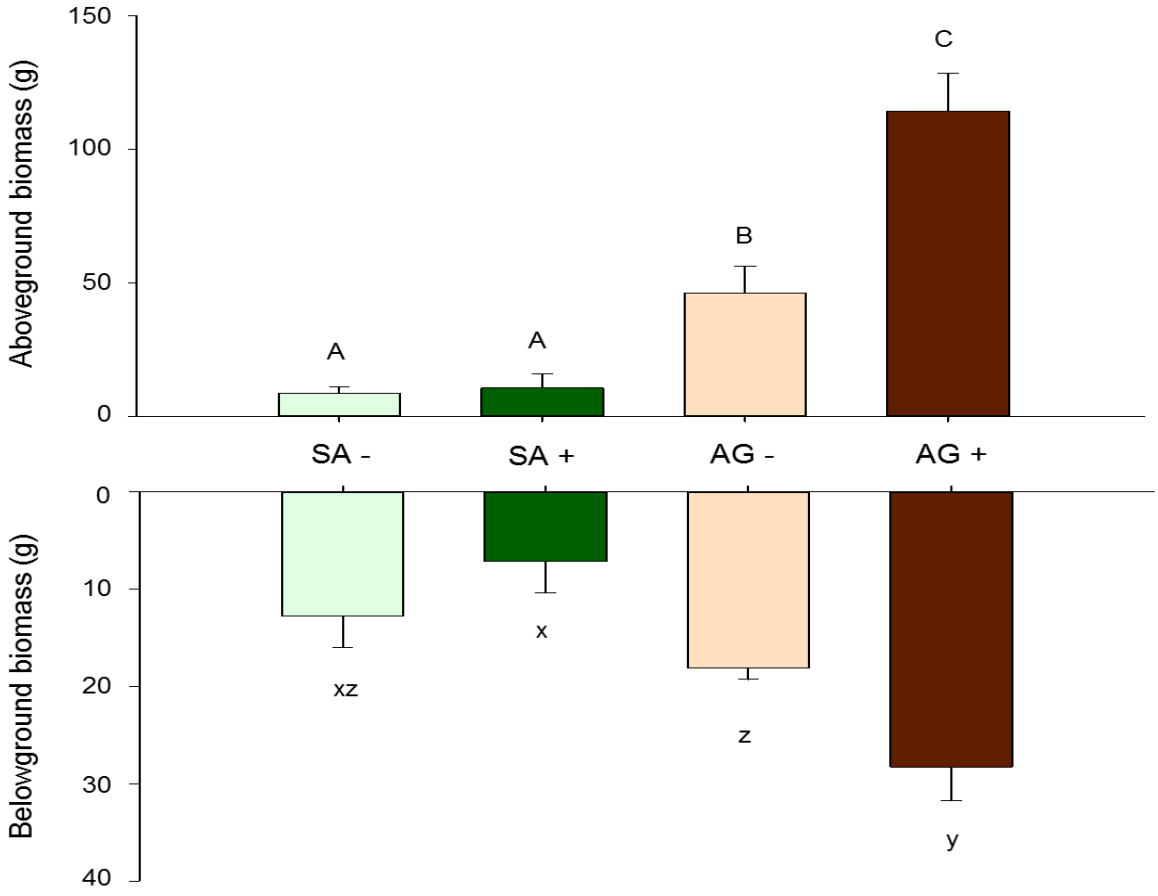

Figure 3.2 Total plant aboveground biomass clipped from 10 cm x 10 cm quadrat and total belowground biomass extracted from a 10 cm diameter (to 20 cm depth) core for *Spartina alterniflora* (smooth cordgrass; SA) and *Avicennia germinans* (black mangrove; AG) patches in control (-) and fertilized (+) treatment plots. Data are mean values ± standard error; n = 6. Different letters indicate significance at perm $p < 0.05$; see Table 3.1 for statistical analyses.

Table 3.1 Results from separate permANOVAs to determine differences in total aboveground biomass (ABV), belowground biomass (BLW), and BLW:ABV. A three-way mixed permANOVA model was utilized: treatment (2 levels: control and fertilized) x vegetation type (2 levels: *Spartina alterniflora* - smooth cordgrass and *Avicennia germinans* - black mangrove) x block (6 levels). Perm *p* values obtained from 9999 unique permutations of the data. * Indicates significance at perm $p < 0.05$.

	Aboveground biomass		**Belowground biomass**		**BLW:ABV**	
	Pseudo F	Perm *p*	Pseudo F	Perm *p*	Pseudo F	Perm *p*
Treatment	2.72	0.11	2.14	0.30	5.03	0.09
Vegetation type	7.23	< 0.01*	4.65	< 0.01*	0.05	0.02
Block	2.57	0.11	2.45	0.16	1.33	0.37
Treatment x vegetation type	2.54	0.15	2.87	0.12	1.83	0.89
Treatment x block	0.97	0.52	1.06	0.42	0.97	0.94
Vegetation type x block	2.61	0.13	2.50	0.18	1.57	0.50

Root biomass was significantly lower within *S. alterniflora* cores compared to *A. germinans* cores in the top 5 cm of sediment (Table 3.2); fertilized *A. germinans* had the most root biomass (Figure 3.4). There were no significant differences in root biomass between vegetation type or treatment within deeper core sections (5-20 cm; Table 3.2).

Total percent phosphorus within enriched *S. alterniflora* roots was significantly higher than in roots in all other treatments, but only within the top core section (Tables 3.2, 3.3). The rest of the nutrient contents within roots did not vary between vegetation types, treatments, or core depth (Table 3.2).

When aboveground biomass plant components were assessed separately, there was no significant effect of fertilization on biomass or average height of *S. alterniflora* live and dead shoots (Figure 3.5 a,c; Tables 3.4, 3.5). *Avicennia germinans* leaf, wood, and pneumatophore biomass were significantly greater in fertilized plots (Figure 3.5 b; Table 3.4). *Avicennia germinans* shrub average height was significantly taller in fertilized plots. *Avicennia germinans* pneumatophore average height did not vary with nutrient treatment (Figure 3.5 d; Table 3.5), but there were significantly more in

fertilized plots (Table 3.5). *Spartina alterniflora* leaf nutrient concentrations did not vary between treatment plots (Tables 3.6, 3.7). Only leaf N:P was significantly higher in fertilized *A. germinans* samples; the remaining leaf nutrient concentrations did not vary with nutrient addition (Tables 3.6, 3.7).

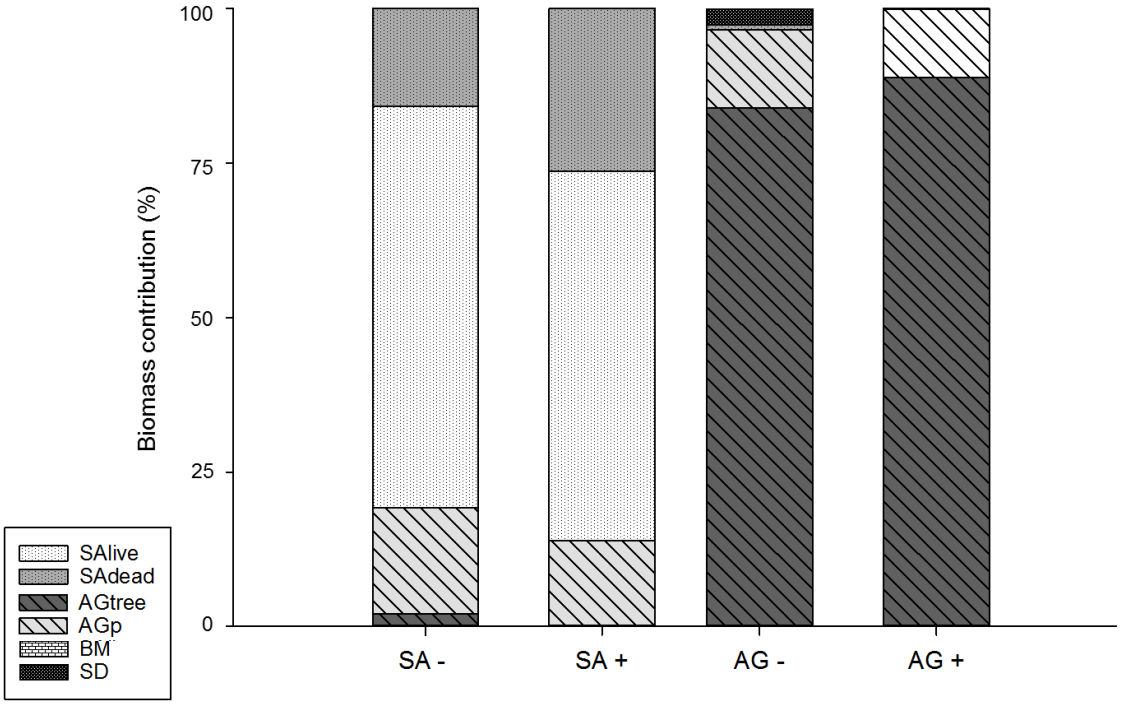

Figure 3.3 Total plant aboveground biomass clipped from 10 cm x 10 cm quadrat broken down by percent contribution of collected material for *Spartina alterniflora* (smooth cordgrass; SA) and *Avicennia germinans* (black mangrove; AG) patches in control (-) and fertilized (+) treatment plots.

Table 3.2 Results from separate permANOVAs to determine differences in root biomass, carbon (% C), nitrogen (% N), phosphorus (% P), carbon to nitrogen (C:N), carbon to phosphorus (C:P), and nitrogen to phosphorus (N:P) for each core segment collected (0-5 cm, 5-10 cm, 10-15 cm, and 15-20 cm) in control and fertilized treatment plots. A three-way mixed permANOVA model was utilized: treatment (2 levels: control and fertilized) x core type (2 levels: *Spartina alterniflora* - smooth cordgrass and *Avicennia germinans* - black mangrove) x block (11 levels). Perm *p* values obtained from 9999 unique permutations of the data. * Indicates significance at perm $p < 0.05$.

		Root biomass		**% C**		**% N**		**% P**		**C:N**		**C:P**		**N:P**	
		Pseudo F	Perm *p*	Pseudo F	Perm *p*	Pseudo F	Perm *p*	Pseudo F	Perm *p*	Pseudo F	Perm *p*	Pseudo F	Perm *p*	Pseudo F	Perm *p*
0 – 5 cm	Treatment	3.94	0.11	1.52	0.27	0.34	0.58	9.59	0.03*	0.01	0.92	1.04	0.35	0.01	0.94
	Core type	12.02	0.03*	0.02	0.89	2.78	0.18	9.81	0.04*	1.24	0.33	0.83	0.39	1.59	0.28
	Block	1.40	0.46	1.20	0.51	0.77	0.65	0.61	0.72	0.23	0.91	0.29	0.89	0.62	0.71
	Treatment x core type	7.13	0.09	3.24	0.22	0.05	0.85	3.90	0.17	0.51	0.55	0.31	0.63	0.13	0.75
	Treatment x block	1.08	0.55	1.16	0.52	0.60	0.73	2.88	0.28	0.53	0.76	0.66	0.69	0.42	0.82
	Core type x block	1.63	0.43	1.33	0.46	0.07	0.98	0.31	0.85	0.27	0.87	0.35	0.83	0.08	0.98
5 – 10 cm	Treatment	2.78	0.16	0.33	0.59	0.62	0.48	0.90	0.39	0.49	0.55	0.63	0.46	1.87	0.24
	Core type	0.04	0.84	9.54	0.05	0.06	0.82	0.83	0.45	0.12	0.72	0.10	0.78	0.41	0.54
	Block	1.57	0.43	0.64	0.73	116.30	0.01*	0.21	0.93	36.42	0.03*	0.32	0.87	8.60	0.10
	Treatment x core type	0.01	0.97	1.67	0.30	3.94	0.18	0.99	0.46	0.05	0.84	0.77	0.51	0.28	0.66
	Treatment x block	0.32	0.87	1.97	0.37	41.65	0.02*	0.05	0.10	4.42	0.19	0.02	1.00	2.28	0.33
	Core type x block	0.80	0.63	0.66	0.67	53.87	0.02*	0.10	0.97	34.46	0.03	0.05	0.99	4.41	0.20
10 – 15 cm	Treatment	3.06	0.14	0.01	0.91	2.54	0.17	0.28	0.62	2.81	0.16	0.10	0.76	3.02	0.15
	Core type	0.16	0.71	6.29	0.08	1.91	0.26	0.48	0.53	1.76	0.28	0.58	0.50	1.78	0.27
	Block	11.14	0.09	1.69	0.40	2.31	0.33	1.84	0.38	331.80	0.01*	1.56	0.44	2.40	0.32
	Treatment x core type	1.92	0.31	0.93	0.44	1.00	0.64	0.41	0.59	67.43	0.02*	0.01	0.96	1.24	0.40
	Treatment x block	1.90	0.37	3.14	0.25	2.14	0.35	1.94	0.37	335.05	0.01*	2.70	0.29	1.80	0.40
	Core type x block	21.98	0.05	0.24	0.90	3.99	0.21	0.39	0.80	665.11	<0.01*	0.42	0.80	4.09	0.21
15 – 20 cm	Treatment	0.97	0.43	4.01	0.07	2.84	0.62	2.11	0.21	0.35	0.57	0.07	0.81	0.49	0.50
	Core type	1.78	0.27	0.47	0.57	- -	- -	1.50	0.29	0.47	0.57	0.63	0.48	0.52	0.51
	Block	141.96	0.01*	27.44	0.04*	- -	- -	1.30	0.49	107.61	0.01*	1.27	0.49	33.45	0.03
	Treatment x core type	2.88	0.24	4.63	0.14	- -	- -	0.61	0.52	4.63	0.14	0.01	0.91	0.21	0.68
	Treatment x block	62.40	0.02*	8.16	0.11	- -	- -	0.26	0.90	147.03	<0.01*	0.41	0.82	39.77	0.03
	Core type x block	22.83	0.05	18.43	0.05	- -	- -	0.84	0.61	18.43	0.05	2.12	0.35	0.83	0.61

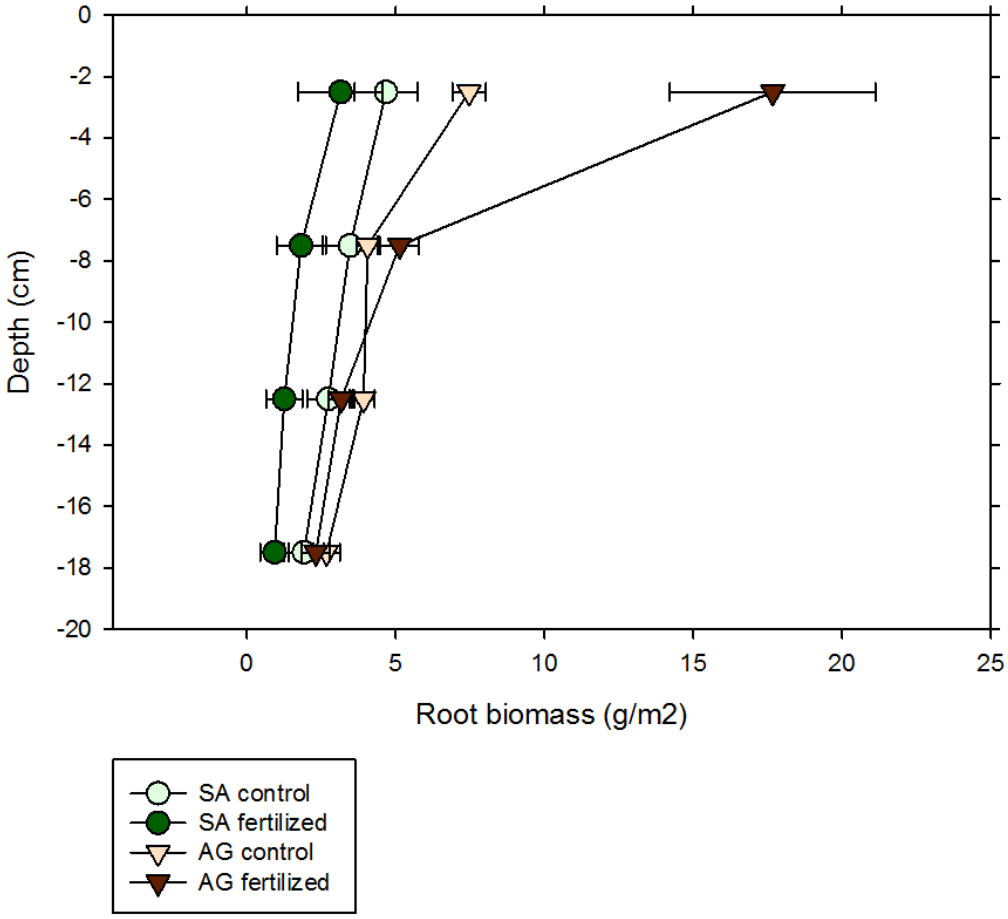

Figure 3.4 Total root biomass (g) extracted from a 10 cm diameter (to 20 cm depth) for *Spartina alterniflora* (smooth cordgrass; SA) and *Avicennia germinans* (black mangrove; AG) in control (closed) and fertilized (open) treatment plots. Data are mean values ± standard error; n = 6. See Table 3.2 for statistical analyses.

3.4 Discussion

Above- and belowground biomass allocations did not differ between species, but total biomass was more than 1.5 times larger in fertilized *A. germinans* samples than the control samples. The greater *A. germinans* biomass indicates enrichment increased mangrove growth and could facilitate increased woody coverage within the marsh-mangrove ecotone. The positive nutrient responses in *A. germinans* characteristics

Table 3.3 Total percent carbon (% C), nitrogen (% N), phosphorus (% P), carbon to nitrogen (C:N), carbon to phosphorus (C:P), and nitrogen to phosphorus (N:P) of *Spartina alterniflora* (smooth cordgrass; marsh) and *Avicennia germinans* (black mangrove; mangrove) roots in control and fertilized treatment plots for each core segment collected (0-5 cm, 5-10 cm, 10-15 cm, and 15-20 cm). Data are mean values ± standard error; n = 6.

		% C	% N	% P	C:N	C:P	N:P
		Avg. (± SE)	Avg. (± SE)	Avg. (± SE)	Avg. (± SE)	Avg. (± SE)	Avg. (± SE)
0 – 5 cm	Marsh – control	22.58 (2.39)	0.22 (0.05)	0.05 (<0.01)	147.39 (32.34)	1128.59 (131.74)	10.00 (4.22)
	Marsh – fertilized	26.67 (0.35)	0.29 (0.09)	0.09 (0.01)	134.86 (44.25)	794.29 (86.26)	10.15 (2.59)
	Mangrove – control	29.25 (2.34)	0.24 (0.11)	0.05 (<0.01)	12032.35 (7761.85)	1476.50 (192.76)	9.32 (2.34)
	Mangrove – fertilized	28.04 (3.58)	0.33 (0.09)	0.07 (0.01)	2163.61 (2044.75)	1058.05 (139.43)	6.99 (1.69)
5 – 10 cm	Marsh – control	20.90 (1.50)	0.08 (0.05)	0.05 (0.01)	8438.39 (4957.16)	1075.30 (159.84)	3.81 (2.10)
	Marsh – fertilized	20.79 (2.06)	0.01 (<0.01)	0.03 (0.01)	17951.14 (8390.84)	3656.64 (2295.23)	2.02 (1.97)
	Mangrove – control	26.25 (2.99)	0.07 (0.06)	0.04 (0.01)	8413.63 (4394.14)	2503.52 (1032.03)	2.49 (1.67)
	Mangrove – fertilized	27.10 (0.99)	0.14 (0.07)	0.05 (<0.01)	10737.50 (6614.59)	1356.11 (75.91)	5.72 (2.87)
10 – 15 cm	Marsh – control	17.58 (2.35)	<0.01 (<0.01)	0.04 (0.01)	20499.89 (2735.56)	1206.16 (208.04)	0.06 (0.01)
	Marsh – fertilized	14.39 (3.03)	<0.01 (<0.01)	0.04 (0.01)	16783.32 (3537.78)	988.20 (189.26)	0.06 (0.01)
	Mangrove – control	18.64 (1.82)	0.06 (0.03)	0.04 (0.01)	9478.27 (4334.65)	1207.98 (142.44)	2.54 (1.46)
	Mangrove – fertilized	21.10 (2.16)	<0.01 (<0.01)	0.06 (0.01)	16212.65 (3245.78)	1199.98 (248.99)	0.21 (0.15)
15 – 20 cm	Marsh – control	11.24 (1.57)	<0.01 (<0.01)	0.05 (0.01)	13102.41 (1826.63)	737.29 (213.29)	0.06 (0.02)
	Marsh – fertilized	11.56 (0.60)	<0.01 (<0.01)	0.11 (0.04)	13478.00 (705.34)	492.28 (292.26)	0.04 (0.02)
	Mangrove – control	15.44 (2.97)	0.04 (0.04)	0.04 (0.01)	15244.88 (4598.35)	982.77 (198.97)	1.69 (1.63)
	Mangrove – fertilized	18.01 (2.98)	0.01 (<0.01)	0.05 (<0.01)	16730.76 (4579.10)	1049.40 (184.72)	0.26 (0.21)

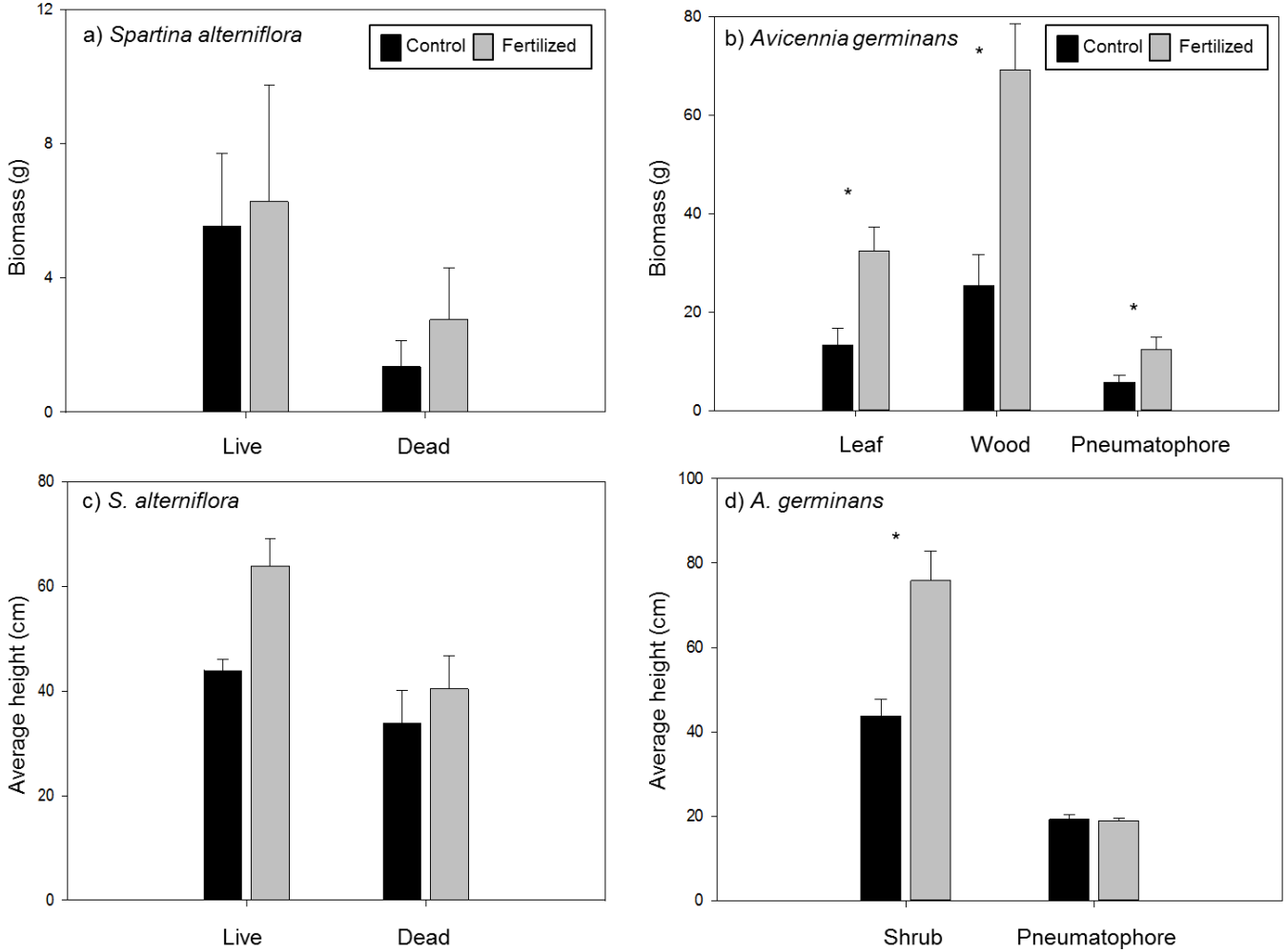

Figure 3.5 *Spartina alterniflora* (smooth cordgrass) live and dead shoot biomass (**a**) and average height (**c**) and *Avicennia germinans* (black mangrove) leaf, wood, and pneumatophore biomass (**b**) and average height (**d**) clipped from 10 cm x 10 cm quadrat in control and fertilized treatment plots. Data are mean values ± standard error; n = 6. * Indicates significance at perm $p < 0.05$; see Tables 3.4 and 3.5for statistical analyses.

Table 3.4 Results from separate permANOVAs to determine differences in *Spartina alterniflora* (smooth cordgrass; top portion) live and dead shoot biomass and *Avicennia germinans* (black mangrove; bottom portion) leaf, wood, and pneumatophore biomass between control and fertilized treatment plots. A two-way mixed permANOVA model was utilized: treatment (2 levels: control and fertilized) x block (6 levels). Perm *p* values obtained from 9999 unique permutations of the data. * Indicates significance at perm $p < 0.05$.

***Spartina alterniflora* (smooth cordgrass)**				
	Live biomass		Dead biomass	
	Pseudo F	Perm *p*	Pseudo F	Perm *p*
Treatment	1.64	0.27	0.01	0.93
Block	2.87	0.09	1.08	0.48

***Avicennia germinans* (black mangrove)**						
	Leaf biomass		Wood biomass		Pneumatophore biomass	
	Pseudo F	Perm *p*	Pseudo F	Perm *p*	Pseudo F	Perm *p*
Treatment	8.51	0.03*	12.93	0.01*	14.46	0.01*
Block	0.64	0.67	0.63	0.67	5.70	0.05

Table 3.5 Results from separate permANOVAs to determine differences in *Spartina alterniflora* (smooth cordgrass; top portion) live and dead average height and *Avicennia germinans* (black mangrove; bottom portion) shrub and pneumatophore average height, and pneumatophore density between control and fertilized treatment plots. A two-way mixed permANOVA model was utilized: treatment (2 levels: control and fertilized) x block (6 levels). Perm *p* values obtained from 9999 unique permutations of the data. * Indicates significance at perm $p < 0.05$.

***Spartina alterniflora* (smooth cordgrass)**				
	Live average height		Dead average height	
	Pseudo F	Perm *p*	Pseudo F	Perm *p*
Treatment	12.26	0.08	7.32	0.09
Block	1.28	0.50	2.52	0.29

***Avicennia germinans* (black mangrove)**						
	Shrub average height		Pneumatophore average height		Pneumatophore density	
	Pseudo F	Perm *p*	Pseudo F	Perm *p*	Pseudo F	Perm *p*
Treatment	15.25	0.02*	0.03	0.87	10.43	0.02*
Block	0.69	0.65	1.30	0.40	3.15	0.13

Table 3.6 Results from separate permANOVAs to determine differences in *Spartina alterniflora* (smooth cordgrass; top portion) and *Avicennia germinans* (black mangrove; bottom portion) live leaf total carbon (% C), nitrogen (% N), phosphorus (% P), carbon to nitrogen (C:N), carbon to phosphorus (C:P), and nitrogen to phosphorus (N:P) between control and fertilized treatment plots. A two-way mixed permANOVA model was utilized: treatment (2 levels: control and fertilized) x block (6 levels). Perm *p* values obtained from 9999 unique permutations of the data. * Indicates significance at perm $p < 0.05$.

***Spartina alterniflora* (smooth cordgrass)**												
	% C		% N		% P		C:N		C:P		N:P	
	Pseudo F	Perm *p*	Pseudo F	Perm *p*	Pseudo F	Perm *p*	Pseudo F	Perm *p*	Pseudo F	Perm *p*	Pseudo F	Perm *p*
Treatment	2.13	0.27	8.07	0.08	0.41	0.61	7.32	0.09	0.50	0.60	2.18	0.28
Block	3.93	0.21	2.70	0.29	1.88	0.37	2.52	0.29	1.70	0.39	0.24	0.89
***Avicennia germinans* (black mangrove)**												
	% C		% N		% P		C:N		C:P		N:P	
	Pseudo F	Perm *p*	Pseudo F	Perm *p*	Pseudo F	Perm *p*	Pseudo F	Perm *p*	Pseudo F	Perm *p*	Pseudo F	Perm *p*
Treatment	1.58	0.26	6.36	0.06	0.01	0.94	2.47	0.18	0.16	0.71	13.26	0.02*
Block	0.59	0.69	0.80	0.58	1.14	0.45	0.65	0.67	0.90	0.55	2.49	0.19

Table 3.7 Total percent carbon (% C), nitrogen (% N), phosphorus (% P), carbon to nitrogen (C:N), carbon to phosphorus (C:P), and nitrogen to phosphorus (N:P) of live *Spartina alterniflora* (smooth cordgrass; top portion) and *Avicennia germinans* (black mangrove; bottom portion) leaves in control and fertilized treatment plots. Data are mean values ± standard error; n = 6.

	***Spartina alterniflora* (smooth cordgrass)**					
	% C	% N	% P	C:N	C:P	N:P
	Avg. (± SE)	Avg. (± SE)	Avg. (± SE)	Avg. (± SE)	Avg. (± SE)	Avg. (± SE)
Control	42.21 (0.25)	1.05 (0.09)	0.13 (0.01)	47.99 (3.74)	858.51 (116.18)	18.04 (2.17)
Fertilized	41.54 (0.34)	0.76 (0.08)	0.15 (0.02)	65.63 (8.12)	757.69 (88.22)	11.63 (1.07)
	***Avicennia germinans* (black mangrove)**					
	% C	% N	% P	C:N	C:P	N:P
	Avg. (± SE)	Avg. (± SE)	Avg. (± SE)	Avg. (± SE)	Avg. (± SE)	Avg. (± SE)
Control	44.38 (0.75)	1.72 (0.05)	0.15 (0.01)	30.26 (1.39)	796.10 (50.21)	26.31 (1.21)
Fertilized	45.73 (0.60)	2.02 (0.10)	0.15 (0.01)	26.74 (1.63)	829.77 (60.45)	31.02 (1.19)

within this study are similar to fertilized mangrove monocultures (e.g., Feller et al. 2007). However, the lack of fertilization effect on *S. alterniflora* biomass contradicts other studies that have reported positive fertilization biomass and height responses in this species (e.g., Pennings et al. 2002, McKee and Rooth 2008). These previous studies were conducted in mesocosms and/or were not fertilized for as long as this study, potentially missing latent woody plant nutrient responses (Michelsen et al. 1999).

In coastal ecosystems, elevated nutrient supply frequently increases aboveground biomass and decreases belowground biomass (Deegan et al. 2012, Poorter et al. 2012). This change in biomass allocation has been reported in marsh (e.g., Valiela et al. 1976) and mangrove (e.g., Naidoo 1987) monospecific fertilization studies. Therefore, it was surprising that neither target species displayed a similar trend. Average BLW:ABV values were lower in fertilized plots compared to control plots for both species, but these trends were not significant. The lack of a difference in BLW:ABV between control and fertilized plots for both target species could indicate that another factor is limiting (Poorter et al. 2012). In other grassland-shrubland systems, factors such as light and soil salinity can limit plant growth and biomass allocation, despite increases in nutrient availability (Lett and Knapp 2003, Bloor et al. 2008, Chen and Ye 2014). Enriched mangroves that have positive growth responses but reduced or no differences in BLW:ABV and leaf nutrient contents, have been found in areas with high soil salinity (Naidoo 1987, Chen and Ye 2014). The results from my study showed a similar pattern, with increases in *A. germinans* height but minimal to no differences in BLW:ABV and tissue (leaf and root) nutrient contents; this pattern may have been influenced by high soil pore water salinity, which can exceed 40 PSU in this area (Guo et al. 2013).

Nutrient enrichment may influence herbaceous and woody plant composition within the marsh-mangrove ecotone by facilitating mangrove growth. This was exhibited in *A. germinans* parameters such as biomass and height, which were significantly greater in fertilized plots. However, the lack of differences in biomass allocation between nutrient treatments suggests other factors such as soil salinity may also be influencing these plant species. Although freezing events are considered to be the main driving

factor in increased mangrove coverage (Osland et al. 2013, Cavanaugh et al. 2014), nutrient enrichment may also accelerate woody plant dominance. In the last few decades, reduced lethal freezing events have facilitated an increase in mangrove vegetation within the marsh-mangrove ecotone often encroaching into salt marsh dominated systems (Saintilan and Rogers 2015). Therefore, it is important to further investigate how nutrient addition may facilitate mangrove growth within mixed stands, in order to better predict the future state of coastal habitat within marsh-mangrove ecotones.

CHAPTER IV
NUTRIENT ENRICHMENT SHIFTS MANGROVE SIZE DISTRIBUTION WITHIN THE MARSH-MANGROVE ECOTONE

4.1 Introduction

Global changes are driving shifts in plant species coverage, phenology, and distribution within multiple biomes around the world (as reviewed by: Walther et al. 2002, Parmesan 2006, Lavergne et al. 2010). Species within ecotones, defined as intermediate areas between different vegetation types, are particularly sensitive to global changes (Risser 1995, Grimm et al. 2013). Many plants within ecotones are considered foundation species, in that the structure and function of the ecosystem are dependent on the presence of these species (Dayton 1972). A shift in dominant vegetation type could dramatically alter associated ecosystem services (Costanza et al. 1997, Scavia et al. 2002). Therefore, it is imperative to understand how global changes may influence species interactions within an ecotone.

Oscillations in dominant vegetation types in the ecotones between grasslands and shrublands can be mediated by many factors (Scholes and Archer 1997). Over the past two centuries woody vegetation has expanded globally in biomass and coverage, often encroaching into grasslands (Archer et al. 1995, Briggs et al. 2005, Saintilan and Rogers 2015). Woody encroachment is influenced by global changes such as increases in temperatures or elevated CO_2 (Briggs et al. 2005, D'Odorico et al. 2012). Other, generally local, factors such as intensified grazing practices and reduced fire occurrence, can further influence this vegetation shift (Van Auken 2009, D'Odorico et al. 2012).

Although most literature has focused on terrestrial woody encroachment, this phenomenon is also occurring along the coast within the marsh-mangrove ecotone (Saintilan and Rogers 2015). Mangroves are woody plants commonly associated with tropical habitats, but are increasing in distribution and coverage in subtropical regions, mainly driven by elevated temperatures (Stuart et al. 2007, Friess et al. 2012, Saintilan et

al. 2014). Like other tropical vegetation, mangroves die if exposed to freezing temperatures; therefore, mangrove distribution and growth is primarily limited by the frequency, duration, and severity of freezing events (Stuart et al. 2007). Over the last 50 years, mangrove stands have proliferated worldwide, often encroaching into salt marshes dominated by herbaceous halophytes (Saintilan et al. 2014, Armitage et al. 2015).

Mangrove encroachment is mainly attributed to alterations in climate, such as increased mean annual minimum temperature (Osland et al. 2013) and decreased freezing event frequency (Cavanaugh et al. 2014). Similar to terrestrial woody expansion, other factors, such as added nutrient resources, may further facilitate this habitat shift. Nutrients from anthropogenic sources enter coastal systems through groundwater inflows, runoff, and wastewater discharge (Vitousek 1997, Boesch 2002), making marsh and mangrove systems susceptible to nutrient input (Gedan et al. 2009, Alongi 2015). In monotypic stands of either marsh or mangrove vegetation, fertilization generally increases plant growth and productivity (e.g., Pennings et al. 2002, Lovelock et al. 2004, Feller et al. 2007, Darby and Turner 2008a, e.g., Naidoo 2009, Fox et al. 2012). Because marsh plants can suppress mangrove growth and survival (Patterson et al. 1993, McKee and Rooth 2008, Simpson et al. 2013), enriched conditions may facilitate more growth in marsh vegetation than in mangroves. Therefore, nutrient enrichment may slow encroaching mangroves and maintain salt marsh dominance. However, these previous enrichment studies focused on younger/shorter mangroves (e.g., McKee and Rooth 2008, Simpson et al. 2013), and suppression by neighboring marsh plants is negated in larger mangroves (Guo et al. 2013). Therefore, how nutrient enrichment may influence mangrove encroachment and marsh displacement within the ecotone could depend on the size distribution of the mangrove population.

Mangroves are able to outcompete salt marsh vegetation for light because of their taller, wider canopies (Smith and Whelan 2006, Stevens et al. 2006). Conversely, marsh vegetation has been reported to reduce the growth and survivability of small mangroves (Patterson et al. 1993, McKee and Rooth 2008, Guo et al. 2013). Along the expanding edge of a mangrove stand, where marsh and mangrove plants co-occur, mangroves are

smaller and may experience negative interactions with neighboring salt marsh plants. Mangroves may eventually grow to a height where negative effects from marsh plants are nullified (Guo et al. 2013). In nutrient enriched conditions, this growth suppression may be further augmented by accelerating marsh plant growth and subsequently maintaining marsh vegetation dominance (McKee and Rooth 2008, Simpson et al. 2013).

To investigate nutrient enrichment effects on mangrove encroachment, I fertilized naturally co-occurring stands on the Texas (USA) coast in the Northern Gulf of Mexico over four growing seasons (2010 – 2013). Plots were placed in an area where mangrove stands are actively increasing and replacing salt marsh (Armitage et al. 2015) to investigate how nutrient addition may influence mangrove encroachment and marsh displacement over time. I expected that nutrient enrichment would augment marsh growth and conversely slow mangrove growth, and that the magnitude of mangrove stand expansion and subsequent marsh displacement would be reduced in fertilized plots.

4.2 Methods

4.2.1 Site description and experimental design

In the Northern Gulf of Mexico, *Avicennia germinans* (black mangrove) is the only mangrove species and has been historically scattered throughout this region (Sherrod and McMillan 1981, Saintilan et al. 2014). The first documentation of mangroves in the area was in 1853, but it was not until the 1930s that reports of this species presence along the Texas coast were continuously documented (Sherrod and McMillan 1981). Although *A. germinans* has a higher cold temperature tolerance than other mangrove species, they are still susceptible to diebacks following freezing events (Markley et al. 1982). Therefore, *A. germinans* in this area are often interspersed with marsh forb and graminoid species, particularly *Spartina alterniflora* (smooth cordgrass) (Montagna et al. 2011).

Port Aransas, TX, USA is one of the locations where *A. germinans* stands on the Texas coast have been documented since the 1930s (Sherrod and McMillan 1981, 1985). A massive mangrove dieback occurred in this region following several freezing events in the early 1980s (Sherrod and McMillan 1981, 1985, Montagna et al. 2011), but since that time, freezing temperatures have not been of sufficient severity or duration to cause substantial dieback, and mangrove stands have increased in areal cover, particularly within the Port Aransas region (Montagna et al. 2011, Armitage et al. 2015). In the last twenty years, mangrove coverage has surpassed the reported accounts in 1979, and most of this increase has been in areas previously dominated by salt marsh species, such as *S. alterniflora* (Montagna et al. 2011, Armitage et al. 2015). Because Port Aransas is within the Gulf of Mexico marsh-mangrove ecotone and is actively experiencing mangrove expansion, it was an ideal location to study how nutrient enrichment may influence this vegetation shift.

In the spring of 2010, at the beginning of the *S. alterniflora* growing season (Kirby and Gosselink 1976, Darby and Turner 2008b), plots were established in Port Aransas (27.9°N, 97.1°W) along the low marsh elevation contour in areas with mixed marsh and mangrove vegetation. Plots were placed along the edge of dense mangrove stands, where *A. germinans* was interspersed with characteristically low elevation marsh vegetation, mainly *S. alterniflora* (e.g., Guo et al. 2013). Plots were placed along the mangrove stand edge in order to measure species interactions where *A. germinans* was expanding into salt marsh. At the time of plot deployment, mangroves were mostly (> 95 %) seedlings but some small shrubs were present. Herein, seedlings refer to mangroves that are < 0.5 m; this classification is based on height similar to other studies (Osland et al. 2015) and not necessarily indicative of a newly established plant. Succulent marsh species, primarily *Batis maritima* (saltwort) and *Salicornia depressa* (Virginia glasswort), were also present in and around the plots.

Plots were placed in a split block design where each of the eleven blocks (no closer than 4 m) contained two 4 m^2 plots, one of each nutrient treatment type: control and fertilized. A randomized block design was used to account for landscape

heterogeneity. Prior to treatment application, there were no significant differences between plots, based on species densities using a two-way mixed permutational analysis of variance (permANOVA; treatment x block). A slow-release fertilizer (Osmocote® Outdoor & Indoor Smart-Release® Plant Food NPK 19-6-12) at a loading rate of 0.342 g N m^{-2} day^{-2} and 0.108 g P m^{-2} day^{-1}, based on previous enrichment experiments in Gulf of Mexico salt marshes (Darby and Turner 2008a, Slocum and Mendelssohn 2008) was used in fertilized plots. Fertilizer was applied by broadcasting pellets onto the sediment surface and was re-applied multiple times to ensure continued enrichment throughout the study period.

4.2.2 Sample collection and analysis

Plots were sampled at peak plant production prior to fall senescence (Kirby and Gosselink 1976, Darby and Turner 2008b) each year from 2010 through 2013 (September – October). Total density of each species present was quantified for the entire plot (2 m x 2 m). For higher densities where total plot quantification was logistically difficult, a subquadrat (30 cm x 30 cm) was used; densities were standardized to # m^{-2}. *Avicennia germinans* densities were recorded in each of three size classes: < 0.5 m, 0.5 m - 1.0 m, and > 1.0 m (herein, seedling, short shrub, and tall shrub, respectively). The maximum height of the tallest *A. germinans* and *S. alterniflora* individuals within each plot was measured. Green leaves (n = 20) were collected from haphazardly selected *S. alterniflora* and *A. germinans* throughout each plot for nutrient content analyses. In the laboratory, leaves were rinsed to remove any adhered sediments, dried to constant mass in a drying oven (60 °C), ground with a Thomas Wiley® Mini-Mill, and sieved through a 60 mesh (0.25 mm) screen. Total carbon (C) and nitrogen (N) content were quantified using a Costech ECS 4010 Elemental Analyzer; analytical variability ranged 2 – 5 %, as determined by running National Institute of Standards and Technology standard reference material (SRM 1941-b). Total phosphorus (P) content was determined via a dry-oxidation, acid hydrolysis extraction followed by a

colorimetric analysis on a Shimadzu UV-1800 Spectrophotometer (Fourqurean et al. 1992).

4.2.3 Data analyses

Individual nutrient responses for each sampling event (i.e., nutrient content, height, and density) were determined with separate three-way permANOVAs where treatment (control and fertilized) and year (2010 – 2013) were fixed factors and block (11 levels) was treated as a random factor. The three-way interaction term (treatment x year x block) was excluded from the model because there was no replication within blocks, typical of randomized block experimental designs. All data were analyzed using PERMANOVA+ version 1.0.5 in PRIMER 6 version 6.1.15 (PRIMER-E Ltd., Plymouth Marine Laboratory, UK; Anderson et al. 2008).

Individual analyses for nutrient content parameters (total %C, %N, %P, C:N, C:P, and N:P) for each species were based on Euclidean distance resemblance. Some *A. germinans* leaves collected in 2013 were contaminated in the laboratory and therefore nutrient data for the 2013 sampling event consisted of only six of the eleven blocks. Maximum height data for both species were square root transformed and a Euclidean distance based resemblance matrix was used. In some plots *S. alterniflora* was not present (particularly in the final sampling event), and therefore those plots were excluded from the nutrient and height analyses.

Density data were fourth root transformed and a Bray Curtis resemblance was used. Total *S. alterniflora* and *A. germinans* densities, as well as *A. germinans* size classes, were analyzed separately. To account for the high number of zeros within the *S. alterniflora* and *A. germinans* size class (seedling, short shrub, and tall shrub) density data, a dummy variable was added to each resemblance matrix. Pair-wise tests were used to determine significant differences between nutrient treatments and sampling events. Significance for analyses was determined using permutation *p* values which were obtained from 9999 unique permutations of the data.

4.3 Results

Avicennia germinans leaf nutrient content metrics, particularly measures of nitrogen content, significantly varied between nutrient treatments, whereas *S. alterniflora* leaf nutrient contents did not (Table 4.1). *Avicennia germinans* had higher total % C in fertilized leaves in the first three years (2010 – 2012; Tables 4.2, 4.3) and total leaf % N, C:N and N:P were significantly different between treatments in the second (2011) and third (2012) years (Tables 4.2, 4.3). Only *A. germinans* total leaf N:P was significantly higher in fertilized plots in the fourth growing season (2013), although total % N was near significant (perm $p < 0.056$; Tables 4.2, 4.3). Total % P was only significantly different between control and fertilized treatments in *A. germinans* leaves collected in 2012 (Tables 4.2, 4.3). Fertilization did not significantly affect any *S. alterniflora* leaf nutrient content variables in any of the sampling years (Tables 4.2, 4.3).

Spartina alterniflora density was not significantly different between treatments but significantly decreased over time in both treatment types; this temporal trend was more pronounced in fertilized plots (Figure 4.1 a; Tables 4.3 – 4.5). Total *A. germinans* density did not change between fertilization treatments or over time (Figure 4.1 b; Tables 4.3 – 4.5). When *A. germinans* plants were divided into size classes (seedling, short shrub, and tall shrub), treatment and temporal trends were evident. *Avicennia germinans* seedlings and short shrub densities were significantly different between treatments (Table 4.4). Fertilization shifted mangroves to taller size classes, as there was lower seedling density (Figure 4.2 a; Tables 4.4, 4.5) and higher short and tall shrubs densities in fertilized plots (Figure 4.2 b, c; Tables 4.4, 4.5). Taller mangroves (short and tall shrubs) were also significantly different between sampling years and treatment (Tables 4.4, 4.5). Mangrove seedling densities within control plots were similar across all four growing seasons, but significantly decreased over time within fertilized plots (Figure 4.2 a). Short shrub density increased over time in both treatments, but was 10x higher in fertilized than control plots in 2012 and 2013 (Figure 4.2 b). Tall shrub density in control plots was constant over time, but significantly increased in fertilized plots throughout the course of the experiment (Figure 4.2 c).

Table 4.1 Results from separate permANOVAs to determine differences in *Spartina alterniflora* (smooth cordgrass; top portion) and *Avicennia germinans* (black mangrove; bottom portion) live leaf total carbon (% C), nitrogen (% N), phosphorus (% P), carbon to nitrogen (C:N), carbon to phosphorus (C:P), and nitrogen to phosphorus (N:P) between control and fertilized treatment plots. A three-way mixed permANOVA model was utilized: treatment (2 levels: control and fertilized) x year (4 levels: 2010-2013) x block (11 levels). Perm *p* values obtained from 9999 unique permutations of the data. * Indicates significance at perm $p < 0.05$.

	***Spartina alterniflora* (smooth cordgrass)**											
	% C		% N		% P		C:N		C:P		N:P	
	Pseudo F	Perm *p*	Pseudo F	Perm *p*	Pseudo F	Perm *p*	Pseudo F	Perm *p*	Pseudo F	Perm *p*	Pseudo F	Perm *p*
Treatment	0.96	0.41	3.01	0.12	1.11	0.35	4.68	0.05	0.91	0.37	4.21	0.07
Year	21.74	< 0.01*	6.05	< 0.01*	9.51	< 0.01*	5.96	< 0.01*	11.21	< 0.01*	11.03	< 0.01*
Block	1.43	0.25	2.93	0.02*	10.05	< 0.01*	3.51	0.01*	8.74	< 0.01*	10.89	< 0.01*
Treatment x year	1.45	0.26	0.50	0.69	0.60	0.64	1.24	0.34	0.62	0.61	0.44	0.73
Year x block	1.27	0.32	1.71	0.13	1.91	0.11	1.59	0.17	1.44	0.23	1.75	0.13
Treatment x block	0.91	0.54	1.38	0.26	1.65	0.19	1.61	0.19	0.63	0.77	1.00	0.48
	***Avicennia germinans* (black mangrove)**											
	% C		% N		% P		C:N		C:P		N:P	
	Pseudo F	Perm *p*	Pseudo F	Perm *p*	Pseudo F	Perm *p*	Pseudo F	Perm *p*	Pseudo F	Perm *p*	Pseudo F	Perm *p*
Treatment	13.65	< 0.01*	42.98	< 0.01*	3.74	0.08	29.59	< 0.01*	0.21	0.66	45.57	< 0.01*
Year	14.64	< 0.01*	8.01	< 0.01*	17.36	< 0.01*	2.60	0.07	7.64	< 0.01*	2.35	0.10
Block	0.86	0.58	4.79	< 0.01*	1.98	0.08	3.95	< 0.01*	1.66	0.15	2.99	0.01*
Treatment x year	0.36	0.78	7.41	< 0.01*	1.50	0.24	5.50	< 0.01*	1.50	0.24	2.54	0.08
Year x block	1.69	0.10	0.93	0.57	0.74	0.76	0.87	0.63	0.79	0.71	0.94	0.56
Treatment x block	1.75	0.13	1.57	0.17	0.74	0.68	1.32	0.27	0.81	0.62	0.67	0.74

Table 4.2 Total percent carbon (% C), nitrogen (% N), phosphorus (% P), carbon to nitrogen (C:N), carbon to phosphorus (C:P), and nitrogen to phosphorus (N:P) of live *Spartina alterniflora* (smooth cordgrass; top portion) and *Avicennia germinans* (black mangrove; bottom portion) leaves in control and fertilized treatment plots within each sampling year (2010 – 2013). Data are mean values ± standard error; n = 11.

***Spartina alterniflora* (smooth cordgrass)**								
Year:	2010		2011		2012		2013	
Treatment:	*Control*	*Fertilized*	*Control*	*Fertilized*	*Control*	*Fertilized*	*Control*	*Fertilized*
% C	40.79 (0.29)	41.47 (0.33)	41.32 (0.34)	40.91 (0.25)	40.20 (0.30)	40.75 (0.45)	42.85 (0.19)	43.28 (0.32)
% N	1.01 (0.07)	1.04 (0.06)	1.09 (0.11)	1.23 (0.05)	0.77 (0.07)	0.95 (0.12)	1.16 (0.07)	1.32 (0.08)
% P	0.13 (0.01)	0.11 (0.01)	0.18 (0.01)	0.19 (0.01)	0.18 (0.03)	0.19 (.02)	0.15 (0.02)	0.17 (0.01)
C:N	49.99 (4.69)	47.98 (2.68)	47.76 (4.33)	39.46 (2.01)	66.51 (7.52)	55.05 (6.08)	44.16 (2.55)	38.98 (1.96)
C:P	854.89 (66.41)	961.73 (54.61)	604.34 (45.44)	592.31 (47.85)	709.25 (102.76)	607.77 (73.49)	775.61 (85.60)	672.00 (56.28)
N:P	17.83 (1.37)	20.53 (1.50)	13.99 (1.65)	15.00 (0.87)	11.95 (2.66)	11.98 (1.91)	60.93 (4.83)	78.46 (6.80)
***Avicennia germinans* (black mangrove)**								
Year:	2010		2011		2012		2013	
Treatment:	*Control*	*Fertilized*	*Control*	*Fertilized*	*Control*	*Fertilized*	*Control*	*Fertilized*
%C	44.44 (0.22)	45.33* (0.30)	45.28 (0.32)	46.25* (0.26)	46.53 (0.18)	47.60* (0.34)	44.38 (0.75)	45.73 (0.60)
%N	1.74 (0.06)	1.84 (0.09)	1.69 (0.07)	2.30* (0.07)	1.77 (0.06)	2.31* (0.09)	1.72 (0.13)	2.03 (0.10)
%P	0.13 (0.01)	0.13 (0.01)	0.13 (0.01)	0.14 (0.01)	0.14 (0.01)	0.16* (0.01)	0.15 (0.01)	0.15 (0.01)
C:N	30.27 (1.17)	29.39 (1.25)	31.86 (1.35)	23.68* (0.90)	31.04 (1.03)	24.40* (0.99)	30.26 (1.39)	26.72 (0.99)
C:P	897.51 (22.13)	912.62 (30.22)	887.28 (27.72)	834.69 (11.51)	836.01 (16.99)	790.58* (10.63)	796.05 (50.42)	829.12 (59.87)
N:P	29.92 (0.88)	31.33 (0.95)	28.20 (1.28)	35.69* (1.42)	27.29 (1.23)	32.91* (1.36)	26.32 (1.22)	31.01* (1.18)

Table 4.3 Results from separate permANOVAs to determine differences in *Spartina alterniflora* (smooth cordgrass; top portion) and *Avicennia germinans* (black mangrove; bottom portion) live leaf total carbon (% C), nitrogen (% N), phosphorus (% P), carbon to nitrogen (C:N), carbon to phosphorus (C:P), nitrogen to phosphorus (N:P), total trunk/stem density (# m^{-2}), and maximum height (cm) between control and fertilized treatment plots and sampling years (2010 – 2013). A three-way mixed permANOVA model was utilized: treatment (2 levels: control and fertilized) x year (4 levels: 2010 – 2013) x block (11 levels). Significance was determined for treatment (control or fertilized) within each sampling year using a pair-wise test (treatment x year). Perm *p* values obtained from 9999 unique permutations of the data. * Indicates significance at perm $p < 0.05$. Total *Avicennia germinans* = all size classes; seedling density = *A. germinans* < 0.5 m; short shrub density = *A. germinans* 0.5 - 1.0 m; tall shrub = *A. germinans* > 1.0 m.

	***Spartina alterniflora* (smooth cordgrass)**							
Year:	2010		2011		2012		2013	
	t	perm *p*	t	perm *p*	t	perm *p*	t	perm *p*
% C	1.89	0.08	0.95	0.37	1.03	0.35	1.24	0.28
% N	0.42	0.69	0.97	0.36	1.38	0.24	1.03	0.37
% P	1.99	0.07	0.01	0.99	0.90	0.43	0.04	0.96
C:N	0.43	0.69	1.64	0.15	1.34	0.25	1.22	0.27
C:P	1.76	0.11	0.30	0.78	0.62	0.54	0.49	0.64
N:P	2.03	0.07	1.02	0.34	2.06	0.10	0.32	0.76
Total density	0.22	0.89	0.74	0.55	0.35	0.86	0.21	0.93
Max height	1.62	0.13	2.92	0.02*	2.87	0.03*	4.82	< 0.01*
	***Avicennia germinans* (black mangrove)**							
Year:	2010		2011		2012		2013	
	t	perm *p*	t	perm *p*	t	perm *p*	t	perm *p*
% C	2.44	0.03*	2.58	0.03*	4.67	< 0.01*	1.26	0.27
% N	1.38	0.21	5.80	< 0.01*	5.98	< 0.01*	2.59	0.06
% P	0.24	0.81	2.15	0.06	3.42	< 0.01*	0.06	0.90
C:N	0.85	0.42	5.02	< 0.01*	5.28	< 0.01*	1.50	0.19
C:P	0.46	0.65	1.64	0.13	2.62	0.03*	0.41	0.69
N:P	1.09	0.31	3.95	< 0.01*	4.68	< 0.01*	3.61	0.02*
Total density	0.31	0.86	0.55	0.60	1.35	0.20	1.97	0.07
Seedling density	0.35	0.78	0.40	0.71	2.74	0.02*	3.29	< 0.01*
Short shrub density	2.16	0.03*	3.57	< 0.01*	1.67	0.11	2.16	0.05
Tall shrub density	0.09	0.77	0.14	0.77	1.71	0.12	4.09	< 0.01*
Max height	2.13	0.06	3.14	0.01*	4.38	< 0.01*	5.82	< 0.01*

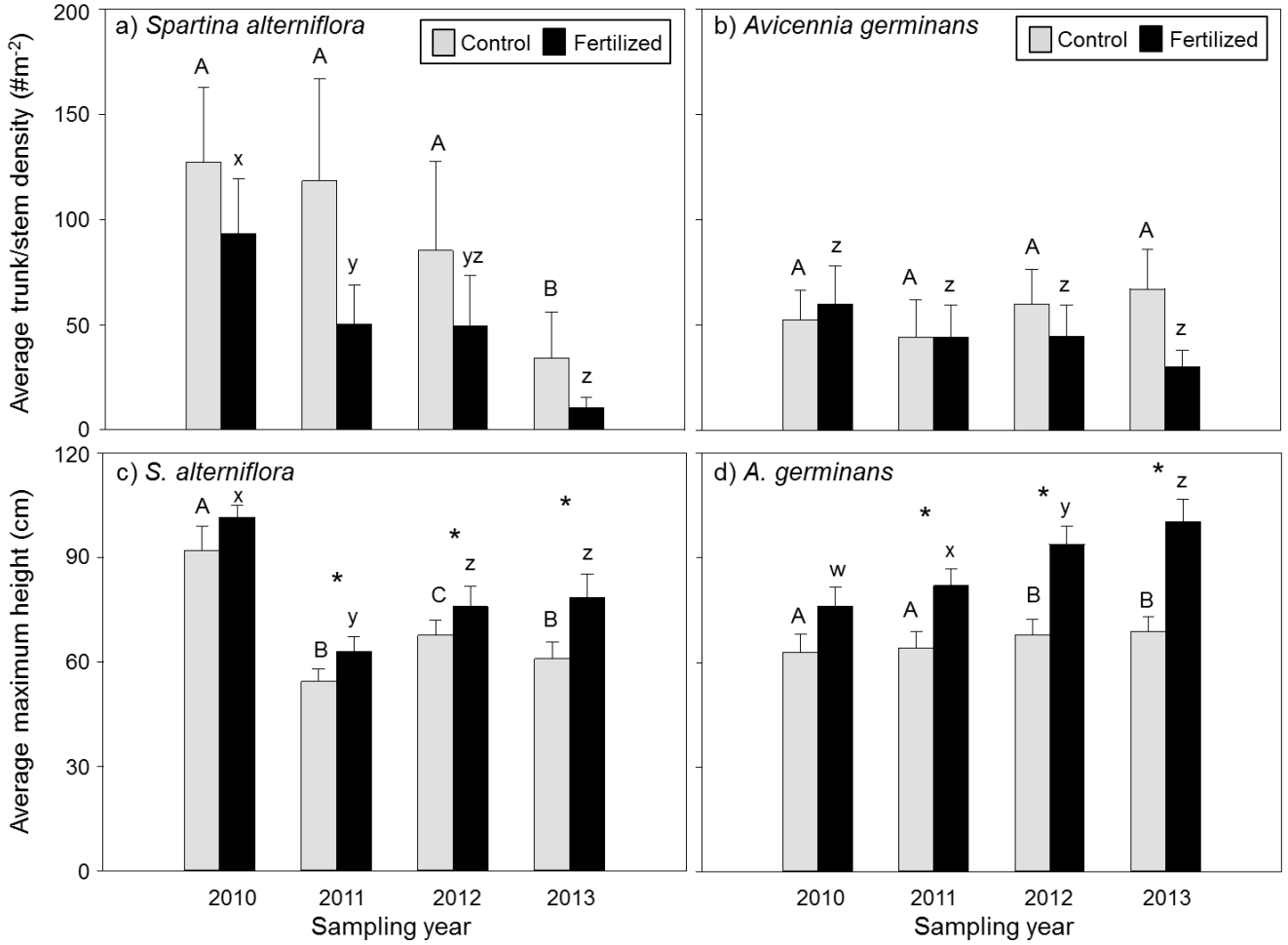

Figure 4.1 Total density (# m^{-2}) and maximum height (cm) within each sampling year (2010 – 2013) for *Spartina alterniflora* (smooth cordgrass; **a**, **c**) and *Avicennia germinans* (black mangrove; **b**, **d**) in control and fertilized treatment plots. Data are mean values ± standard error; n = 6. Upper case letters indicate temporal trends between control plots; lower case letters indicate temporal trends between fertilized plots. Different letters indicate significance at perm $p < 0.05$; see Tables 4.3-4.5 for statistical analyses.

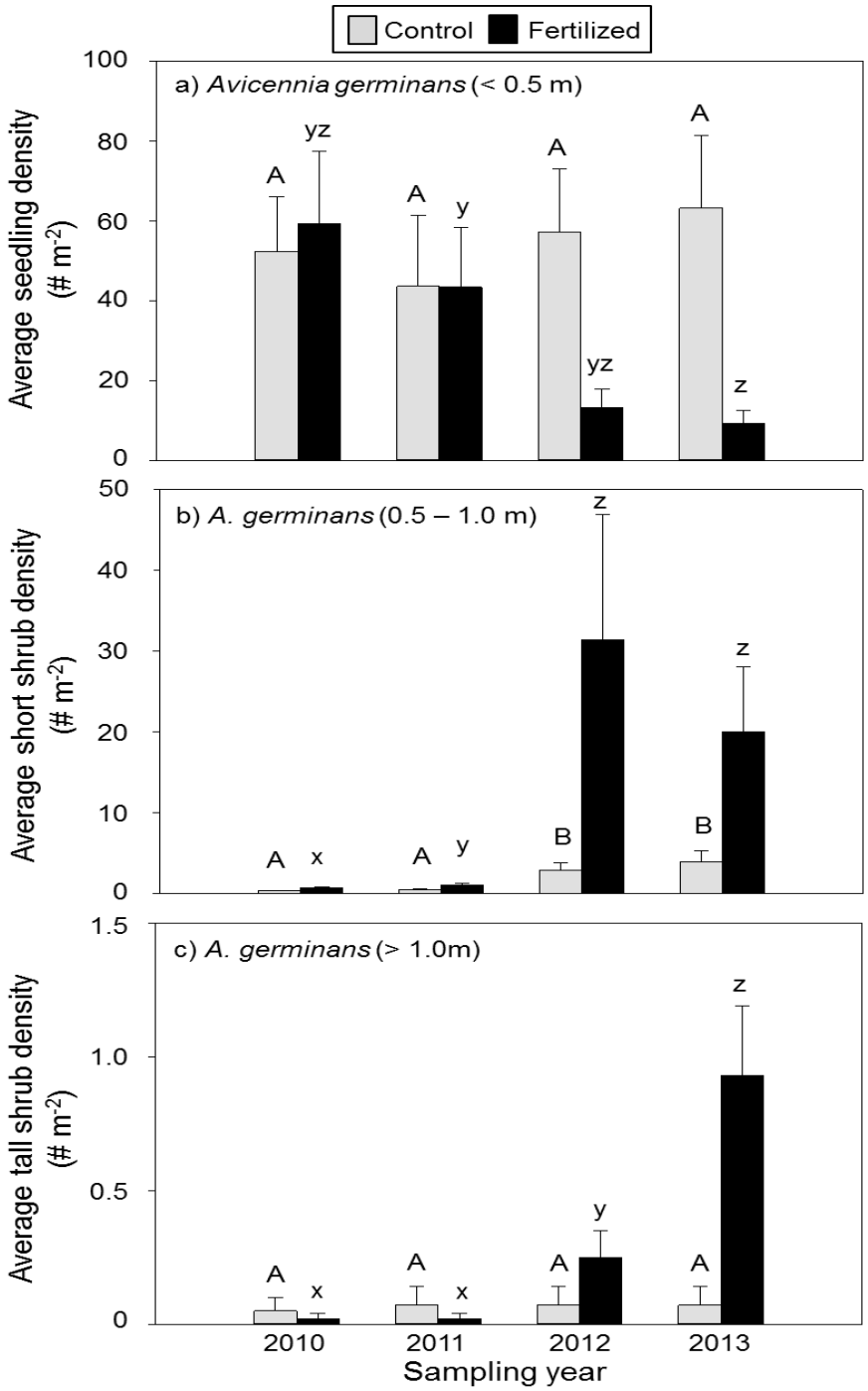

Figure 4.2 Density (# m-2) within each sampling year (2010 – 2013) for three *Avicennia germinans* (black mangrove) size classes: seedling = *A. germinans* < 0.5 m (**a**); short shrub = *A. germinans* 0.5 - 1.0 m (**b**); tall shrub = *A. germinans* > 1.0 m (**c**) in control and fertilized treatment plots. Data are mean values ± standard error; n = 6. Upper case letters indicate temporal trends between control plots; lower case letters indicate temporal trends between fertilized plots. Different letters indicate significance at perm $p < 0.05$; see Tables 4.3-4.5 for statistical analyses.

Table 4.4 Results from separate permANOVAs to determine differences in *Spartina alterniflora* (smooth cordgrass; left portion) and *Avicennia germinans* (black mangrove; right portion) maximum height (cm) and density (# m^{-2}) between control and fertilized treatment plots and sampling years (2010 – 2013). Mangrove density is divided into: total black mangrove (*A. germinans*; all size classes) density, seedling density (*A. germinans*; < 0.5 m), short shrub density (*A. germinans*; 0.5 - 1.0 m), and tall shrub density (*A. germinans*; > 1.0 m). A three-way mixed permANOVA model was utilized: treatment (2 levels: control and fertilized) x year (4 levels: 2010 – 2013) x block (11 levels). Perm *p* values obtained from 9999 unique permutations of the data. * Indicates significance at perm $p < 0.05$.

	***Spartina alterniflora* (smooth cordgrass)**				***Avicennia germinans* (black mangrove)**									
	Maximum height		Total density		Maximum height		Total density		Seedling density		Short shrub density		Tall shrub density	
	Pseudo F	Perm *p*	Pseudo F	Perm *p*	Pseudo F	Perm *p*	Pseudo F	Perm *p*	Pseudo F	Perm *p*	Pseudo F	Perm *p*	Pseudo F	Perm *p*
Treatment	23.203	< 0.01*	0.12	0.83	15.92	< 0.01*	0.47	0.53	7.23	0.02*	23.203	< 0.01*	0.12	0.83
Year	42.26	< 0.01*	10.97	< 0.01*	22.70	< 0.01*	1.38	0.26	1.12	0.34	42.26	< 0.01*	10.97	< 0.01*
Block	14.39	< 0.01*	10.64	< 0.01*	43.88	< 0.01*	4.38	< 0.01*	2.46	0.02*	14.39	< 0.01*	10.64	< 0.01*
Treatment x year	2.29	0.11	0.30	0.93	8.31	< 0.01*	1.11	0.36	4.43	< 0.01*	2.29	0.11	0.30	0.93
Year x block	1.77	0.11	1.29	0.18	1.39	1.19	1.41	0.16	0.97	0.54	1.77	0.11	1.29	0.18
Treatment x block	2.00	0.10	9.23	< 0.01*	20.55	< 0.01*	2.57	0.02*	1.58	0.14	2.00	0.10	9.23	< 0.01*

Table 4.5 Results from separate permANOVAs to determine differences in *Spartina alterniflora* (smooth cordgrass; top portion) and *Avicennia germinans* (black mangrove; bottom portion) *Spartina alterniflora* (smooth cordgrass; SA) total stem density (# m^{-2}), SA maximum height (cm), *Avicennia germinans* (black mangrove; AG) total, seedling (< 0.5 m), short shrub (0.5 - 1.0 m), and tall shrub (> 1.0 m) trunk density (# m^{-2}), and AG maximum height (cm) height (cm) between control and fertilized treatment plots and sampling years (2010 – 2013). A three-way mixed permANOVA model was utilized: treatment (2 levels: control and fertilized) x year (4 levels: 2010 – 2013) x block (11 levels). Significance was determined for sampling year (2010-2013) within each treatment type (control vs fertilized) using a pair-wise test (year x treatment). Perm *p* values obtained from 9999 unique permutations of the data. * Indicates significance at perm $p < 0.0$; - - - indicates "t" was not able to be calculated because a zero was present in the denominator (numbers were the same between years) and therefore a perm *p* was not assigned.

Control plots

Year:	2010 x 2011		2010 x 2012		2010 x 2013		2011 x 2012		2011 x 2013		2012 x 2013	
	t	Perm *p*	t	Perm *p*	t	Perm *p*	t	Perm *p*	t	Perm *p*	t	Perm *p*
SA total density	1.65	0.27	1.88	0.06	2.95	0.01*	1.65	0.08	3.11	< 0.01*	2.55	< 0.01*
SA max height	6.53	< 0.01*	5.29	< 0.01*	6.70	< 0.01*	3.70	< 0.01*	1.59	0.16	2.58	0.04*
AG total density	0.81	0.43	0.83	0.45	0.86	0.44	1.99	0.07	2.11	0.06	0.45	0.67
AG seedling density	0.79	0.44	0.79	0.45	0.83	0.43	1.94	0.08	2.07	0.07	0.38	0.72
AG short shrub density	1.47	0.19	4.60	< 0.01*	4.48	< 0.01*	4.51	< 0.01*	4.39	< 0.01*	0.90	0.42
AG tall shrub density	1.00	0.52	1.00	0.52	1.00	0.52	- - -	- - -	- - -	- - -	- - -	- - -
AG max height	1.48	0.18	2.39	0.03*	2.69	0.02*	2.59	0.03*	2.69	0.01*	0.90	0.39

Fertilized plots

Year:	2010 x 2011		2010 x 2012		2010 x 2013		2011 x 2012		2011 x 2013		2012 x 2013	
	t	Perm *p*	t	Perm *p*	t	Perm *p*	t	Perm *p*	t	Perm *p*	t	Perm *p*
SA total density	2.28	0.04*	2.88	< 0.01*	4.07	< 0.01*	0.83	0.51	2.26	0.02*	1.46	0.13
SA max height	8.42	< 0.01*	4.19	< 0.01*	3.77	< 0.01*	2.69	0.03*	3.55	< 0.01*	0.69	0.51
AG total density	0.37	0.76	0.59	0.59	0.83	0.44	0.62	0.59	0.99	0.39	0.66	0.59
AG seedling density	0.34	0.77	1.54	0.15	2.10	0.06	1.61	0.13	2.45	0.03*	1.46	0.18
AG short shrub density	4.47	< 0.01*	3.46	< 0.01*	4.40	< 0.01*	3.17	0.01*	3.93	< 0.01*	1.03	0.42
AG tall shrub density	- - -	- - -	2.38	0.04*	4.15	< 0.01*	2.38	0.04*	4.15	< 0.01*	2.47	0.03*
AG max height	5.30	< 0.01*	4.52	< 0.01*	5.21	< 0.01*	3.57	< 0.01*	4.58	< 0.01*	2.54	0.03*

Avicennia germinans maximum height in fertilized plots was significantly higher than the control in all years except the first sampling event (2010; Figure 4.1 c; Tables 4.3 – 4.5). A strong temporal trend was evident in fertilized plots as maximum height of fertilized *A. germinans* significantly increased each year of the experiment (Figure 4.1c and Table 4.5). In control plots, *A. germinans* maximum height also increased over time, but by a much smaller margin than the fertilized counterparts; *A. germinans* maximum height significantly increased only between 2011 and 2012 (Figure 4.1 c; Table 4.2). Maximum height was the only measured *S. alterniflora* parameter that significantly differed between nutrient treatments. Fertilized *S. alterniflora* were significantly taller than in control plots in all years following the first sampling event (Figure 4.1 d; Tables 4.3, 4.4). In both control and fertilized plots, *Spartina* was significantly taller in the first year (2010) than the subsequent sampling years (Figure 4.1 d; Table 4.5).

4.4 Discussion

4.4.1 Species responses to nutrient addition

Throughout four growing seasons of continuous enrichment, *S. alterniflora* leaf nutrient content was unchanged, whereas *A. germinans* leaf nutrient metrics, particularly those containing nitrogen, varied between treatment plots. The positive fertilization responses in this study's *A. germinans* leaves are similar to other mangrove-focused nutrient addition studies (e.g., Feller et al. 2007, Naidoo 2009). However, the lack of enrichment response in *S. alterniflora* leaf nutrient contents within this study does not correspond with other *S. alterniflora* fertilization studies which have reported significant increases in nutrient concentrations (Pennings et al. 2002, Darby and Turner 2008a).

Individuals of both species were significantly taller in fertilized plots relative to controls in all years except 2010. Maximum height was the only *S. alterniflora* parameter that significantly responded to the nutrient enrichment treatment. An increase in height following fertilization is a common outcome in other *S. alterniflora* enrichment studies (e.g., Valiela et al. 1978, Buresh et al. 1980) as well as within grassland studies (e.g., Bloor et al. 2008). *A. germinans* maximum height was also positively affected by

nutrient addition, and, in fertilized plots, increased each year. In contrast, control *A. germinans* maximum height increased only slightly, with a significant increase only between years two (2011) and three (2012). The small increase in height within control plots is most likely characteristic of typical canopy growth patterns in this region.

It should be noted that a drought occurred in Texas in 2011, during the second growing season (Nielsen-Gammon 2012). This drought was linked to the reduction of emergent vegetation height in other Texas salt marshes (Kinney et al. 2014), and potentially may have influenced the significant decrease in *S. alterniflora* maximum height within this study. *Spartina alterniflora* heights in subsequent sampling events were more similar to the drought year (2011) than pre-drought (2010), suggesting a continued drought effect. However, *S. alterniflora* is quite resilient to droughts and generally show no difference in density and percent cover in growing seasons following a drought (Armitage, unpublished data). Additionally, an even more severe drought occurred along the Texas coast in 2009 (Nielsen-Gammon 2012), the year prior to the start of the enrichment experiment. If drought effects manifest for subsequent growing seasons, and if 2009 was a record-setting drought for Texas coastal habitats, then it seems unlikely 2010 would have had the tallest *S. alterniflora* heights recorded. Therefore, the decreased *S. alterniflora* heights within this study may have been less related to latent drought effects and more related to other factors such as limit limitation by increased *A. germinans* canopy height.

Spartina alterniflora and A. germinans (total and each size class) densities for each treatment varied between years but not between treatments of individual sampling events. *Spartina alterniflora* density decreased throughout the study, suggesting marsh loss or displacement over time. Nearly each year there were significantly fewer *S. alterniflora* shoots in fertilized plots, whereas the decrease in control plots was slower to manifest and density only significantly decreased between the last two sampling events (2012 – 2013). Density had high variability, particularly in control *S. alterniflora* which may have manifested from the subquadrat method of estimating densities which was not utilized in all plots. Mangroves of each size class within control plots had very little

annual variation. However, the total number of *A. germinans* seedlings in enriched plots decreased over time, suggesting that individuals grew into the next size class in response to fertilization.

4.4.2 Implications for mangrove encroachment

These results reveal that within fertilized plots, as the enrichment period progressed, there were more mangroves classified as short and tall shrubs. The positive responses in mangrove height suggest a mechanism by which nutrient enrichment may facilitate mangrove encroachment. Tree seedlings generally have low growth rates and neighboring grass species can suppress seedling growth and reduce survivability (Patterson et al. 1993, Coll et al. 2004). However, once seedlings reach a height that surpasses neighboring plants, growth rates increase. As trees become taller, the growth suppression from grass species diminishes (Hill et al. 1995, Guo et al. 2013). In fact, as tree height increases, a reversal in growth suppression can occur. Taller trees are correlated with wider canopies and greater aboveground biomass, thereby increasing competition for light, nutrient resources, and space, particularly with herbaceous plant competitors (Scholes and Archer 1997, Smith and Whelan 2006, Eldridge et al. 2011).

At the beginning of this enrichment experiment, using maximum height as a proxy for canopy height, *A. germinans* in both control and fertilized plots were shorter than *S. alterniflora*. In subsequent sampling events, *S. alterniflora* and *A. germinans* maximum heights were relatively similar in control plots. However, fertilization promoted mangrove growth, and by the end of the second growing season, *A. germinans* was taller than *S. alterniflora*. Once mangrove height emerged from the *S. alterniflora* canopy, growth rates may have increased, as manifested by more trees entering taller size classes than control plots after each growing season. Nutrient enrichment promoted *A. germinans* growth and produced a higher quantity of taller mangroves sooner than in ambient conditions. Therefore, nutrient addition may accelerate a shift in mangrove population size distribution, potentially leading to a faster decrease in *S. alterniflora* density.

4.4.3 Positive feedback

Paired effects, such as increased precipitation and nitrogen deposition (Köchy and Wilson 2001) or increased carbon dioxide and nitrogen enrichment (Ratajczak et al. 2011), have facilitated terrestrial woody plant expansion into grasslands. Woody encroachment is often linked to a large-scale exogenic driver (e.g., raised CO_2 levels) which changes the competitive advantage in favor of the woody plant (D'Odorico et al. 2012). Some local endogenic factors (e.g., grazing) can act as a positive feedback, perpetuating the effects of the exogenic driver (D'Odorico et al. 2012). Here I propose that reduced freezing events act as the exogenic driver of mangrove encroachment, and that increased nutrient resource availability is an endogenic factor.

Based on these results, nutrient enrichment could perpetuate climate-driven mangrove encroachment within the marsh-mangrove ecotone. I hypothesize the following pathway: 1) decreased duration and frequency of freezing events will reduce mangrove diebacks, thereby promoting mangrove growth and stand expansion into salt marsh dominated areas, 2) nutrient enrichment will stimulate mangrove growth, particularly canopy height, reducing any negative interactions from neighboring marsh plants and augmenting the ability to outcompete marsh plants for resources such as space and light, reducing marsh plant growth and distribution, 3) reduced marsh plant cover will lessen growth suppression of mangrove seedlings, thereby increasing seedling establishment, survival, and growth, leading to mangrove stand expansion.

4.4.4 Conclusions

In this study, *S. alterniflora* and *A. germinans* were fertilized in naturally co-occurring plots in a coastal area currently experiencing mangrove encroachment. *Avicennia germinans* responded positively to fertilization with increases in leaf nutrient contents and maximum height. *Spartina alterniflora* maximum height was positively influenced by nutrient treatment, but no other parameters were significantly affected by fertilization. After four growing seasons, *S. alterniflora* density was reduced in both control and fertilized plots, with fertilized plots decreasing in density after the first

growing season. Most notably, densities of mangroves in larger size classes (i.e., short and tall shrubs) significantly increased in fertilized plots over the course of the experiment. Contrary to predictions, this trend was constant over time, indicating that *S. alterniflora* did not inhibit *A. germinans* growth within the plots. These results establish that nutrient enrichment enhanced *A. germinans* growth, which produced a higher quantity of taller mangroves in fertilized plots. The increase in the number and height of shrubs suggests that additional nutrient resources could facilitate mangrove stand growth and expansion, subsequently accelerating marsh grass displacement.

In many woody encroachment scenarios, various factors, such as nutrient enrichment, have been presented as additive effects that perpetuate woody vegetation establishment and expansion. Increased minimum winter temperatures and reduced lethal freezing events are the main drivers of mangrove expansion (Osland et al. 2013, Cavanaugh et al. 2014), but additional nutrient resources may facilitate this transition. I propose that nutrient enrichment serves as a positive feedback for mangrove encroachment into salt marshes by increasing mangrove canopy height, therefore augmenting competitive shading of salt marsh plants. Mangrove encroachment and many other of the displaced marsh plants are considered foundation species (Osland et al. 2013); a shift in species dominance could have a substantial impact on ecosystem structure and function (Dayton 1972). Shifts within ecotones, such as woody encroachment into grass-dominated habitats, are sensitive to global changes, such as elevated temperatures and CO_2 levels (Risser 1995, Grimm et al. 2013). It is important to identify and understand factors serving as feedbacks in order to better predict how ecosystem components will be influenced in various global change scenarios.

CHAPTER V
CONCLUSION

Freezing temperatures play a major role in governing herbaceous and woody coverage within the marsh-mangrove ecotone by causing major mangrove damage and death, thereby creating gaps for marsh vegetation and maintaining mixed plant composition (Stevens et al. 2006, Friess et al. 2012). Recent (past 50 years) increases in mangrove stand sizes within the ecotone have largely been attributed to the reduced frequency and severity of freezing events (Osland et al. 2013, Cavanaugh et al. 2014). However, other environmental conditions may also influence the composition of this ecotone by negatively or positively impacting mangrove encroachment. Because these coastal systems are susceptible to anthropogenic nutrient enrichment (Boesch 2002), I investigated how added nutrient resources might influence this vegetation shift. I assessed nutrient dynamics by fertilizing naturally, co-occurring *Spartina alterniflora* (smooth cordgrass) and *Avicennia germinans* (black mangrove) stands within the Northern Gulf of Mexico marsh-mangrove ecotone. I measured above- and belowground plant metrics and assessed plot-level dynamics to determine if nutrients would favor herbaceous or woody vegetation and to investigate how enrichment may alter mangrove encroachment and subsequent marsh displacement.

5.1 Overall findings

Both *S. alterniflora* and *A. germinans* responded positively to nutrient enrichment within monotypic stands (e.g., Pennings et al. 2002, Feller et al. 2007). In other studies within the marsh-mangrove ecotone, *S. alterniflora* has reduced *A. germinans* growth and survivability (McKee and Rooth 2008, Perry and Mendelssohn 2009, Simpson et al. 2013). Therefore, I expected that *S. alterniflora* growth would positively respond to enrichment, whereas *A. germinans* would have minimal to no nutrient response. I further hypothesized that fertilized conditions would augment *S. alterniflora* productivity, leading to decreased *A. germinans* growth and survivability,

and thereby slowing mangrove encroachment. However, I found the opposite to be true: my results suggest that nutrient enrichment promotes *A. germinans* growth, which could subsequently lead to accelerated mangrove stand expansion and subsequent displacement of *S. alterniflora*.

Nearly all *A. germinans* plant metrics were significantly different between control and fertilized plots, indicating that nutrient enrichment facilitated growth in *A. germinans*. Maximum *A. germinans* height was taller and above- and belowground biomass were greater in enriched conditions. Additionally, *A. germinans* leaf carbon, nitrogen, phosphorus, and chlorophyll *a* concentrations were elevated in fertilized plots, indicating higher nutrient storage and photosynthetic potential (Lovelock and Feller 2003). Larger leaf surface area in enriched *A. germinans* could also indicate higher photosynthetic rates because of increased light absorption potential (Lovelock et al. 2004).

Conversely, *S. alterniflora* plant metrics had very little response to nutrient enrichment. Leaf nutrient contents, chlorophyll *a* levels, aboveground biomass, belowground biomass, and biomass allocations did not differ between treatments. Maximum height and leaf surface area, however, were significantly greater in fertilized plots. The differences in these metrics suggest that *S. alterniflora* may also be responding positively to nutrient enrichment. Although maximum height and leaf surface area were larger in fertilized plots, total aboveground biomass was not different, due to a decrease in stem density. Therefore, the shoot elongation and greater leaf area of enriched *S. alterniflora* may be more of a response to increased shading by the taller *A. germinans* present within the fertilized plots.

Plot wide metrics also indicated that nutrient enrichment favors mangrove encroachment. Total *S. alterniflora* and *A. germinans* density did not differ between control and fertilized plots, but mangrove size distribution shifted with nutrient addition. After four growing seasons of continued enrichment, there were significantly more mangroves taller than 50 cm in fertilized plots than in control plots. There was significantly lower *S. alterniflora* density in both treatments from the first to the fourth

growing season. Although high variability within each sampling event obscured fertilization treatment effects, temporal trends were present and *S. alterniflora* density decreased in fertilized plots nearly every growing season.

Collectively, these data indicate that *A. germinans* growth was facilitated by nutrient enrichment, whereas *S. alterniflora* exhibited few fertilization responses. More specifically, mangrove height distribution significantly increased within fertilized plots, potentially augmenting its ability to displace neighboring marsh plants. Mangrove height is positively correlated with canopy width, which can greatly increase light interception for vegetation at lower canopy levels (Smith and Whelan Peltzer and Köchy 2001, 2006, Stevens et al. 2006). These results suggest, contrary to previous studies, that *A. germinans* growth may benefit more from nutrient enrichment than *S. alterniflora* in the marsh-mangrove ecotone. Although nutrient enrichment is not the main factor driving mangrove encroachment, fertilization may have the potential to accelerate the increase in mangrove coverage and subsequent marsh displacement.

5.2 Mangrove height implications

Mangrove height, or specifically height thresholds, may be the component that can best explain how nutrient enrichment influences this vegetation shift. Mangrove heights above a certain threshold can increase mangrove tree resiliency (ability to recover) from freeze damage (Osland et al. 2015). Negative marsh neighbor effects that reduce mangrove growth and survivability (Patterson et al. 1993, Simpson et al. 2013) are also lessened or even reversed after mangroves exceed a certain height (Guo et al. 2013). Therefore, mangroves may have species specific height thresholds where freezing conditions and competitor effects have lowered negative impacts on mangrove growth and survivability.

In terrestrial systems, when a tree surpasses a height threshold, its ability to withstand deleterious effects from disturbances such as fire strengthens; this threshold is referred to as its "escape height" (Bond 2008). I propose that the "escape height" concept can also be applied to mixed stands within the marsh-mangrove ecotone.

Nutrient addition, by increasing mangrove size distribution, can drive mangroves to their "escape height" faster than in control conditions. Reaching these threshold heights will reduce negative impacts from freezing temperatures and neighboring plants. Therefore, enriched conditions could reverse mangrove growth suppression, diebacks, and seedling mortality, subsequently facilitating mangrove stand establishment and accelerating expansion (Figure 5.1).

5.3 Ecological implications

A shift from marsh to mangrove vegetation, which may be augmented by anthropogenic nutrient enrichment, could have large implications for ecosystem processes. Mangrove systems tend to have higher carbon sequestration rates than salt marshes (Bianchi et al. 2013, Saintilan and Rogers 2015). Mangrove dominated areas also tend to have greater rates of accretion and elevation gain, and higher soil shear strength, potentially reducing erosion and increasing coastal resiliency in response to near-term sea level rise (Rogers et al. 2005, Comeaux et al. 2012). Although decomposition rates are similar between marsh and mangrove areas, nutrient enrichment increases mangrove leaf litter quality and decomposition rates, potentially contributing more to the detrital food web (Perry and Mendelssohn 2009, Keuskamp et al. 2015).

Although marsh and mangrove habitats often have similar abundance and richness in marine fauna (e.g., crustaceans, birds, and fish), these systems support different assemblages with little overlap (Guest and Connolly 2004, Mazumder et al. 2006; unpublished data). This could be a consequence of different habitat usage or related more to the food web, as dietary carbon sources can be distinct between marshes and mangroves with little mixing, even in transitional areas (Guest and Connolly 2004). An increase in mangrove cover could have particularly negative effects on salt-marsh dependent, commercially important species such as brown shrimp (NOAA 2015).

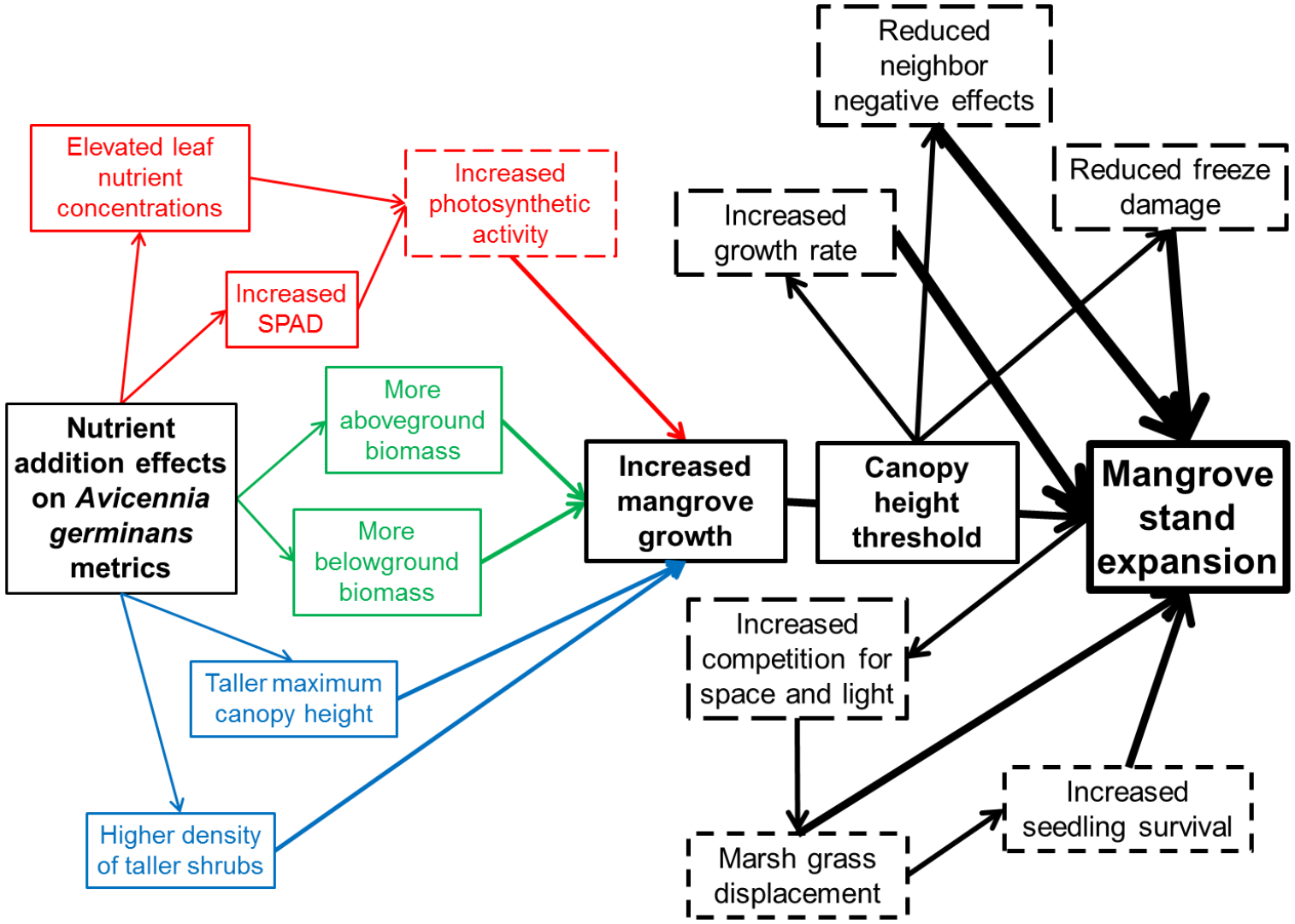

Figure 5.1 A conceptual diagram illustrating how nutrient addition may facilitate mangrove stand expansion.

5.4 Management implications

Within marsh-mangrove ecotones worldwide, mangroves are increasing in coverage. As the climate warms, and the severity and duration of freezing events declines, this encroachment is likely to continue, leading to ecosystem shifts in structure and function. Understanding the mechanisms of this coastal woody encroachment and its impact on ecosystem processes is needed, particularly for those making coastal management decisions. My dissertation results provide insight into how nutrient enrichment may facilitate this increase in coastal woody plant coverage. Areas within the marsh-mangrove ecotone that have higher potential for anthropogenic nutrient enrichment could be more susceptible to mangrove encroachment and implementation of management strategies such as reduction in nutrient loading rates, may need to be prioritized.

5.5 Concluding remarks

Anthropogenic nutrient enrichment is not the main driver of mangrove encroachment within the marsh-mangrove ecotone. Large scale factors such as freezing temperatures and elevated atmospheric CO_2 levels have a substantial impact on coastal herbaceous and woody plant composition (Osland et al. 2013, Saintilan and Rogers 2015). However, mangrove canopy height may increase and reach the "escape height" more rapidly in enriched conditions. Mangrove coverage will then proliferate because taller mangroves have enhanced competitive advantages for light, diminished growth reduction from neighboring marsh plants, and higher resiliency from freeze damage. Therefore, nutrient enrichment can be considered a positive feedback for mangrove stand expansion, as it can further perpetuate climate-driven woody encroachment within the marsh-mangrove ecotone.

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