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Decade-scale Nutrient Enrichment Effects on Wetland Plant Community Structure, Function, and Stability

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DECADE-SCALE NUTRIENT ENRICHMENT EFFECTS ON WETLAND PLANT COMMUNITY STRUCTURE, FUNCTION, AND STABILITY

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ABSTRACT

Human activities have increased the supply of nitrogen (N) and phosphorus (P) to coastal waters worldwide, threatening coastal wetlands with excess nutrient loading and subsequent eutrophication. In this dissertation, I present results from two decade-scale fertilization experiments in a *Sagittaria lancifolia* dominated oligohaline marsh that examined the species-, community-, and ecosystem-level effects of nutrient enrichment. My objectives were to determine (1) which nutrient limits primary production, (2) how increased supply of the limiting nutrient affects plant community structure and function, both above- and belowground, and (3) whether nutrient over-enrichment compromises ecosystem stability. Overall, significant changes in plant growth occurred with N enrichment only. Aboveground, N enrichment stimulated primary production and altered plant tissue nutrient ratios, nutrient resorption efficiencies, and species dominance. Belowground, excess N simultaneously increased live root biomass accumulation in unexploited soil and reduced *in situ* live root standing crop. The rate of marsh elevation change was unaffected by nutrient enrichment due to an apparent compensatory effect on marsh accretionary processes whereby nutrient-induced shallow subsidence, attributed to reduced live root standing crop, was balanced by nutrient-enhanced soil accretion resulting from greater organic matter accumulation at the soil surface. In addition, the structural integrity of the soil matrix did not deteriorate under elevated nutrient conditions; decomposition rates were similar to control plots, and although root standing crop was reduced, the root system were evidently stronger as soil shear strength tended to increase rather than decrease. Based on these results, I conclude that this oligohaline marsh is N-limited, and that N enrichment beyond the assimilation capacity of the vegetation drives changes in plant community structure and function caused by altered plant nutrient cycling. Eutrophic conditions were both beneficial and

detrimental to ecosystem function, and therefore represent an unlikely destabilizing mechanism in this coastal marsh and possibly others due to counterbalancing effects on plant growth above and below the soil surface. However, additional long-term research is required in a diverse range of habitats and environmental settings before broad-based, general conclusions concerning the effects of nutrient enrichment on coastal wetland stability can be made with a high degree of certainty.

CHAPTER 1
INTRODUCTION

1.1 Introduction

Coastal wetlands provide a wide variety of ecologically and economically important services including nutrient removal from surface waters, carbon sequestration, storm protection for coastal communities, and habitat for numerous species of birds, invertebrates, fish, and mammals (Barbier *et al*. 2011). However, these environments are becoming increasingly impacted by human population growth, for example, loss rates currently range from 1 to 3% yr^{-1} (Pendleton *et al*. 2012) as urbanization, industrial expansions, and agricultural activities increase (MEA 2005). In addition, anthropogenic sources have more than doubled the global supply rates of nitrogen (N) and phosphorus (P), and nearly tripled their delivery to coastal waters (Meybeck 1982, Bennett *et al.* 2001, Green *et al*. 2003, Schlesinger 2009), creating the foremost water quality problem affecting estuarine ecosystems worldwide (Bricker *et al.* 2008, MEA 2005, Diaz & Rosenberg 2008, Smith & Schindler 2009).

Although coastal wetlands are widely recognized for their capacity to assimilate, transform, and bury nutrients (DeLaune *et al*. 1981, White & Howes 1994, Sundareshwar & Morris 1999, Davis *et al.* 2004, Drake *et al.* 2009, Koop-Jakobsen & Giblin 2010), excess nutrient loading threatens to alleviate nutrient limitation in these systems, and thereby compromise wetland plant community structure, function, and stability. However, nutrient enrichment may differentially influence coastal wetlands depending on type and related nutrient limitation status (DiTommaso & Aarssen 1989, Bedford *et al.* 1999). Previous research has established that plant growth in brackish and salt marshes is primarily N limited (Tyler 1967, Sullivan & Daiber 1974, Valiela & Teal 1974, Patrick & Delaune 1976, Jefferies & Perkins

1977, Mendelssohn 1979, Boyer *et al.* 2001, Wigand *et al.* 2004, Crain 2007), whereas mangroves, another coastal wetland community, can be limited by either N, P, or both (McKee *et al.* 2007, Feller *et al.* 2003, Castañeda-Moya *et al.* 2011). In comparison, there has been only one assessment of nutrient limitation in oligohaline wetlands (Crain 2007). Yet, it is these low salinity wetlands that can dominate the landscape where large rivers deliver freshwater, and elevated nutrient loads, to coastal environments, such as in the Mississippi River Delta (Sasser *et al.* 2008). Hence, general conclusions concerning nutrient limitation and the consequences of enhanced nutrient supply are lacking.

Unlike salt marshes and mangroves that contain relatively few plant species, oligohaline wetlands are typically highly diverse communities (Visser *et al.* 1998). However, evidence from numerous ecosystems shows that as nutrient availability increases, species richness declines (Smith *et al.* 1999, Bedford *et al.* 1999, Bobbink *et al.* 2010). These shifts in community composition often result in the dominance of a few highly productive species (Suding *et al.* 2005), where under eutrophic conditions nutrient-aggressive or invasive plants reduce species richness by displacement (Chambers *et al.* 1999, Silliman & Bertness 2004, Tyler *et al.* 2007, Bobbink *et al.* 2010). Consequently, increased plant biomass resulting from greater nutrient inputs could modify biological diversity and result in unanticipated or unwanted changes to the valuable services that these coastal wetlands provide.

Moreover, as global climate change threatens coastlines around the world with inundation caused by sea level rise and increasing storm intensity (IPCC 2007, Knutson *et al*. 2010), ecosystem stability may be further compromised by increased nutrient loading. The effects of nutrient enrichment on belowground plant growth are of particular concern due to the important role it has in regulating soil organic matter accumulation (Rasse *et al.* 2005), microbial biomass

(Fierer *et al.* 2009), and biogeochemical cycling (Freschet *et al.* 2013). For instance, the nutrient-drowning hypothesis, proposed by Turner *et al.* (2009), predicts that eutrophic conditions contribute to coastal wetland inundation by reducing the rate of soil organic matter accumulation. This implication highlights the importance of understanding how nutrient enrichment alters complex feedbacks that occur in the soil environment. Plant production influences wetland stability directly through mineral sediment and organic matter accumulation (Hatton *et al.* 1983, Nyman *et al.* 1990, Calloway *et al.* 1996, Turner *et al.* 2004), and indirectly through feedbacks on microbial nutrient cycling (Jordan *et al.* 1989, DeLaune & Patrick 1990, VanZomeren *et al.* 2013). Consequently, the effects of nutrient enrichment on plant growth and subsequent decay may affect elevation dynamics (Morris *et al.* 2002, Cahoon *et al.* 2004, McKee *et al.* 2007, Day *et al.* 2011, Baustian *et al.* 2012) and soil shear strength (Howes et al. 2010, Turner 2011), which in turn, may compromise the ability of coastal wetlands to keep pace with sea level rise and resist the erosive forces of extreme meteorologic events. However, the link between nutrient enrichment, altered function, and ecosystem stability is currently not well established due to limited data from long-term experiments and inconsistent findings from investigations thus far. Therefore, additional information is required to provide more comprehensive answers to fundamental questions concerning the future stability of coastal wetlands as the climate changes.

1.2 Research Objectives

The overall goals of this research were to (1) identify the species-, community-, and ecosystem-level effects of nutrient enrichment and (2) determine how nutrient enrichment affects plant community structure, function, and stability. To address these objectives, I carried out two long-term fertilization experiments in an oligohaline (i.e., intermediate-brackish) marsh located on the north shore of Lake Pontchartrain, LA, USA (Figure 1.1). In one study, I enriched

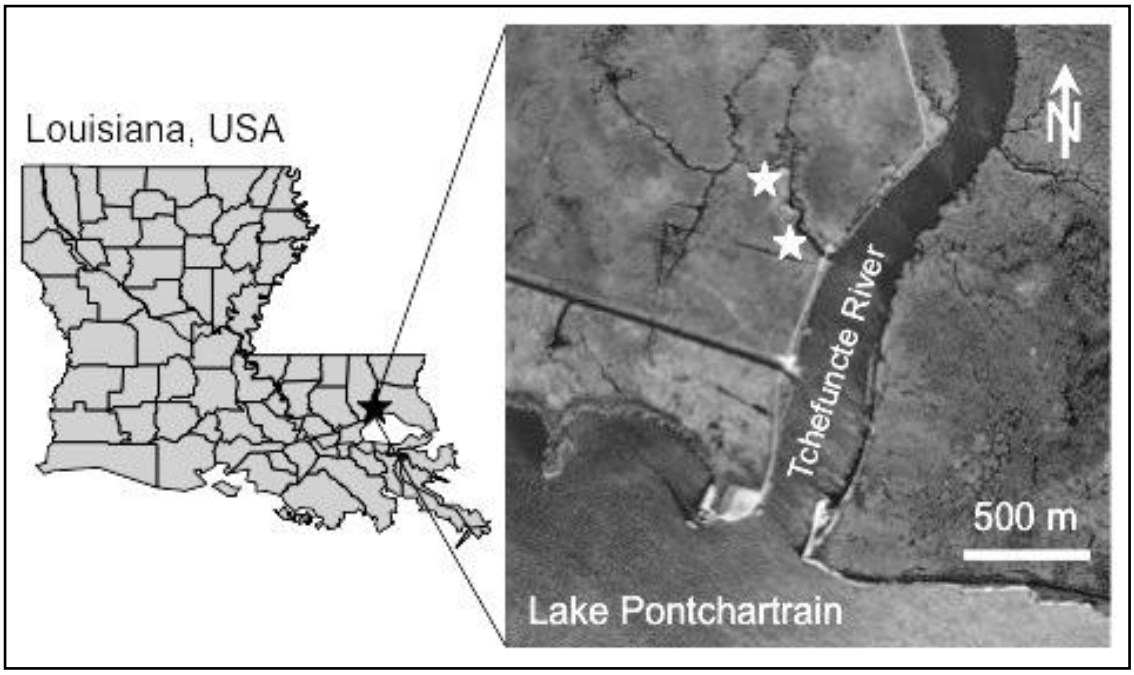

Figure 1.1 Site location map. White stars identify study sites.

experimental plots for nine years with one of four levels of N (0, 50, 200, or 1200 kg N ha^{-1} yr^{-1}) in combination with one of two levels of P (0 or 131 kg P ha^{-1} yr^{-1}), while in a companion study, I fertilized a nearby location within the same contiguous marsh for 13 years with three levels of N-P- Potassium (K) (unfertilized control; medium: 200 kg N ha^{-1} yr^{-1}, 51 kg P ha^{-1} yr^{-1}, and 99 kg K ha^{-1} yr^{-1}; and high: 1200 kg N ha^{-1} yr^{-1}, 306 kg P ha^{-1} yr^{-1}, and 594 kg K ha^{-1} yr^{-1}). My specific research questions included:

1. Which nutrient or nutrient combination limits oligohaline marsh primary production?
2. How does increased supply of the limiting nutrient(s) affect plant community structure and function, both above- and belowground?
3. Does nutrient enrichment compromise ecosystem stability in relation to global climate change?

I hypothesized that (1) N limits plant growth, but P becomes secondarily limiting at high N loading rates, (2) nutrient enrichment will elicit diverse effects on some aspects of ecosystem

structure and function (e.g., enhanced aboveground production, reduced belowground standing crop, altered plant nutrient cycling and community composition), but not others (e.g., organic matter decomposition), and (3) the effects of nutrient enrichment on ecosystem function will negatively impact soil shear strength while surface elevation change will remain unaffected.

1.3 Synopsis of Chapters

My primary research questions and over-arching hypotheses were addressed in three chapters. Chapter 2 investigates the effects of multiple levels of N and/or P enrichment on aboveground net primary production, plant nutrient cycling, and community composition, and identifies the nutrient that limits plant growth. Chapter 3 follows by determining how excess N and/or P loading affects belowground plant growth. In a separate experiment, Chapter 4 identifies the effects of combined N-P-K enrichment on a suite of belowground processes that regulate soil surface elevation change and soil shear strength (i.e., wetland stability), and determines whether eutrophic conditions compromises the ability to keep pace with sea level rise and resist erosion. A summary of my results, overall conclusions, and their management implications are presented in Chapter 5.

1.4 References

Barbier EB, Hacker SD, Kennedy C, Koch EW, Stier AC, Silliman BR (2011) The value of estuarine and coastal ecosystem services. *Ecol Monogr* 81:169-193.

Baustian JJ, Mendelssohn IA, Hester MW (2012) Vegetation's importance in regulating surface elevation in a coastal salt marsh facing elevated rates of sea level rise. *Glob Change Biol* 18:3377-3382.

Bedford BL, Walbridge MR, Aldous A (1999) Patterns in nutrient availability and plant diversity of temperate North American wetlands. *Ecology* 80:2151-2169.

Bennett EM, Carpenter SR, Caraco NF (2001) Human impact on erodable phosphorus and eutrophication: A global perspective. *Bioscience* 51:227-234.

Bobbink R, Hicks K, Galloway J, Spranger T, Alkemade R, Ashmore M, Bustamante M, Cinderby S, Davidson E, Dentener F, Emmett B, Erisman JW, Fenn M, Gilliam F, Nordin A, Pardo L, De Vries W (2010) Global assessment of nitrogen deposition effects on terrestrial plant diversity: a synthesis. *Ecol Appl* 20:30-59.

Boyer KE, Fong P, Vance RR, Ambrose RF (2001) *Salicornia virginica* in a southern California salt marsh: seasonal patterns and a nutrient-enrichment experiment. *Wetlands* 21:315-326.

Bricker SB, Longstaff B, Dennison W, Jones A, Boicourt K, Wicks C, Woerner J (2008) Effects of nutrient enrichment in the nation's estuaries: a decade of change. *Harmful Algae* 8:21-32.

Cahoon DR, Ford MA, Hensel PF (2004) Ecogeomorphology of *Spartina patens*-dominated tidal marshes: soil organic matter accumulation, marsh elevation dynamics, and disturbance. In: Faghaerazzi S, Marani M, Blum LK (eds) *The Ecogeomorphology of Tidal Marshes*. American Geophysical Union Coastal and Estuarine Studies, Volume 59, Washington, DC, p 247-265.

Callaway JC, Nyman JA, DeLaune RD (1996) Sediment accretion in coastal wetlands: a review and a simulation model of processes. *Curr Top Wetland Biochemistry* 2:2-23.

Castañeda-Moya E, Twilley RR, Rivera-Monroy VH, Marx BD, Coronado-Molina C, Ewe SML (2011) Patterns of root dynamics in mangrove forests along environmental gradients in the Florida coastal Everglades, USA. *Ecosystems* 14:1178-1195.

Chambers RM, Meyerson LA, Saltonstall K (1999) Expansion of *Phragmites australis* into tidal wetlands of North America. *Aquat Bot* 64:261-273.

Crain CM (2007) Shifting nutrient limitation and eutrophication effects in marsh vegetation across estuarine salinity gradients. *Estuaries Coasts* 30:26-34.

Davis JL, Nowicki B, Wigand C (2004) Denitrification in fringing salt marshes of Narragansett Bay, Rhode Island, USA. *Wetlands* 24:870-878.

Day JW, Kemp GP, Reed DJ, Cahoon DR, Boumans RM, Suhayda JM, Gambrell R (2011) Vegetation death and rapid loss of surface elevation in two contrasting Mississippi delta salt marshes: The role of sedimentation, autocompaction and sea-level rise. *Ecol Eng* 37:229-240.

Delaune RD, Patrick WH (1990) Nitrogen cycling in Louisiana Gulf Coast brackish marshes. *Hydrobiologia* 199:73-79.

DeLaune RD, Reddy CN, Patrick WH (1981) Accumulation of plant nutrients and heavy metals through sedimentation processes and accretion in a Louisiana salt marsh. *Estuaries* 4:328-334.

Diaz RJ, Rosenberg R (2008) Spreading dead zones and consequences for marine ecosystems. *Science* 321:926-929.

DiTommaso A, Aarssen LW (1989) Resource manipulations in natural vegetation: a review. *Vegetatio* 84:9-29.

Drake DC, Peterson BJ, Galván KA, Deegan LA, Hopkinson C, Johnson JM, Koop-Jakobsen K, Lemay LE, Picard C (2009) Salt marsh ecosystem biogeochemical responses to nutrient enrichment: a paired 15N tracer study. *Ecology* 90:2535-2546.

Green PA, Vörösmarty CJ, Meybeck M, Galloway JN, Peterson BJ, Boyer EW (2004) Pre-industrial and contemporary fluxes of nitrogen through rivers: a global assessment based on typology. *Biogeochemistry* 68:71-105.

Feller IC, McKee KL, Whigham DF, O'Neill JP (2003) Nitrogen vs. phosphorus limitation across an ecotonal gradient in a mangrove forest. *Biogeochemistry* 62:145-175.

Fierer N, Strickland MS, Liptzin D, Bradford MA, Cleveland CC (2009) Global patterns in belowground communities. *Ecol Lett* 12:1238-1249.

Freschet GT, Cornwell WK, Wardle DA, Elumeeva TG, Liu W, Jackson BG, Onipchenko VG, Soudzilovskaia NA, Tao J, Cornelissen JHC (2013) Linking litter decomposition of above- and below-ground organs to plant–soil feedbacks worldwide. *J Ecol* 101:943-952.

Hatton RS, Delaune RD, Patrick WH (1983) Sedimentation, accretion, and subsidence in marshes of Barataria Basin, Louisiana. *Limnol Oceanogr* 28:494-502.

Howes NC, FitzGerald DM, Hughes ZJ, Georgiou IY, Kulp MA, Miner MD, Smith JM, Barras JA (2010) Hurricane-induced failure of low salinity wetlands. *Proc Natl Acad Sci USA* 107:14014-14019.

IPCC [Intergovernmental Panel on Climate Change] (2007) Climate change 2007: the physical science basis. Contributions of working group I to the fourth assessment report of the intergovernmental panel on climate change. In: Solomon S, Qin D, Manning M, Chen Z, Marquis M, Averyt KB, Tignor M, Miller HL (eds). Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.

Jefferies RL, Perkins N (1977) The effects on the vegetation of the additions of inorganic nutrients to salt marsh soils at Stiffkey, Norfolk. *J Ecol* 65:867-88.

Jordan TE, Whigham DF, Correll DL (1989) The role of litter in nutrient cycling in a brackish tidal marsh. *Ecology* 70:1906-1915.

Koop-Jakobsen K, Giblin AE (2010) The effect of increased nitrate loading on nitrate reduction via denitrification and DNRA in salt marsh sediments. *Limnol Oceanogr* 55:789-802.

Knutson TR, McBride JL, Chan J, Emanuel K, Holland G, Landsea C, Held I, Kossin JP, Srivastava AK, Sugi M (2010) Tropical cyclones and climate change. *Nat Geosci* 3:157-163.

McKee KL, Cahoon DR, Feller IC (2007) Caribbean mangroves adjust to rising sea level through biotic controls on change in soil elevation. *Glob Ecol Biogeogr* 16:545-556.

MEA [Millennium Ecosystem Assessment] (2005) *Ecosystems and human well-being: synthesis*. World Resources Institute, Washington, DC.

Mendelssohn IA (1979) Influence of nitrogen level, form, and application method on the growth-response of *Spartina alterniflora* in North Carolina. *Estuaries* 2:106-112.

Meybeck M (1982) Carbon, nitrogen, and phosphorus transport by world rivers. *Am J Sci* 282:401-450.

Morris JT, Sundareshwar PV, Nietch CT, Kjerfve B, Cahoon DR (2002) Responses of coastal wetlands to rising sea level. *Ecology* 83:2869-2877.

Nyman JA, Delaune RD, Patrick WH (1990) Wetland soil formation in the rapidly subsiding Mississippi River Deltaic Plain: mineral and organic matter relationships. *Estuar Coast Shelf Sci* 31:57-69.

Patrick WH, Delaune RD (1976) Nitrogen and phosphorus utilization by Spartina alterniflora in a salt marsh in Barataria Bay, Louisiana. *Estuar Coast Mar Sci* 4:59-64.

Pendleton L, Donato DC, Murray BC, Crooks S, Jenkins WA, Sifleet S, Craft C, Fourqurean JW, Kauffman JB, Marbà N, Megonigal P, Pidgeon E, Herr D, Gordon D, Baldera A (2012) Estimating global "blue carbon" emissions from conversion and degradation of vegetated coastal ecosystems. PLoS ONE 7:e43542.

Rasse DP, Rumpel C, Dignac M-F (2005) Is soil carbon mostly root carbon? Mechanisms for a specific stabilisation. *Plant Soil* 269:341-356.

Sasser CE, Visser JM, Mouton E, Linscombe J Hartley SB (2008) Vegetation types in coastal Louisiana in 2007. U.S. Geological Survery Open-File Report 2008-1224, 1 sheet, scale 1:550,000.

Schlesinger WH (2009) On the fate of anthropogenic nitrogen. *Proc Natl Acad Sci USA* 106:203-208.

Silliman BR, Bertness MD (2004) Shoreline development drives invasion of Phragmites australis and the loss of plant diversity on New England salt marshes. *Conserv Biol* 18:1424-1434.

Smith VH, Schindler DW (2009) Eutrophication science: where do we go from here? *Trends Ecol Evol* 24:201-207.

Smith VH, Tilman GD, Nekola JC (1999) Eutrophication: impacts of excess nutrient inputs on freshwater, marine, and terrestrial ecosystems. *Environ Pollut* 100:179-196.

Suding KN, Collins SL, Gough L, Clark C, Cleland EE, Gross KL, Milchunas DG, Pennings S (2005) Functional- and abundance-based mechanisms explain diversity loss due to N fertilization. *Proc Natl Acad Sci USA* 102:4387-4392.

Sullivan MJ, Daiber FC (1974) Response in production of cord grass, *Spartina alterniflora*, to inorganic nitrogen and phosphorus fertilizer. *Chesapeake Sci* 15:121-123

Turner RE, Howes BL, Teal JM, Milan CS, Swenson EM, Goehringer-Toner DD (2009) Salt marshes and eutrophication: an unsustainable outcome. *Limnol Oceanogr* 54:1634-1642.

Turner RE, Swenson EM, Milan CS, Lee JM, Oswald TA (2004) Below-ground biomass in healthy and impaired salt marshes. *Ecol Res* 19:29-35.

Turner RE (2011) Beneath the salt marsh canopy: loss of soil strength with increasing nutrient loads. *Estuaries Coasts* 34:1084-1093.

Tyler AC, Lambrinos JG, Grosholz ED (2007) Nitrogen inputs promote the spread of an invasive marsh grass. *Ecol Appl* 17:1886-1898.

Tyler G (1967) On the effect of phosphorus and nitrogen supplied to Baltic shore-meadow vegetation. *Bot Notiser* 120:433-447.

Valiela I, Teal JM (1974) Nutrient limitation in salt marsh vegetation. In: Reimold RJ, Queen WH (eds) *Ecology of halophytes*. Academic Press, New York, NY.

VanZomeren CM, White JR, DeLaune RD (2013) Ammonification and denitrification rates in coastal Louisiana bayou sediment and marsh soil: Implications for Mississippi river diversion management. *Ecol Eng* 54:77-81.

Visser JM, Sasser CE, Chabreck RH, Linscombe RG (1998) Marsh vegetation types of the Mississippi River Deltaic Plain. *Estuaries* 21:818-828.

White DS, Howes BL (1994) Long-term 15N-nitrogen retention in the vegetated sediments of a New England salt marsh. *Limnol Oceanogr* 39:1878-1892.

Wigand C, Thursby GB, McKinney RA, Santos AF (2004) Response of *Spartina patens* to dissolved inorganic nutrient additions in the field. *J Coast Res* 45:134-149.

CHAPTER 2
MULTIPLE LEVELS OF NITROGEN APPLIED TO AN OLIGOHALINE MARSH IDENTIFY A PLANT COMMUNITY RESPONSE SEQUENCE TO EUTROPHICATION[1]

2.1 Introduction

Human activities and the resulting manipulation of the global environment have greatly altered the flow and cycling of nutrients across the land-sea margin. Global inputs of reactive nitrogen (N) to coastal waters have increased twenty fold since 1860 (Galloway & Cowling 2002), and the flux of phosphorus (P) to the world's oceans has increased by nearly three fold in modern times (Howarth *et al.* 1995, Bennett *et al.* 2001). As a result, cultural eutrophication, or the biological response to human induced nutrient over-enrichment, is affecting more than 400 coastal areas around the world (Diaz & Rosenberg 2008). In the U.S. alone, two-thirds of 141 estuaries, representing more than 90% of the conterminous U.S. estuarine surface area, are moderately to severely affected by eutrophic conditions (Bricker et. al. 2008).

The ecological effects of eutrophication are readily apparent in coastal wetlands. Documented effects of nutrient over-enrichment include increased primary production, community metabolism, and consumer activity, reduced species richness and carbon sequestration, altered nutrient cycling and species composition, and expansions of invasive species, to name a few (Mendelssohn 1979, Whigham & Nusser 1990, Morris & Bradley 1999, Chambers *et al.* 1999, Pennings *et al.* 2002, Silliman & Bertness 2004, DeLaune *et al.* 2005, Bertness *et al.* 2008, and more). A large body of literature supports the proposition that in

[1]This chapter previously appeared as Graham SA, Mendelssohn IA (2010) Multiple levels of nitrogen applied to an oligohaline marsh identify a plant community response sequence to eutrophication, Marine Ecology Progress Series, 417:73-82. It is reprinted by permission of Inter-Research. Some references have been updated.

mesohaline (brackish) and polyhaline (salt) marshes these changes in ecosystem structure and function result from greater N loading because plant growth in these systems is N limited (Tyler 1967, Sullivan & Daiber 1974, Valiela & Teal 1974, Broome *et al.* 1975, Patrick & Delaune 1976, Jefferies & Perkins 1977, Boyer *et al.* 2001, Wigand *et al.* 2004). In comparison, oligohaline (intermediate) marshes have received much less attention, and general conclusions concerning the effects of coastal eutrophication on them are lacking.

Although a few published oligohaline marsh fertilization studies have documented altered plant community structure and/or function induced by N (DeLaune & Lindau 1990) and N-P-potassium (K) enrichment (Gough & Grace 1997, Slocum & Mendelssohn 2008), only Crain (2007) applied fully crossed treatments of N and P to determine the growth limiting nutrient or nutrient combination that affects change, in this case N+P co-limitation. Furthermore, no study has emphasized plant community- and species-level response trajectories resulting from the application of multiple nutrient levels in oligohaline marshes. Therefore, additional information is needed to provide more comprehensive answers to fundamental questions concerning the dynamics of oligohaline marsh ecology, including 'Are oligohaline marshes in general co-limited by N and P?' and 'What drives ecosystem change once nutrient over-enrichment occurs?'

Though global and national inventories of oligohaline marshes do not exist, the latter represent a major coastal marsh type that requires more intensive study. For example, oligohaline marshes are the dominant wetland type in coastal Louisiana, representing 26% (~422,000 ha) of the total wetland area (~1.65 million ha) (Sasser *et al.* 2008). This amount alone accounts for nearly 4% of the total coastal wetland area in the entire conterminous United States (Field *et al.* 1991). These marshes typically have much greater plant species diversity than their more saline counterparts (Visser *et al.* 1998, Crain 2007). Multiple studies have documented up to 30

species growing within relatively small sampling areas (Brewer & Grace 1990, Baldwin & Mendelssohn 1998). Nutrient over-enrichment is of particular concern in species-rich oligohaline marshes because nutrient excess may alter competitive hierarchies (Brewer & Grace 1990) and thereby reduce or otherwise modify biodiversity.

The objectives of this research were to determine (1) the nutrient or nutrient combination (N, P or both) that limits primary productivity in an oligohaline marsh, (2) how experimental nutrient manipulation alters plant nutrient cycling and community composition, and (3) whether these changes occur simultaneously or at different rates depending upon the level of nutrient enrichment applied. By addressing these objectives, I tested the general hypothesis that N and P co-limit oligohaline marsh primary production. I also identified how enrichment with the limiting nutrient(s) affects individual component species and the vegetative community as a whole, and determined the factors driving changes in community composition following nutrient enrichment. Specifically, I hypothesized that N limits aboveground biomass production, and because of this, N enrichment increases aboveground production, alters plant nutrient dynamics, and affects overall community composition. At high N loading, I hypothesized that P becomes secondarily limiting when N limitation is relieved by fertilization, and enrichment with P in combination with high N induces additional changes in ecosystem structure and function. I also hypothesized that altered ecosystem structure will result from altered plant nutrient cycling once maximum aboveground primary production is achieved.

2.2 Materials and Methods

2.2.1 Study Area

The coastal waters of Louisiana receive approximately 1.6 million mt N yr^{-1} and 136,500 mt P yr^{-1} from the Mississippi-Atchafalaya River complex (Goolsby *et al.* 1999). The nitrate

load (~950,000 mt yr^{-1}; Goolsby *et al.* 1999) is now more than twice that discharged in the 1950s (Turner & Rabalais 1991), driving the development of a persistent and reoccurring near-shore hypoxic area that can exceed 20,000 km^2 (Rabalais 2002, Turner *et al.* 2006, www.gulfhypoxia.net Accessed 15 Aug 2013). Prior to human modification of the Lower Mississippi River, much of this water would follow distributaries and crevasses through the vast coastal wetlands of Louisiana's delta before entering the Gulf of Mexico (Welder 1959). However, following the great flood of 1927, flood-control levees were constructed almost continuously to the mouth of the River, and today most of Louisiana's wetlands remain hydrologically isolated from the Mississippi River (Kesel 1988, 2003). Due to this and a number of other related factors (see Day *et al.* 2007), approximately 5400 km^2 of wetlands were lost in coastal Louisiana between 1930 and 2010 (Couvillion *et al.* 2011).

One proposed method for reducing the nutrient load to the northern Gulf and restoring Louisiana's coastal wetlands is to reconnect the Mississippi River to its delta through river diversions (Mitsch *et al.* 2001, CPRA 2007, 2012). The rationale is that the freshwater, nutrients, and sediment in the river water will stimulate plant growth, accretion, and sedimentation, and reduce wetland loss while simultaneously reducing the nutrient load to the Gulf of Mexico (see Day et al. 2007). Although the stability of Louisiana's wetlands is dependent upon sediment and nutrients, the elevated nutrient load in the Mississippi River is considered to be a potential driver of coastal wetland eutrophication over the long-term (Parsons et al. 2006).

2.2.2 Study Site

To address this concern and my objectives, I investigated the effects of nutrient enrichment in a river-fed oligohaline marsh along the west bank of the Tchefuncte River (N 30° 23.205', W 90° 09.551'), approximately 1 km north of Lake Pontchartrain. Soil at the site is

classified as a Kenner series Histosol (euic, thermic Fluvaquentic Medisaprist) formed from herbaceous plant material and characterized as "very poorly drained, rapidly permeable organic soil" (Trahan *et al.* 1990). The plant community is highly diverse (Slocum & Mendelssohn 2008) and is representative of the Oligohaline Mix vegetation type described by Visser *et al.* (1998). The species mix is dominated by *Sagittaria lancifolia* L., *Eleocharis fallax* Weatherby, and *Polygonum punctatum* Ell. All three dominants are perennial (clonal) herbs that emerge, flower, and senesce at similar times during the growing season at this site.

The marsh floods from water level fluctuations in the Tchefuncte River and Lake Pontchartrain caused primarily by wind shifts during frontal passages, although a 10 cm microtidal range also affects hydrology (Swenson & Chuang 1983). Average surface water salinity (1999-2006) was approximately 1.6 g L^{-1} (LADEQ 2006) indicating oligohaline estuarine conditions (Odum 1988). Nutrient loading to the study marsh is affected by residential development throughout the Tchefuncte River watershed and agriculture in the upper reaches (Table 2.1). However, the Tchefuncte River water has much lower concentrations of inorganic N (NH_3+NO_3+NO_2), total N, and total P when compared to the Mississippi River – 6, 3, and 2 times lower, respectively (Table 2.1).

2.2.3 Design and Sampling

I applied granulated slow-release fertilizer by surface broadcast for four years to 1 m^2 oligohaline marsh plots. Each plot received one of four N levels (0, 50, 200, or 1200 kg N ha^{-1} yr^{-1} applied as Nutralene Methylene Urea 40-0-0) in combination with one of two P levels (0 or 131 kg P ha^{-1} yr^{-1} applied as Humaphos 0-5-0) to yield eight treatment combinations in a completely randomized block design. Treatment combinations were replicated in five locations (i.e., blocks) spaced 5-10 m apart and parallel to a small drainage canal. Plots were fertilized

Table 2.1 Average ambient water quality condition of the Tchefuncte River at Madisonville, LA (LA040802_00, Site 0106; LADEQ 2006) and the Mississippi River at St. Francisville, LA (LA070201_00, Site 0055; LADEQ 2006) from 1999 to 2006.

Constituent	Sample Size	Concentration (mg N or P L^{-1})	Potential Loading Rate (kg ha^{-1} yr^{-1})*
Tchefuncte River			
NH_3-N	n=64	0.14 ± 0.01	41
NO_3+NO_2-N	n=81	0.12 ± 0.01	40
Total N	n=82	0.79 ± 0.03	231
Total P	n=90	0.11 ± 0.004	32
Mississippi River			
NH_3-N	n=42	0.15 ± 0.02**	***
NO_3+NO_2-N	n=91	1.39 ± 0.06	
Total N	n=91	2.26 ± 0.07	
Total P	n=89	0.21 ± 0.01	

*Potential nutrient loading rate to the study marsh was estimated using water level measurements collected on September 22, 2006 at three locations within each 1 m^2 plot at mid-tide. Average water level for each plot was correlated by time with 15-minute river gauge height data for the Tchefuncte River at Madisonville, approximately 1 km north of the study site (USGS Station #07375230) to obtain the plot surface elevation relative to the river stage. Relative surface elevation was then averaged across all plots and subtracted from the gauge height data to calculate the mean flood depth per plot (0.137 m m^{-2}) and flooding frequency (0.59 day^{-1}) for the period of record February 2004 to November 2006. Potential loading rate for each nutrient shown above was then calculated using appropriate unit conversions and the following equation: [nutrient] x flood depth x plot area x flooding frequency.

**Average NH_3 concentration from 1999 to 2004.

*** To calculate the hypothetical potential nutrient loading rate to this marsh from diverted Mississippi River water, assuming similar hydrologic conditions, plug the Mississippi River nutrient concentrations in to the potential loading rate equation described above (see *).

twice during the growing season in April and July of 2002 through 2005. I delayed sampling until the third and fourth growing seasons after the initiation of nutrient additions (i.e., 2004 or 2005) to increase the chances of detecting treatment effects, as multiple studies have shown that

the effects of nutrient enrichment on wetland plant communities become more pronounced with each year of continued enrichment (Valiela *et al.* 1975, Craft *et al.* 1995, Kiehl *et al.* 1997, Crain 2007, Frost *et al.* 2009). However, I should note that Lindig-Cisneros *et al.* (2003) found reduced effects of nutrient enrichment on *Spartina foliosa* total stem length over multiple years of fertilization.

To address objective 1, I estimated net aboveground primary productivity (NAPP) during the 2005 growing season. Vegetation at this site senesces each winter (Baldwin & Mendelssohn 1998, S. A. Graham – personal observation), so plant biomass at the beginning of the growing season was zero. Each 1 m^2 plot was divided into four 0.25 m^2 sub-plots, and all aboveground biomass within a single randomly chosen sub-plot was clipped approximately every six weeks, for a total of four biomass harvests per plot. Clipped plant biomass was then separated into live and dead categories, dried to a constant weight at 60^{o}C, and weighed. Estimates of NAPP were calculated using the Smalley Method, which uses changes in both live and dead biomass over time to determine plant production (Smalley 1959). This method is the most widely used in salt marshes for estimating net production, although it is limited in ability to account for biomass export due to tidal flushing and shoot mortality and decomposition between sampling periods (Linthurst & Reimold 1978). While absolute production may be underestimated (Daoust & Childers 1998), relative differences in production accurately reflect treatment effects.

To address objectives 2 and 3, I harvested end-of-season aboveground biomass in October 2004. This end of the growth season biomass harvest had no affect on the following year's NAPP estimation because vegetation at the site senesces each winter. Plant material within a 0.5 m^2 quadrat placed in the center of each 1 m^2 plot was clipped to the ground surface and separated into live or dead categories by species, then dried to a constant weight at 60^{o}C and

weighed. Species richness was determined as the total number of species per clipped plot. I calculated species relative dominance as the percent of specie-specific biomass per clipped plot. Differential biomass among species is a good measure of dominance, especially when the plants have similar growth forms. However, because many species occurred at low frequency, I statistically analyzed relative dominance of the three dominant species only.

Dried green and senesced leaf and stem tissue from the plant community dominant, *S. lancifolia*, was ground using a Wiley mill and analyzed for total N using a Costech 4010 CHNS/O Elemental Combustion System and total P using Inductively Coupled Plasma (ICP) Spectrometry (Spectro Ciros) following nitric acid digestion. Tissue N and P concentrations were then used to calculate N:P ratios (mol:mol) and dry weight based nutrient resorption efficiencies (i.e., the relative percent difference between live and dead tissue nutrient concentrations; van Heerwaarden *et al.* 2003).

2.2.4 Statistical Analysis

All statistical analyses were conducted using SAS (Statistical Analysis Systems, version 9.1.3, SAS Institute, Inc., Cary, NC). I used MANOVA (PROC GLM) to determine overall effects of N, P, and their interaction (N x P) on the following dependent variables as a group: net above-ground primary production, species richness, relative dominance of the 3 most dominant species (*Sagittaria lancifolia, Eleocharis fallax, and Polygonum punctatum),* and *S. lancifolia* tissue N and P concentrations, ratios, and resorption efficiencies. Overall treatments effects were determined using the Wilks' Lambda test statistic. Where a significant overall effect was identified, individual mixed-model ANOVAs were used to identify the specific dependent variables that contributed to the significant overall effect. Treatment means were tested using the least squares (LS) means procedure with a Tukey-Kramer adjustment to maintain an experiment-

wise error rate of 5%. I also used linear and curvilinear regression analyses to identify predictive relationships with increasing N enrichment, and Pearson Correlation Analysis to identify bivariate relationships among calculated nutrient resorption efficiencies. When necessary, these data were logarithmically or square root transformed prior to analysis to improve homogeneity of variance and goodness of fit to a normal distribution. All measures of significance were identified at $p < 0.05$.

2.3 Results

MANOVA results indicated a significant overall N effect on the various measures of oligohaline marsh ecosystem structure (e.g., species richness and percent dominance) and function (e.g., NAPP and *S. lancifolia* tissue nutrient concentrations, ratios, and resorption efficiencies) (Table 2.2). Phosphorus had no significant effect alone or in interaction with N. Thus, only N-effects are discussed. ANOVA results showing the individual dependent variables that contributed to the significant overall N effect are displayed in Table 2.2.

2.3.1 Net Aboveground Primary Production

Primary production increased from 1243 ± 75 g m^{-2} yr^{-1} to 1912 ± 152 g m^{-2} yr^{-1} with increasing N enrichment, and enrichment with 200 kg N ha^{-1} yr^{-1} resulted in significantly greater NAPP compared to control plots (see [a] in Figure 2.1). Further enrichment with 1200 kg N ha^{-1} yr^{-1} also increased NAPP compared to the control, but had no additional affect when compared to the 200 kg N ha^{-1} yr^{-1} treatment. Rather, NAPP increased asymptotically with N enrichment. The quadratic regression suggests that maximum community primary production occurred within my range of N enrichment treatments.

Table 2.2 MANOVA results showing the overall effects of nitrogen (N), phosphorus (P), and their interaction (N x P) on all (n=10) aboveground dependent variables as a group. Significant ($p<0.05$) treatments effects were determined using Wilks' Lambda test statistic. Individual ANOVA results are displayed for the N effect only to identify the specific dependent variables that contributed to the significant overall N effect. P-values in bold indicate a significant effect.

	Num DF, Den DF	P-value
MANOVA		
N	30, 56.4	**0.0001**
P	10, 19	0.21
N x P	30, 56.4	0.78
ANOVA (N Effect)		
Net aboveground primary production	3, 28	**0.0006**
Species richness	3, 28	0.87
Relative dominance:		
Sagittaria lancifolia	3, 28	0.08
Eleocharis fallax	3, 28	**0.0004**
Polygonum punctatum	3, 28	**0.001**
Sagittaria lancifolia tissue nutrients:		
Green tissue N	3, 28	**0.03**
Green tissue P	3, 32*	0.36
Green tissue molar N:P ratio	3, 28	**0.0003**
N resorption efficiency	3, 28	**0.01**
P resorption efficiency	3, 28	**0.004**

*Den DF = 32 because the block covariance parameter estimate, $\sigma_\rho^2 = 0$. Covariance parameters with zero variance do not contribute to degrees of freedom computed by Satterthwaite.

2.3.2 Community Composition

Within the plant community, I distinguished 20 distinct taxonomic categories representing 17 identified species in 11 families (Table 2.3). Nitrogen enrichment had no significant effect on species richness. The total number of species per 0.5 m^2 clip plot averaged 7.9 ± 0.2 regardless of treatment. Although most species were rare and represented only a small

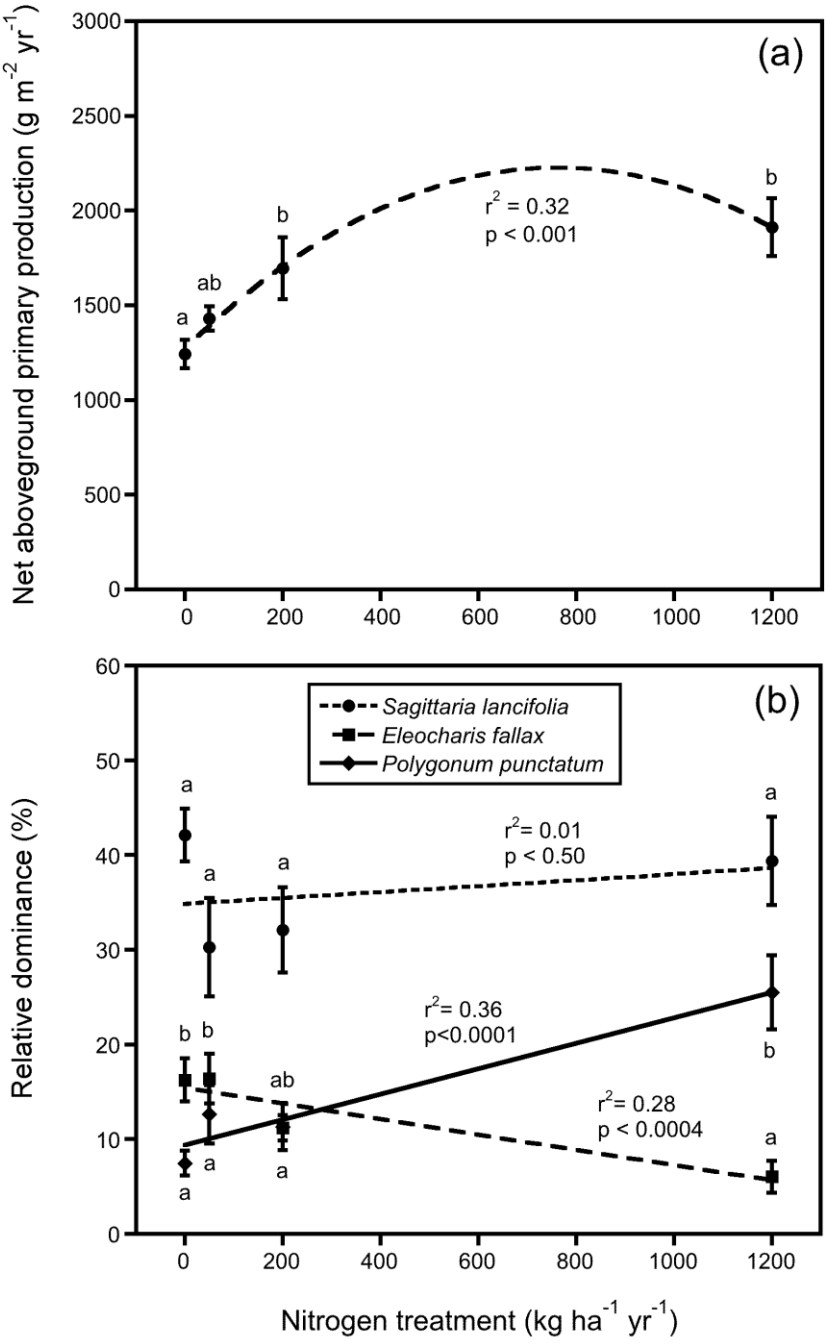

Figure 2.1 (a) Net aboveground primary production and (b) the relative dominance of the three dominant plant species. Means (n=10, ± 1 SE) are averaged over phosphorus treatment-levels (no significant P- or N x P-effect). Means separated by different letters are significantly different at $p \leq 0.05$ based on the Tukey-Kramer multiple comparison test. Different letters in (b) indicate significant differences in relative dominance within (not across) species. Coefficients of determination (r^2) and p-values represent the best fit to (a) $y = -0.00165x^2 + 2.5091x + 1270.3$ (NAPP), and (b) $y = 0.00319x + 34.810$ (*Sagittaria lancifolia*), $y = -0.00812x + 15.410$ (*Eleocharis fallax*), and $y = 0.01344x + 9.353$ (*Polygonum punctatum*).

Table 2.3 Plant species identified within study plots and their relative dominance presented as the average (range) for all plots.

Species	Family	Relative Dominance (%)
Sagittaria lancifolia	Alismataceae	36 (12-60)
Polygonum punctatum	Polygonaceae	14 (0-48)*
Eleocharis fallax	Cyperaceae	12 (1-31)
Alternanthera philoxeroides	Ameranthaceae	9 (0-29)
Symphyotrichum subulatum	Asteraceae	4 (0-18)
Vigna luteola	Fabaceae	2 (0-6)
Lythrum lineare	Lythraceae	2 (0-34)
Echinochloa crus-galli	Poaceae	1 (0-4)
Panicum dichotomiflorum	Poaceae	<1 (0-6)
Ipomoea sagittata	Convolvulaceae	<1 (0-3)
Galium tinctorium	Rubiaceae	<1 (0-<1)
Phyla nodiflora	Verbenaceae	<1 (0-<1)
Cyperus odoratus	Cyperaceae	<1 (0-<1)
Dioda virginiana	Rubiaceae	<1 (0-<1)
Schoenoplectus tabernaemontani	Cyperaceae	<1 (0-<1)
Spartina patens	Poaceae	<1 (0-<1)
Ambrosia artemisiifolia	Asteraceae	<1 (0-<1)
Unknown Grass (1)**	Poaceae	<1 (0-<1)
Unknown Grass (2)**	Poaceae	<1 (0-<1)
Unknown spp.**	Unknown	<1 (0-<1)

* *Polygonum punctatum* was present in all but one plot.
** Unidentifiable plant fragments.

fraction of each plot's total biomass, the three dominant plant species (*S. lancifolia*, *E. fallax*, and *P. punctatum*) were present in almost every plot, and when combined, accounted for >60% of the total aboveground biomass on average. *Sagittaria lancifolia* was clearly the dominant plant, representing 36.0 ± 2.2 % of the total biomass, regardless of treatment (see [b] in Figure 2.1). Regression analysis did not identify any significant trends or any variability in *S. lancifolia* relative dominance due to N enrichment, which indicates that *S. lancifolia* biomass increased at

the same rate as the plant community. However, in plots receiving 1200 kg N ha^{-1} yr^{-1}, the relative dominance of *E. fallax* fell by 10% while the dominance of *P. punctatum* increased by 18% compared to control plots. N-induced changes in the relative dominance of both species displayed highly significant linear trends that ultimately resulted in a shift in dominance between the two species at the greatest N enrichment level of 1200 kg ha^{-1} yr^{-1}.

2.3.3 *Sagittaria lancifolia* Tissue Nutrients

Although the main effect of N enrichment on green tissue N was significant (Table 2.2), no N treatment level was significantly different from the control (see [a] in Figure 2.2). Tissue N was significantly higher only in plots enriched with 1200 kg N ha^{-1} yr^{-1} compared to plots enriched with 200 kg N ha^{-1} yr^{-1}, but both were similar to the control. Green tissue P content did not change significantly following enrichment with N. However, on average, green tissue N and P concentrations were approximately 10% higher and 13% lower, respectively, in plots enriched with 1200 kg N ha^{-1} yr^{-1} compared to control plots. Overall, tissue N had a significant positive linear relationship with N enrichment, but the regression analysis accounted for only 21% of the variability. The linear relationship between tissue P and N enrichment was not significant.

S. lancifolia N:P ratios increased significantly with 1200 kg N ha^{-1} yr^{-1} compared to the control due to the combined non-significant changes in tissue N and P (see [b] in Figure 2.2). Plots enriched with intermediate levels of N (50 and 200 kg ha^{-1} yr^{-1}) contained *S. lancifolia* plants with intermediate tissue N:P ratios that were not statistically different from either the control or the high N plots. A highly significant positive linear relationship with increasing N enrichment accounted for 41% of the variability in tissue N:P.

In controls plots, resorption of N (NRE) to perennating structures by *S. lancifolia* during senescence was nearly half as efficient as P resorption (PRE): NRE = 35% and PRE = 64%. Both

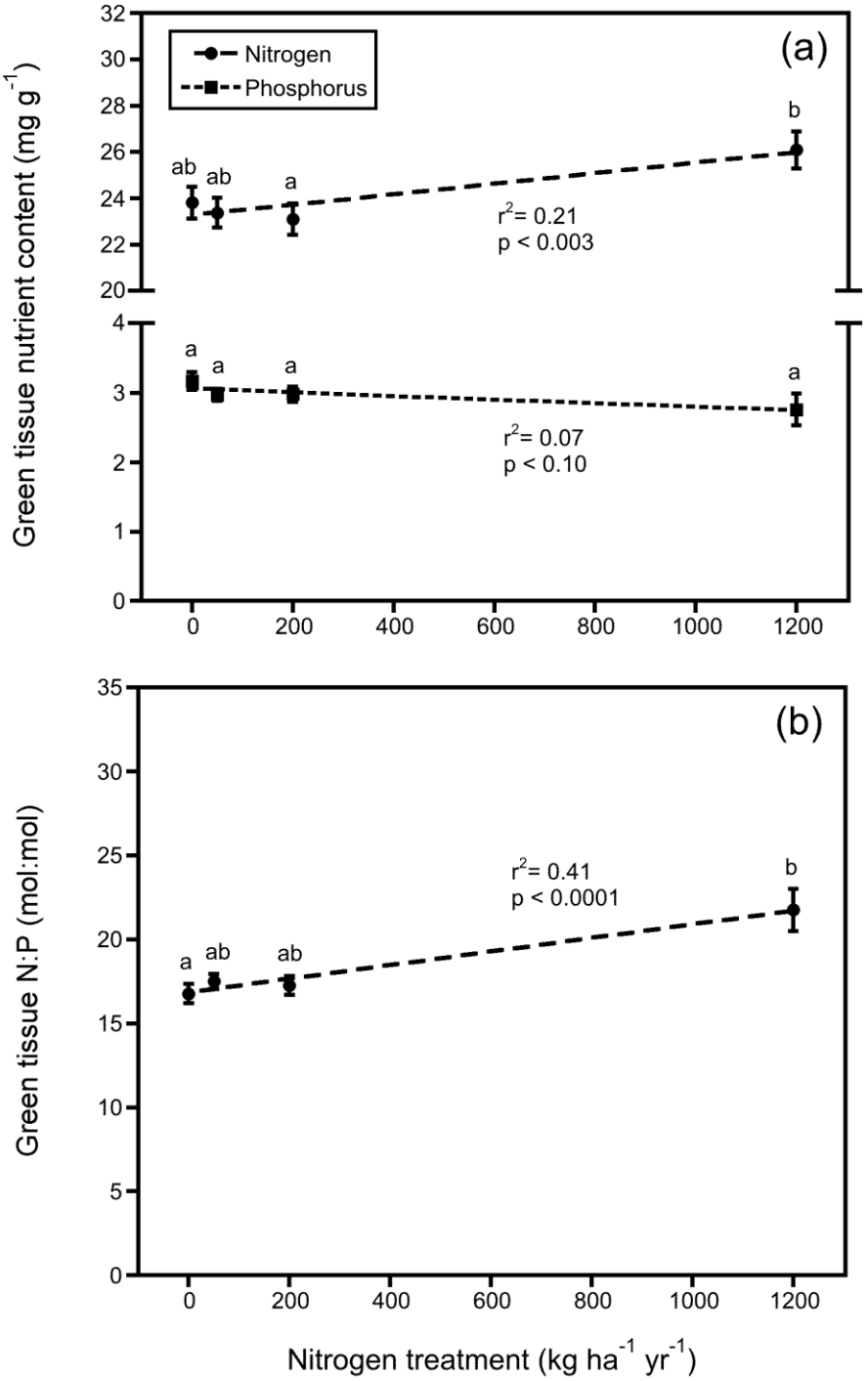

Figure 2.2 *Sagittaria lancifolia* green tissue (a) N and P content and (b) N:P. Means (n=10, ± 1 SE) are averaged over phosphorus treatment-levels (no significant P- or N x P-effect). Means separated by different letters are significantly different at $p \leq 0.05$ based on the Tukey-Kramer multiple comparison test. Coefficients of determination (r^2) and p-values are displayed for (a) y = 0.00226x + 23.271 (N content) and y = -0.00026x + 3.064 (P content), and (b) y = 0.00404x + 16.853 (N:P).

NRE and PRE decreased following enrichment with 1200 kg N ha^{-1} yr^{-1} to where *S. lancifolia* resorbed significantly less N and P when compared to the control plots (Figure 2.3). Linear regressions for both NRE and PRE were highly significant, but each explained only 19% of the variability in nutrient resorption with increasing N enrichment. There was also a strong correlation between *S. lancifolia* NRE and PRE (r=0.68, $p < 0.0001$; data not shown). Higher NRE corresponded to higher PRE although not at a 1:1 relationship. On average, each 10% change in NRE corresponded to a 6% change in PRE.

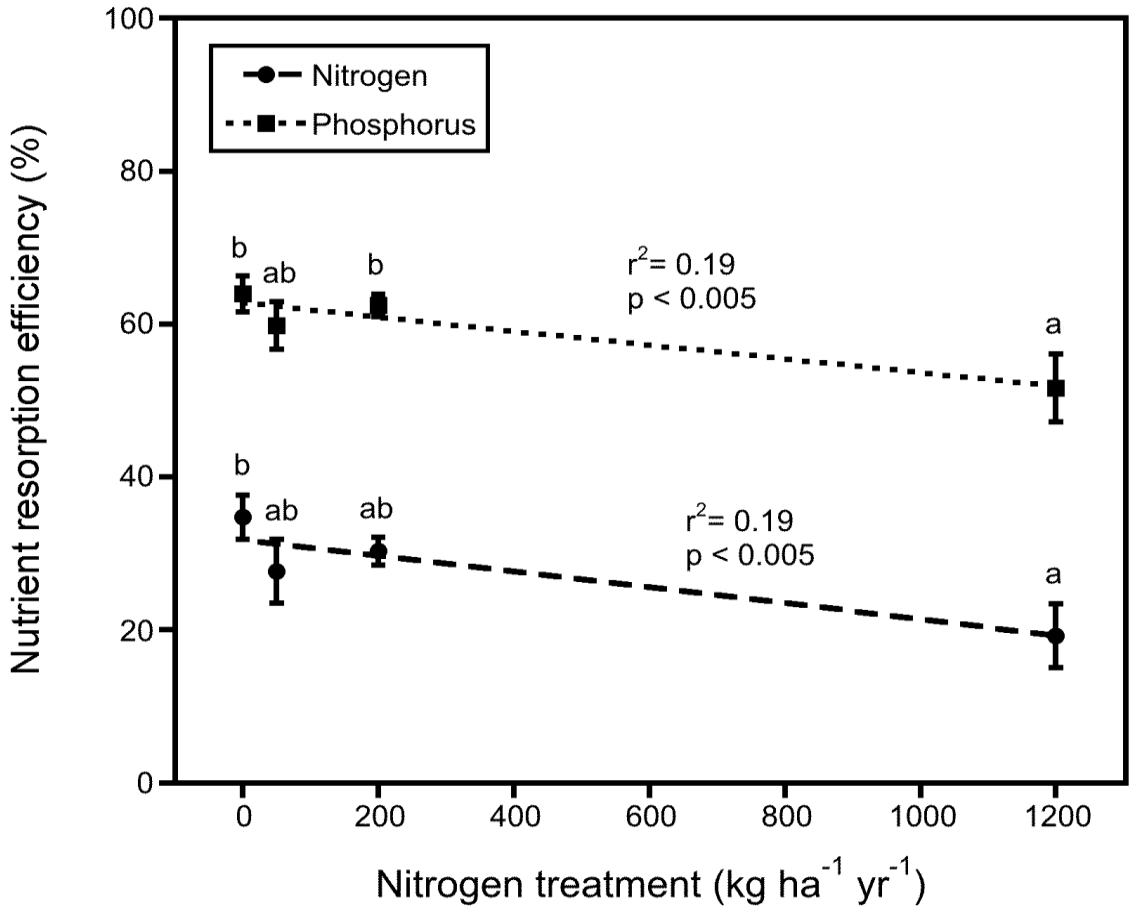

Figure 2.3 *Sagittaria lancifolia* nutrient resorption efficiencies (PRE and NRE, respectively). Means (n=10, ± 1 SE) are averaged over phosphorus treatment-levels (no significant P- or N x P-effect). Means separated by different letters are significantly different at $p \leq 0.05$ based on the Tukey-Kramer multiple comparison test. Coefficients of determination (r^2) and p-values represent the best fit to y = -0.00915x + 62.778 (PRE) and y = -0.01048x + 31.796 (NRE).

2.4 Discussion

2.4.1 Nutrient Limitation Status

The results from this study confirm my hypothesis that N limits primary production in this oligohaline marsh, but refutes the hypothesis of secondary P limitation. This conclusion is based primarily on the significant increase (36-54%) in NAPP after four years of N enrichment; no changes were detected following P enrichment, and no additional changes were detected following N+P enrichment. My findings are consistent with a number of experiments in mesohaline (brackish) and polyhaline (salt) marsh systems that have documented increased plant primary production or standing crop following N enrichment (Valiela & Teal 1974, Sullivan & Daiber 1974, Cargill & Jefferies 1984, Boyer *et al.* 2001, Wigand *et al.* 2004). My results are also consistent with studies that have applied N to oligohaline marshes in Louisiana. Fertilizing with N only, DeLaune & Lindau (1990) doubled the biomass of *S. lancifolia* dominated marsh. Outside of Louisiana, N enrichment increased *Zizaniopsis miliacea* (Giant Cutgrass) biomass by 2-fold in a Georgia tidal freshwater marsh after two years of N and/or P enrichment (Frost *et al.* 2009).

Although my results are consistent with more saline ecosystems in general, and other oligohaline marshes in Louisiana, they are contrary to results from the only other oligohaline marsh fertilization study designed to determine oligohaline marsh nutrient limitation. Crain (2007) concluded that oligohaline marshes along two estuaries in southern Maine were co-limited by N and P after three years of combined nutrient enrichment increased aboveground biomass by 300%. This inconsistency indicates that the relative importance of P to oligohaline marsh primary production differs between locations. Potential factors contributing to differential

nutrient loading include tidal flushing, nutrient source, eutrophic condition, and relative nutrient inputs (Bricker *et al.* 1999).

Compared to results from other oligohaline marsh fertilization studies (DeLaune & Lindau 1990, Crain 2007), I observed a relatively small, but significant, increase in NAPP following enrichment, which can most likely be attributed to differences in ambient marsh productivity. Ambient potential nutrient loading rates calculated for this marsh in Table 2.1 coupled with high NAPP (~1250 g m^{-2} yr^{-1}) in control plots indicate that fertile conditions existed at my site prior to N and/or P enrichment. Furthermore, control plots at my site had approximately 16% and 35-80% more biomass than the control plots harvested by DeLaune & Lindau (1990) and Crain (2007), respectively, during comparable biomass harvests in mid-summer. Güsewell & Bollens (2003) determined that the absolute supply of the limiting nutrient was most important when ambient marsh fertility was low. Therefore, nutrient enrichment should have less of an impact on more productive marshes than those that are naturally less productive.

Under the current assemblage of plant species, NAPP appears to have reached a maximum at enrichment levels $\geq$ 200 kg N ha^{-1} yr^{-1}. NAPP in plots enriched with 200 kg N ha^{-1} yr^{-1} was not significantly different from NAPP in plots enriched with 1200 kg N ha^{-1} yr^{-1}, but both were significantly greater than the control. However, I cannot eliminate the possibility that plant production could have been greater at some level of enrichment between 200 and 1200 kg N ha^{-1} yr^{-1} as suggested by the regression analysis (see [a] in Figure 2.1). According to the quadratic fit, NAPP would have peaked at approximately 2220 g m^{-2} yr^{-1} with 760 g N ha^{-1} yr^{-1} enrichment. Regardless, the observed reduction in the rate that NAPP increased when

enrichment exceeded 200 kg N ha^{-1} yr^{-1} is an indication that N limitation was alleviated and the vegetation's nutrient assimilation capacity was surpassed.

2.4.2 Plant Nutrient Cycling and Community Composition

I measured *S. lancifolia* green tissue N:P ratios of approximately 17 (mol:mol) in control plots, corroborating that N was the primary limiting nutrient under ambient conditions (N:P < 31 mol:mol; Koerselman & Meuleman 1996). Adding additional N increased tissue N:P ratios to approximately 22 (mol:mol), but the ratio remained well below that necessary to indicate P limitation (N:P > 36 mol:mol; Koerselman & Meuleman 1996), even though NAPP measurements indicate that N limitation was alleviated. Similarly, Frost *et al.* (2009) were unable to increase *Z. miliacea* N:P ratios in an N limited tidal freshwater marsh to a level beyond the P limitation threshold. Others have found that vegetation N:P ratios do not correspond with nutrient limitation determined by fertilization, suggesting that N:P ratios have limited application in understanding the nutrient dynamics of these and perhaps other systems (Crain 2007, Morse *et al.* 2004). During the present study, a corresponding decrease in N and P resorption occurred as tissue N:P ratios increased with increasing N enrichment. Thus, under conditions of high N loading, *S. lancifolia* conserves less N and P and returns more to the soil during senescence, which may explain why tissue N:P ratios did not exceed the P limitation threshold.

Reduced nutrient resorption efficiency is also an indication that *S. lancifolia's* optimal N supply rate was surpassed. A meta-analysis of fertilization experiments by Aerts (1996) revealed that only about one third of the species tested responded to increased nutrient availability by lowering nutrient resorption. Concurrent changes in relative dominance of the two sub-dominant species at the 1200 kg N ha^{-1} yr^{-1} enrichment level suggest that these species have different optimal N supply rates than *S. lancifolia* and the plant community as a whole (Bedford *et al.*

1999), and may explain why the meta-analysis by Aerts (1996) revealed that only a small portion of plant species reduce nutrient resorption under conditions of higher nutrient availability. The linear increase in *P. punctatum* dominance with increasing N enrichment indicates that this species prefers a higher nutrient environment, and therefore, would not down-regulate nutrient resorption under such conditions (see [b] in Figure 2.1). If this linear trend persists with further N enrichment in time or quantity, a shift in species dominance is likely to occur. *Polygonum* could eventually achieve competitive advantage and possibly reduce species richness by displacing species that utilize nutrients less efficiently or that prefer a lower nutrient environment (e.g., *Eleocharis*). Studies have shown that nutrient aggressive plants such as *Typha* and *Phragmites* are capable of reducing species richness by displacement under high nutrient loading rates (Chambers *et al.* 1999, Pennings *et al.* 2002). In fact, increased *Polygonum* dominance relative to other component species characterized nutrient enrichment in areas of the Florida Everglades (Vaithiyanathan & Richardson 1999).

2.4.3 Eutrophication Potential

When viewed collectively, these data show that N enrichment affects different aspects of the plant community at different rates depending on the loading rate (Table 2.4). Adding 200 kg N ha^{-1} yr^{-1} to this marsh stimulated NAPP, but no other significant changes were detected, showing that at this level of enrichment the additional N was utilized primarily to increase plant production. Adding 1200 kg N ha^{-1} yr^{-1}, on the other hand, not only stimulated NAPP to a similar degree as with 200 kg N ha^{-1} yr^{-1}, but also increased *S. lancifolia* tissue N:P ratios, decreased *S. lancifolia* nutrient resorption efficiencies, and altered the relative dominance of the dominant species. Therefore, N enrichment beyond that which contributes to plant growth is

Table 2.4 Eutrophication potential of aboveground functional and structural vegetative characteristics. Initial response level indicates the nitrogen (N) enrichment level at which a significant ($p<0.05$) change first occurred based on the Tukey-Kramer multiple comparison test.

Vegetation Parameter	Initial Response Level
Net aboveground primary productivity	200 kg N ha^{-1} yr^{-1}
Tissue N:P ratio	1200 kg N ha^{-1} yr^{-1}
N and P resorption efficiency	1200 kg N ha^{-1} yr^{-1}
Relative dominance	1200 kg N ha^{-1} yr^{-1}
Species richness	No Response

stored in plant tissues, which in turn, alters plant nutrient-utilization strategies, and ultimately results in changes in plant community structure. Although I detected no significant changes in species richness, Slocum & Mendelssohn (2008) observed reduced species richness at a nearby site within the same contiguous marsh receiving the same N loading rate (1200 kg N ha^{-1} yr^{-1}) applied as N-P-K over a comparable time frame.

Similar to the results from this study, Aerts *et al.* (1992) explained that the eutrophication process in N limited European *Sphagnum* bogs can be viewed as a chronosequence. The results from their study showed that nitrogen enrichment initially increased *Sphagnum* production and tissue N:P ratios. With further N enrichment in time or quantity, the N:P ratio became so high that P became limiting, ultimately reducing *Sphagnum* growth and leading to its disappearance. Contrary to the sequence described by Aerts *et al.* (1992), I observed an increase in production prior to detecting any changes in nutrient utilization and storage. Furthermore, *S. lancifolia's* N:P ratio never indicated P limitation, despite the fact that NAPP measurements indicated that N limitation had been alleviated. Unlike vascular plants, *Sphagnum* mosses have no root system, and therefore, are unable to translocate nutrients during

senescence via nutrient resorption, at least in the traditional sense (see Rydin & Clymo 1989). The observed reduction in nutrient resorption in this study may be a mechanism by which the effects of elevated nutrient loading are counteracted, slowing this sequence of eutrophication and helping *S. lancifolia* maintain relative dominance in the short-term. Though over the long-term, reduced nutrient resorption could potentially create a positive feedback loop and accelerate eutrophication by returning more N and P to the soil during senescence.

2.5 Conclusions

I conclude that this marsh and possibly others dominated by *S. lancifolia* in Louisiana are N limited. Therefore, my results refute generalized N+P co-limitation of oligohaline marsh primary production and suggest that local factors, such as ambient marsh fertility, may dictate the relative importance of N and P in these systems. The various measured plant characteristics indicate that a sequence of eutrophication occurs as enrichment with the limiting nutrient increases. This marsh is capable of assimilating at least 200 kg ha^{-1} yr^{-1} more N than the current loading rate without significant changes to ecosystem structure. Increased NAPP following N addition, while significant, was rather small in magnitude, and there were signs that N limitation was alleviated. Nitrogen enrichment above this amount (i.e., 1200 kg N ha^{-1} yr^{-1}) surpassed the nutrient assimilation capacity of the vegetation, altering plant nutrient cycling, which caused changes in ecosystem structure. This suggests that oligohaline marshes such as this one may have limited potential for removing excess nutrients, even if some of the species relax their nutrient resorption efficiencies. Although it is unlikely that natural sources of eutrophication (e.g., the Mississippi River) will surpass my experimental N loading rates in magnitude, the cumulative load over the long-term (decades) could be much higher, and result in equivalent or more pronounced changes in ecosystem structure and function.

2.6 References

Aerts R, Wallen B, Malmer N (1992) Growth-limiting nutrients in *Sphagnum*-dominated bogs subject to low and high atmospheric nitrogen supply. *J Ecol* 80:131-140.

Aerts R (1996) Nutrient resorption from senescing leaves of perennials: are there general patterns? *J Ecol* 84:597-608.

Baldwin AH, Mendelssohn IA (1998) Response of two oligohaline marsh communities to lethal and nonlethal disturbance. *Oecologia* 116:543-555.

Bedford BL, Walbridge MR, Aldous A (1999) Patterns in nutrient availability and plant diversity of temperate North American wetlands. *Ecology* 80:2151-2169.

Bennett EM, Carpenter SR, Caraco NF (2001) Human impact on erodable phosphorus and eutrophication: A global perspective. *Bioscience* 51:227-234.

Bertness MD, Crain C, Holdredge C, Sala N (2008) Eutrophication and consumer control of New England salt marsh primary productivity. *Conserv Biol* 22:131-139.

Boyer KE, Fong P, Vance RR, Ambrose RF (2001) *Salicornia virginica* in a southern California salt marsh: seasonal patterns and a nutrient-enrichment experiment. *Wetlands* 21:315-326.

Brewer JS, Grace JB (1990) Plant community structure in an oligohaline tidal marsh. *Vegetatio* 90:93-107.

Bricker SB, Longstaff B, Dennison W, Jones A, Boicourt K, Wicks C, Woerner J (2008) Effects of nutrient enrichment in the nation's estuaries: a decade of change. *Harmful Algae* 8:21-32.

Broome SW, Woodhouse WW, Seneca ED (1975) Relationship of mineral nutrients to growth of Spartina alterniflora in North Carolina. 2. Effects of N, P, and Fe fertilizers. *Soil Sci Soc Am J* 39:301-307.

Cargill SM, Jefferies RL (1984) Nutrient limitation of primary production in a sub-arctic salt-marsh. *J Appl Ecol* 21:657-668.

Chambers RM, Meyerson LA, Saltonstall K (1999) Expansion of *Phragmites australis* into tidal wetlands of North America. *Aquat Bot* 64:261-273.

CPRA [Coastal Protection and Restoration Authority] of Louisiana (2007) Integrated ecosystem restoration and hurricane protection: Louisiana's comprehensive master plan for a sustainable coast. Baton Rouge, LA.

CPRA [Coastal Protection and Restoration Authority] of Louisiana (2012) Louisiana's comprehensive master plan for a sustainable coast. Baton Rouge, LA.

Couvillion BR, Barras JA, Steyer GD, Sleavin W, Fischer M, Beck H, Trahan N, Griffin B, Heckman D (2011) Land Area Change in Coastal Louisiana from 1932 to 2010. US Geological Survey. Scientific Investigations Map no. 3164.

Crain CM (2007) Shifting nutrient limitation and eutrophication effects in marsh vegetation across estuarine salinity gradients. *Estuaries Coasts* 30:26-34.

Daoust RJ, Childers DL (1998) Quantifying aboveground biomass and estimating net aboveground primary production for wetland macrophytes using a non-destructive phenometric technique. *Aquat Bot* 62:115-133.

Day JW, Boesch DF, Clairain EJ, Kemp GP, Laska SB, Mitsch WJ, Orth K, Mashriqui H, Reed DJ, Shabman L, Simenstad CA, Streever BJ, Twilley RR, Watson CC, Wells JT, Whigham DF (2007) Restoration of the Mississippi Delta: lessons from hurricanes Katrina and Rita. *Science* 315:1679-1684.

DeLaune RD, Lindau CW (1990) Fate of ^{15}N labeled nitrogen in a *Sagittaria lancifolia* L. Gulf Coast marsh. *J Freshw Ecol* 5:265-268.

DeLaune RD, Pezeshki SR, Jugsujinda A (2005) Impact of Mississippi River freshwater reintroduction on *Spartina patens* marshes: responses to nutrient input and lowering of salinity. *Wetlands* 25:155-161.

Diaz RJ, Rosenberg R (2008) Spreading dead zones and consequences for marine ecosystems. *Science* 321:926-929.

Field DW, Reyer AJ, Genoves PA, Shearer BD (1991) Coastal wetlands of the United States: an accounting of a valuable national resource. Special NOAA 20th Anniversary Report. National Oceanic and Atmospheric Administration and United States Fish and Wildlife Service, Washington, D.C.

Frost JW, Schleicher T, Craft C (2009) Effects of nitrogen and phosphorus additions on primary production and invertebrate densities in a Georgia (USA) tidal freshwater marsh. *Wetlands* 29:196-203.

Galloway JN, Cowling EB (2002) Reactive nitrogen and the world: 200 years of change. *Ambio* 31:64-71.

Goolsby DA, Battaglin WA, Lawrence GB, Artz RS, Aulenbach BT, Hooper RP, Keeney DR, Stensland GJ (1999) Flux and sources of nutrients in the Mississippi-Atchafalaya River basin. *Topic 3 Report for the Integrated Assessment of Hypoxia in the Gulf of Mexico*. National Oceanic and Atmospheric Administration Coastal Ocean Program, Silver Springs, Maryland.

Gough L, Grace JB (1997) The influence of vines on an oligohaline marsh community: results of a removal and fertilization study. *Oecologia* 112:403-411.

Güsewell S, Bollens U (2003) Composition of plant species mixtures grown at various N:P ratios and levels of nutrient supply. *Basic Appl Ecol* 4:453-466.

Howarth R, Jensen WH, Marino R, Postma H (1995) Transport to and processing of P in near-shore and oceanic waters. In: Tiessen H (ed) *Phosphorus in the global environment: transfers, cycles and management*. Scope 54, Wiley, Chichester, UK.

Jefferies RL, Perkins N (1977) The effects on the vegetation of the additions of inorganic nutrients to salt marsh soils at Stiffkey, Norfolk. *J Ecol* 65:867-882

Kesel RH (1988) The decline in the suspended-load of the lower Mississippi River and its Influence on adjacent wetlands. *Environ Geol Water* Sci 11:271-281.

Kesel RH (2003) Human modifications to the sediment regime of the lower Mississippi River flood plain. *Geomorphology* 56:325-334.

Kiehl K, Esselink P, Bakker JP (1997) Nutrient limitation and plant species composition in temperate salt marshes. *Oecologia* 111:325-330.

Koerselman W, Meuleman AFM (1996) The vegetation N:P ratio: a new tool to detect the nature of nutrient limitation. *J Appl Ecol* 33:1441-1450.

LADEQ [Louisiana Department of Environmental Quality] (2006) Ambient surface water quality monitoring network. Water Quality Assessment Division. Baton Rouge, LA.

Lindig-Cisneros R, Desmond J, Boyer KE, Zedler JB (2003) Wetland restoration thresholds: can a degradation transition be reversed with increased effort? *Ecol Appl* 13:193-205.

Linthurst RA, Reimold RJ (1978) Evaluation of methods for estimating the net aerial primary productivity of estuarine angiosperms. *J Appl Ecol* 15:919-931.

Mendelssohn IA (1979) Influence of nitrogen level, form, and application method on the growth-response of Spartina alterniflora in North Carolina. *Estuaries* 2:106-112.

Mitsch WJ, Day JW, Gilliam JW, Groffman PM, Hey DL, Randall GW, Wang NM (2001) Reducing nitrogen loading to the Gulf of Mexico from the Mississippi River Basin: strategies to counter a persistent ecological problem. *Bioscience* 51:373-388.

Morris JT, Bradley PM (1999) Effects of nutrient loading on the carbon balance of coastal wetland sediments. *Limnol Oceanogr* 44:699-702.

Morse, JL, Megonigal JP, Walbridge MR (2004) Sediment nutrient accumulation and nutrient availability in two tidal freshwater marshes along the Mattaponi River, Virginia, USA. *Biogeochemistry* 69:175–206.

Odum WE (1988) Comparative Ecology of Tidal Freshwater and Salt Marshes. *Annu Rev Ecol Syst* 19:147-176.

Parsons ML, Dortch Q, Turner RE, Rabalais NR (2006) Reconstructing the development of eutrophication in Louisiana salt marshes. *Limnol Oceanogr* 51:534-544.

Patrick WH, Delaune RD (1976) Nitrogen and phosphorus utilization by *Spartina alterniflora* in a salt marsh in Barataria Bay, Louisiana. *Estuar Coast Mar Sci* 4:59-64.

Pennings SC, Stanton LE, Brewer JS (2002) Nutrient effects on the composition of salt marsh plant communities along the southern Atlantic and Gulf coasts of the United States. *Estuaries* 25:1164-1173.

Rabalais NN (2002) Nitrogen in aquatic ecosystems. *Ambio* 31:102–112.

Rydin H, Clymo RS (1989) Transport of carbon and phosphorus compounds about Sphagnum. *Proc R Soc Lond* 237:63-84.

Sasser CE, Visser JM, Mouton E, Linscombe J Hartley SB (2008) Vegetation types in coastal Louisiana in 2007. U.S. Geological Survery Open-File Report 2008-1224, 1 sheet, scale 1:550,000.

Silliman BR, Bertness MD (2004) Shoreline development drives invasion of *Phragmites australis* and the loss of plant diversity on New England salt marshes. Conserv Biol 18:1424-1434.

Slocum MG, Mendelssohn IA (2008) Effects of three stressors on vegetation in an oligohaline marsh. *Freshw Biol* 53:1783-1796.

Smalley AE (1959) The role of two invertebrate populations, *Littorina irrorata* and *Orchelimum fidicinum* in the energy flow of a salt marsh ecosystem. PhD dissertation, University of Georgia, Athens, GA.

Sullivan MJ, Daiber FC (1974) Response in production of cord grass, *Spartina alterniflora*, to inorganic nitrogen and phosphorus fertilizer. *Chesap Sci* 15:121-123.

Swenson EM, Chuang WS (1983) Tidal and subtidal water volume exchange in an estuarine system. *Estuar Coast Shelf Sci* 16:229-240.

Trahan L, Bradley JJ, Morris L (1990) *Soil survey of St. Tammany Parish, Louisiana*. U.S. Department of Agriculture, Soil Conservation Service, Washington D.C., USA. In

cooperation with Louisiana Agricultural Experiment Station and Louisiana State Soil and Water Conservation Committee, Baton Rouge, LA, USA.

Turner RE, Rabalais NN (1991) Changes in Mississippi River water quality this century. *Bioscience* 41:140-147.

Turner RE, Rabalais NN, Justic D (2006) Predicting summer hypoxia in the northern Gulf of Mexico: Riverine N, P, and Si loading. *Mar Pollut Bull* 52:139-148.

Tyler G (1967) On the effect of phosphorus and nitrogen supplied to Baltic shore-meadow vegetation. *Bot Notiser* 120:433-447.

Vaithiyanathan P, Richardson CJ (1999) Macrophyte species changes in the everglades: examination along a eutrophication gradient. *J Environ Qual* 28:1347-1358.

Valiela I, Teal JM (1974) Nutrient limitation in salt marsh vegetation. In: Reimold RJ, Queen WH (eds) *Ecology of halophytes*. Academic Press, New York, NY.

Valiela I, Teal JM, Sass WJ (1975) Production and dynamics of salt-marsh vegetation and effects of experimental treatment with sewage sludge – biomass, production, and species composition. *J Appl Ecol* 12:973-981.

van Heerwaarden LM, Toet S, Aerts R (2003) Nitrogen and phosphorus resorption efficiency and proficiency in six sub-arctic bog species after 4 years of nitrogen fertilization. *J Ecol* 91:1060-1070.

Visser JM, Sasser CE, Chabreck RH, Linscombe RG (1998) Marsh vegetation types of the Mississippi River Deltaic Plain. *Estuaries* 21:818-828.

Welder FA (1959) Processes of deltaic sedimentation in the lower Mississippi River. Louisiana State University, Coastal Studies Institute Technical Report, Baton Rouge, LA, USA.

Whigham DF, Nusser SM (1990) The response of *Distichlis spicata* (L.) Green and *Spartina patens* (Ait.) Muhl. to nitrogen fertilization in hydrologically altered wetlands. In: Whigham DF *et al.* (eds) *Wetland ecology and management: case studies*. Kluwer Academic Publishers, Netherlands.

Wigand C, Thursby GB, McKinney RA, Santos AF (2004) Response of Spartina patens to dissolved inorganic nutrient additions in the field. *J Coast Res*:134-149.

CHAPTER 3
CONTRASTING EFFECTS OF LONG-TERM NUTRIENT ENRICHMENT ON BELOWGROUND BIOMASS IN COASTAL WETLANDS

3.1 Introduction

Nutrient-induced changes in plant growth resulting from anthropogenically-enhanced nitrogen (N) and phosphorus (P) availability elicit diverse effects on ecosystem structure and function. Numerous studies in a variety of habitats around the world have shown conclusively that excess nutrients stimulate the biomass of plants aboveground (Elser *et al.* 2007), and that enhanced plant growth can have cascading effects on nutrient cycling, competitive hierarchies, community composition, and biodiversity (Bedford et al. 1999, Smith *et al.* 1999, Suding *et al.* 2005, Bobbink *et al.* 2010). In contrast, similar perspectives of eutrophic expressions occurring in the soil environment are currently lacking, despite the important role belowground plant biomass has in regulating, for example, soil organic matter accumulation (Rasse *et al.* 2005), microbial biomass (Fierer *et al.* 2009), and biogeochemical cycling (Freschet *et al.* 2013). Thus, the implications of enhanced nutrient supply on valuable ecosystem services derived from soil processes, such carbon sequestration (Lu *et al.* 2013), are currently under debate.

Although the belowground plant response to nutrient over-enrichment, as well as any consequential effects on soil organic matter, is clearly important on a global scale, it can be argued that understanding soil-nutrient dynamics is more critical in coastal wetlands than in any other ecosystem. High rates of plant production combined with slow decomposition promote organic matter (carbon) accumulation in coastal wetland soils rivaling that of terrestrial forests, despite representing only a fraction of the land surface area by comparison (Mcleod *et al.* 2011). In addition, belowground biomass is an essential determinant of coastal wetland stability that not only helps counterbalance the effects of sea level rise by modulating surface elevation change

through contributions to soil volume (McKee *et al.* 2007, Kirwan & Mudd 2012), but plant roots and rhizomes also stabilize the soil matrix during extreme meteorologic events such as hurricanes (Howes *et al*. 2010). At the same time, coastal wetlands are also particularly vulnerable to eutrophication due to their low elevational position along coastal margins and hydrologic forcings from both the land and sea that serve as direct vectors for nutrient input. Consequently, recent research has focused considerable efforts to identify the effects of nutrient enrichment on soil processes, though it currently remains unclear why nutrient additions can have either positive, negative, or no effect on belowground biomass (e.g., Tyler *et al.* 2007, Darby & Turner 2008a, b, Hunter *et al.* 2008, Langley *et al.* 2009, Anisfeld & Hill 2011, Ket *et al.* 2011, Deegan *et al.* 2012, Nelson & Zavaleta 2012).

Surprisingly, little attention has been given to methodological differences that may be affecting overall conclusions concerning the belowground effects of nutrient enrichment. Even though dissimilarities among methods have been identified as a source of variation along other resource gradients, such as soil moisture availability (Hendricks *et al.* 2006), it is currently commonplace for the effects of nutrient enrichment on belowground biomass to be assessed using both the ingrowth and standing crop methods. However, the use of different assessment procedures necessitates interpretations that distinguish the distinct aspects of plant growth being measured; the ingrowth method measures new belowground growth into unexploited substrate after intact roots and rhizomes are severed and removed, whereas the standing crop method measures *in situ*, or maintenance, belowground biomass of plants in equilibrium with their environment. Although previous work by Neill (1992) concluded that under ambient conditions these methods offered "reasonably similar estimates of annual belowground net primary production," actual patterns of belowground biomass measured over time contrasted

substantially between the two methods. For example, *in situ* root standing crop remained at near steady state, while root accumulation in ingrowth cores increased steadily from zero. Subsequent work by Hendricks *et al*. (2006) also showed this same difference in root biomass temporal pattern between the two methods. To date, results obtained using these methods have not been critically examined under elevated nutrient conditions or ruled out as a potential source of error. Furthermore, regardless of the method used, greater measurement resolution, that results from estimates of individual organic matter pools, increases the ability to discern nutrient-induced responses specific to a particular belowground biomass component (e.g., live roots) that may be unidentifiable with bulk measurements of total (live + dead) belowground biomass within the timeframe of most fertilization studies. To my knowledge, Valiela *et al.* (1976) is the only study to use both of these methods to investigate the direct effects of coastal wetland nutrient enrichment on the various belowground biomass pools individually, and their results suggested different responses occurred for living belowground biomass components depending on method used, although no explanation was offered as to why this occurred.

The present research addresses these methodological considerations that may constrain a detailed understanding of the effects of nutrient enrichment on belowground plant biomass by comparing results obtained using different belowground biomass estimation techniques and assessing the extent to which measurement of different biomass pools confounds overall findings. Although much of the controversial literature derives from salt marsh research (e.g. Darby & Turner 2008a, b, Anisfeld & Hill 2012), it is low salinity wetlands that dominate the landscape where rivers deliver freshwater, and elevated nutrient loads, to coastal environments, such as in the Mississippi River Delta (Sasser *et al.* 2008). Therefore, I quantified live and dead belowground biomass components using the ingrowth and standing crop methods within an

oligohaline marsh that has been fertilized with a factorial combination of N and P for nine years. I propose that lack of consensus in the belowground plant response to nutrient enrichment stems from the measurement of different organic matter pools using different methods that require different interpretations: (1) the ingrowth method serves as a proxy for belowground growth into unexploited soil or open habitat, (2) the standing crop method represents the quantity of belowground biomass required to sustain the nutritional needs of established plants, and (3) regardless of method used, the measurement of component biomass pools increases the ability to identify nutrient enrichment effects occurring belowground. I hypothesized that N enrichment simultaneously increases live belowground biomass accumulation in ingrowth cores containing root/rhizome free soil and decreases *in situ* live standing crop, but has no effect on total belowground biomass within my measurement timeframe.

3.2 Materials and Methods

3.2.1 Site Description and Experimental Design

My study site is a species rich, *Sagittaria lancifolia* L. dominated, oligohaline (i.e., intermediate-brackish) marsh located along the west bank of the Tchefuncte River, approximately 1 km north of Lake Pontchartrain, LA, USA, (30° 23.205'N, 90° 09.551' W). Soil at the site is classified as a Kenner series Histosol (euic, thermic Fluvaquentic Medisaprist) formed from herbaceous plant material and characterized as "very poorly drained, rapidly permeable organic soil" (Trahan *et al.* 1990). Marsh flooding results from a combination of microtidal influence (10 cm tide range; Swenson & Chuang, 1983) and wind shifts during frontal passages that affect water levels in Lake Pontchartrain and the Tchefuncte River. Previous research at this site determined that soil surface inundation occurs on average (1999-2006) approximately every other day to a depth of 15 cm with surface water that has the following

water quality characteristics: 1.6 g L^{-1} salinity, 0.26 mg inorganic N L^{-1}, 0.79 mg total N L^{-1}, and 0.11 mg total P L^{-1} (Chapter 2).

My fertilization experiment consisted of eight 1-m^2 plots replicated at five locations spaced 5-10 m apart parallel to a small drainage canal (i.e., 40 plots total). At each location, plots were fertilized with one of four N levels (0, 50, 200, or 1200 kg N ha^{-1} yr^{-1} applied as Nutralene Methylene Urea 40-0-0) in combination with one of two levels of P (0 or 131 kg P ha^{-1} yr^{-1} applied as Humaphos 0-5-0), yielding eight treatment combinations (n = 5 per treatment) within a randomized complete block design. Treatment levels were maintained for nine years by applying granulated, slow-release fertilizer in April and July of 2002 through 2010.

3.2.2 Sample Collection

I used the ingrowth method to estimated belowground plant biomass accumulation in root and rhizome free sediment over a three-year period from 2005 to 2008, four to seven years after initiating fertilization treatments. The native soil at 5 locations within each plot was removed to a depth of 30 cm using a 7.62 cm diameter aluminum core tube and replaced with creek bank sediment collected from a nearby marsh with a similar vegetative community. Roots and rhizomes, both live and dead, were hand picked from the creek bank sediment prior to installation, but the sediment was not sieved in order to recreate a soil environment that contained a natural mix of soil organic matter similar to the study marsh. Upon installation in June 2005, the location of each ingrowth core was marked with a 4 cm long by 7.62 cm diameter PVC collar inserted approximately 2 cm into the soil surface. A randomly selected ingrowth core was then extracted from each plot using the same aluminum core tube after 0.5, 1, 1.5, 2, and 3 yr (n = 5 per treatment at each time period). The extracted ingrowth cores were sieved over a 2 mm mesh screen and the live (roots + rhizomes) material was separated, dried to a constant

weight at 60^{o}C, and weighed. On the final measurement in June 2008, after three years of soil incubation, live roots, live rhizomes, and dead (roots + rhizomes) were categorized separately. In all samplings, live material was separated using a combination of characteristics that included color, turgidity, and evidence of decomposition (e.g., epidermal lesions and resistance to breakage). When necessary, a dissecting microscope (3x magnification) was used to examine the belowground material in more detail.

To estimate belowground standing crop, I extracted a 7.62 cm diameter by 30 cm long soil core from each plot using an aluminum core tube at the end of the growing season in 2006, 2008, and 2010, corresponding with year five, seven, and nine of fertilization. In all years, total (live + dead) belowground standing crop was estimated by washing each core over a 2 mm mesh sieve. In 2008, the belowground material was further categorized as live roots, live rhizomes, and dead (roots + rhizomes) standing crop using the separation method described above. After sieving and/or sorting, all material was dried to a constant weight at 60^{o}C, and weighed.

3.2.3 Statistical Analysis

All statistical analyses were conducted using SAS (Statistical Analysis Systems, version 9.2, SAS Institute, Inc., Cary, NC). I used univariate two- and three-way mixed-model ANOVA (PROC MIXED) to identify the effects of N, P, time (as a repeated measure when applicable), and their interactions on belowground biomass pools quantified using the ingrowth and standing crop methods separately, including live root biomass, live rhizome biomass, live (root + rhizome) biomass, dead (root + rhizome) biomass, and total (live + dead) biomass. I verified ANOVA assumptions of normality and homoscedasticity by examining normal probability plots and residual plots, respectively (PROC UNIVARIATE). When necessary, these data were logarithmically or square root transformed prior to analysis to validate model assumptions. Upon

finding a fixed effect at $p \leq 0.10$, differences among treatment means were tested using the Tukey-Kramer multiple comparison test, which maintains an experiment-wise error rate for all pair-wise comparisons as specified.

3.3 Results

3.3.1 Belowground Biomass Accumulation (Ingrowth)

Live (roots + rhizomes) biomass accumulation in ingrowth cores increased significantly over the three-year period of measurement from June 2005 to June 2008 (see [a] in Figure 3.1). New growth obtained a maximum of 433 ± 51 g m^{-2} in year two, with no additional live biomass accumulation occurring in year three. Nutrient enrichment had no significant effect on the accumulation of live biomass over time ($p \geq 0.12$; Table 3.1), although on average, plots that received 1200 kg N ha^{-1} yr^{-1} were consistently higher than the controls, especially after 2 and 3 years of ingrowth (year 2: 495 ± 133 g m^{-2} vs. 346 ± 57 g m^{-2}; year 3: 561 ± 169 g m^{-2} vs. 225 ± 56 g m^{-2}, respectively). However, a significant N effect on live root biomass was observed when roots were separated from rhizomes in the final year of measurement (2008), after three years of ingrowth (Table 3.1). Live root accumulation increased with increasing N enrichment to where ingrowth cores in plots receiving 1200 kg N ha^{-1} yr^{-2} had significantly greater root biomass than control plots (see [b] in Figure 3.1). Live root accumulation was also influenced by P enrichment when applied in combination with N ($p = 0.08$; Table 3.1), but the overall trend was similar to that which occurred with N enrichment alone. The N x P interaction was driven by a single positive response to P at the 50 kg N ha^{-1} yr^{-2} enrichment level in contrast to the other N enrichment treatments that had greater live root accumulation when P was not applied in combination, though these N x P treatment means were not significantly different from each other ($p \geq 0.24$, Tukey-Kramer multiple comparison test; data not shown). Nutrient enrichment

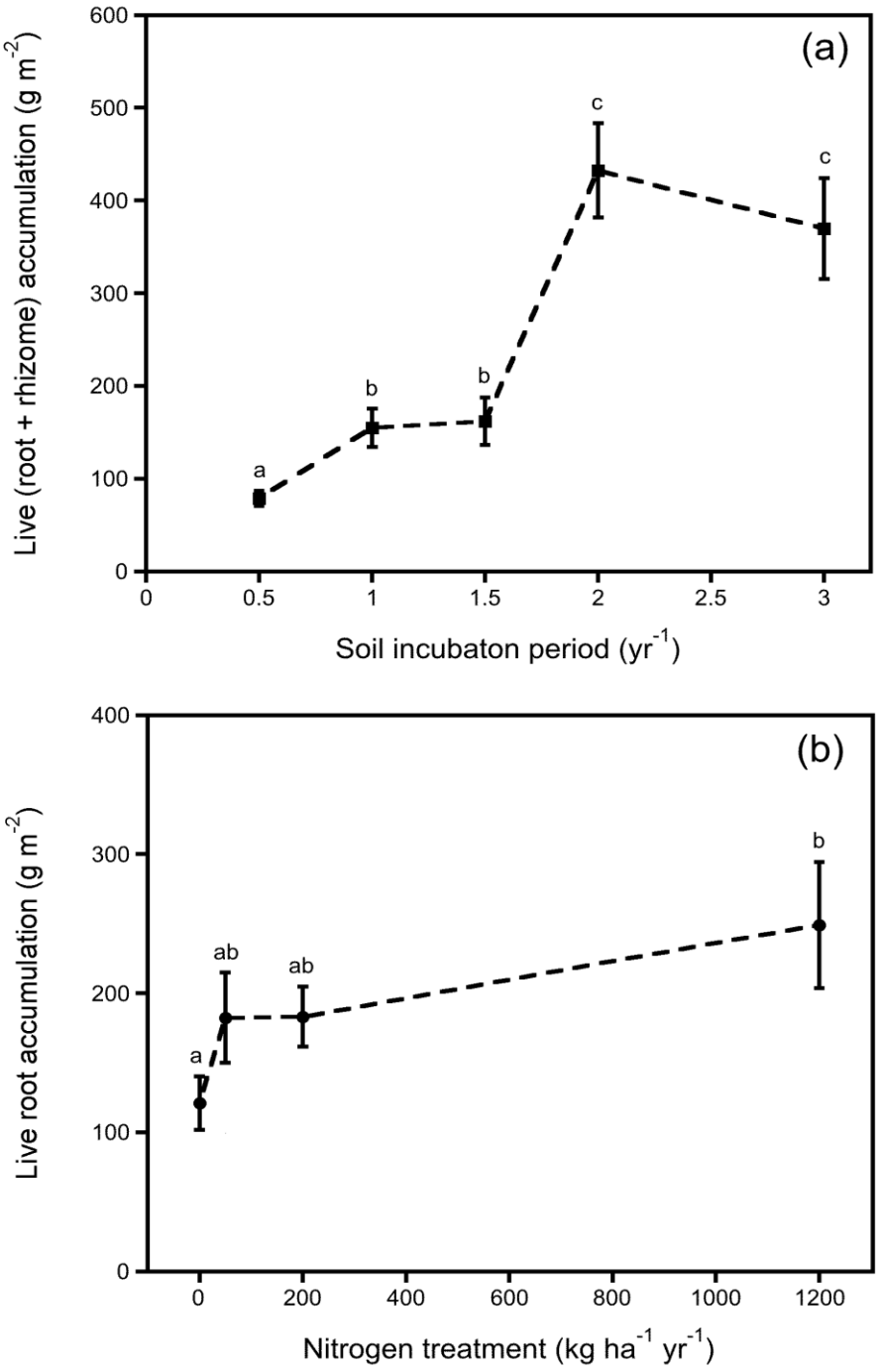

Figure 3.1 (a) Live (root + rhizome) and (b) live root biomass accumulation in ingrowth cores. Values in (a) are means (± SE; n = 40), averaged over all treatment levels (i.e., no significant N-, P-, or N x P-effect) for each time period (June 2005 through June 2008), whereas values in (b) are N-treatment means (± SE; n = 10) averaged over P treatment levels (i.e., no significant P- or N x P-effect) after three years of ingrowth (June 2008). Means identified by different letters are significantly different ($p \leq 0.05$, Tukey-Kramer multiple comparison test).

Table 3.1 Summary of 2- and 3-way ANOVAs showing the effects of nitrogen (N), phosphorus (P), time (as a repeated measure where applicable), and their interactions on belowground biomass accumulation in ingrowth cores. Live (root + rhizome) biomass was measured five times from 2005 to 2008. Live roots, live rhizomes, dead (roots + rhizomes), and total (live + dead) biomass were measured separately in 2008 only. Superscripted dependent variables were natural log (ln) or square root (sr) transformed prior to analysis to meet ANOVA assumptions of normality and homoscedasticity. Values are *F*-ratios, with associated numerator and denominator degrees of freedom subscripted in parentheses, and *P*-values that are underlined when significant ($p \leq 0.05$). When no significant treatment effect existed, the overall mean (± SE) is displayed; otherwise the figure that displays the significantly different treatment means is referenced.

	2005-2008		2008							
	Live (Roots + Rhizomes)^{ln}		Live Roots		Live Rhizomes^{sr}		Dead (Roots + Rhizomes)		Total (Live + Dead)^{ln}	
Model Source	$F_{(ndf, ddf)}$	*P*	$F_{(ndf, ddf)}$	*P*	$F_{(ndf, ddf)}$	*P*	$F_{(ndf, ddf)}$	*P*	$F_{(ndf, ddf)}$	*P*
N	$2.14_{(3, 28.5)}$	0.12	$3.39_{(3, 27.2)}$	<u>0.03</u>	$0.94_{(3, 27.3)}$	0.43	$0.71_{(3,31)}$	0.55	$1.65_{(3,27.4)}$	0.20
P	$0.01_{(1, 28.5)}$	0.91	$0.12_{(1, 27.2)}$	0.73	$0.83_{(1, 27.3)}$	0.37	$0.05_{(1,31)}$	0.82	$0.05_{(1,27.4)}$	0.82
N x P	$2.10_{(3, 28.5)}$	0.12	$2.49_{(3, 27.2)}$	0.08	$0.87_{(3, 27.3)}$	0.47	$1.42_{(3,31)}$	0.26	$2.07_{(3,27.4)}$	0.13
Time	$25.04_{(4, 97.5)}$	<u><0.0001</u>								
N x Time	$0.38_{(12, 108)}$	0.97								
P x Time	$0.26_{(4, 97.4)}$	0.90								
N x P x Time	$0.98_{(12, 108)}$	0.47								
Mean ± SE (g m^{-2})	*Figure 3.1(a)*		*Figure 3.1(b)*		186 ± 41		178 ± 20		548 ± 61	

had no effect on live rhizome biomass, dead (roots + rhizomes) biomass, or total (live + dead) biomass accumulation in ingrowth cores extracted in 2008 (Table 3.1).

3.3.2 Belowground Standing Crop

Total (live + dead) belowground standing crop measured in 2006, 2008, and 2010 was not significantly affected by nutrient enrichment and did not vary significantly from year to year (Table 3.2). However, an N x P interaction ($p = 0.08$; Table 3.2) tended to suggest that P enrichment in combination with 1200 kg N ha^{-1} yr^{-1} increased total standing crop (3803 ± 368 g m^{-2}) when compared to enrichment with only 1200 kg N ha^{-1} yr^{-1} (2946 ± 201 g m^{-2}), though these treatment combinations were not significantly different from the control (3310 ± 334 g m^{-2}) or each other ($p \geq 0.30$, Tukey-Kramer multiple comparison test). In contrast, when total belowground standing crop was further separated into component biomass pools in 2008, live root standing crop decreased with increasing N enrichment to where a significant reduction compared to the control was observed with N enrichment ≥ 200 kg N ha^{-1} yr^{-1} (Figure 3.2). Nitrogen enrichment had a similar effect on live (root + rhizome) standing crop ($p = 0.08$; Table 3.2), which tended to decrease with increasing N enrichment (control = 456 ± 159 g m^{-2} vs. 1200 kg N ha^{-1} yr^{-1} = 184 ± 77 g m^{-2}; $p = 0.08$, Tukey-Kramer multiple comparison test). Although, this trend was likely driven by the significantly lower root standing crop since nutrient enrichment did not have an effect on the standing crop of live rhizomes (Table 3.2). Dead standing crop was also unaffected by nutrient enrichment (Table 3.2).

3.4 Discussion

As predicted, my results show that living belowground plant biomass in this oligohaline marsh is significantly affected by long-term N enrichment, but not by P enrichment or its interaction with N enrichment. Significant changes in live root biomass were observed in

Table 3.2 Summary of 2- and 3-way ANOVAs showing the effects of nitrogen (N), phosphorus (P), year (as a repeated measure where applicable), and their interactions on *in situ* belowground standing crop biomass. Total (live + dead) standing crop was measured in 2006, 2008, and 2010. Live (roots + rhizomes), live roots, live rhizomes, and dead (roots + rhizomes) were measured separately in 2008 only. Superscripted dependent variables were natural log (ln) transformed prior to analysis to meet ANOVA assumptions of normality and homoscedasticity. Values are *F*-ratios, with associated numerator and denominator degrees of freedom subscripted in parentheses, and *P*-values that are underlined when significant ($p \leq 0.05$). When no significant treatment effect existed, the overall mean (± SE) is displayed; otherwise the figure that displays the significantly different treatment means is referenced.

	2006, 2008, 2010		2008							
	Total (Live + Dead)^{ln}		Live (Roots + Rhizomes)^{ln}		Live Roots^{ln}		Live Rhizomes^{ln}		Dead (Roots + Rhizomes)	
Model Source	$F_{(ndf, ddf)}$	*P*	$F_{(ndf, ddf)}$	*P*	$F_{(ndf, ddf)}$	*P*	$F_{(ndf, ddf)}$	*P*	$F_{(ndf, ddf)}$	*P*
N	$0.61_{(3, 39.6)}$	0.61	$2.56_{(3, 28)}$	0.08	$4.07_{(3, 28)}$	<u>0.02</u>	$1.56_{(3, 28)}$	0.22	$0.66_{(3, 28)}$	0.58
P	$0.09_{(1, 39.6)}$	0.77	$0.01_{(1, 28)}$	0.91	$2.19_{(1, 28)}$	0.15	$0.44_{(1, 28)}$	0.51	$0.36_{(1, 28)}$	0.55
N x P	$2.45_{(3, 39.6)}$	0.08	$1.31_{(3, 28)}$	0.29	$0.18_{(3,28)}$	0.91	$1.67_{(3, 28)}$	0.20	$0.76_{(3, 28)}$	0.52
Year	$0.37_{(2, 62.8)}$	0.69								
N x Year	$1.20_{(6, 66.1)}$	0.32								
P x Year	$0.17_{(2, 62.8)}$	0.85								
N x P x Year	$0.54_{(6, 66.1)}$	0.78								
Mean ± SE (g m^{-2})	3162 ± 102		293 ± 56		*Figure 3.2*		240 ± 55		2782 ± 140	

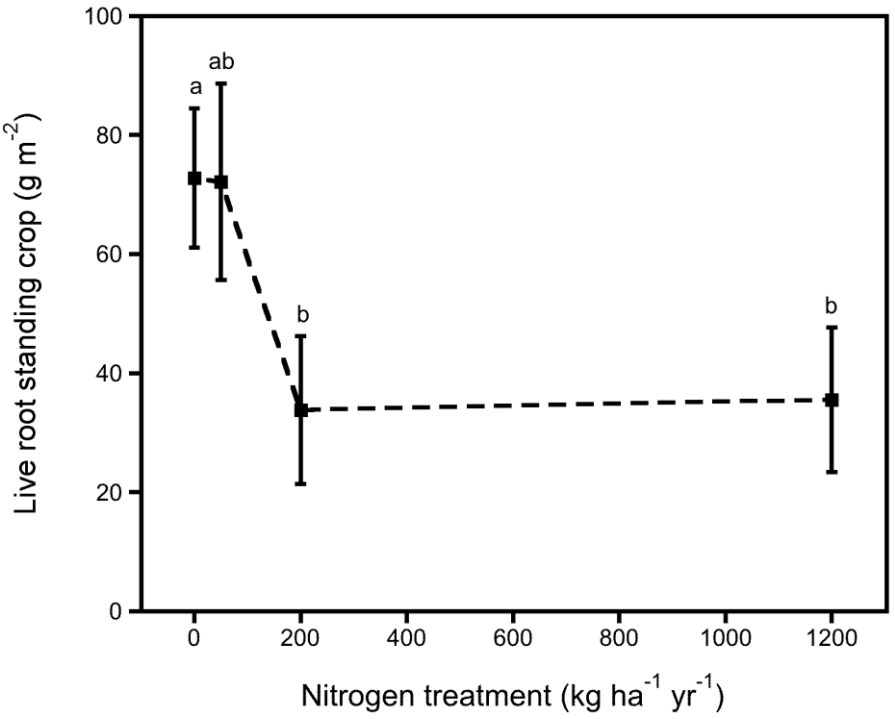

Figure 3.2 Live root standing crop. Values are N-treatment means (± SE; n = 10), averaged over P treatment levels (i.e., no significant P- or N x P-effect) for cores collected in 2008. Means identified by different letters are significantly different ($p \leq 0.05$, Tukey-Kramer multiple comparison test).

response to N enrichment using both the ingrowth method and the standing crop method. This result of N-affected belowground plant growth is consistent with documented aboveground N-limitation in this marsh (Chapter 2) and numerous other coastal marshes (Sullivan & Daiber 1974, Valiela & Teal 1974, Mendelssohn 1979, Cargill & Jefferies 1984, Boyer *et al.* 2001, Wigand *et al.* 2004, Frost *et al*. 2009, Anisfeld & Hill 2011, Fox *et al.* 2012, and many more). Previous research in this marsh also shows that significant changes in belowground biomass do not occur until maximum aboveground production is achieved through N enrichment (i.e., $\geq$ 200 kg N ha^{-1} yr^{-1}; Chapter 2). My results are also consistent with previous research that identified N as the primary nutrient affecting belowground biomass when applied independently and in combination with P (Valiela *et al.* 1976, Hines *et al.* 2006, Tyler *et al.* 2007, Davey *et al*. 2011, Ket *et al.* 2011, Nelson & Zavaleta 2012), although belowground biomass responses due only to

P enrichment have been observed in salt marshes (Darby & Turner 2008a, b). The present research also suggests possible N x P interactions, which further suggests that the importance of P in regulating belowground biomass may vary locally or regionally depending on relative N and P loading rates. Nonetheless, these results collectively indicate that N is the primary nutrient regulating plant growth both above- and belowground in coastal marshes, with P playing a lesser role.

Also occurring as predicted, N-induced belowground response trajectories in the present study were dependent upon both method used and belowground biomass pool measured. Nitrogen enrichment affected only live roots (i.e., the primary nutrient acquisition structures), but when compared across the two commonly used belowground biomass estimation techniques (i.e., the ingrowth method vs. the standing crop method), the effect of N enrichment was incongruent. Even though live root biomass in unfertilized control plots were similar on average among the two methods (see [b] in Figure 3.1, and Figure 3.2), live root accumulation in ingrowth cores increased with increasing N availability, while live root standing crop decreased following N enrichment. Moreover, nutrient enrichment had no significant effect on other belowground biomass pools (e.g., live rhizomes, dead, and total biomass) using either method. In fact, my results are strikingly similar to the first measurements using both the ingrowth and standing crop methods simultaneously to investigate belowground responses to nutrient enrichment in a salt marsh (Valiela *et al.* 1976).

In general, the different N-enrichment results that occurred in the present study between the ingrowth and standing crop methods are consistent with previously published coastal wetland fertilization experiments that have used these methods over the course of more than 35 years of belowground research (Tables 3.3 & 3.4). Of the five studies (present included) that used the

Table 3.3 Coastal wetland belowground response to nutrient enrichment as determined by the ingrowth method.

Reference[1]	Location	Site Vegetation[2]	Years Fertilized	Nutrients Applied[3]	Core Incubation	Biomass Pool[4]
Evidence of Reduced Biomass						
None						
Evidence of Increased Biomass						
Valiela *et al.* (1976)[5]	Massachusetts	SPAL, SPPA	2 or 3	Urea (N), N+P+K	2 months	Roots, Rhizomes
McKee *et al.* (2007)[6]	Belize	RHMA (2)	8	N, P	6 months	Total roots
This study	Louisiana	SALA	7	N, P, N+P	3 years	Roots
Evidence of No Biomass Response						
McKee *et al.* (2007)[6]	Belize	RHMA	8	N, P	6 months	Total roots
Langley *et al.* (2009)	Maryland	SCAM/SPPA/DISP	2	N	1 year	Total fine roots
Anisfeld & Hill (2011)	Connecticut	SPAL	5	N, P, N+P	6 months	Total

[1]References listed are peer reviewed journal articles that established a cause and effect relationship by applying fertilizer to attached wetland substrates in which the natural plant community grew under ambient field conditions in oligohaline marsh (this study), brackish marsh (Langley *et al.*, 2009), salt marsh (Valiela *et al.*, 1976; Anisfeld & Hill, 2011), and mangrove (McKee *et al.*, 2007) environments.

[2]A comma (,) separates dominant plant species growing in different plant communities, whereas a solidus (/) separates co-dominants growing within the same plant community. A number contained within parentheses indicates that multiple sites within similar plant communities were fertilized. Plant species abbreviations are as follows: DISP = *Distichlis spicata*, RHMA = *Rhizophora mangle*, SALA = *Sagittaria lancifolia*, SCAM = *Schoenoplectus americanus*, SPAL = *Spartina alterniflora*, SPPA = *Spartina patens*.

[3]Applied nutrients are nitrogen (N), phosphorus (P), and potassium (K). Nutrient additions that caused a belowground response are underlined.

(Table 3.3 continued)

[4]Belowground biomass pools affected by nutrient enrichment are identified. If no effect was observed, the measured pools are identified. Biomass descriptors are as follows: Roots = living root biomass, Rhizomes = living rhizome biomass, Total roots = live + dead root biomass, Total fine roots = live + dead root biomass < 2 mm, Total = live + dead root and rhizome biomass.

[5]Urea (N) was applied for 2 years while treated municipal sludge (N+P+K) was applied for 3 years. Two-month core incubations were repeated throughout the growing season from March to September to obtain cumulative estimates of annual production. Results were not statistically analyzed; evidence of nutrient effects on belowground biomass was based on the authors' written interpretation of the data and by examining Table 1 in Valiela *et al*. (1976). Note: nutrient additions had no obvious effect on SPPA root biomass.

[6]Sites with similar plant communities were fertilized in fringe, transition, and interior mangrove forests. Six-month core incubations were summed to obtain annual root accumulation over a 3-year period. Transition and interior mangroves responded to fertilization but fringe mangroves did not.

Table 3.4 Coastal wetland belowground response to nutrient enrichment as determined by the standing crop method.

Reference[1]	Location	Site Vegetation[2]	Years Fertilized	Nutrients Applied[3]	Biomass Pool[4]
Evidence of Reduced Biomass					
Valiela *et al.* (1976)[5]	Massachusetts	SPAL, SPPA	2 or 3	Urea (N), N+P+K	Roots
Morris & Bradley (1999)	South Carolina	SPAL	13	N+P	Total
Hines *et al.* (2006)[6]	New Jersey	SPAL	2	N	Roots
Darby & Turner (2008a)	Louisiana	SPAL	1	N, P, Fe, N+P, N+Fe, P+Fe, N+P+Fe	Live
Darby & Turner (2008b)[7]	Louisiana	SPAL (4)	1	N+P	Live
Darby & Turner (2008b)[8]	Massachusetts	SPAL	1	N+P, P	Live
Darby & Turner (2008b)	Nova Scotia	SPAL	1	N+P	Live
Darby & Turner (2008b)	Virginia	SPAL (2)	1	N+P, P	Live
Ket *et al*. (2011)	Georgia	ZIMI	5	N, P, N+P	Rhizomes, Total
Davey et al. (2011)	South Carolina	SPAL	13	N, P, N+P	Total fine roots
Deegan *et al*. (2012)[9]	Massachusetts	SPAL	7	N+P	Live
This study	Louisiana	SALA	7	N, P, NxP	Roots
Evidence of Increased Biomass					
Valiela *et al*. (1976)[5]	Massachusetts	SPPA	2 or 3	Urea (N), N+P+K	Rhizomes
Tyler *et al*. (2007)	Washington	SPAL (3)	1	N	Live
Darby & Turner (2008b)[8]	Massachusetts	SPAL	1	N+P, P	Live
Hunter *et a*l. (2008)	Alabama	SABI	1	N+P	Total
Nelson & Zavaleta (2012)[10]	California	SAPA	2	N	Total

(Table 3.4 continued)

Reference[1]	Location	Site Vegetation[2]	Years Fertilized	Nutrients Applied[3]	Biomass Pool[4]
Evidence of No Biomass Response					
Gallagher (1975)[11]	Georgia	SPAL	1	N	Total
Wigand *et al*. (2004)	Rhode Island	SPPA	3	N, P, NxP	Total
Tyler *et al*. (2007)[12]	California	SPHY (3)	1	N	Live
Hunter *et al*. (2008)	Alabama	DISP, DISP/SABI	1	N+P	Total
Anisfeld & Hill (2011)	Connecticut	SPAL	5	N, P, N+P	Total

[1]References listed are peer reviewed journal articles that established a cause and effect relationship by applying fertilizer to attached wetland substrates in which the natural plant community grew under ambient field conditions in tidal freshwater marsh (Ket *et al*., 2011), oligohaline marsh (this study), and salt marsh (13 references) environments. Where applicable, distinct locations and plant communities are identified separately for each reference.

[2]A comma (,) separates dominant plant species growing in different plant communities, whereas a solidus (/) separates co-dominants growing within the same plant community. A number contained within parentheses indicates that multiple sites within similar plant communities were fertilized. Plant species abbreviations are as follows: DISP = *Distichlis spicata*, SALA = *Sagittaria lancifolia*, SABI = *Salicornia bigelovii*, SAVI = *Salicornia virginica*, SAPA = *Sarcocornia pacifica*, SPAL = *Spartina alterniflora*, SPFO = *Spartina foliosa*, SPHY = *Spartina alterniflora x foliosa* (hybrid), SPPA = *Spartina patens*, ZIMI = *Zizaniopsis miliacea*.

[3]Applied nutrients are nitrogen (N), phosphorus (P), potassium (K), and iron (Fe). Nutrient additions that caused a belowground response are underlined.

[4]Belowground biomass pools affected by nutrient enrichment are identified. If no effect was observed, the measured pools are identified. Biomass descriptors are as follows: Roots = living root biomass, Rhizomes = living rhizome biomass, Live = living root + living rhizome biomass, Total fine roots = live + dead root biomass < 1 mm, Total = live + dead root and rhizome biomass.

[5]Urea (N) was applied for 2 years while treated municipal sludge (N+P+K) was applied for 3 years. Results were not statistically analyzed; evidence of nutrient effects on belowground biomass was based on the authors' written interpretation of the data and by examining Figure 3 in Valiela *et al*. (1976). Note: urea had no obvious effect on SPAL root biomass.

(Table 3.4 continued)

[6]$p = 0.06$.

[7]Results from Darby & Turner (2008a) were excluded to avoid duplicating results.

[8]Results from Valiela *et al.* (1976) were excluded to avoid duplicating results.

[9]$p = 0.08$.

[10]November 2009 measurements ($p = 0.09$); July 2009 measurements ($p = 0.47$).

[11]No effect overall (0-55 cm) or incrementally (0-15 cm, 35-55 cm); $p = 0.10$ at the 15-35 cm depth increment.

[12]The three sites fertilized in California had different sub-dominant vegetation representing different habitat types invaded by SPHY: mudflat, SPFO-dominated marsh, and SAVI-dominated marsh.

ingrowth method at eight coastal wetland sites in five different geographic locations, three studies measured an increase in live root and/or rhizome biomass accumulation at five sites in three different locations, while three studies at three sites in three locations found no effect from nutrient enrichment (Table 3.3). Interestingly, not one of the ingrowth studies measured a reduction in belowground biomass following nutrient enrichment relative to unfertilized control plots. By comparison, I identified 15 studies (31 sites in 12 locations) that used the standing crop method to investigate the effects of fertilization on belowground biomass in coastal wetlands (Table 3.4). Although belowground standing crop results are more variable than those obtained using the ingrowth method, the majority show a negative effect following fertilization: nine studies measured a decrease in some component of belowground standing crop at 17 sites in seven different locations, while five studies measured an increase at seven sites in four locations, and five studies found no effect at eight sites in five locations. Across both methods, the effects of nutrient enrichment on belowground biomass were primarily associated with the live portion of belowground biomass or it's component roots and rhizomes rather than dead or total (live + dead) biomass pools (Table 3.3). All eight fertilization studies that distinguished live biomass, or it's components, from dead biomass observed a response to nutrient enrichment, although this was the case at only one of two locations fertilized by Tyler *et al*. (2007). In contrast, only six of 17 studies, less one of three sites fertilized by McKee *et al*. (2007), observed a belowground response to nutrient enrichment without separating live from dead, though several (three) did differentiate total root biomass, and most (four) were long-term fertilization experiments.

Collectively, these results show that nutrient over-enrichment has contrasting effects on belowground biomass in coastal wetlands depending on the method of measurement and biomass pool measured. The ingrowth method obviously measures new belowground plant growth into

unvegetated soil; my results show that new live biomass accumulation in ingrowth cores can occur over a 2-year period before equilibrium is established (see [a] in Figure 3.1). Therefore, since none of the other references in Table 3.3 incubated cores for more than one year, it is likely that all were measuring the effects of nutrient enrichment on new belowground growth as it replenishes an under utilized volume of soil. Thus, in situations where plants can exploit unvegetated soil, nutrient enrichment generally results in enhanced live belowground biomass accumulation. In contrast, nutrient enrichment generally reduces live belowground biomass in situations where established plant communities sustain nutritional requirements via maintenance growth, as evidenced using the standing crop method (Table 3.4). When both measurement method and biomass pool measured are considered, the research presented in Tables 3.3 & 3.4 provides strong evidence supporting my hypothesis for this disparity in the belowground response to nutrient enrichment (88% by reference, 86% by location, and 74% by site), and minimal evidence in opposition (29%, 29%, and 29%, respectively).

Even with such strong support, my hypothesis becomes more apparent when some of the belowground responses presented in Table 3.4 are interpreted as representing new growth into unexploited soil as well. For example, Hunter *et al*. (2008) found that nutrient enrichment stimulated belowground standing crop of the annual plant *Salicornia bigelovii* but not the perennial plant *Distichlis spicata* or mixed *S. bigelovii/D. spicata* stands, which indicates that nutrient enrichment facilitates plant establishment and new belowground growth that occurs when plants propagate from seed. In a second example, Nelson & Zavaleta (2012) determined that enhanced root standing crop coincided with high soil mineral matter content (75% by weight) resulting from a constant sedimentation rate of 2-5 mm yr^{-1}, which again supports my hypothesis that nutrient enrichment stimulates new growth into unexploited substrate. Thirdly,

Tyler *et al*. (2007) found that N enrichment increased *Spartina alterniflora* and *Spartina* hybrid (*alterniflora x foliosa*) invasion of mud flats in Willapa Bay, WA and in San Francisco Bay, CA, respectively. Although live belowground standing crop increased with nutrient enrichment at their Washington sites only, the authors reasoned that a lack of response at the San Francisco Bay sites may have been due to already eutrophic conditions. Similarly, Darby & Turner (2008b) concluded that plants are more responsive to nutrient enrichment when nutrient availability is low. Nonetheless, when considered together, these examples illustrate that when nutrient loading is high, belowground plant biomass is enhanced in unexploited soil and sediment, as indicated by the present study and others (Table 3.3). Accordingly, when these case studies are interpreted in the manner described, evidence supporting my hypothesis that nutrient enrichment has contrasting effects belowground increases to 100%, 100%, and 89% when considered by reference, location, and site, respectively, while evidence in opposition decreases to 19%, 21%, and 14%, respectively.

Results similar those presented in Tables 3.3 & 3.4 demonstrating the contrasting effects of nutrient enrichment on belowground biomass have also been found using the ingrowth and/or standing crop methods along nutrient availability gradients in coastal and other wetland types, and following fertilization in similar, as well as diverse, ecosystems. In fact, Castañeda-Moya *et al.* (2011) determined that *in situ* root standing crop decreased while root accumulation in ingrowth cores simultaneously increased along a mangrove forest nutrient availability gradient in the Everglades (FL, USA). Greater root biomass accumulation was also observed using the ingrowth method in fertilized coastal dunes (Stevenson & Day 1996), tallgrass prairie (Owensby *et al.* 1994), and a wide variety of forested ecosystems (Cuevas & Madina 1988, Raich *et al.* 1994, Helmisaari & Hallbäcken 1999, Davis *et al.* 2004, Gress *et al.* 2007, Gleeson & Good

2010). Indeed, I am aware of only one case where fertilization reduced root biomass accumulation in ingrowth cores (Cheng & Bledsoe 2002), though this negative response was isolated to one of several species (*Quercus douglasii*) contributing to overall ingrowth at only one of three sites. Furthermore, root ingrowth of other species (all annual grasses) increased significantly, and overall root accumulation from all species combined was unaffected, if not higher on average, in fertilized plots. However, root ingrowth does not always respond to fertilization, as was shown to be the case in wet tundra (Nadelhoffer *et al.* 2002), coniferous forest (Clemensson-Lindell & Persson 1995, Ahlström *et al.* 1988, Boxman *et al.* 1998, Smith *et al.* 2005), and pine plantation (Lee & Jose 2003) sites. In addition, total (live + dead) ingrowth was not affected by fertilization in tallgrass prairie restoration sites (Camill *et al.* 2004), or along a wet grassland nutrient gradient (Kaplova *et al.* 2011). Thus, in some cases, factors other than nutrients may control biomass accumulation, or alternatively, the quantity of nutrients supplied and/or the measurement timeframe were not sufficient to detect a significant response. Nonetheless, the 23 references presented herein that used the ingrowth method, in addition to the present research, show that greater root accumulation in unexploited soil is the most likely response trajectory when nutrient availability is enhanced.

Additional evidence in a variety of habitats also supports my hypothesis that excess nutrients induce a contrasting, negative response in root and/or rhizome standing crop. Miao & Sklar (1998) found that *Typha domingensis* growing in the Everglades allocated less root standing crop to total belowground standing crop when P was available in excess. Using phytometer species, Pauli *et al*. (2002) observed a reduction in *Succisa pratensis* root + rhizome standing crop in N fertilized calcareous fens. Yet, as hypothesized, greater nutrient availability had no effect on total belowground standing crop in a fresh marsh receiving sewage effluent

(Bayley *et al.* 1985), a constructed salt marsh fertilized with urea (Boyer *et al.* 2000), or salt marshes along a N loading gradient (Wigand 2008). In contrast to my hypothesis, Daoust & Childers (2004) increased total belowground standing crop by applying low-level P enrichment ($\leq$ 400 mg P m^{-2} yr^{-1}) to oligotrophic wet prairie and sawgrass marsh sites in the Everglades. However, these low-level P treatments were not sufficient to alter porewater P concentrations, which suggests that this growth-limiting nutrient was not supplied in excess. Therefore, a reduction in belowground standing crop would not be expected to occur. Izdepski *et al*. (2009) also measured enhanced belowground standing crop along a nutrient gradient in a floating marsh receiving municipal effluent, although the floatant was still developing vertically and going through the process of plant succession. Thus, their result of nutrient-enhanced standing crop may be more accurately interpreted as that which occurs during a habitat invasion or via habitat creation rather than that of an established plant community in equilibrium with its environment.

Beyond the wetland environment, numerous studies in a variety of forested habitats have investigated the belowground effects of nutrient enrichment; hence a full review of this literature was beyond the scope of the present research. Nevertheless, fertilizer-induced reductions in live fine root standing crop (generally roots < 1-2 mm) have been observed in pine plantations (Haynes & Gower 1995) and hardwood (Phillips & Fahey, 2007), coniferous (Clemensson-Lindell & Persson 1995), and tropical montane (Gower & Vitousek 1989) forests. Similar reductions in the number of root tips (Farrell & Leaf 1974) and fine root production estimates obtained using sequential coring techniques (Gower et al. 1992) have also been measured when excess nutrients were supplied. However, root standing crop responses to nutrient enrichment were mixed in similar forested environments depending on site and nutrient applied (Alexander & Fairley 1985, Ostertag 2001, Helmisaari & Hallbäcken 1999), and some research shows that

fertilization can increase root standing crop in forests (Smith *et al.* 1994, Nadelhoffer *et al.* 1999, Ostertag, 2001). Similarly, chronic N additions increased total root standing crop in prairie grasslands, although it was unclear if this response resulted from an N-induced shift in plant composition from native to naturalized species, which accounted for as much as 67% of the aboveground plant biomass in fertilized plots (Fornara & Tilman, 2002) Thus, this finding may emphasize the ability of nutrient enrichment to facilitate plant invasions, as observed by Tyler *et al*. (2007), rather than stimulating root standing crop of a community containing the same assemblage of plant species. Further, meta-analytic reviews that combined results from multiple terrestrial ecosystems came to different conclusions concerning the effect of nutrient enrichment on root standing crop depending on size class. Xia & Wan (2008) determined that N enrichment stimulated total root biomass, while Liu & Greaver (2010) found that N enrichment had no effect on fine root biomass. Although, neither meta-analysis categorized source data by estimation method (i.e., ingrowth vs. standing crop), which is a distinction that should be considered in future reviews according to results from the present study.

The present research in combination with results from previous research in this marsh and other fertilized coastal wetlands, fertilization experiments in other ecosystems, and investigations along nutrient gradients in wetlands, offers a general framework through which the effects of nutrient over-enrichment on belowground plant biomass can be viewed. Conceptually, these effects can be described by the following response sequence: (1) plant establishment and initial belowground growth into unvegetated soil are facilitated by nutrient enrichment, (2) when the plant community becomes established and maximum aboveground growth is achieved through nutrient enrichment, plants equilibrate to excess nutrients by reducing nutrient foraging efforts compared to that which would occur under nutrient limited

conditions, (3) initially, these effects are measurable only in the living belowground components that assimilate and store nutrients (i.e. roots and rhizomes, respectively), and (4) with more time, chronic nutrient over-enrichment can potentially result in reduced total belowground biomass. As such, the timeframe of my investigation, even at nearly a decade in duration, may not have been sufficient to detect a response to N enrichment using bulk measurements due to the fact that live roots represent such a small fraction of total biomass (2.3%) in this particular marsh (Table 3.2, Figure 3.2). However, given that rhizome standing crop was not affected in the present study, it is logical to assume that the approximately 50% reduction in root standing crop observed herein will eventually result in reduced soil organic matter content unless the effect of nutrient enrichment on other processes, such as enhanced aboveground biomass, offset this loss as some research suggests (Anisfeld & Hill 2011, Fox *et al.* 2012). In fact, a more recent interpretation of the significant reduction in total belowground biomass (0-5 cm) observed by Morris & Bradley (1999) after 13 years of fertilization suggests that this response may have been due to dilution of soil organic matter resulting from enhanced mineral sedimentation caused by a fertilization-induced increase in aboveground stem density (Morris *et al*. 2002).

3.5 Conclusions

Belowground biomass is a primary determinant of ecosystem function, which is of critical importance from ecological, economic, and social perspectives worldwide. Based on the present research, in combination with previous studies investigating the effects of nutrients on belowground biomass in coastal wetlands and other ecosystems, I conclude that eutrophic conditions can affect ecosystem function beneficially by stimulating belowground plant growth in previously unexploited soil, or detrimentally by reducing the belowground standing crop required to sustain the nutritional needs of established plants in mature communities. In support

of this conclusion, I have shown that nutrient-enhanced belowground growth can occur when sedimentation rates are constant or when soil mineral matter content is high, where annual plants dominate or propagation by seed is prevalent, during the process of plant succession, and when plants invade or create new habitats. In all of these cases, enhanced belowground growth is sustained by greater nutrient availability in under- or unutilized soil that is capable of supporting plant growth, holding constant all other factors that affect plant growth. Conversely, reduced belowground biomass is expected in chronically eutrophic areas that maintain established, mature plant communities and have perennial plants that store nutrients in belowground structures during non-growing seasons. In these instances, reduced belowground growth occurs when nutrients are available in excess because plants can acquire the nutrients necessary to sustain maximum growth with fewer roots.

3.6 References

Ahlström K, Persson H, Börjesson I (1988) Fertilization in a mature Scots pine (*Pinus sylvestris* L.) stand – effects on fine roots. *Plant Soil* 106:179-190.

Alexander IJ, Fairley RI (1983) Effects of N fertilisation on populations of fine roots and mycorrhizas in spruce humus. *Plant Soil* 71:49-53.

Anisfeld S, Hill T (2011) Fertilization effects on elevation change and belowground carbon balance in a long island sound tidal marsh. *Estuaries Coasts* 35:201-211.

Bayley SE, Zoltek J, Jr., Hermann AJ, Dolan TJ, Tortora L (1985) Experimental manipulation of nutrients and water in a freshwater marsh: effects on biomass, decomposition, and nutrient accumulation. *Limnol Oceanogr* 30:500-512.

Bedford BL, Walbridge MR, Aldous A (1999) Patterns in nutrient availability and plant diversity of temperate North American wetlands. *Ecology* 80:2151-2169.

Bobbink R, Hicks K, Galloway J, Spranger T, Alkemade R, Ashmore M, Bustamante M, Cinderby S, Davidson E, Dentener F, Emmett B, Erisman JW, Fenn M, Gilliam F, Nordin A, Pardo L, De Vries W (2010) Global assessment of nitrogen deposition effects on terrestrial plant diversity: a synthesis. *Ecol Appl* 20:30-59.

Boxman AW, Blanck K, Brandrud T, Emmett BA, Gundersen P, Hogervorst RF, Kjønaas OJ, Persson H, Timmermann V (1998) Vegetation and soil biota response to experimentally-changed nitrogen inputs in coniferous forest ecosystems of the NITREX project. *Forest Ecol Manag* 101:65-79.

Boyer KE, Callaway JC, Zedler JB (2000) Evaluating the progress of restored cordgrass (*Spartina foliosa*) marshes: belowground biomass and tissue nitrogen. *Estuaries* 23:711-721.

Boyer KE, Fong P, Vance RR, Ambrose RF (2001) *Salicornia virginica* in a southern California salt marsh: seasonal patterns and a nutrient-enrichment experiment. *Wetlands* 21:315-326.

Camill P, McKone MJ, Sturges ST, Severud WJ, Ellis E, Limmer J, Martin CB, Navratil RT, Purdie AJ, Sandel BS, Talukder S, Trout A (2004) Community- and ecosystem-level changes in a species-rich tallgrass prairie restoration. *Ecol Appl* 14:1680-1694.

Cargill SM, Jefferies RL (1984) Nutrient limitation of primary production in a sub-arctic salt-marsh. *J Appl Ecol* 21:657-668.

Castañeda-Moya E, Twilley RR, Rivera-Monroy VH, Marx BD, Coronado-Molina C, Ewe SML (2011) Patterns of root dynamics in mangrove forests along environmental gradients in the Florida coastal Everglades, USA. *Ecosystems* 14:1178-1195.

Cheng X, Bledsoe CS (2002) Contrasting seasonal patterns of fine root production for blue oaks (*Quercus douglasii*) and annual grasses in California oak woodland. *Plant Soil* 240:263-274.

Clemensson-Lindell A, Persson H (1995) The effects of nitrogen addition and removal on Norway spruce fine-root vitality and distribution in three catchment areas at Gårdsjön. *Forest Ecol Manag* 71:123-131.

Cuevas E, Medina E (1988) Nutrient dynamics within amazonian forests. *Oecologia* 76:222-235.

Daoust RJ, Childers DL (2004) Ecological effects of low-level phosphorus additions on two plant communities in a neotropical freshwater wetland ecosystem. *Oecologia* 141:672-686.

Darby FA, Turner RE (2008a) Below- and aboveground biomass of Spartina alterniflora: response to nutrient addition in a Louisiana salt marsh. *Estuaries Coasts* 31:326-334.

Darby FA, Turner RE (2008b) Effects of eutrophication on salt marsh root and rhizome biomass accumulation. *Mar Ecol Prog Ser* 363:63-70.

Davey E, Wigand C, Johnson R, Sundberg K, Morris J, Roman CT (2011) Use of computed tomography imaging for quantifying coarse roots, rhizomes, peat, and particle densities in marsh soils. *Ecol Appl* 21:2156-2171.

Davis MR, Allen RB, Clinton PW (2004) The influence of N addition on nutrient content, leaf carbon isotope ratio, and productivity in a *Nothofagus* forest during stand development. *Can J Forest Res* 34:2037-2048.

Deegan LA, Johnson DS, Warren RS, Peterson BJ, Fleeger JW, Fagherazzi S, Wollheim WM (2012) Coastal eutrophication as a driver of salt marsh loss. *Nature* 490:388-392.

Elser JJ, Bracken MES, Cleland EE, Gruner DS, Harpole WS, Hillebrand H, Ngai JT, Seabloom EW, Shurin JB, Smith JE (2007) Global analysis of nitrogen and phosphorus limitation of primary producers in freshwater, marine and terrestrial ecosystems. *Ecol Lett* 10:1135-1142.

Farrell EP, Leaf AL (1974) Effects of fertilization and irrigation on root numbers in a red pine plantation. *Can J Forest Res* 4:366-371.

Fierer N, Strickland MS, Liptzin D, Bradford MA, Cleveland CC (2009) Global patterns in belowground communities. *Ecol Lett* 12:1238-1249.

Fornara DA, Tilman D (2012) Soil carbon sequestration in prairie grasslands increased by chronic nitrogen addition. *Ecology* 93:2030-2036.

Fox L, Valiela I, Kinney E (2012) Vegetation cover and elevation in long-term experimental nutrient-enrichment plots in Great Sippewissett Salt Marsh, Cape Cod, Massachusetts: implications for eutrophication and sea level rise. *Estuaries Coasts* 35:445-458.

Freschet GT, Cornwell WK, Wardle DA, Elumeeva TG, Liu W, Jackson BG, Onipchenko VG, Soudzilovskaia NA, Tao J, Cornelissen JHC (2013) Linking litter decomposition of above- and below-ground organs to plant–soil feedbacks worldwide. *J Ecol* 101:943-952

Frost JW, Schleicher T, Craft C (2009) Effects of nitrogen and phosphorus additions on primary production and invertebrate densities in a Georgia (USA) tidal freshwater marsh. *Wetlands* 29:196-203.

Gallagher JL (1975) Effect of an ammonium-nitrate pulse on growth and elemental composition of natural stands of *Spartina alterniflora* and *Juncus roemerianus*. *Am J Bot* 62:644-648

Gleeson SK, Good RE (2010) Root growth response to water and nutrients in the New Jersey Pinelands. *Can J Forest Res* 40:167-172.

Gower ST, Vitousek PM (1989) Effects of nutrient amendments on fine root biomass in a primary successional forest in Hawai'i. *Oecologia* 81:566-568.

Gower ST, Vogt KA, Grier CC (1992) Carbon dynamics of rocky Mountain Douglas-fir: influence of water and nutrient availability. *Ecol Monogr* 62:43-65.

Gress SE, Nichols TD, Northcraft CC, Peterjohn WT (2007) Nutrient limitation in soils exhibiting differing nitrogen availabilities: what lies beyond nitrogen saturation? *Ecology* 88:119-130.

Haynes BE, Gower ST (1995) Belowground carbon allocation in unfertilized and fertilized red pine plantations in northern Wisconsin. *Tree Physiology* 15:317-325.

Helmisaari HS, Hallbäcken L (1999) Fine-root biomass and necromass in limed and fertilized Norway spruce (*Picea abies* (L.) Karst.) stands. *Forest Ecol Manag* 119:99-110.

Hendricks JJ, Hendrick RL, Wilson CA, Mitchell RJ, Pecot SD, Guo D (2006) Assessing the patterns and controls of fine root dynamics: an empirical test and methodological review. *J Ecol* 94:40-57.

Hines J, Megonigal JP, Denno RF (2006) Nutrient subsidies to belowground microbes impact aboveground food web interactions. *Ecology* 87:1542-1555.

Howes NC, FitzGerald DM, Hughes ZJ, Georgiou IY, Kulp MA, Miner MD, Smith JM, Barras JA (2010) Hurricane-induced failure of low salinity wetlands. *Proc Natl Acad Sci USA* 107:14014-14019.

Hunter A, Morris NMB, Lafabrie C, Cebrian J (2008) Effects of nutrient enrichment on *Distichlis spicata* and *Salicornia bigelovii* in a marsh salt pan. *Wetlands* 28:760-775.

Izdepski CW, Day JW, Sasser CE, Fry B (2009) Early floating marsh establishment and growth dynamics in a nutrient amended wetland in the lower Mississippi Delta. *Wetlands* 29:1004-1013.

Kaplova M, Edwards KR, Kvet J (2011) The effect of nutrient level on plant structure and production in a wet grassland: a field study. *Plant Ecol* 212:809-819.

Ket WA, Schubauer-Berigan JP, Craft CB (2011) Effects of five years of nitrogen and phosphorus additions on a *Zizaniopsis miliacea* tidal freshwater marsh. *Aquat Bot* 95:17-23.

Kirwan ML, Mudd SM (2012) Response of salt-marsh carbon accumulation to climate change. *Nature* 489:550-553.

Langley JA, McKee KL, Cahoon DR, Cherry JA, Megonigal JP (2009) Elevated CO2 stimulates marsh elevation gain, counterbalancing sea-level rise. *Proc Natl Acad Sci USA* 106:6182-6186.

Lebauer DS, Treseder KK (2008) Nitrogen limitation of net primary productivity in terrestrial ecosystems is globally distributed. *Ecology* 89:371-379.

Lee KH, Jose S (2003) Soil respiration, fine root production, and microbial biomass in cottonwood and loblolly pine plantations along a nitrogen fertilization gradient. *Forest Ecol Manag* 185:263-273.

Liu L, Greaver TL (2010) A global perspective on belowground carbon dynamics under nitrogen enrichment. *Ecol Lett* 13:819-828.

Lu M, Zhou X, Yang Q, Li H, Luo Y, Fang C, Chen J, Yang X, Li B (2012) Responses of ecosystem carbon cycle to experimental warming: a meta-analysis. *Ecology* 94:726-738.

McKee KL, Cahoon DR, Feller IC (2007) Caribbean mangroves adjust to rising sea level through biotic controls on change in soil elevation. *Glob Ecol Biogeogr* 16:545-556.

McLeod E, Chmura GL, Bouillon S, Salm R, Björk M, Duarte CM, Lovelock CE, Schlesinger WH, Silliman BR (2011) A blueprint for blue carbon: toward an improved understanding of the role of vegetated coastal habitats in sequestering CO_2. *Front Ecol Environ* 9:552-560.

Mendelssohn IA (1979) Influence of nitrogen level, form, and application method on the growth-response of *Spartina alterniflora* in North Carolina. *Estuaries* 2:106-112

Miao SL, Sklar FH (1997) Biomass and nutrient allocation of sawgrass and cattail along a nutrient gradient in the Florida Everglades. *Wetl Ecol Manag* 5:245-264.

Morris JT, Bradley PM (1999) Effects of nutrient loading on the carbon balance of coastal wetland sediments. *Limnol Oceanogr* 44:699-702.

Morris JT, Sundareshwar PV, Nietch CT, Kjerfve B, Cahoon DR (2002) Responses of coastal wetlands to rising sea level. *Ecology* 83:2869-2877.

Nadelhoffer KJ, Downs MR, Fry B (1999) Sinks for 15N-enriched additions to an oak forest and a red pine plantation. *Ecol Appl* 9:72-86

Nadelhoffer KJ, Johnson L, Laundre J, Giblin AE, Shaver GR (2002) Fine root production and nutrient content in wet and moist arctic tundras as influenced by chronic fertilization. *Plant Soil* 242:107-113.

Neill C (1992) Comparison of soil coring and ingrowth methods for measuring belowground production. Ecology 73:1918-1921.

Nelson JL, Zavaleta ES (2012) Salt marsh as a coastal filter for the oceans: changes in function with experimental increases in nitrogen loading and sea-level rise. *PLoS ONE* 7:e38558.

Ostertag R (2001) Effects of nitrogen and phosphorus availability on fine-root dynamics in Hawaiian montane forests. *Ecology* 82:485-499

Owensby CE, Auen LM, Coyne PI (1994) Biomass production in a nitrogen-fertilized, tallgrass prairie ecosystem exposed to ambient and elevated levels of CO_2. *Plant Soil* 165:105-113.

Pauli D, Peintinger M, Schmid B (2002) Nutrient enrichment in calcareous fens: effects on plant species and community structure. *Basic Appl Ecol* 3:255-266.

Phillips RP, Fahey TJ (2007) Fertilization effects on fineroot biomass, rhizosphere microbes and respiratory fluxes in hardwood forest soils. *New Phytol* 176:655-664.

Raich JW, Riley RH, Vitousek PM (1994) Use of root-ingrowth cores to assess nutrient limitations in forest ecosystems. *Can J Forest Res* 24:2135-2138.

Rasse DP, Rumpel C, Dignac M-F (2005) Is soil carbon mostly root carbon? Mechanisms for a specific stabilisation. *Plant Soil* 269:341-356.

Sasser CE, Visser JM, Mouton E, Linscombe J Hartley SB (2008) Vegetation types in coastal Louisiana in 2007. U.S. Geological Survery Open-File Report 2008-1224, 1 sheet, scale 1:550,000.

Smith CK, Coyea MR, Munson AD (2005) Response of fine roots to fertilized ingrowth cores in burned and harvested black spruce ecosystems. *Commun Soil Sci Plant Anal* 36:9-10.

Smith VH, Tilman GD, Nekola JC (1999) Eutrophication: impacts of excess nutrient inputs on freshwater, marine, and terrestrial ecosystems. *Environ Pollut* 100:179-196.

Stevenson MJ, Day FP (1996) Fine-root biomass distribution and production along a barrier island chronosequence. *Am Midl Nat* 135:205-217.

Suding KN, Collins SL, Gough L, Clark C, Cleland EE, Gross KL, Milchunas DG, Pennings S (2005) Functional- and abundance-based mechanisms explain diversity loss due to N fertilization. *Proc Natl Acad Sci USA* 102:4387-4392.

Sullivan MJ, Daiber FC (1974) Response in production of cord grass, *Spartina alterniflora*, to inorganic nitrogen and phosphorus fertilizer. *Chesapeake Sci* 15:121-123.

Swenson EM, Chuang WS (1983) Tidal and subtidal water volume exchange in an estuarine system. *Estuar Coast Shelf Sci* 16:229-240.

Trahan L, Bradley JJ, Morris L, Nolde R (1990) Soil Survey of St. Tammany Parish, Louisiana U.S. Department of Agriculture, Soil Conservation Service, Washington D.C., USA. In cooperation with Louisiana Agricultural Experiment Station and Louisiana State Soil and Water Conservation Committee, Baton Rouge, LA, USA.

Tyler AC, Lambrinos JG, Grosholz ED (2007) Nitrogen inputs promote the spread of an invasive marsh grass. *Ecol Appl* 17:1886-1898.

Valiela I, Teal JM (1974) Nutrient limitation in salt marsh vegetation. In: Reimold RJ, Queen WH (eds) *Ecology of Halophytes*. Academic Press, New York.

Valiela I, Teal JM, Persson NY (1976) Production and dynamics of experimentally enriched salt marsh vegetation: belowground biomass. *Limnol Oceanogr* 21:245-252.

Wigand C, Thursby GB, McKinney RA, Santos AF (2004) Response of *Spartina patens* to dissolved inorganic nutrient additions in the field. *J Coast Res* 45:134-149.

Wigand C (2008) Coastal salt marsh community change in Narragansett Bay in response to cultural eutrophication. In: Desbonnt A, Costa-Pierce BA (eds) *Science for Ecosystem-based Management*. Springer, New York, NY.

Xia J, Wan S (2008) Global response patterns of terrestrial plant species to nitrogen addition. *New Phytol* 179:428-439.

CHAPTER 4
OLIGOHALINE MARSH STABILITY MAINTAINED THROUGH COUNTERBALANCING ACCRETIONARY PROCESSES AFTER MORE THAN A DECADE OF EXPERIMENTAL NUTRIENT ENRICHMENT

4.1 Introduction

Anthropogenic alterations to global nutrient budgets are coupled to an array of ecological issues currently affecting estuarine and near-shore marine environments around the world (Diaz & Rosenberg 2008). Consequently, a growing number of valuable ecosystem services are at risk of being compromised by nutrient-induced degradation of coastal habitats (MEA 2005, Barbier *et al.* 2011). Coastal wetlands occupy a critical interface between upland nutrient sources and near-shore receiving basins, serving as both nutrient sink and buffer for adjacent ecosystems (Fisher & Acreman 1999, Valiela & Cole 2002, Sousa *et al.* 2008). Numerous fertilization experiments have demonstrated the nutrient assimilation and transformation capacity of these ecologically important ecosystems via enhanced plant growth and nutrient uptake (Sullivan & Daiber 1974, Mendelssohn 1979, Cargill & Jefferies 1984, Boyer *et al.* 2001, Frost *et al.* 2009, Drake *et al.* 2009, Chapter 2) and microbial-mediated processes such as denitrification (Davis *et al.* 2004, Koop-Jakobsen & Giblin 2010, Gardner & White 2010, VanZomeren *et al.* 2012). Soil sorption, sediment burial, and organic matter accumulation have also been identified as important long-term nutrient sinks as well (DeLaune *et al*. 1981, White & Howes 1994, Sundareshwar & Morris 1999, Drake *et al.* 2009, Loomis & Craft 2010).

Although coastal wetlands are important regulators of near-shore water quality, they are also vulnerable to eutrophication. Previous research has shown that nutrient over-enrichment can influence an array of functional and structural characteristics including primary production, community metabolism, consumer activity, carbon sequestration, competitive hierarchies, and

species composition, to name a few (Morris & Bradley 1999, Pennings *et al*. 2002, Crain 2007, Bertness *et al*. 2008, Slocum & Mendelssohn 2008, Frost *et al.* 2009, Ramirez *et al.* 2012, Chapter 2). Eutrophic conditions can further influence ecosystem structure and function by creating an environment that is prone to species invasions and loss of biodiversity (Chambers *et al*. 1999, Silliman & Bertness 2004, Tyler *et al.* 2007). These implications underscore the importance of understanding how nutrient availability affects complex feedbacks that regulate coastal wetland stability as global climate change threatens coastal wetlands around the world with inundation due to a higher sea level and more intense storms (IPCC 2007, Knutson *et al*. 2010). Yet, the link between nutrient enrichment, altered structure and function, and ecosystem stability is currently not well established.

To remain stable, coastal wetlands must (1) maintain an intertidal elevation by accreting soil vertically at a rate that paces relative sea level rise, and (2) resist the erosive forces of storm-generated waves and surge, both of which are mediated in large part by soil organic matter (Nyman *et al.* 2006, McKee *et al.* 2007, Howes *et al.* 2010, Kirwan & Mudd 2012). However, recent research provides conflicting evidence as to the fate of coastal wetlands enduring chronically eutrophic conditions. Some have concluded that excess nutrient availability causes coastal wetland deterioration, a result of reduced belowground plant biomass, enhanced organic matter decomposition, and consequently, increased vulnerability to tidal inundation and extreme meteorological events (Turner *et al.* 2009, Kearney *et al.* 2011, Deegan *et al.* 2012). Others have observed no net deleterious effect of enhanced nutrient supply on ecosystem function or the ability of coastal wetlands to keep pace with sea level rise (Langley *et al.* 2009, Anisfeld & Hill 2011, Day *et al.* 2013), and in a few cases, even shown that nutrient enrichment stimulates accretionary processes due to enhanced aboveground growth (Morris *et al.* 2002, Fox *et al.*

2012). Furthermore, direct evidence supporting the proposition that nutrient enrichment creates a soil environment that is more susceptible to erosion is limited in scope (Turner *et al.* 2009, Turner 2011). Thus, accurately predicting the consequences of coastal wetland eutrophication and mitigating against any potential losses of important ecological and economic services caused by coastal wetland degradation requires a better understanding of the effects of nutrient enrichment on ecosystem stability.

In this chapter, I present results from a 13-year oligohaline marsh fertilization experiment that assessed changes in belowground ecosystem function caused by excess nutrient loading. My objectives were to (1) identify the effects of nutrient enrichment on belowground processes that regulate marsh elevation and soil shear strength (i.e., belowground standing crop, belowground decomposition, organic and mineral matter accumulation, soil accretion, and shallow subsidence), and (2) determine whether eutrophic conditions compromise ecosystem stability, defined here as the capacity to maintain an intertidal elevation and resist erosion. I hypothesized based on previously published research that eutrophic conditions enhance organic and mineral matter deposition at the soil surface (a result of increased aboveground biomass and stem density), reduce belowground plant growth (i.e., living root and/or rhizome standing crop), but have no effect on the rate of belowground organic matter decomposition. As a result of the combined effects on these individual processes, I predicted that nutrient enrichment has no adverse effect on the rate of soil surface elevation change. However, as a consequence of reduced belowground standing crop, I predicted that enhanced nutrient availability does compromise the integrity of the soil matrix by reducing soil shear strength.

4.2 Materials and Methods

4.2.1 Site Description and Experimental Design

To accomplish my objectives, I utilized a long-term fertilization experiment in a *Sagittaria lancifolia* L. dominated oligohaline (aka: intermediate-brackish) marsh located along the Tchefuncte River approximately 1 km north of its point of drainage into Lake Pontchartrain, LA, USA (N 30° 23'21", W 90° 09'37"). This study site is a fertile, river-fed coastal wetland with a diverse plant community (Slocum & Mendelssohn 2008) and Histosol soil (Kenner Series; Trahan *et al.* 1990). Marsh flooding results from a combination of microtidal influence (10 cm tide range; Swenson & Chuang 1983) and wind shifts during frontal passages that affect water levels in Lake Pontchartrain and the Tchefuncte River. Hydrologic characteristics of a nearby site within the same contiguous marsh indicate that soil surface inundation at this site occurs on average (1999-2006) approximately every other day to a depth of 15 cm with surface water that has the following water quality characteristics: 1.6 g L^{-1} salinity, 0.26 mg inorganic nitrogen (N) L^{-1}, 0.79 mg total N L^{-1}, and 0.11 mg total phosphorus (P) L^{-1} (see Chapter 2).

In July 1999, five locations were randomly selected along a 200 m transect paralleling a small bayou that drains into the Tchefuncte River. Each location initially received three N-P-potassium (K) fertilization treatments that were randomly applied to 2 x 2 m plots (n=15). Five plots (i.e., one per location) received a 'medium' fertilization treatment consisting of 200 kg N ha^{-1} yr^{-1}, 51 kg P ha^{-1} yr^{-1}, and 99 kg K ha^{-1} yr^{-1}, five plots received a 'high' fertilization treatment consisting of 1200 kg N ha^{-1} yr^{-1}, 306 kg P ha^{-1} yr^{-1}, and 594 kg K ha^{-1} yr^{-1}, and five plots were unfertilized (i.e., controls). These nutrient enrichment levels were maintained for 13 years using slow-release fertilizer applied twice during the growing season by surface broadcast in April and July of 1999 through 2011.

In August 2001, each 2 x 2 m plot was split into two 1 x 2 m plots to identify the possible effects of herbivory. Exclusion fencing (2.5 cm^2 mesh, plastic-coated wire) was installed around one of the two split-plots chosen at random to exclude medium- to large-sized ground dwelling herbivores (e.g., nutria). Fertilization continued as before, regardless of fencing, thus creating a nutrient enrichment by herbivory factorial treatment arrangement with six treatment combinations replicated at five locations. However, only the effects of nutrient enrichment will be discussed in this chapter, as described in the statistical analysis section.

4.2.2 Surface Elevation Change, Soil Accretion, and Shallow Subsidence

I measured soil surface elevation change over a five-year period from 2005 to 2010 (7 to 12 years after initiating fertilization) using the rod surface-elevation table (rSET; Cahoon *et al.* 2002). A total of 15 rSETs (one per plot) were established in July 2004 along the centerline separating the exclosed split-plots from the non-exclosed split-plots by pounding a stainless steel benchmark rod into the marsh soil approximately 6 to 10 m until refusal, attaching a stainless steel receiver to the surface exposed portion of the benchmark rod, and cementing the connection in place inside a 15 cm diameter x 30 cm long PVC pipe. Each rSET station was then allowed approximately one year to equilibrate, at which point initial baseline rSET measurements were made on June 27, 2005. Subsequent measurements were repeated every 6 to 12 months thereafter for a total of 8 readings over an 1823-day (5 year) time period ending on June 24, 2010. During each reading, a removable collar and leveling arm were attached to the receiver of each rSET, nine fiberglass pins were lowered to the soil surface through evenly spaced holes drilled in the arm, and the portion of each pin remaining above the leveled arm was measured to the nearest millimeter. This procedure was repeated along six directional headings within each plot (three directions per split-plot, each separated by 45°). Cumulative elevation change over time was then

calculated for each arm-direction as the pin-averaged difference between the baseline readings and each successive measurement.

I determined soil accretion as the vertical accumulation of mineral sediment and organic matter above two 0.25 m x 0.25 m feldspar marker horizons laid down in each plot (one per split-plot) at the time of the baseline rSET readings on June 24, 2010. All subsequent accretion measurements coincided with each successive elevation change measurement. For each accretion measurement, soil cores that penetrated the feldspar layer were extracted using a cryogenic coring apparatus (Cahoon *et al.* 1996), which prevents compaction and preserves an intact (i.e., frozen) feldspar layer. Upon extraction, the height of material above the feldspar layer was measured to the nearest mm at three to four locations around each core and averaged.

Simultaneous measurements of surface elevation change and soil accretion were utilized to determine shallow subsidence. Prior to calculation, elevation change measurements by arm-direction were averaged to obtain a single value for each split-plot (2 per fertilization treatment) for each time period. Shallow subsidence was then calculated by difference (i.e., accretion – elevation change = subsidence). This method accounts for subsidence that occurs between the bottom of the benchmark rod and the feldspar marker horizon (initially at the soil surface) but not at depths below the benchmark rods. However, deep subsidence was assumed to be uniform across the study area during the measurement timeframe and considered negligible compared to shallow subsidence (Törnqvist *et al.* 2006).

4.2.3 Organic Matter and Mineral Sediment Accumulation

In conjunction with each accretion measurement, except on April 2007, organic and mineral matter accumulation were determined by collecting a separate core to the same depth as the feldspar horizon using a 7.62 cm diameter aluminum core tube. The contents of each core

were dried and weighed to determined soil bulk density (Blake & Hartge 1986), and then ground and combusted at 550 ºC to determine organic/mineral matter content (Christensen & Malmros 1982). The product of vertical accretion, soil bulk density, and soil organic/mineral matter content was then utilized to calculate organic and mineral matter accumulation over time.

4.2.4 Belowground Organic Matter Decomposition

I measured belowground decomposition using litterbags (6 cm wide x 30 cm long, 1-mm^2 nylon mesh) filled with material collected from a nearby location within the study marsh that contained a naturally occurring mix of soil macro-organic matter. The belowground organic matter was manually homogenized and air-dried, and a representative 1-g sample inserted in to each bag. To determine the initial oven-dried weight of material in the litterbags, ten 1-g air-dried samples of the fill material were dried at 60 ^{o}C and weighed ($\bar{x}$ = 0.89 ± 0.005 g). In August 2005 (year 7 of fertilization), 16 litterbags were inserted into the soil of each plot (eight per split-plot) to a depth of 15 cm using a hand trowel so that the material within each bag was positioned between 10 and 15 cm below the soil surface. Bags were removed after approximately 1 wk, 3 wk, 6 wk, 3 mo, 6 mo, 1 yr, 1.5 yr, and 2 yr. The material remaining inside each bag at the time of each removal was rinsed over a 1-mm mesh screen and any obvious ingrown roots or rhizomes were removed and discarded. The remaining material was then dried to constant weight at 60 ^{o}C and weighed.

4.2.5 Soil Shear Strength

I determined soil shear strength in both the dormant and active growing seasons (February and September 2011; 12 and 13 years after initiating fertilization, respectively). During each season, soil shear strength was measured at four locations in each plot (two per split-plot) from the soil surface to a depth of 50 cm in 5 cm intervals. Measurements were made

using a 5 cm long shear vane attached to a direct reading torque gauge (Geotechnics Ltd., Auckland, NZ). The procedure consisted of inserting the shear vane into the soil, twisting the vane until soil failure, pushing the vane in to the soil an additional 5 cm, and repeating.

4.2.6 Belowground Standing Crop

I determined belowground standing crop by extracting two 7.62 cm diameter by 50 cm long soil cores from each plot (one per split-plot) in September 2011, after 13 years of fertilization. Upon removal, cores were sectioned in to 5 cm increments and subsequently sieved through a 2 mm mesh screen to remove mineral sediment and fine particulate organic matter. The material from each increment was then categorized as live roots, live rhizomes, and dead roots + rhizomes using a combination of characteristics that included color, turgidity, and evidence of decomposition (e.g., epidermal lesions and resistance to breakage). When necessary, a dissecting microscope (3x magnification) was used to examine the belowground material in more detail. After sieving and sorting, all material was dried to constant weight at 60 ^{o}C and weighed. The belowground material was then assessed in three different ways: by depth as collected, summed over the top 30 cm of soil (i.e., the active rooting zone), and in the top 5 cm of soil only (i.e., the most likely region of observable treatment effects). The maximum depth increment to which living roots and rhizomes extended (i.e., rooting depth) was also determined.

4.2.7 Statistical Analysis

I performed all data analyses using SAS (Statistical Analysis Systems, version 9.3, SAS Institute, Inc., Cary, NC). Prior to statistical analysis, temporal measurements of organic matter and mineral sediment accumulation, soil accretion, shallow subsidence, and elevation change by arm-direction were converted to rates using linear regression forced through the origin.

Decomposition rates (i.e., k constants) were obtained in the same manner after data were natural log transformed to fit an exponential decay curve.

As previously stated, only the effects of nutrient enrichment are presented, although an herbivory treatment was included in the experimental design. To justify doing so, I used multivariate analysis of variance (MANOVA) to test the overall effects of nutrient enrichment, herbivory, and their interaction on all dependent variables as a group. Overall, the MANOVA test statistic (Wilks' lambda) indicated that the effect of nutrient enrichment ($p = 0.06$) and herbivory ($p = 0.07$) warranted further independent investigation, but the nutrient x herbivory interaction was not significant ($p = 0.68$). Furthermore, previously published work at this site also determined that the effects of nutrient enrichment on aboveground ecosystem structure and function did not interact significantly with herbivory (Slocum & Mendelssohn 2008). Thus, in this chapter my results and discussion include only the effects of nutrient enrichment.

Although my presentation of results was restricted to effects occurring in response to nutrient enrichment only, the statistical model included the complete experimental design, which tested the effects of nutrient enrichment, herbivory, soil depth (as a repeated measure) and season when applicable, and their interactions using mixed model analysis of variance (ANOVA). For all tests (including MANOVA), assumptions of normality and homoscedasticity were verified by examining normal probability and residual plots, respectively. When necessary, these data were logarithmically, square root, or cube root transformed prior to analysis to validate model assumptions. Upon finding a significant ($p \leq 0.05$) or marginally significant ($0.05 < p^* \leq 0.10$) nutrient enrichment effect with ANOVA, differences among treatment means were tested using the Tukey-Kramer multiple comparison test, which maintains an experiment-wise error rate for all pair-wise comparisons as specified.

4.3 Results

4.3.1 Belowground Standing Crop

The standing crops of live roots and rhizomes decreased significantly with increasing depth, but dead (root + rhizome) standing crop was relatively uniform throughout the 50 cm soil profile (Figure 4.1). The depth distribution of living roots was similar in the 0-5 cm and 5-10 cm depth increments, significantly lower in the 10-15 cm depth increment, negligible from 15 to 35 cm in depth (i.e., ≤ 3 g m^{-2} per 5 cm depth increment), and absent at deeper depths to 50 cm. Live rhizomes followed a similar depth distribution as roots, but masses were uniform to a slightly deeper depth of 15 cm. Below 15 cm, rhizomes decreased rapidly, becoming absent below 20 cm.

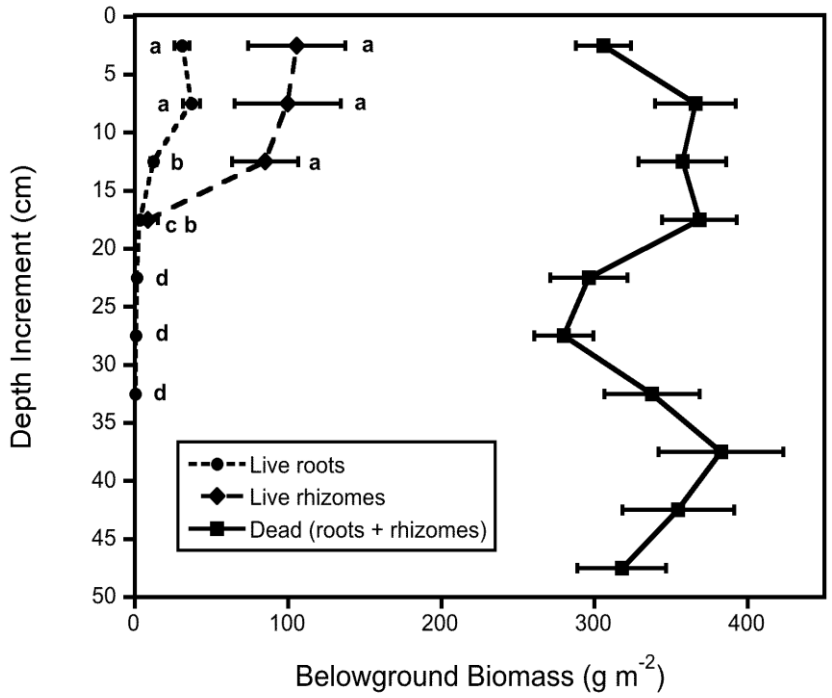

Figure 4.1 Live root, live rhizome, and dead (root + rhizome) standing crop depth distributions in the top 50 cm of soil. Values are means (± SE) by depth, averaged over herbivory and nutrient enrichment treatments. Means identified by different letters are significantly different ($p \leq 0.05$, Tukey-Kramer multiple comparison test). At the 15-20 cm depth increment where two letters are positioned side-by-side, the letter on the left corresponds with live roots and the letter on the right corresponds with live rhizomes. At depths below 20 cm, live rhizomes were not present and letters correspond only to living roots. Below 35 cm, living roots were absent.

Rooting depth (i.e., the depth of living roots and rhizomes) increased, on average, from 18 ± 2 cm in control plots to 24 ± 3 cm in plots receiving the high nutrient enrichment treatment ($p = 0.11$, Tukey-Kramer multiple comparison test). In contrast, the standing crop of live roots decreased significantly with increasing nutrient enrichment in the top 5 cm, and was similarly lower, on average, in the top 30 cm of soil (Table 4.1). However, live rhizome, live and dead (roots + rhizomes), and total (live + dead) standing crops in both the top 5 cm and the top 30 cm of soil were unaffected by nutrient enrichment.

4.3.2 Belowground Organic Matter Decomposition

Belowground organic matter decomposition in litterbags was generally slow, but followed a typical exponential decay curve (see [a] in Figure 4.2). An initial loss in mass of

Table 4.1 Effects of nutrient enrichment on belowground standing crop (g m^{-2}) in the top 5 cm and top 30 cm of soil. Treatment means (± SE) are presented and identified as significantly different by different superscripted lowercase letters ($p \leq 0.05$, Tukey-Kramer multiple comparison test).

	Control	Medium	High
<u>0 – 5 cm</u>			
Live roots	45 ± 11^{a}	33 ± 6^{ab}	14 ± 3^{b}
Live rhizomes	97 ± 32	182 ± 86	39 ± 15
Live (roots + rhizomes)	142 ± 37	215 ± 89	53 ± 15
Dead (roots + rhizomes)	307 ± 34	315 ± 28	294 ± 35
Total (live + dead)	450 ± 59	530 ± 102	347 ± 43
<u>0 – 30 cm</u>			
Live roots	112 ± 25	90 ± 18	55 ± 9
Live rhizomes	253 ± 76	367 ± 146	277 ± 126
Live (roots + rhizomes)	365 ± 85	457 ± 158	332 ± 130
Dead (roots + rhizomes)	2097 ± 150	1928 ± 146	1897 ± 104
Total (live + dead)	2462 ± 188	2385 ± 223	2229 ± 110

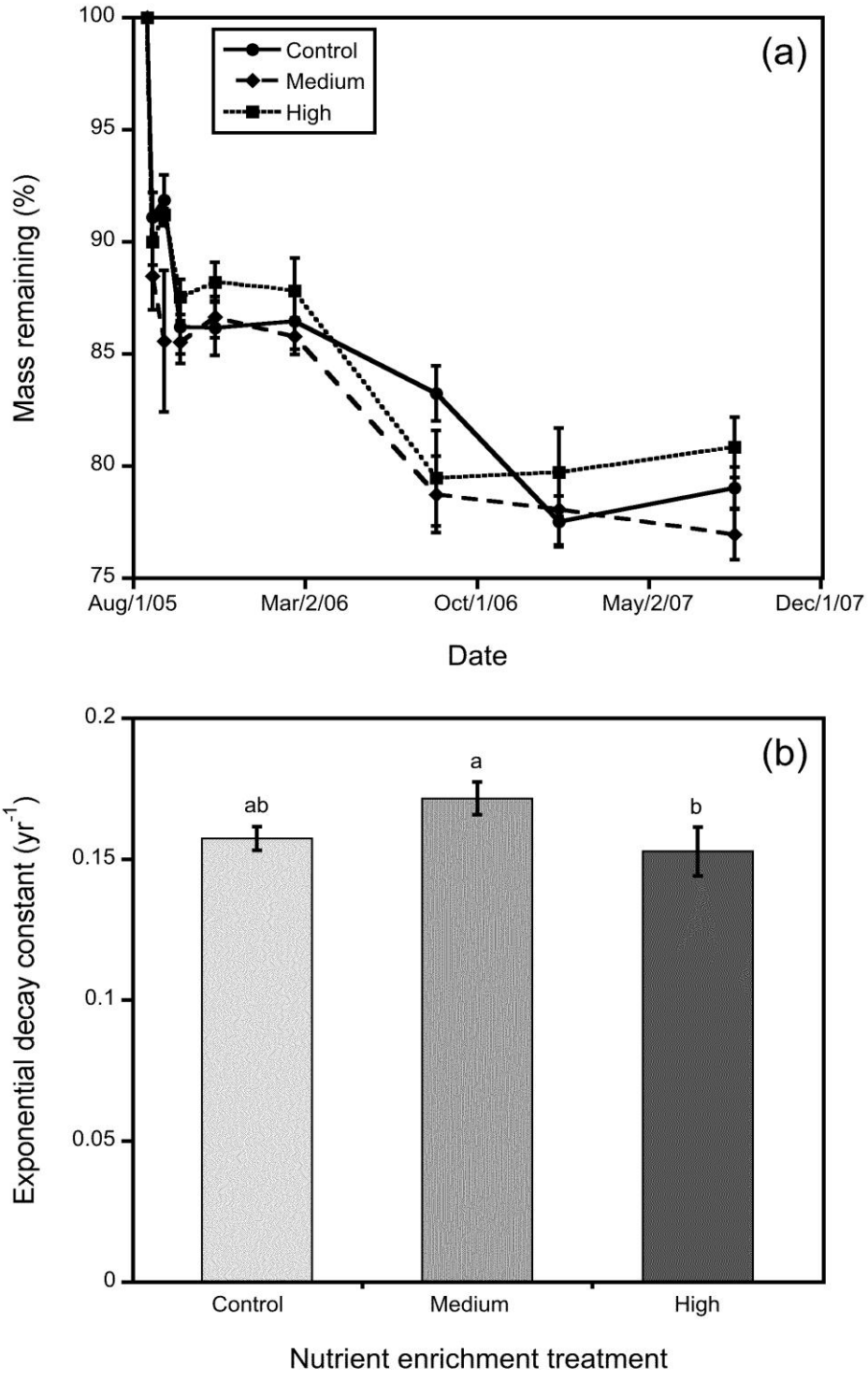

Figure 4.2 (a) Percent mass remaining over time and (b) exponential decay rates of belowground organic matter in litterbags. Values are nutrient enrichment treatment-means (± SE), averaged over herbivory treatments. Means in (b) are significantly different when identified by different letters ($p \leq 0.05$, Tukey-Kramer multiple comparison test).

approximately 10% occurred for all treatments in the first week, but this was followed by additional losses of less than 15% over the next two years. Greater than 75% of the material remained at the end of the incubation period, regardless of treatment. Decay constants (k) were similar among fertilized and control plots, though the rate of decomposition was significantly higher in moderately enriched plots compared to those receiving the high nutrient enrichment treatment (see [b] in Figure 4.2).

4.3.3 Soil Shear Strength

Depth profiles of soil shear strength were significant, ranging from 10.0 ± 0.4 to 6.6 ± 0.3 kPa (see [a] in Figure 4.3). Soil strength was greatest in the 10 to 15 cm depth range and decreased uniformly with increasing depth to 30 cm, below which it was relatively constant. The surface soil to a depth of 10 cm had intermediate strength compared to the 10-15 cm depth increment. Soil strength was also significantly greater in the dormant season compared to the growing season ($p = 0.0001$; 8.7 ± 0.2 kPa vs. 7.4 ± 0.2 kPa, respectively). In addition, soil strength tended to increase with increasing nutrient enrichment ($p = 0.10$; see [b] in Figure 4.3).

4.3.4 Organic Matter and Mineral Sediment Accumulation

Organic matter and mineral sediment accumulation on the soil surface (i.e., above the feldspar marker horizon) was approximately equal in mass, but each followed a distinctly different pattern over time. Organic matter accumulation increased uniformly and similarly among plots through the first three years of measurement, after which the high nutrient enrichment plots diverged positively (see [a] in Figure 4.4). As a result of this divergence after three years, and greater accumulation on average throughout the study period, the overall rate of organic matter accumulation was significantly greater in the high nutrient enrichment plots compared to the control (see [b] in Figure 4.4).

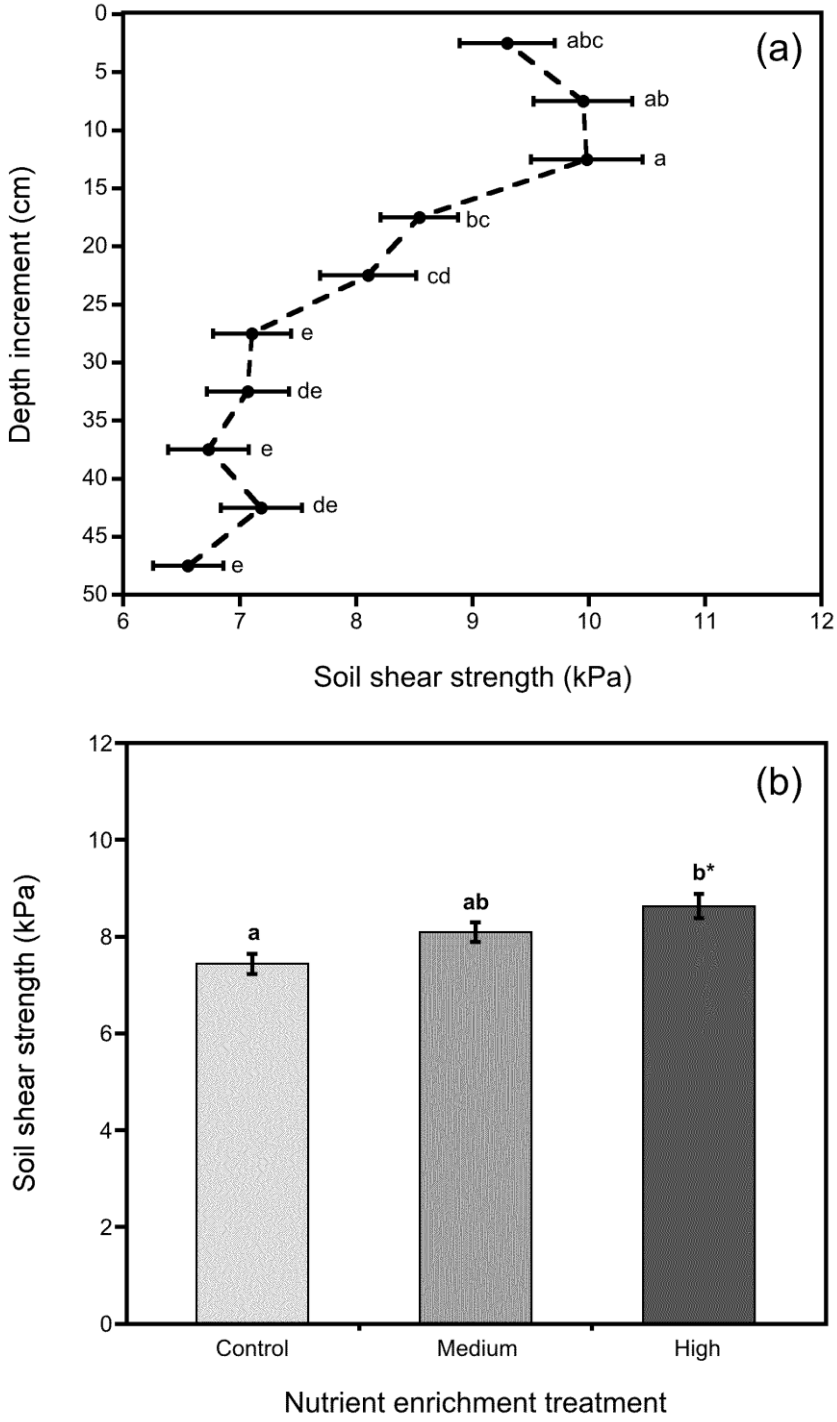

Figure 4.3 Soil shear strength (a) depth distribution and (b) depth-averaged effects of nutrient enrichment. Values in (a) are means (± SE) by depth, averaged over herbivory and nutrient treatments and season. Values in (b) are nutrient enrichment treatment-means (± SE) averaged over herbivory treatments, season, and depth. Different letters identify significantly different means ($p \leq 0.05$, $0.05 < p^* \leq 0.10$, Tukey-Kramer multiple comparison test).

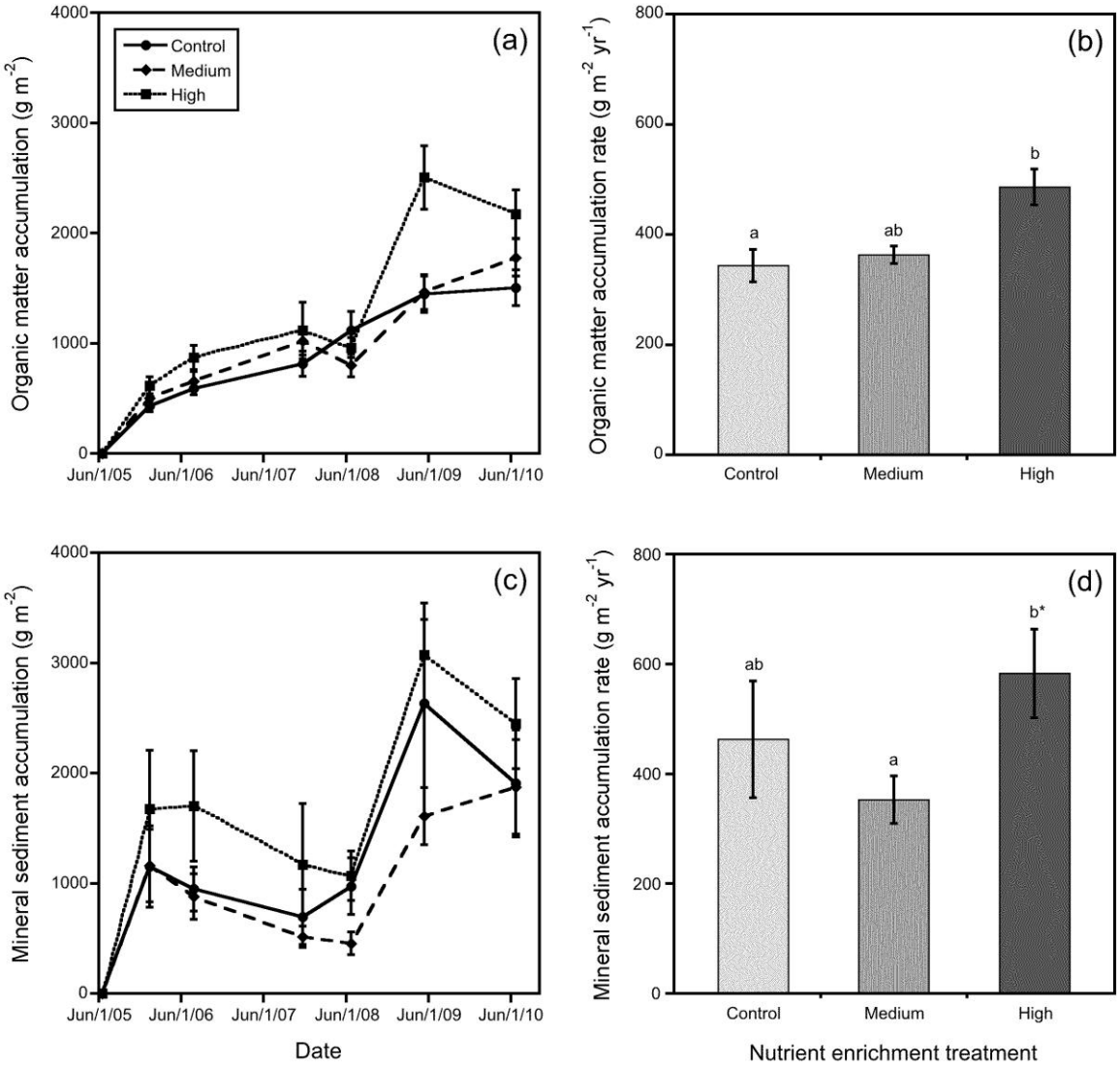

Figure 4.4 Temporal measurements and rates of (a, b) organic matter and (c, d) mineral sediment accumulation. Values are nutrient enrichment treatment-means (± SE), averaged over herbivory treatments. Significantly different means in (b) and (d) are identified by different letters ($p \leq 0.05$, $0.05 < p^* \leq 0.10$, Tukey-Kramer multiple comparison test).

Mineral sediment accumulation followed a pattern consistent with sediment deposition accompanying the landfall of Hurricanes Katrina and Gustav on August 29, 2005 and September 1, 2008, respectively (see [c] in Figure 4.4). In all plots, both post-hurricane measurements of soil surface mineral matter content were elevated initially and diluted over time. High nutrient enrichment plots tended to have higher rates of mineral matter accumulation compared to

moderately enriched plots ($p = 0.06$, Tukey-Kramer multiple comparison test), but neither treatment was different from the control (see [d] in Figure 4.4).

4.3.5 Accretion, Elevation Change, and Shallow Subsidence

Patterns of soil accretion, elevation change, and shallow subsidence (i.e., accretion minus elevation change) were consistent throughout the study (Figure 4.5). Surface accretion increased steadily in all plots over time, but at rates that were significantly greater with increasing nutrient enrichment (see [a, b] in Figure 4.5). However, nutrient-enhanced accretion did not influence elevation change, which was similar among all plots throughout the study period, with corresponding rates that were not significantly affected by nutrient enrichment (see [c, d] in Figure 4.5). On average, shallow subsidence rates increased with increasing nutrient enrichment, though treatment means were not significantly different ($p = 0.18$, Tukey-Kramer multiple comparison test) due to high variability across time periods as well as nutrient enrichment treatments (see [e, f] in Figure 4.5).

4.4 Discussion

4.4.1 Belowground Standing Crop, Decomposition, and Soil Shear Strength

As hypothesized, increasing the availability of nutrients decreased live root standing crop to where it was significantly lower in the top 5 cm of soil, and lower on average in the top 30 cm of soil. This response is consistent with the proposition that plants equilibrate to excess nutrients by reducing nutrient foraging efforts compared to that occurring under nutrient limited conditions (Darby & Turner 2008b). In contrast, enhanced nutrient supply had no effect on the standing crop of living rhizomes or dead roots plus rhizomes. Furthermore, the reduction in live root biomass was not sufficient to manifest itself in the combined biomass pools, such as live (root + rhizome) or total (live + dead) standing crops.

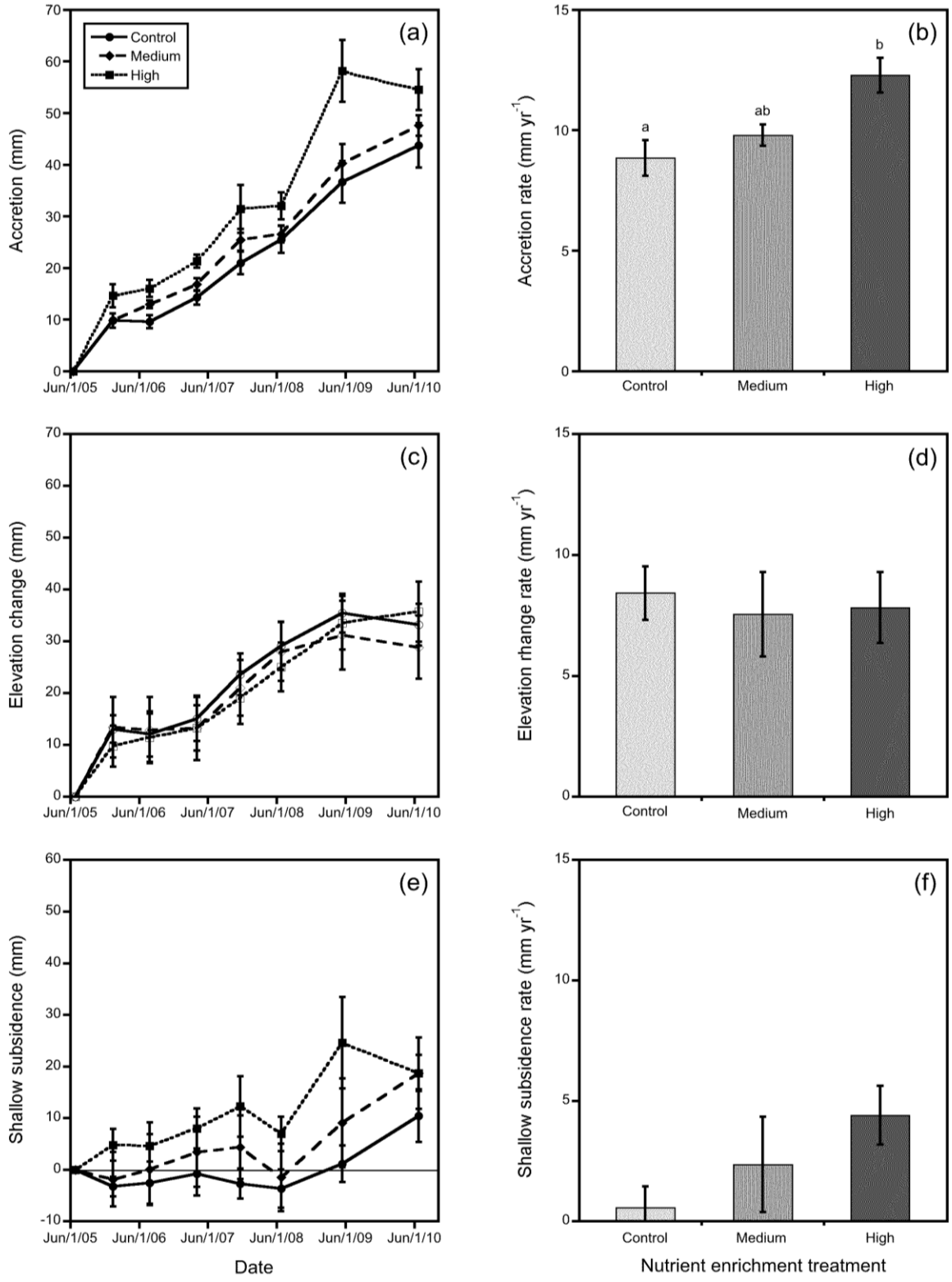

Figure 4.5 Temporal measurements and rates of soil accretion (a, c), elevation change (b, d), and shallow subsidence (e, f). Values are nutrient enrichment treatment-means (± SE), averaged over herbivory treatments. Significantly different means in (b), (d), and (f) are identified by different letters ($p \leq 0.05$, Tukey-Kramer multiple comparison test).

These results are supported by a growing body of scientific literature that has also shown eutrophic conditions can reduce living belowground standing crop, and more specifically the standing crop of live roots (Valiela *et al.* 1976, Hines *et al.* 2006, Darby & Turner 2008a, Darby & Turner 2008b, Davey *et al.* 2011, Deegan *et al.* 2012, see also Chapter 3). Nutrient-induced reductions in rhizome standing crop have been observed to a far lesser extent (Valiela *et al.* 1976, Ket *et al.* 2011). Thus, reduced root growth is a likely consequence when nutrients are available in excess because plants can acquire the nutrients necessary to sustain maximum growth with fewer roots. However, this effect is apparently quantifiable only in the living belowground components. I am aware of only two instances of reduced total (live + dead) standing crop resulting from nutrient enrichment in coastal wetlands (Morris & Bradley 1999, Ket *et al.* 2011). By comparison, many more studies have observed no nutrient effect on total standing crop (Gallagher 1975, Wigand *et al.* 2004, Hunter *et al.* 2008, Anisfeld & Hill 2011). In fact, a more recent interpretation by Morris et al. (2002) suggests that the significant reduction in total belowground biomass observed by Morris & Bradley (1999) after 13 years of fertilization may have been due to dilution of soil organic matter resulting from enhanced mineral sedimentation caused by a fertilization-induced increase in aboveground stem density.

In this oligohaline marsh, the quantity of dead belowground biomass was an order of magnitude greater than the quantity of live biomass. Thus, the effect of nutrient enrichment on the rate of belowground organic matter decomposition represents a major pathway for altered ecosystem function. However, as predicted, soil organic matter decomposing in litterbags for two years was unaffected by nutrient enrichment when compared to the control. These results were corroborated by my dead belowground standing crop estimates, which showed no changes in mass after 13 years of nutrient additions.

My litterbag results are similar to other coastal wetland fertilization studies that have utilized this method as a direct measure of decomposition in nutrient amended soil (Valiela et al. 1985, Jordon *et al.* 1989, Feller *et al.* 1999, Rybczyk *et al.* 2002, McKee *et al.* 2007, Anisfeld & Hill 2011). However, of these studies, only Valiela *et al.* 1985 and Feller *et al.* (1999) used organic matter that was internally enriched with nutrients, while the remainder, including the present study, incubated unenriched organic matter in nutrient enriched soil (i.e., external enrichment). A previous review of wetland decomposition experiments by Rybczyk *et al.* (1996) concluded that external nutrient enrichment generally has no effect on rates of organic matter decomposition, while internal enrichment of plant tissue can accelerate decay rates, but only during the initial stages of decomposition. For example, Valiela *et al.* (1985) found that aboveground *Spartina alterniflora* and *Spartina patens* tissue grown in N fertilized plots lost more mass during the first year of decomposition when excess nutrients were provided, but mass losses were similar to control treatments at the end of the 700-day study. These results also correspond with a more recent meta-analysis that showed external N enrichment stimulates the decomposition of high quality organic matter but inhibits the breakdown of recalcitrant tissue, with no overall significant effect (Knorr *et al.* 2005). Ramirez *et al.* (2012) further determined that N enrichment consistently altered microbial community structure, depressed microbial activity, and reduced the ability of extracellular enzymes to decompose recalcitrant organic matter in soils from a broad range of ecosystems, including wetlands and coastal environments. Overall, my decomposition results, as well as my standing crop estimates, support these conclusions.

To some extent, the results described above are in contrast to those that have found nutrient-enhanced decomposition using indirect measures such as soil respiration (Morris &

Bradley 1999, Wigand *et al.* 2009, Anisfeld & Hill 2011) and the cotton-strip assay (Fellers *et al.* 1999, Turner 2011). These contrasting responses likely result from a number of methodological artifacts. For instance, soil respiration is a cumulative measure of CO_2 efflux that is produced both by microbes and plant roots. In forest soils, the contribution of root respiration to total soil respiration can range from as little as 10% to as much as 90% (Hanson *et al.* 2000), and thus could potentially represent a sizable portion of soil respiration in wetland soils as well. Furthermore, cotton strips, which are composed almost entirely of cellulose (i.e., a relatively labile carbon source; Slocum *et al.* 2009), do not reflect the heterogeneity of substrate quality in soil organic matter undergoing decomposition. A prime example of this inconsistency was presented in the study by Fellers *et al.* (1999), where nutrient enrichment accelerated the decomposition rate of cotton strips, but plant tissue simultaneously decomposing in litterbags was unaffected by nutrient additions. However, results obtained using both indirect methods (i.e., soil respiration and cotton strips) do reinforce findings that suggest a shift in microbial activity occurs with nutrient enrichment to the preferential decomposition of labile organic matter pools (Ramirez *et al.* 2012).

Based on the assumption that the quantity of belowground biomass and rate of organic matter decomposition are directly related to soil shear strength, I expected the nutrient-induced reduction in live root standing crop to be accompanied by a concomitant reduction in the force required to induce soil failure. Unlike mineral sediment, both living and dead roots and rhizomes form an interconnected network that can effectively dissipate shear stress to a larger volume of soil. However, in contrast to my hypothesis, reduced living root standing crop resulting from nutrient enrichment had no negative affect on soil strength. In fact, the opposite occurred; the force required to induce soil failure tended to increase with increasing nutrient enrichment. These

results suggest that even though fewer roots were present in fertilized plots, the root system tended to be stronger, possibly due to enhanced tissue quality associated with higher nutrient conditions, though increased rooting depth, on average, may have also played a role (see Howes *et al.* 2010). My results also show that regardless of nutrient enrichment treatment, significant reductions in soil shear strength with depth occurred only where living roots and rhizomes decreased to negligible quantities (i.e., between 15 and 25 cm). Further, at depths below 25 cm where living biomass was either present in such small quantities to likely have little influence on soil strength, or was entirely absent, soil strength was relatively constant. Therefore, since the depth distribution of dead standing crop was relatively uniform throughout the soil column to a depth of 50 cm and there was no significant effect of nutrient enrichment on the rate organic matter decomposition, soil shear strength appears to be directly related to the quantity of living belowground biomass.

The tendency for nutrient enrichment to enhance soil strength was unexpected, somewhat counterintuitive, and in opposition to the results of others. Turner et al. (2009) and Turner (2010) concluded that greater nutrient availability reduces soil shear strength, and thereby increases the vulnerability of coastal wetlands to erosion. Although, in both studies, significantly lower soil shear strength due to nutrient enrichment was identified only at depths where it is likely that living roots and rhizomes were present in minimal quantities compared to surface depths, or not at all (i.e., 25-30 cm and 60-100 cm, respectively for the two studies). Hence, their results suggest that nutrient enrichment affects the strength of dead biomass by stimulating decomposition of recalcitrant organic matter, which is contrary to my findings and those of others, though different processes may be occurring at deeper depths. Swarzenski *et al.* (2008) also found that lower soil shear strength in an oligohaline marsh receiving nutrient-laden

Mississippi-Atchafalaya River water coincided with a more decomposed substrate compared to a similar marsh that did not receive diverted river water. However, in their study, marsh soils with lower shear strength had higher porewater sulfide concentrations and lower soil redox potential in addition to having higher porewater N and P concentrations. Thus, in this case, reduced soil strength may be more likely attributable to changes in hydrology caused by river water influx or the effect of increased sulfate loading on soil organic matter dynamics rather than increased N and P availability.

4.4.2 Accretion, Elevation Change, and Subsidence

Previous research at the present site (Slocum & Mendelssohn 2008), as well as at a nearby site within the same contiguous marsh (Chapter 2), documented that nutrient enrichment, specifically N enrichment, increases aboveground standing crop and primary production. Therefore, it was no surprise that organic matter accumulation at the soil surface was also significantly higher when additional nutrients were supplied. However, nutrient enrichment did not enhance the vegetation's ability to trap mineral sediment; there were no significant differences in the rate of mineral sediment accumulation between fertilized plots and control plots as observed by others (e.g., Morris et al. 2002). In the present study, sediment accumulation appeared to be strongly influenced by two hurricanes that made landfall near the site (Katrina in 2005 and Gustav in 2008), which corresponds with the proposition that hurricanes can be a major sediment source (Turner *et al.* 2006). Nonetheless, soil accretion increased with increasing nutrient enrichment as a result of greater organic matter accumulation rates. These results, in combination with those that show reduced root standing crop and unaffected organic matter decomposition (relative to the control) following nutrient enrichment, suggest that organic matter deposition from aboveground sources is the dominant process driving soil accretion in this

oligohaline marsh. Previous research in salt marshes has also found that nutrient-enhanced aboveground plant growth corresponds with enhanced accretion (Anisfeld & Hill 2011, Fox et al. 2012), which brings into question the theory that belowground biomass is the primary regulator of soil organic matter accumulation (Turner *et al.* 2004, Kearney et al. 2011), especially under elevated nutrient conditions.

Interestingly, enhanced soil accretion in nutrient amended plots did not coincide with enhanced surface elevation change. Anisfeld & Hill (2011) observed a similar response in nutrient amended salt marsh plots, although an increasing trend, on average, was apparent in their results. In contrast, there were no elevation-trends across nutrient enrichment treatments in the present study; elevation change was similar during all sampling periods, and overall rates of change were not significantly different. Instead, I observed a 7-fold increase in shallow subsidence rates, on average, with increasing nutrient enrichment, which offset the nutrient-enhanced accretion rates.

Since no other measured processes were negatively affected by nutrient enrichment, the most likely mechanism driving higher average rates of shallow subsidence was lower root standing crop in the surface soil of fertilized plots. Living roots are turgid structures, and even small reductions in mass can have potentially important implications for soil volume, especially in coastal wetlands with organic soil (Nyman *et al.* 1990). In fact, McKee *et al.* (2007) found that root volume was directly related to shallow subsidence in mangroves. Others have calculated that one-gram of soil organic matter in coastal wetland soil is volumetrically equivalent to 4 – 22 g of mineral sediment (Turner *et al.* 2000, Neubauer *et al.* 2008). Thus, the loss of root mass and the corresponding effects on soil volume are likely contributors to observed increases in shallow subsidence following nutrient enrichment.

4.5 Conclusions

By the end of this century, global mean sea level is projected to increase by 22-44 cm (A1B scenario; IPCC 2007), and warmer sea surface temperatures combined with greater atmospheric moisture content are anticipated to increase average storm intensity by 2 to11% (Knutson *et al*. 2010). For coastal wetlands, it has been suggested that enhanced nutrient supply may intensify the effects of global climate change by contributing to altered ecosystem function that facilitates instability (e.g., Turner *et al.* 2009, Kearney *et al.* 2011, Deegan *et al.* 2012). However, I observed an apparent compensatory effect of nutrient-enhanced organic matter accumulation at the soil surface whereby nutrient-induced soil volumetric changes associated with reduced root standing crop were negated. As a result, the capacity to maintain an intertidal elevation did not diminish, even after more than a of decade experimental nutrient additions. Furthermore, I observed no evidence that elevated nutrient conditions negatively affect the integrity of the soil matrix, even though root biomass was reduced by nutrient enrichment. The root system was evidently stronger, and soil strength tended to increase rather than decrease after additional nutrients were provided. In sum, I observed no negative changes in the stability of this oligohaline marsh after 13 years of nutrient enrichment, which is among the longest coastal wetland fertilization experiments to date. Based on these results, the ability of this marsh, and possibly others, to keep pace with sea level rise and resist the erosive forces of extreme meteorologic events will likely not be compromised by enhanced nutrient loading. However, before broad-based, general conclusions concerning the effects of nutrient enrichment on ecosystem stability can be made with a high degree of certainty, similar long-term research will be required in a diverse range of coastal wetlands that differ by salinity, species composition, hydrology, morphology, and specific nutrient inputs, among other factors.

4.6 References

Anisfeld S, Hill T (2011) Fertilization effects on elevation change and belowground carbon balance in a long island sound tidal marsh. *Estuaries Coasts* 35:201-211.

Barbier EB, Hacker SD, Kennedy C, Koch EW, Stier AC, Silliman BR (2011) The value of estuarine and coastal ecosystem services. *Ecol Monogr* 81:169-193.

Blake GR, Hartge KH (1986) Bulk density. In: Klute A (ed) Methods of Soil Analysis, Part 1 Physical and Mineralogical Methods. American Society of Agronomy - Soil Science Society of America, Madison, Wisconsin, USA.

Boyer KE, Fong P, Vance RR, Ambrose RF (2001) *Salicornia virginica* in a southern California salt marsh: seasonal patterns and a nutrient-enrichment experiment. *Wetlands* 21:315-326.

Cahoon DR, Lynch JC, Knaus RM (1996) Improved cryogenic coring device for sampling wetland soils. *J Sediment Res* 66:1025-1027.

Cahoon DR, Lynch JC, Perez BC, Segura B, Holland RD, Stelly C, Stephenson G, Hensel P (2002) High-precision measurements of wetland sediment elevation: II. The rod surface elevation table. *J Sediment Res* 72:734-739.

Cargill SM, Jefferies RL (1984) Nutrient limitation of primary production in a sub-arctic salt-marsh. *J Appl Ecol* 21:657-668.

Chambers RM, Meyerson LA, Saltonstall K (1999) Expansion of *Phragmites australis* into tidal wetlands of North America. *Aquat Bot* 64:261-273.

Christenson BT, Malmros PA (1982) Loss-on-ignition and carbon content in a beech forest soil profile. *Holarctic Ecol* 5:376-380.

Crain CM (2007) Shifting nutrient limitation and eutrophication effects in marsh vegetation across estuarine salinity gradients. *Estuaries Coasts* 30:26-34.

Darby FA, Turner RE (2008a) Below- and aboveground biomass of Spartina alterniflora: response to nutrient addition in a Louisiana salt marsh. *Estuaries Coasts* 31:326-334.

Darby FA, Turner RE (2008b) Effects of eutrophication on salt marsh root and rhizome biomass accumulation. *Mar Ecol Prog Ser* 363:63-70.

Davey E, Wigand C, Johnson R, Sundberg K, Morris J, Roman CT (2011) Use of computed tomography imaging for quantifying coarse roots, rhizomes, peat, and particle densities in marsh soils. *Ecol Appl* 21:2156-2171.

Davis JL, Nowicki B, Wigand C (2004) Denitrification in fringing salt marshes of Narragansett Bay, Rhode Island, USA. *Wetlands* 24:870-878.

Day J, Lane R, Moerschbaecher M, DeLaune R, Mendelssohn I, Baustian J, Twilley R (2013) Vegetation and soil dynamics of a Louisiana estuary receiving pulsed Mississippi River water following Hurricane Katrina. *Estuaries Coasts* 36:665-682.

Deegan LA, Johnson DS, Warren RS, Peterson BJ, Fleeger JW, Fagherazzi S, Wollheim WM (2012) Coastal eutrophication as a driver of salt marsh loss. *Nature* 490:388-392.

DeLaune RD, Reddy CN, Patrick WH (1981) Accumulation of plant nutrients and heavy metals through sedimentation processes and accretion in a Louisiana salt marsh. *Estuaries* 4:328-334.

Diaz RJ, Rosenberg R (2008) Spreading dead zones and consequences for marine ecosystems. *Science* 321:926-929.

Drake DC, Peterson BJ, Galván KA, Deegan LA, Hopkinson C, Johnson JM, Koop-Jakobsen K, Lemay LE, Picard C (2009) Salt marsh ecosystem biogeochemical responses to nutrient enrichment: a paired 15N tracer study. *Ecology* 90:2535-2546.

Feller IC, Whigham DF, O'Neill JP, McKee KL (1999) Effects of nutrient enrichment on within-stand cycling in a mangrove forest. *Ecology* 80:2193-2205.

Fisher J, Acreman MC (1999) Wetland nutrient removal: a review of the evidence. *Hydrol Earth Syst Sci* 8:673-685.

Fox L, Valiela I, Kinney E (2012) Vegetation cover and elevation in long-term experimental nutrient-enrichment plots in Great Sippewissett Salt Marsh, Cape Cod, Massachusetts: implications for eutrophication and sea level rise. *Estuaries Coasts* 35:445-458.

Frost JW, Schleicher T, Craft C (2009) Effects of nitrogen and phosphorus additions on primary production and invertebrate densities in a Georgia (USA) tidal freshwater marsh. *Wetlands* 29:196-203.

Gallagher JL (1975) Effect of an ammonium-nitrate pulse on growth and elemental composition of natural stands of *Spartina alterniflora* and *Juncus roemerianus*. *Am J Bot* 62:644-648.

Gardner LM, White JR (2010) Denitrification Enzyme Activity as an Indicator of Nitrate Movement through a Diversion Wetland. *Soil Sci Soc Am J* 74:1037-1047.

Hanson PJ, Edwards NT, Garten CT, Andrews JA (2000) Separating root and soil microbial contributions to soil respiration: A review of methods and observations. *Biogeochemistry* 48:115-146.

Hines J, Megonigal JP, Denno RF (2006) Nutrient subsidies to belowground microbes impact aboveground food web interactions. *Ecology* 87:1542-1555.

Howes NC, FitzGerald DM, Hughes ZJ, Georgiou IY, Kulp MA, Miner MD, Smith JM, Barras JA (2010) Hurricane-induced failure of low salinity wetlands. *Proc Natl Acad Sci USA* 107:14014-14019.

Hunter A, Morris NMB, Lafabrie C, Cebrian J (2008) Effects of nutrient enrichment on *Distichlis spicata* and *Salicornia bigelovii* in a marsh salt pan. *Wetlands* 28:760-775.

IPCC [Intergovernmental Panel on Climate Change] (2007) Climate change 2007: the physical science basis. Contributions of working group I to the fourth assessment report of the intergovernmental panel on climate change. Solomon S, Qin D, Manning M, Chen Z, Marquis M, Averyt KB, Tignor M, Miller HL (eds). Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.

Jordan TE, Whigham DF, Correll DL (1989) The role of litter in nutrient cycling in a brackish tidal marsh. *Ecology* 70:1906-1915.

Kearney MS, Riter JCA, Turner RE (2011) Freshwater river diversions for marsh restoration in Louisiana: twenty-six years of changing vegetative cover and marsh area. *Geophys Res Lett* 38:L16405.

Ket WA, Schubauer-Berigan JP, Craft CB (2011) Effects of five years of nitrogen and phosphorus additions on a *Zizaniopsis miliacea* tidal freshwater marsh. *Aquat Bot* 95:17-23.

Kirwan ML, Mudd SM (2012) Response of salt-marsh carbon accumulation to climate change. Nature 489:550-553.

Knorr M, Frey SD, Curtis PS (2005) Nitrogen additions and litter decomposition: a meta-analysis. Ecology 86:3252-3257.

Knutson TR, McBride JL, Chan J, Emanuel K, Holland G, Landsea C, Held I, Kossin JP, Srivastava AK, Sugi M (2010) Tropical cyclones and climate change. *Nat Geosci* 3:157-163.

Koop-Jakobsen K, Giblin AE (2010) The effect of increased nitrate loading on nitrate reduction via denitrification and DNRA in salt marsh sediments. *Limnol Oceanogr* 55:789-802.

Langley JA, McKee KL, Cahoon DR, Cherry JA, Megonigal JP (2009) Elevated CO2 stimulates marsh elevation gain, counterbalancing sea-level rise. *Proc Natl Acad Sci USA* 106:6182-6186.

Loomis MJ, Craft CB (2010) Carbon sequestration and nutrient (nitrogen, phosphorus) accumulation in river-dominated tidal marshes, Georgia, USA. *Soil Sci Soc Am J* 74:1028-1036

McKee KL, Cahoon DR, Feller IC (2007) Caribbean mangroves adjust to rising sea level through biotic controls on change in soil elevation. Glob *Ecol Biogeogr* 16:545-556.

Mendelssohn IA (1979) Influence of nitrogen level, form, and application method on the growth-response of *Spartina alterniflora* in North Carolina. *Estuaries* 2:106-112

MEA [Millennium Ecosystem Assessment] (2005) Ecosystems and human well-being: synthesis. World Resources Institute, Washington, DC.

Morris JT, Bradley PM (1999) Effects of nutrient loading on the carbon balance of coastal wetland sediments. *Limnol Oceanogr* 44:699-702.

Morris JT, Sundareshwar PV, Nietch CT, Kjerfve B, Cahoon DR (2002) Responses of coastal wetlands to rising sea level. *Ecology* 83:2869-2877

Neubauer SC (2008) Contributions of mineral and organic components to tidal freshwater marsh accretion. *Estuar Coast Shelf Sci* 78:78-88.

Nyman JA, Delaune RD, Patrick WH (1990) Wetland soil formation in the rapidly subsiding Mississippi River Deltaic Plain: mineral and organic matter relationships. *Estuar Coast Shelf Sci* 31:57-69.

Nyman JA, Walters RJ, Delaune RD, Patrick WH (2006) Marsh vertical accretion via vegetative growth. *Estuar Coast Shelf Sci* 69:370-380

Pennings SC, Stanton LE, Brewer JS (2002) Nutrient effects on the composition of salt marsh plant communities along the southern Atlantic and Gulf coasts of the United States. *Estuaries* 25:1164-1173.

Ramirez KS, Craine JM, Fierer N (2012) Consistent effects of nitrogen amendments on soil microbial communities and processes across biomes. *Glob Change Biol* 18:1918-1927.

Rybczyk JM, Day JW, Conner WH (2002) The impact of wastewater effluent on accretion and decomposition in a subsiding forested wetland. *Wetlands* 22:18-32.

Rybczyk JM, Garson G, Day JW (1996) Nutrient enrichment and decomposition in wetland ecosystems: models, analyses and effects. *Curr Top Wetland Biochemistry* 2:52-72.

Silliman BR, Bertness MD (2004) Shoreline development drives invasion of Phragmites australis and the loss of plant diversity on New England salt marshes. *Conserv Biol* 18:1424-1434.

Slocum MG, Mendelssohn IA (2008) Effects of three stressors on vegetation in an oligohaline marsh. *Freshw Biol* 53:1783-1796.

Slocum MG, Roberts J, Mendelssohn IA (2009) Artist canvas as a new standard for the cotton-strip assay. *J Plant Nutr Soil Sci* 172:71-74.

Sousa AI, Lillebø AI, Caçador I, Pardal MA (2008) Contribution of *Spartina maritima* to the reduction of eutrophication in estuarine systems. *Environ Pollut* 156:628-635.

Sullivan MJ, Daiber FC (1974) Response in production of cord grass, *Spartina alterniflora*, to inorganic nitrogen and phosphorus fertilizer. *Chesapeake Sci* 15:121-123.

Sundareshwar PV, Morris JT (1999) Phosphorus sorption characteristics of intertidal marsh sediments along an estuarine salinity gradient. *Limnol Oceanogr* 44:1693-1701.

Swarzenski CM, Doyle TW, Fry B, Hargis TG (2008) Biogeochemical response of organic-rich freshwater marshes in the Louisiana delta plain to chronic river water influx. *Biogeochemistry* 90:49-63.

Swenson EM, Chuang WS (1983) Tidal and subtidal water volume exchange in an estuarine system. *Estuar Coast Shelf Sci* 16:229-240.

Trahan L, Bradley JJ, Morris L, Nolde R (1990) Soil Survey of St. Tammany Parish, Louisiana. U.S. Department of Agriculture, Soil Conservation Service, Washington D.C., USA. In cooperation with Louisiana Agricultural Experiment Station and Louisiana State Soil and Water Conservation Committee, Baton Rouge, LA, USA.

Turner RE (2011) Beneath the salt marsh canopy: loss of soil strength with increasing nutrient loads. *Estuaries Coasts* 34: 1084-1093.

Turner RE, Baustian JJ, Swenson EM, Spicer JS (2006) Wetland sedimentation from Hurricanes Katrina and Rita. *Science* 314: 449-452.

Turner RE, Howes BL, Teal JM, Milan CS, Swenson EM, Goehringer-Toner DD (2009) Salt marshes and eutrophication: an unsustainable outcome. *Limnol Oceanogr* 54:1634-1642.

Turner RE, Swenson EM, Milan CS (2000) Organic and inorganic contributions to vertical accretion in salt marsh sediments. In: Weinstein M, Kreeger K (eds) Concepts and controversies in tidal marsh ecology. Kluwer Academic Publishing, Dordrecht, Netherlands.

Turner RE, Swenson EM, Milan CS, Lee JM, Oswald TA (2004) Below-ground biomass in healthy and impaired salt marshes. *Ecol Res* 19:29-35.

Tyler AC, Lambrinos JG, Grosholz ED (2007) Nitrogen inputs promote the spread of an invasive marsh grass. *Ecol Appl* 17:1886-1898.

Valiela I, Cole ML (2002) Comparative evidence that salt marshes and mangroves may protect seagrass meadows from land-derived nitrogen loads. *Ecosystems* 5:92-102.

Valiela I, Teal JM, Allen SD, Van Etten R, Goehringer D, Volkmann S (1985) Decomposition in salt marsh ecosystems: the phases and major factors affecting disappearance of above-ground organic matter. *J Exp Mar Biol Ecol* 89:29-54.

Valiela I, Teal JM, Persson NY (1976) Production and dynamics of experimentally enriched salt marsh vegetation: belowground biomass. *Limnol Oceanogr* 21:245-252.

VanZomeren CM, White JR, DeLaune RD (2012) Fate of Nitrate in Vegetated Brackish Coastal Marsh. *Soil Sci Soc Am J* 76:1919-1927.

White DS, Howes BL (1994) Long-term 15N-nitrogen retention in the vegetated sediments of a New England salt marsh. *Limnol Oceanogr* 39:1878-1892.

Wigand C, Brennan P, Stolt M, Holt M, Ryba S (2009) Soil respiration rates in coastal marshes subject to increasing watershed nitrogen loads in southern New England, USA. *Wetlands* 29:952-963.

Wigand C, Thursby GB, McKinney RA, Santos AF (2004) Response of *Spartina patens* to dissolved inorganic nutrient additions in the field. *J Coast Res* 45:134-149.

CHAPTER 5
CONCLUSIONS

5.1 Summary and Conclusions

The overall objectives of this research were to (1) identify the species-, community-, and ecosystem-level effects of nutrient enrichment and (2) determine how nutrient enrichment affects plant community structure, function, and stability. To address these objectives, I conducted two long-term fertilization studies in a species rich, *Sagittaria lancifolia* dominated oligohaline marsh located on the north shore of Lake Pontchartrain, LA, USA. In one study, experimental plots were enriched for nine years with one of four N levels (0, 50, 200, or 1200 kg N ha^{-1} yr^{-1}) in combination with one of two levels of P (0 or 131 kg P ha^{-1} yr^{-1}) to investigate nutrient limitation of primary production and plant community- and species-level responses to nutrient enrichment. In a companion study, a nearby location within the same contiguous marsh was fertilized for 13 years with three levels of N-P-K (unfertilized control; medium: 200 kg N ha^{-1} yr^{-1}, 51 kg P ha^{-1} yr^{-1}, and 99 kg K ha^{-1} yr^{-1}; and high: 1200 kg N ha^{-1} yr^{-1}, 306 kg P ha^{-1} yr^{-1}, and 594 kg K ha^{-1} yr^{-1}) to determine whether eutrophic conditions compromise ecosystem stability.

The first study revealed that changes in ecosystem structure and function were driven by N enrichment only; P enrichment had no significant effect alone or in interaction with N. Both 200 and 1200 kg N ha^{-1} yr^{-1} stimulated aboveground plant production, while enrichment with 1200 kg N ha^{-1} yr^{-1} also increased *S. lancifolia* tissue N:P, reduced *S. lancifolia* N and P resorption during senescence, and altered the relative dominance of the three dominant species, but had no affect on species richness. Belowground response trajectories were dependent upon method used and organic matter pool measured. Excess N simultaneously increased live root

biomass accumulation in ingrowth cores and reduced *in situ* live root standing crop, but had no effect on other belowground biomass pools using either method.

The companion study identified an apparent compensatory effect of nutrient enrichment on marsh accretionary processes. Nutrient-induced shallow subsidence attributed to reduced live root standing crop was balanced by nutrient-enhanced soil accretion resulting from greater organic matter accumulation at the soil surface most likely due to greater aboveground plant growth. Consequently, the rate of marsh elevation change measured over a five-year period was unaffected after 12 years of experimental nutrient enrichment. Furthermore, elevated nutrient conditions had no negative affect on the structural integrity of the soil matrix. Decomposition rates were similar among control and fertilized plots, and although live root biomass was reduced by nutrient enrichment, the root system was evidently stronger as soil strength tended to increase rather than decrease after nutrients were provided for 13 years.

Based on these results, I can draw several conclusions as to the effects of nutrient enrichment on ecosystem structure, function, and stability. First, I conclude that N limits primary production in this oligohaline marsh. This finding is consistent with previous research in brackish and salt marsh systems that have documented increased plant primary production or standing crop following N enrichment (e.g., Patrick & Delaune 1976, Mendelssohn 1979, Cargill & Jefferies 1984, Wigand *et al.* 2004, Crain 2007), as well as studies that have applied N to oligohaline marshes in Louisiana (DeLaune & Lindau 1990). However, outside of Louisiana, Crain (2007) observed N + P co-limitation, suggesting that the relative importance of P to oligohaline primary production may be dependent upon local factors such as hydrology and relative nutrient loading rates.

Second, I conclude that the aboveground plant response to N enrichment is sequential: moderate N loading stimulated plant production only, while high N loading maintained the elevated production and also altered plant tissue nutrients and species dominance, but not species richness. Thus, N enrichment beyond the assimilation capacity of the vegetation drives changes in ecosystem structure caused by altered plant nutrient cycling. Altered community composition is a well-documented consequence of nutrient over-enrichment (DiTommaso & Aarssen 1989, Suding *et al.* 2005), which can lead to loss of species diversity and expansions of invasive species (Chambers *et al.* 1999, Silliman & Bertness 2004, Tyler *et al.* 2007, Bobbink *et al.* 2010). Therefore, in the present study, linear changes in species dominance with increasing N enrichment suggest that further nutrient enrichment in time or quantity may result in a shift in species dominance and reduced species richness.

Third, I conclude that eutrophic conditions facilitate initial belowground growth into unvegetated soil, as evidenced using the in growth method (Valiela *et al.* 1976, McKee *et al.* 2007). These data further suggest that nutrient-enhanced belowground growth can occur when sedimentation rates are constant or when soil mineral matter content is high (Nelson & Zavaleta 2012), where annual plants dominate or propagation by seed is prevalent (Hunter *et al.* 2008), during the process of plant succession (Izdepski *et al.* 2009), and when plants invade or create new habitats (Tyler *et al.* 2007). However, when the plant community becomes established and maximum aboveground growth is achieved through nutrient enrichment, plants equilibrate to excess nutrients by reducing nutrient foraging efforts (i.e., live root standing crop) compared to that which would occur under nutrient limited conditions (Valiela *et al.* 1976, Hines *et al.* 2006, Darby & Turner 2008a, b, Deegan *et al.* 2012). Consequently, reduced root biomass is expected in chronically eutrophic areas that maintain established, mature plant communities and have

perennial plants that store nutrients in belowground structures during non-growing seasons. In these instances, reduced belowground growth occurs when nutrients are available in excess because plants can acquire the nutrients necessary to sustain maximum growth with fewer roots.

Finally, I conclude that enhanced nutrient loading is an unlikely destabilizing mechanism in this coastal marsh and possibly others due to counterbalancing effects on stability-regulating accretionary processes. However, additional long-term research will be required before broad-based, general conclusions concerning the effects of nutrient enrichment on coastal wetland stability can be made with a high degree of certainty. While my results confirm enhanced aboveground plant growth and greater soil accretion as nutrient availability increases (DeLaune *et al.* 2003, Lane *et al.* 2006, Anisfeld & Hill 2011), they also show that lower root input contributes to shallow subsidence (McKee *et al.* 2007). Therefore, in coastal wetlands where detrital export exceeds deposition or belowground resource allocation is relatively high, nutrient enrichment may negatively affect elevation dynamics due to soil volumetric changes caused by altered root growth (Nyman *et al.* 1990, Turner *et al.* 2000, and Neubauer *et al.* 2008). Furthermore, my results are inconsistent with other research findings that have shown nutrient enrichment creates a soil environment that is more susceptible to erosion by reducing soil shear strength (Turner *et al.* 2009, Turner 2011). Rather, my results suggest that factors other than nutrient enrichment my be responsible for these findings, such as altered hydrology and increased sulfate loading (Swarzenski *et al.* 2008).

Overall, this dissertation illustrates the diverse effects of chronic nutrient enrichment on coastal wetland structure and function. While some effects enhanced stability, others were detrimental. Therefore, strategies that reduce excess nutrient loading to coastal ecosystems should be employed.

5.2 References

Anisfeld S, Hill T (2011) Fertilization effects on elevation change and belowground carbon balance in a long island sound tidal marsh. *Estuaries Coasts* 35:201-211.

Bobbink R, Hicks K, Galloway J, Spranger T, Alkemade R, Ashmore M, Bustamante M, Cinderby S, Davidson E, Dentener F, Emmett B, Erisman JW, Fenn M, Gilliam F, Nordin A, Pardo L, De Vries W (2010) Global assessment of nitrogen deposition effects on terrestrial plant diversity: a synthesis. Ecol Appl 20:30-59.

Cargill SM, Jefferies RL (1984) Nutrient limitation of primary production in a sub-arctic salt marsh. *J Appl Ecol* 21:657-668.

Chambers RM, Meyerson LA, Saltonstall K (1999) Expansion of *Phragmites australis* into tidal wetlands of North America. *Aquat Bot* 64:261-273.

Crain CM (2007) Shifting nutrient limitation and eutrophication effects in marsh vegetation across estuarine salinity gradients. *Estuaries Coasts* 30:26-34.

Darby FA, Turner RE (2008a) Below- and aboveground biomass of Spartina alterniflora: response to nutrient addition in a Louisiana salt marsh. *Estuaries Coasts* 31:326-334.

Darby FA, Turner RE (2008b) Effects of eutrophication on salt marsh root and rhizome biomass accumulation. *Mar Ecol Prog Ser* 363:63-70.

Deegan LA, Johnson DS, Warren RS, Peterson BJ, Fleeger JW, Fagherazzi S, Wollheim WM (2012) Coastal eutrophication as a driver of salt marsh loss. *Nature* 490:388-392.

DeLaune RD, Jugsujinda A, Peterson GW, Patrick WH (2003) Impact of Mississippi River freshwater reintroduction on enhancing marsh accretionary processes in a Louisiana estuary. *Estuar Coast Shelf Sci* 58:653-662.

DeLaune RD, Lindau CW (1990) Fate of ^{15}N labeled nitrogen in a *Sagittaria lancifolia* L. Gulf Coast marsh. *J Freshw Ecol* 5:265-268.

DiTommaso A, Aarssen LW (1989) Resource manipulations in natural vegetation: a review. *Vegetatio* 84:9-29.

Hines J, Megonigal JP, Denno RF (2006) Nutrient subsidies to belowground microbes impact aboveground food web interactions. *Ecology* 87:1542-1555.

Hunter A, Morris NMB, Lafabrie C, Cebrian J (2008) Effects of nutrient enrichment on *Distichlis spicata* and *Salicornia bigelovii* in a marsh salt pan. *Wetlands* 28:760-775.

Izdepski CW, Day JW, Sasser CE, Fry B (2009) Early floating marsh establishment and growth dynamics in a nutrient amended wetland in the lower Mississippi Delta. *Wetlands* 29:1004-1013.

Lane RR, Day JW, Day JN (2006) Wetland surface elevation, vertical accretion, and subsidence at three Louisiana estuaries receiving diverted Mississippi River water. *Wetlands* 26:1130-1142.

McKee KL, Cahoon DR, Feller IC (2007) Caribbean mangroves adjust to rising sea level through biotic controls on change in soil elevation. *Glob Ecol Biogeogr* 16:545-556.

Mendelssohn IA (1979) Influence of nitrogen level, form, and application method on the growth-response of Spartina alterniflora in North Carolina. *Estuaries* 2:106-112.

Neubauer SC (2008) Contributions of mineral and organic components to tidal freshwater marsh accretion. *Estuar Coast Shelf Sci* 78:78-88.

Nyman JA, Delaune RD, Patrick WH (1990) Wetland soil formation in the rapidly subsiding Mississippi River Deltaic Plain: mineral and organic matter relationships. *Estuar Coast Shelf Sci* 31:57-69.

Patrick WH, Delaune RD (1976) Nitrogen and phosphorus utilization by *Spartina alterniflora* in a salt marsh in Barataria Bay, Louisiana. *Estuar Coast Mar Sci* 4:59-64.

Silliman BR, Bertness MD (2004) Shoreline development drives invasion of Phragmites australis and the loss of plant diversity on New England salt marshes. *Conserv Biol* 18:1424-1434.

Suding KN, Collins SL, Gough L, Clark C, Cleland EE, Gross KL, Milchunas DG, Pennings S (2005) Functional- and abundance-based mechanisms explain diversity loss due to N fertilization. *Proc Natl Acad Sci USA* 102:4387-4392.

Swarzenski CM, Doyle TW, Fry B, Hargis TG (2008) Biogeochemical response of organic-rich freshwater marshes in the Louisiana delta plain to chronic river water influx. *Biogeochemistry* 90:49-63.

Turner RE (2011) Beneath the salt marsh canopy: loss of soil strength with increasing nutrient loads. *Estuaries Coasts* 34: 1084-1093.

Turner RE, Swenson EM, Milan CS (2000) Organic and inorganic contributions to vertical accretion in salt marsh sediments. In: Weinstein M, Kreeger K (eds) *Concepts and controversies in tidal marsh ecology*. Kluwer Academic Publishing, Dordrecht, Netherlands.

Tyler AC, Lambrinos JG, Grosholz ED (2007) Nitrogen inputs promote the spread of an invasive marsh grass. *Ecol Appl* 17:1886-1898.

Valiela I, Teal JM, Persson NY (1976) Production and dynamics of experimentally enriched salt marsh vegetation: belowground biomass. Limnol Oceanogr 21:245-252.

Wigand C, Thursby GB, McKinney RA, Santos AF (2004) Response of Spartina patens to dissolved inorganic nutrient additions in the field. J Coast Res:134-149.

APPENDIX
PERMISSION TO RE-PRINT CHAPTER 2

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>
> Thank you for you assistance.
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>
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VITA

Sean Allen Graham was born in Lexington, Kentucky, and raised on a small farm in Crossville, Tennessee. His time spent on the farm fostered an appreciation for nature and instilled in him a philosophy of environmental stewardship. Upon graduating from Cumberland County High School in 1993, he enrolled at the University of Tennessee, earning a Bachelor of Arts degree in environmental studies in 1998. In 2001, he was accepted into the graduate program at Indiana University under the guidance of Dr. Christopher Craft. Sean earned a Master's of Science degree in environmental science in 2003 through research evaluating the ability of natural and restored wetlands to sequester nutrients and improve water quality. After receiving his Master's, he continued his wetland investigations at Indiana University for another year to help develop and validate soil- and vegetation-based indicators of wetland nutrient condition, as well as assist in assessing coastal wetland stability as part of the National Science Foundation's Long-term Ecological Research Program. In 2004, he accepted a research associate position in the Department of Oceanography and Coastal Sciences at Louisiana State University (LSU), where his research has investigated the response of coastal wetlands to various restoration techniques, environmental stressors, and meteorologic/climatic phenomenon. While maintaining his full-time research responsibilities, he was accepted into the doctoral program at LSU in 2008 under the guidance of Dr. Irving Mendelssohn. Sean earned a Doctor of Philosophy degree in oceanography and coastal sciences in 2013 and presently continues his research on coastal wetlands at LSU.