RESPONSE OF A *SPARTINA PATENS*-DOMINATED OLIGOHALINE MARSH TO NITROGEN ENRICHMENT IN COASTAL NORTH CAROLINA, USA.

By: Mark Wejrowski

December, 2013

Director of Thesis: Enrique Reyes, PhD

Major Department: Biology

Coastal marshes are highly productive ecosystems that play a significant role in the global carbon budget. Anthropogenic alterations to coastal landscapes can significantly impact these marsh ecosystems, though the actual loss of ecosystem functioning may depend on the type of marsh being impacted. Nitrogen loading into coastal environments has accelerated with increased use of fertilizers for agricultural production. Previous work has demonstrated that some marsh plants respond to nitrogen inputs by allocating more biomass into aboveground stems and leaves while reducing belowground biomass. These changes could diminish the organic matter pool in coastal marshes while also making them more susceptible to erosion. The goal of this study was to fertilize plots in a *Spartina patens*-dominated oligohaline marsh with varying concentrations of urea applied throughout one growing season and assess the response in aboveground and belowground plant biomass and decomposition. Aboveground plant clippings and soil cores were collected to assess the changes in above- and belowground biomass among the treatments throughout time and to also assess tissue nitrogen and organic matter content. Litter bags were also placed at the soil surface of the experimental plots to determine rates of decomposition throughout the study. Neither aboveground nor belowground biomass was significantly affected by nitrogen application, and nitrogen assimilation into plant tissue did not

vary across the treatments. Decomposition was also relatively similar across the treatments, though there were seasonal effects on litter mass loss. Our findings suggest that oligohaline marshes, specifically those dominated by *S. patens*, are not limited by nitrogen to the same extent as salt marshes.

RESPONSE OF A *SPARTINA PATENS*-DOMINATED OLIGOHALINE MARSH TO

NITROGEN ENRICHMENT IN COASTAL NORTH CAROLINA, USA.

A Thesis

Presented To

The Faculty of the Department of Biology

East Carolina University

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

by

Mark Wejrowski

December, 2013

RESPONSE OF A *SPARTINA PATENS*-DOMINATED OLIGOHALINE MARSH TO

NITROGEN ENRICHMENT IN COASTAL NORTH CAROLINA, USA.

by

Mark Wejrowski

APPROVED BY:

DIRECTOR OF THESIS:_____

Enrique Reyes, PhD

COMMITTEE MEMBER:_____

Robert Christian, PhD

COMMITTEE MEMBER:_____

Marcelo Ardón, PhD

COMMITTEE MEMBER:_____

Siddhartha Mitra, PhD

CHAIR OF THE DEPARTMENT OF BIOLOGY:

_____

Jeffrey McKinnon, PhD

DEAN OF THE GRADUATE SCHOOL:

_____

Paul J. Gemperline, PhD

ACKNOWLEDGEMENTS

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

CHAPTER 1

Introduction

Coastal ecosystems are heavily impacted by human development. Many of the world's most populated areas exist near estuaries or within coastal watersheds (McGranahan et al. 2007), and anthropogenic stressors associated with urbanization and agricultural expansion have threatened or destroyed key components of coastal ecosystem functioning (Lee et al. 2006). These stressors include, among others: eutrophication, landscape manipulation for agriculture, and altered hydrologic regimes from water extraction or diversion (Kennish 2002, Nicholls et al. 2008). Coastal marshes are among the most heavily impacted habitats from coastal development, and it is estimated that marsh ecosystems are being lost at a global rate of 1-2% each year (Bridgham et al. 2006, Mcleod et al. 2011).

The degradation of these coastal marshes is significant as they are among the most highly productive ecosystems in the world; their nutrient-rich soils and periodic inundation allow for the rapid growth of a broad array of salt and flood/drought tolerant macrophytes (Keefe 1972, Vernberg 1993, Odum et al. 1995). Furthermore, water saturation and correlated soil hypoxia hinder belowground microbial decomposition (Laffoley and Grimsditch 2009), leading to carbon-rich peat accumulation. While other highly-productive terrestrial ecosystems such as rainforests sequester large amounts of organic carbon over short periods of time, marshes, due to their reduced rates of soil decomposition, can accumulate organic carbon for several millennia (Duarte et al. 2003, Chmura et al. 2003, Reddy and DeLaune 2008, Mcleod et al. 2011).

In addition to their exceptional carbon storage capacities, coastal marshes transform or retain large amounts of nitrogen and can serve as the final buffer to sensitive estuaries. The retention or transformation of nitrogen can be significantly altered with changes in water depth and associated soil oxygen levels, plant and microbe community composition, and anthropogenic

nutrient inputs (Richardson and Vepraskas 2001, Wigand et al. 2004, Lee et al. 2006). Soil

microbes are responsible for fixing atmospheric nitrogen into organic nitrogen and converting

organic nitrogen to ammonium (ammonification) and ammonium to nitrate (nitrification), which

can then be used by plants to support cell growth and development. When plant tissues are

decomposed, the organic nitrogen is converted back to ammonium and nitrate, and in anoxic

soils, denitrification returns nitrate molecules to gaseous forms (N_2, N_2O) that are released to the

atmosphere (Mitsch and Gosselink 2007). While this process occurs naturally in all wetland

environments, external inputs of nitrogen are likely to alter the balance of nitrogen retention and

release (Deegan et al. 2012).

Since the introduction of the Haber-Bosch process for the industrial production of

ammonia in the early twentieth century, worldwide application of nitrogen has exponentially

increased (Galloway and Cowling 2002). Human populations have followed the same rapid

growth trends during this time, and as human demands for food have steadily risen, so too has

the application of fertilizers to maintain crop production efficiency. Coupled with atmospheric

deposition through fossil fuel combustion, the yearly anthropogenic contribution of N to the

biosphere is estimated to be over $16*10^9$ Kg (Gruber and Galloway 2008), and much of the

fertilizer applied to crops near watersheds is transported into surrounding land and water (Gilbert

et al. 2006, Lee et al. 2006). Wigand et al. (2003) estimated that marshes throughout the

Narragansett Bay in New England receive nitrogen loads of up to 1024 g N m^{-2} each year.

Coastal marshes are recognized for their ability to effectively sequester and transform nutrients

before they percolate into aquatic ecosystems (Valiela and Cole 2002, Reddy and DeLaune

2008). However, the impacts of nutrient additions on marsh biogeochemical processes are more

uncertain.

The general paradigm among wetland scientists is that nitrogen is a major limiting nutrient for macrophyte growth in marsh systems (Valiela et al. 1976, Kiehl et al. 1997, Wigand et al. 2004, Darby and Turner 2008a,b, Olcott 2011). Darby and Turner (2008a) found that aboveground biomass of *Spartina alterniflora*, a common species of salt marsh grass, significantly increased when exposed to nitrogen fertilizers. Similar studies have documented the same nitrogen limitation in freshwater marshes (Frost et al. 2009, Ket et al. 2011). There is no clear consensus, however, on the response of belowground marsh plant biomass when introduced to external sources of nitrogen. Some studies have found that relatively small additions of nitrogen can significantly reduce macrophyte root and rhizome biomass (Valiela et al. 1976, Darby and Turner 2008c, Ket et al. 2011, Deegan et al. 2012). This would most likely occur because of the reduced need of the plants to acquire natural sources of nitrogen in soils (Darby and Turner 2008c). However, in a recent study conducted in a tidal salt marsh, Anisfeld and Hill (2012) found that there was no significant effect of the addition of nitrogen fertilizers to the belowground biomass of enriched *Spartina alterniflora* plants.

Previous marsh fertilization research has had a significant focus on tidal, low marsh systems (Blum 1993, Darby and Turner 2008a,b,c, Turner 2011, Anisfeld and Hill 2012, Deegan et al. 2012). However, the effects of nitrogen enrichment on high marsh systems with less frequent inundation, lower salinities, and perennial grass dominance have not been well-documented (Wigand et al. 2004, Graham and Mendelssohn 2010). While some studies have found that *Spartina patens*, a common high marsh grass, experiences increased aboveground biomass (Wigand et al. 2004, Crain 2007) and reduced belowground biomass (Valiela et. al 1976) with the addition of nitrogen, other studies have found no response in belowground (VanZomeren et al. 2011) or aboveground biomass (Etheridge et al. 2012). Both aboveground and belowground plant biomass contribute to the overall organic carbon pool within marsh

ecosystems, so altering the dynamic of either is likely to affect long-term organic matter accumulation. In addition, roots and rhizomes contribute to the stability of marsh soils, and a loss in belowground biomass would likely lead to higher rates of erosion, especially in the face of rising sea levels (Deegan et al. 2012).

As nitrogen is a major component in commercial agricultural fertilizers, it is important to understand not only the fate of this nitrogen in coastal watersheds, but to also understand the ensuing ecological impacts from runoff. The goal of this project was to measure the response of plants in an oligohaline marsh to varying concentrations of urea, a common agricultural fertilizer that provides the plants with a directly usable form of ammonium-nitrogen. Because marsh ecosystems are an integral component of the global carbon cycle, and because high marsh regions are not well-represented in marsh fertilization research, it is important to understand how both the aboveground and belowground stocks of organic carbon in a high marsh are affected by watershed nutrient pollution.

Throughout one growing season, samples of grasses and soil were collected to assess aboveground biomass, belowground biomass, and plant tissue nitrogen and organic matter concentrations. Leaf litter bags were placed at the soil surface to determine aboveground decomposition. It was hypothesized that when exposed to higher concentrations of fertilizer: 1. aboveground plant production would increase across the nutrient gradient, 2. belowground plant biomass would not vary among treatments, and 3. soil-surface decomposition rates would also not vary across the nutrient gradient as phosphorus has been demonstrated to be the limiting resource for microbial activity across marsh landscapes (Sundareshwar et al. 2003, Rejmánková and Houdková 2006).

This project followed the experimental design of two previous studies conducted in the Delmarva Peninsula, USA. The first of these studies was an undergraduate thesis project

developed by Chris Olcott under the direction of Dr. Linda Blum at the University of Virginia

(Olcott 2011). The second study was developed and carried out by both Brooke Costanza and

Sherer Etheridge under the direction of Dr. Robert Christian at East Carolina University

(Etheridge et al. 2012).

Study Site

All field work was performed in an oligohaline marsh on mainland coastal North

Carolina, USA in the Alligator River National Wildlife Refuge (35°46'05.86"N,

75°45'00.92"W)(Figure 1) from April to December 2012. The site consisted of a large strip of

coastal marshland with little to no hydrodynamic or agricultural disturbance from surrounding

sources. The experimental units were placed in an area of high marsh roughly 1km from the

Atlantic Coast. The area is dominated by *S. patens*, although a few other high marsh grass

species also persist in the area in small patches, such as *Phragmites australis, Distichlis spicata*,

and *Cladium mariscus jamaicense*. Tidal action is essentially absent in the area, and most of the

inundation occurs due to either precipitation or ground water supply.

Experimental Design

A drainage canal ran perpendicular to the study site and potentially contributed a source

of unmeasured variation to the experimental plots, such as nutrient runoff or excess soil

saturation. To account for this variation, a randomized block design (Gotelli and Ellison 2004)

was implemented in which three replicate blocks were placed at varying distances from the

drainage canal to obtain replicates along a potential environmental gradient (Figure 2). The three

replicate blocks were placed along a transect approximately 850m inland from the sound. The

5

study plots were placed at distances of 20 m (block A), 50 m (block B), and 90 m (block C) from the canal (Figure 2).

Within each block, six 9-m^2 plots were set up to serve as the experimental units. Each plot was assigned to a different treatment group, to which a specific concentration of 46:0:0 fertilizer with N as urea (Valley Fertilizer & Chemical Co. Inc.) was applied each month during the first 7 months of the sampling period, with the total applied concentrations in each plot being either: 0 g N m^{-2} (C), 3.45 g N m^{-2} (N1), 6.9 g N m^{-2} (N2), 13.8 g N m^{-2} (N3), 27.6 g N m^{-2} (N4) or 46.0 g N m^{-2} (N5) (Table 1). The urea was dissolved in approximately 7 liters of water obtained from the nearby drainage canal and applied evenly across the plots, while the control plots (C) received an equal application of unfertilized canal water. Though the canal water contained ammonium and phosphate, the total contribution of both of these minerals to the treatment plots was negligible (NH_4^+ < 0.015 g m^{-2}, PO_4^{3-} < 0.0001g m^{-2})(Table 2).

One of the goals of this project was to determine the minimal concentration of fertilizer needed to affect plant growth as Olcott (2011) found that low concentrations (30 g N m^{-2}) of urea-fertilizer elicited a response in the aboveground biomass of *Spartina alterniflora* plants. The concentrations applied in this study were chosen as they provided the plants with relatively low levels of fertilizer compared to previous studies (Table 3). Urea was chosen as the source of nitrogen as it is among the most common forms of fertilizer used for agricultural purposes (Gilbert et al. 2006).

<div align="center">Methods</div>

Aboveground Biomass

All plots were sampled for aboveground biomass once each month from April to December 2012 (Table 4). A smaller 4-m^2 quadrat was placed in the center of each 9-m^2 plot

from which grass samples were collected. Two smaller 25 x 25 cm quadrats were randomly selected within the 4-m^2 quadrat, and all aboveground leaves and stems were clipped at the soil surface. The plants were placed in separate, marked bags and transported to East Carolina University's Department of Biology in Greenville, NC for refrigerated storage and analysis. Samples were dried to a constant weight in the lab for 72 hours at 65° C. Any leaves or stems that were green in color were separated as live tissue, and both live and dead tissues were individually weighed to the nearest 0.1 g. Live and dead grass materials were stored separately in sealed bags. Subsamples of the live grasses were homogenized in a Thomas Wiley Grinding Mill (40 mesh size), transferred into 25 mL scintillation vials, and frozen until further analysis for organic matter and nitrogen content.

To assess total aboveground production throughout the growing season, the Smalley method was implemented (Smalley 1959). This procedure calculates the aboveground net primary production (ANPP) of the marsh plots by summing the change in living and dead plant material from the beginning of the growing season to the end using biomass values from each sampling interval. The average ANPP for the growing season was assessed for both blocks and treatments.

Belowground Biomass

Soil cores were collected in April, June, August, November, and December from the C, N2, and N5 plots. The cores were extracted from the fringe border between the larger 9-m^2 plots and the smaller 4-m^2 quadrats to ensure that the removal of the cores did not interfere with the aboveground experimental areas. Two 30 cm deep cores were removed from the plots using a soil borer (diameter = 4 cm). All contents of the cores were placed into separate sealed bags and stored in a freezer until further analysis for biomass, organic matter, and nitrogen content.

Each frozen soil core was thawed to room temperature immediately before biomass analysis. The thawed cores were wet sieved first through a 4.76 mm mesh screen to separate coarse root and rhizome material, then further through a finer 1.18 mm mesh screen to retain fine organic constituents. The contents of each sieve were dried to a constant weight at 65° C and weighed separately. Material that passed through the larger screen was categorized as macro-organic matter (MOM) consisting of mostly live roots and rhizomes, while the finer sediments were classified as particulate-organic patter (POM) made up of finer, predominantly dead plant materials. The plant material was then ground and homogenized (40 mesh size), placed into scintillation vials, and frozen until further analysis for organic matter and nitrogen content.

The total, MOM, and POM dry masses from each core were expressed in units of g m^{-2} to maintain consistency with the aboveground biomass analysis. To estimate biomass per square meter, the dry mass of the plant materials collected from each core (area = 0.0013 m^2) were multiplied by 795.50. Belowground NPP was estimated as the difference in total biomass between the period with the highest biomass values (June) and lowest biomass values (April). It should be noted that while this allowed for a general analysis of belowground primary production throughout the sampling period, it provides only a rough estimate as the samples were very small and were collected infrequently.

Above- and Belowground Nitrogen and Organic Matter Content

Total organic content of subsamples from both the live aboveground and belowground (MOM and POM) plant tissue was determined using loss on ignition (LOI) values. Roughly 0.5 g of the dried, ground plant material was weighed to the nearest 0.0001 g and combusted at 500° C for 5 hours in ceramic crucibles to remove all combustible organic matter. The remains were reweighed, and the loss in mass was used to determine the initial organic content of the tissues.

Nitrogen concentrations in live aboveground and belowground plant tissue were determined using the Total Kjeldahl Nitrigen (TKN) procedure (Eastin 1977, Raveh and Avnimelech 1979). The TKN method used for this study was as follows:

1. 25 mg of the dried, ground grass samples were transferred into individual glass flasks containing a solution of 9.5 g cupric sulfate, 268 g potassium sulfate, 1.5 L deionized water, and 268 mL of 18 M sulfuric acid.

2. The flasks containing plant samples and solution were then placed in a Kjeldahl block digester and heated to 200° C for 60 minutes, then to 380° C for 90 minutes, then cooled at ambient room temperature for 12 hours.

3. The digested samples were processed using a SmartChem 200 analyzer (Westco Scientific Instruments) which is an automated spectrophotometer that reads the absorbance of the samples. Throughout the digestion process, the samples become visibly blue. Samples containing higher concentrations of nitrogen take on a darker shade of blue, and the SmartChem 200 analyzer reads the absorbance (color) of the samples at 660 nm. The absorbance of the final sample is directly proportional to the total concentration of nitrogen contained within the original sample.

Aboveground tissue nitrogen content was only determined for all of the C, N4, and N5 treatment plots. Each month, two random treatments from each block were selected to be analyzed in addition to the other three. All belowground plant tissues were analyzed for nitrogen content.

Soil-Surface Decomposition

Aboveground plant samples were collected from the marsh before the first application of fertilizer. The samples were brought back to the lab and dried at 65° C to a constant weight. Approximately 15 g of dried litter was weighed to the nearest 0.01 g and placed into 48 mesh

decomposition bags and returned to the field site. For a first deployment, four decomposition bags were placed in the fringe of each plot within blocks A and C at the surface of the soil in April. After every 2 months following the first deployment, 1 decomposition bag from each plot was collected. The contents were dried to constant weight, and the remaining mass was used to determine the total decomposition and decomposition rate by dividing mass loss by time.

To compare the decomposition rates throughout the experiment, new leaf litter bags were deployed in the marsh every two months following the initial deployment. In June, 3 decomposition bags were placed in each plot within block A (18 bags total) during a second deployment, 2 bags were placed in each plot within block B in August (12 bags total) for a third deployment, and 1 bag was placed in each plot within block C in November (6 bags total) for the final deployment (Table 5). Grass samples for these second, third, and fourth deployments were collected approximately one week before being placed in the field. This design allowed for the determination of any seasonal trends in decomposition rate throughout the sampling season.

<u>Statistical analysis</u>

The data were analyzed using IBM SPSS Statistics version 20. Because samples were collected at various times throughout the growing season, a repeated measures analysis of variance (ANOVA) was performed to determine responses of above- and belowground biomass and above- and belowground organic and nitrogen content to both the within-subject effects (time) and between-subject effects (treatment). Separate repeated measures ANOVAs were performed to determine significant block effects. A one-way ANOVA was performed on the live aboveground biomass collected in July, October, and December to determine a significant treatment trend for these collections. A post-hoc Fisher's Least Significant Difference multiple comparison analysis was implemented to determine significant mean differences between

treatments at the 0.05 alpha-level as post-hoc adjustments yielded non-significant results. Where Mauchly's Test for Sphericity failed to yield significant results, a Huynh-Feldt correction was used to adjust p-values.

To determine the variability in growth rate throughout the growing season for each of the treatments, a linear regression analysis was performed using live aboveground biomass values across time. The linear contrasts of each of the six treatment plots were compared to determine the rate of change throughout the sampling period.

Results

Aboveground Biomass

Live aboveground biomass increased from 43 ± 30 g m^{-2} (mean; SD) in April to 1080 ± 485 g m^{-2} in early December (Figure 3). The average ANPP for the growing season, calculated across all blocks and treatments using the Smalley procedure, was 2011 ± 758 g m^{-2} (Table 6). Though there was not a significant difference in ANPP across the treatments, there was a trend (p= 0.073). The N4 treatment plots had more growth than all other treatments (2955 ± 374 g m^{-2}), though the ANPP in these plots was only significantly greater than the N1 (1548 ± 153 g m^{-2}, p = 0.016) and N2 (1302 ± 350 g m^{-2}, p = 0.007) treatments (Figure 4). No other treatments had significantly different values of primary production.

There was a high amount of variability in production across the three blocks, although there was not a significant block effect (Figure 5). The production values for the C and N3 plots ranged in value from 976 g m^{-2} to 2711 g m^{-2} and 1434 g m^{-2} to 3408 g m^{-2}, respectively. ANPP was highest for the N3 plot in block A and lowest in the C plot, highest in the N4 plot and lowest in the N2 plot in block B, and highest in the C plot and lowest in the N2 plot in block C.

Live aboveground biomass was significantly different among the treatments in July, October, and December and the N4 treatment plots contained the highest live biomass values during these months (Figure 3). However, there was no significant effect of treatment on either total or live biomass throughout the entire sampling period as the month-to-month variability was very high. The C plots contained the highest live biomass values of all treatments in November (1158 ± 559 g m^{-2}). Aboveground live and total biomasses were not highest within the N5 treatment plots at any sampling interval.

Biomass increased linearly across all six treatment plots throughout the growing season (Table 7). Growth rate was lowest in the N1 and N2 treatment plots. Both the highest growth rate and largest R^2 value (the correlation of plant biomass and time) occurred in the N4 treatment plots. The C treatments had the second highest growth rate, followed by the N6 treatments. The positive, linear relationship of live plant biomass versus time was significant ($p < 0.01$) for all treatments.

Aboveground Nitrogen and Organic Matter Content

Aboveground tissue N content ranged from 0.37% to 1.63% (Figure 6). The highest average nitrogen content across all treatments was in April ($1.06 \pm 0.07\%$) and lowest in August ($0.61 \pm 0.05\%$) with similar low values for the three subsequent samplings. There was a highly significant ($p < 0.01$) linear decline in tissue N content throughout the sampling period. Percent N declined consistently from April to July, at which point nitrogen content remained relatively constant at approximately 0.60% through the rest of the sampling period. The N-content was higher in the N5 treatment plots than all other treatments in the June, October, and November sampling months, though no significant differences were computed between any treatments. All treatment plots followed relatively similar within-month trends in nitrogen content.

Tissue organic matter content showed a similar monthly trend in that the overall average across all six treatments was highest in April (97.0 ± 0.66%) and ranged between 95.6 and 96.6% in the following months (Figure 6). The control plots had the lowest organic content of all treatments in both July and November (94.61 ± 1.38% and 94.48 ± 1.83% respectively). There was a positive relationship in organic content with treatment during the July, October, and November months, while there was no discernible relationship during the other sampling periods.

There was no common trend in the relationship between organic content and tissue nitrogen content in samples across the six treatments, and none of the treatments followed a significantly linear trend (Figure 7). The relationship was slightly positive in the N1 (slope = 0.048, R^2 = 0.068) and N4 (slope = 0.028, R^2 = 0.021) treatments, essentially neutral in the N3 (slope = -0.002, R^2 < 0.001) and N5 (slope = 0.012, R^2 = 0.007) treatments, and negative in the N2 treatment plots (slope = -0.097, R^2 = 0.104).

Belowground Biomass

No significant trends were found in total root and rhizome biomass across nitrogen treatments (Figure 8). Sample biomass levels doubled from roughly 1700 g m^{-2} in April to 3700 g m^{-2} in June, then decreased to 1700 g cm^{-2} in December. Total belowground biomass varied across the sampling period with significance (p < 0.001) and was highest in June and August. The belowground NPP across all plots was estimated to be 2121 ± 770 g m^{-2}. While the N2 treatment plots had higher values of belowground biomass than the other treatments in August, November, and December, the control plots had the greatest values of biomass of any treatment throughout the sampling period in June (4110 ± 864g m^{-2}). In contrast with the aboveground NPP, belowground NPP did vary significantly (p = 0.04) across the three blocks, and production was higher in block A than both blocks B (p = 0.02) and C (p = 0.04). However, there was no

significant block effect on belowground biomass when assessed for the entire sampling period using a repeated measures ANOVA.

POM masses were consistently higher and had a larger range (1087 to 2700 g m^{-2}) than the MOM masses (544 to 959 g m^{-2}), and accounted for roughly 71% of the overall total belowground biomass throughout the growing season. Treatment had a significant effect on MOM biomass (p = 0.02), though only the N2 treatment plots had significantly higher biomass than the C plots (p < 0.01). There were no significant differences between the C and N5 or N2 and N5 plots. POM was not significantly different across the treatments.

Belowground Nitrogen and Organic Matter Content

Belowground tissue nitrogen content decreased significantly throughout the growing season in both POM (p < 0.001) and MOM (p = 0.001) (Figure 9). The MOM nitrogen content ranged from 0.71 ± 0.07% in April to 0.50 ± 0.04% in December. Similar to the overall trend in belowground biomass, these values were lower than those of the POM tissue, which ranged from 1.09 ± 0.09% in April to 0.66 ± 0.03% in December. There were no significant differences in nitrogen content across the C, N2, or N5 treatments for either POM or MOM.

Belowground tissue organic matter content displayed an inverse trend to that of the nitrogen content; both the POM and MOM increased in organic matter throughout the growing season, although the organic content only varied by month significantly for the POM (p = 0.025) (Figure 9). Sample organic content ranged from 88.3% to 95.5% for the MOM and 83.2% to 94.8% for the POM. No significant differences were found in organic content between the C, N2, and N5 treatments.

Decomposition

All treatment plots across all three blocks displayed similar rates of decomposition relative to seasonal trends (Figure 10). Bags deployed in June experienced the greatest loss in

leaf litter mass (mean = 31.07 ± 5.59% mass loss), and almost all of this decomposition occurred within the first two months of deployment (Table 8). The bags that were deployed in April (mean = 26.86 ± 4.50% mass loss) followed a similar trend of rapid decomposition through the late summer months. However, the rate at which the grass in these bags decomposed (rate = 0.16 ± 0.04 g day^{-1}) was much more gradual than those that were deployed in June (rate = 0.31 ± 0.14 g day^{-1}). Also, the bags that were deployed in block C in October experienced lower rates of decomposition than all other sets of bags that were in the field for fewer than 7 months (rate = 0.11 ± 0.06 g day^{-1}).

Out of 14 sets of decomposition bags, grouped by their respective deployment and pickup dates, 10 showed a positive trend in overall mass loss with nitrogen treatment (Figure 10). Additionally, the bags that were deployed in June showed the highest correlation of treatment and decomposition (Figure 11). In block B, the bags left in the field for 71 days showed a negative relationship with treatment (slope = -0.302, R^2 = 0.068), while those placed in the field for a total of 106 days showed a positive relationship (slope = 2.120, R^2 = 0.832). Two sets of bags from block C displayed reduced decomposition with increased treatment; those that were in the field for 126 days from April to August (slope = -0.131, R^2 = 0.006) and those left for 35 days from November to December (slope = -0.513, R^2 = 0.204).

<div align="center">Discussion</div>

Aboveground Biomass

The total live biomass within the study site was much higher in the fall months than expected; typically marsh plants senesce after the peak of the growing season in August or September (Valiela et. al 1976, Roman and Daiber 1984, Roberts 2000, Wigand et. al 2004, Darby and Turner 2008a). However, annual marsh species like *S. alterniflora* senesce earlier in

<div align="center">15</div>

the season (Connor and Chmura 2000) and other studies have found similarly high amounts of aerial live biomass in plots of *S. patens* from October to December (Gosselink et al. 1977, Pezeshki and DeLaune 1991, Connor and Chmura 2000). The control plots had higher values of live aboveground biomass in November than all other treatments and higher values than the N1, N2, and N3 treatment plots in December, so it can be assumed that the fertilization treatment was not a main factor contributing to continued plant growth in the early winter.

While it was expected that the hurricane that passed through the marsh at the end of October would have had a negative impact on plant growth (high inundation, higher salinities, etc.) plants remained highly productive for the remainder of the study. It is possible that the hurricane weather provided water to the plants during the late fall months which are typically known to be relatively dry. Roberts (2000) found that *S. patens* plant growth within a Virginia marsh was significantly different between two growing seasons as low precipitation throughout one season hindered plant productivity.

Although aboveground biomass was somewhat higher in the plots receiving higher nitrogen treatments, our hypothesis that biomass would increase in relation to treatment level was not supported. While the plots receiving 27.6 g N m^{-2} had higher growth than all other treatments, growth in these plots was not significantly greater than growth in the control plots. In addition, the plots receiving the highest nitrogen treatment had only slightly higher biomass values than the control plots and had less NPP than both the N3 and N4 treatments. This suggests that the addition of relatively small concentrations of nitrogen may reduce nutrient stress for *Spartina patens* plants, but that nutrient stress may not be a significant limiting factor for *S. patens* growth in high marsh regions. Our findings coincide with the observations by Crain (2007) which implied that salinity drives nitrogen limitation; *S. patens* plants subjected to higher

salinities require more nitrogen to tolerate the salinity stress. The salinity in our study site was relatively low (Table 2).

Spartina patens has been shown to have a competitive advantage over other marsh plants like S. alterniflora, D. spicata, and P. australis when nutrient availability is low (Bertness et al. 2002, Pennings et al. 2002), which would support the findings that reducing nutrient stress in marsh regions dominated by S. patens would have little effect on plant growth. Rather, it can be expected that species of plants that are less able to compete for nutrients under oligotrophic conditions would displace S. patens over time upon nutrient enrichment. Bertness et al. (2002) found that nitrogen enrichment due to increased coastal development led to the displacement of S. patens on the seaward border by S. alterniflora and by P. australis on the terrestrial border in a New England salt marsh. Our study did not find similar trends in competitive displacement. Because the region of marsh sampled in our study was about 1km from the seaward border, no S. alterniflora plants were present to be affected by enrichment. Other common high marsh plants were collected within the plots throughout the growing season, though these plants represented only 26 of 314 (8%) collected samples and were most prevalent in the C, N1, and N3 treatment plots (Table 9). No plants other than S. patens were collected in any of the N4 plots, which had the highest ANPP.

The observed aboveground tissue nitrogen concentrations are similar to those found in previous studies, both in terms of overall range and seasonal trends (Table 10). The nitrogen content of the sampled tissues was relatively high in the N5 treatment plots throughout most of the latter portion of the growing season. However, this did not translate to significantly higher values of aboveground biomass. Also, the relationship of nitrogen to organic content was essentially neutral in all of the plots. The lack of significance in the nitrogen concentrations between the treatments is consistent with the lack of a clear trend in aboveground biomass.

17

Previous studies on various marsh grass species have found that as biomass increases in response to fertilization, so too does the nitrogen content of plant tissues (Wigand et al. 2004, Darby and Turner 2008b). This study did not find significant differences in either aboveground biomass or nitrogen concentrations across treatments.

Marsh ecosystems experience considerable temporal and spatial variability, and plant dynamics such as species composition and primary production can be heavily influenced by changes in abiotic factors like seasonal trends, weather, and elevation. *S. patens* plants are more productive when growing in soils with higher elevation and lower depths of standing water (Roberts 2000) as they are not as efficient at oxygenating their root systems as other high marsh grasses (Anderson 1973). It is possible that factors such as precipitation influenced plant growth to a higher extent than fertilization, though weather patterns are expected to have influenced each of the study plots to the same extent. However, microtopography did vary among the study plots, and there were times in which inundation was greater in some areas than others. This could have had an influence on plant growth, and while there was no difference in primary production among the three replicate blocks, it is not known whether natural variation in growth patterns among the treatment plots within each block were influenced by elevation/inundation gradients.

Belowground biomass

Belowground biomass showed a similar seasonal trend to that of other studies in that peak biomass occurred in June and declined thereafter (Valiela et al. 1976, Hackney and de la Cruz 1986, Connor and Chmura 2000) (see Table 11 for production values from previous studies). The majority of variability in root and rhizome biomass was in the form of the fine, particulate material suggesting that turnover either occurred due to belowground decomposition of dead tissue or reallocation of biomass into aboveground tissues (Connor and Chmura 2000). Because belowground biomass was reduced throughout the rest of the growing season, it is likely

that the plants expended more energy into producing roots and rhizomes to acquire nutrients in the early spring and then shifted biomass production to aboveground stems and leaves for increased light absorption in the summer and fall.

The estimated belowground to aboveground biomass ratio, commonly referred to as the "root to shoot ratio," in this study was roughly 1. This ratio is considerably lower than those calculated in previous *S. patens* studies (Roman and Daiber 1984, Wigand et al. 2004, Elsey-Quirk et al. 2011), though Windham (2001) found a root to shoot ratio of 1.1 in *S. patens* plants in a New Jersey marsh. Though the Smalley procedure used in our study to estimate aboveground NPP underestimates production because it does not account for losses through decomposition, the maximum-minimum method implemented for belowground NPP estimation is assumed to be even less conservative. Therefore, our estimated belowground biomass values are likely lower than the actual production values.

While differences in belowground biomass were not significant between treatments, the control plots had the highest biomass levels in June, when overall biomass was highest in the plots. It is possible that the nutrient enrichment reduced the need for the higher treatment plots to produce as much biomass in the form of roots as nutrients were more readily available. However, this was not clearly demonstrated due to the lack of significance and relatively small sample size. Also, it is unlikely that a significant trend would have been observed in such a short period of time as other studies have found that belowground plant biomass may not respond to nutrient additions until 1-2 years after treatment (Wigand et al. 2004). Though MOM biomass was significantly higher in the N2 treatment plots than both the C and N5 treatment plots, a corresponding trend in increased nitrogen uptake within these tissues was not demonstrated. As with aboveground plant growth, belowground biomass was also likely affected by elevation and inundation.

Belowground nitrogen concentrations followed the same decline that biomass displayed throughout the growing season. With the exception of the MOM from the N5 treatment plots, all other treatments experienced reduced nitrogen concentrations with increased tissue organic matter content. It is possible that the plants within the N5 plots sequestered more nitrogen into their belowground tissues relative to plants within the other treatment plots. However, nitrogen concentrations within the N5 plots were not higher than those within the other treatment plots, and the small sample size made it difficult to infer significant trends across any of the treatments.

Decomposition

Seasonality had a discernible impact on decomposition. The greatest rates of mass loss were observed during the warmest months of the experiment, June-August (Table 8). During this period the average daily temperature was approximately 26° C, and these temperatures most likely provided ideal conditions for microbial activity. In addition, the bags that were deployed in June had a much more rapid initial phase of mass loss compared to those that were placed in the field in April. Decomposition usually occurs most rapidly during the first several months of exposure in an initial leaching phase, after which microbes begin to consume the remaining tissues (Hodson et al. 1984, Valiela et al. 1985, Foote and Reynolds 1997, Rejmánková and Sirová 2007). Valiela et al. (1985) found that temperature does not affect the rate of decay during the initial leaching phase, so it is possible that the microbial and leaching phases overlapped when the bags were placed in the field in June, when microbial activity was likely higher than in April.

Although statistical significance could not be determined for the effects of treatment on decomposition due to lack of replication, it did not appear that there was a significant correlation in mass loss among the treatments. This is consistent with previous findings that phosphorus, not nitrogen, limits microbial activity in coastal marshes (Sundareshwar et al. 2003, Rejmánková and

Houdková 2006). It was expected that the microbial phase would be the period of decomposition that would experience the greatest variability in decay rate across the treatments. Although the results from block B support this case - the bags that were left in block B for 106 days displayed a positive trend in decomposition rate with higher treatment dosage while those that were in the field for 71 days did not - the observations from blocks A and C did not confirm this trend.

It should also be noted that because this study focused on N-treatment effects on decomposition, litter quality data (i.e. carbon, nitrogen, and phosphorus content) were not obtained. While these factors can be very important drivers of decomposition rates in marsh systems (Enríquez et al. 1993, Rejmánková and Houdková 2006), litter was collected from isolated stands of *S. patens* grasses with relatively similar characteristics (height, diameter, percent green leaves, etc.) so that sets of bags contained similar detritus.

Another important factor in organic carbon burial processes that was not central to this study is the efflux of gasses in response to nutrient enrichment. Marsh soils contain complex community structures in which biogeochemical processes can be altered drastically in response to amount and frequency of inundation, salinity, temperature, and available nutrients. Previous studies have found that the introduction of nutrients to marsh plots increases soil respiration by enhancing belowground decomposition (Wigand et. al 2009), though some have suggested that phosphorus is the limiting nutrient for belowground microbial activity in anoxic soils (Sundareshwar et al. 2003). A complete analysis of organic carbon burial in response to nutrient enrichment should include measurements of belowground decomposition rates as this component of organic carbon loss could significantly offset enhanced rates of aboveground and belowground production. In addition, the rate of denitrification should be measured to assess microbial transformation of the nitrogen added to these systems as N_2O, a byproduct of the denitrification process, is a potent greenhouse gas (Smeets et al. 2009).

<u>Conclusions</u>

Future agricultural management should focus on limiting total nutrient loading into watersheds that connect to coastal waters. While marshes are valued for their ability to intercept these nutrients (Valiela and Cole 2002), the changes that occur to marsh biological processes under eutrophic conditions is not well understood. In addition, marsh regions likely respond differently under eutrophic conditions, so determining best management practices is an even more difficult task. The high marsh zone has received far less attention than low, tidal marsh regions. Much focus should be placed on determining the variability in plant responses to nutrient enrichment across different marsh landscapes.

One of the more substantial, yet often overlooked, values of marsh ecosystems is their ability to store organic carbon more effectively than other terrestrial ecosystems (Mcleod et al. 2011). The implication from many previous marsh fertilization studies is that the addition of nitrogen to these typically nitrogen-limited ecosystems results in an overall loss of organic carbon by reducing belowground biomass and enhancing denitrification rates (Valiela et al. 1976, Darby and Turner 2008 c, Wigand et al. 2009, Deegan et al. 2012). The removal of nutrients from coastal regions by marshes may be mitigating off-shore eutrophication at the expense of long-term organic carbon sequestration.

This study did not provide enough evidence to suggest that the addition of urea-fertilizer affects the production or decomposition of plants in a high marsh. These findings are somewhat contradictory to the overall paradigm of nitrogen limitation in coastal marshes. However, high marsh zones have not been well-represented in the literature, and the effects of eutrophication in these areas should be the subject of further study. The plants in this study did not undergo significant physiological changes when exposed to external sources of nitrogen. While we expected the high marsh zone to serve as a nutrient buffer to the low marsh by sequestering

nitrogen in plant tissues without significant changes to plant production, nitrogen concentrations in both aboveground and belowground tissues were not affected by fertilization. Future studies should focus on determining the fate of this nitrogen that is not sequestered into plant tissues and replicating these results with varying concentrations and forms of fertilizer.

Figure 1. (**a,b**) Google Earth image showing the location of the study site on the coast of North Carolina, USA (copyright 2013 Google). (**c**) Study site layout.
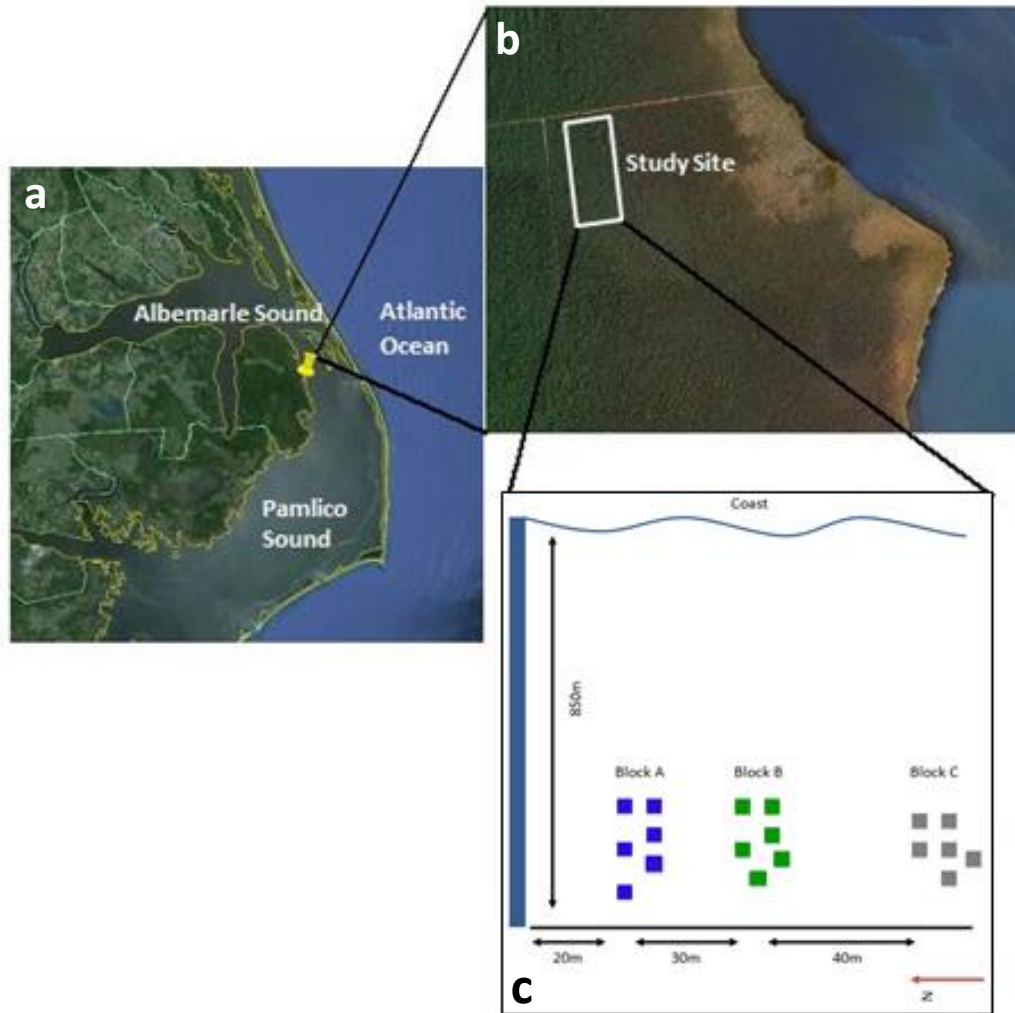
Figure 2. Diagram of the study site showing the location and orientation of the three blocks (A, B, and C) along a transect (horizontal black line). The distance between the blocks and between block A and the drainage creek (vertical blue line) are shown below the transect line. The vertical black arrow displays the distance from the transect line to the coast.
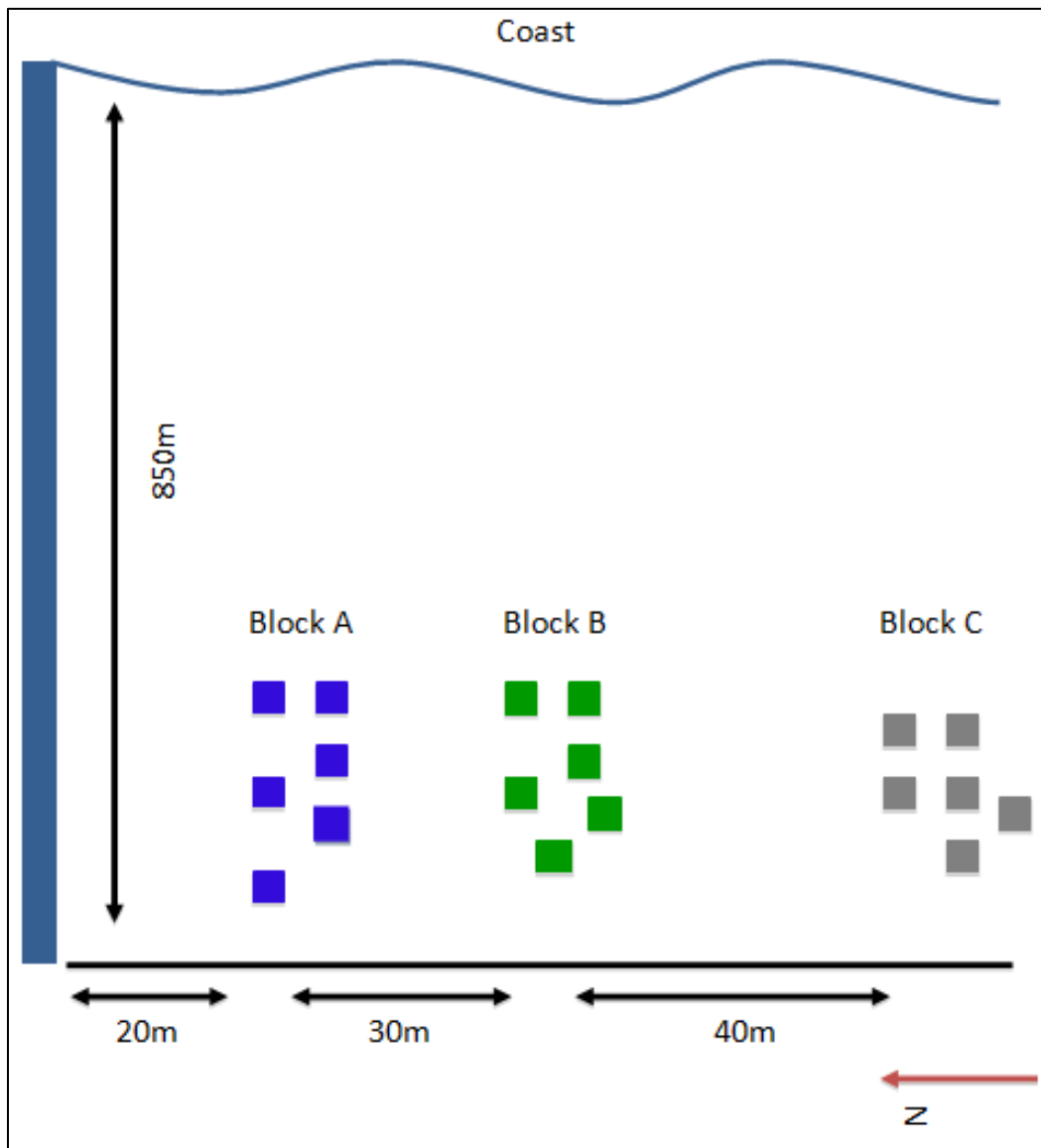
Figure 3. Mean aboveground (**a**) live and (**b**) dead biomass (g m^{-2}; mean ± 1 SE) of three replicates of six N-fertilizer treatments. Sample biomass did not differ significantly across treatments at the $p = 0.05$ significance level based on a repeated measures ANOVA. Asterisks indicate the months in which live biomass was significantly different among the treatments ($p < 0.05$) based on a one-way ANOVA.
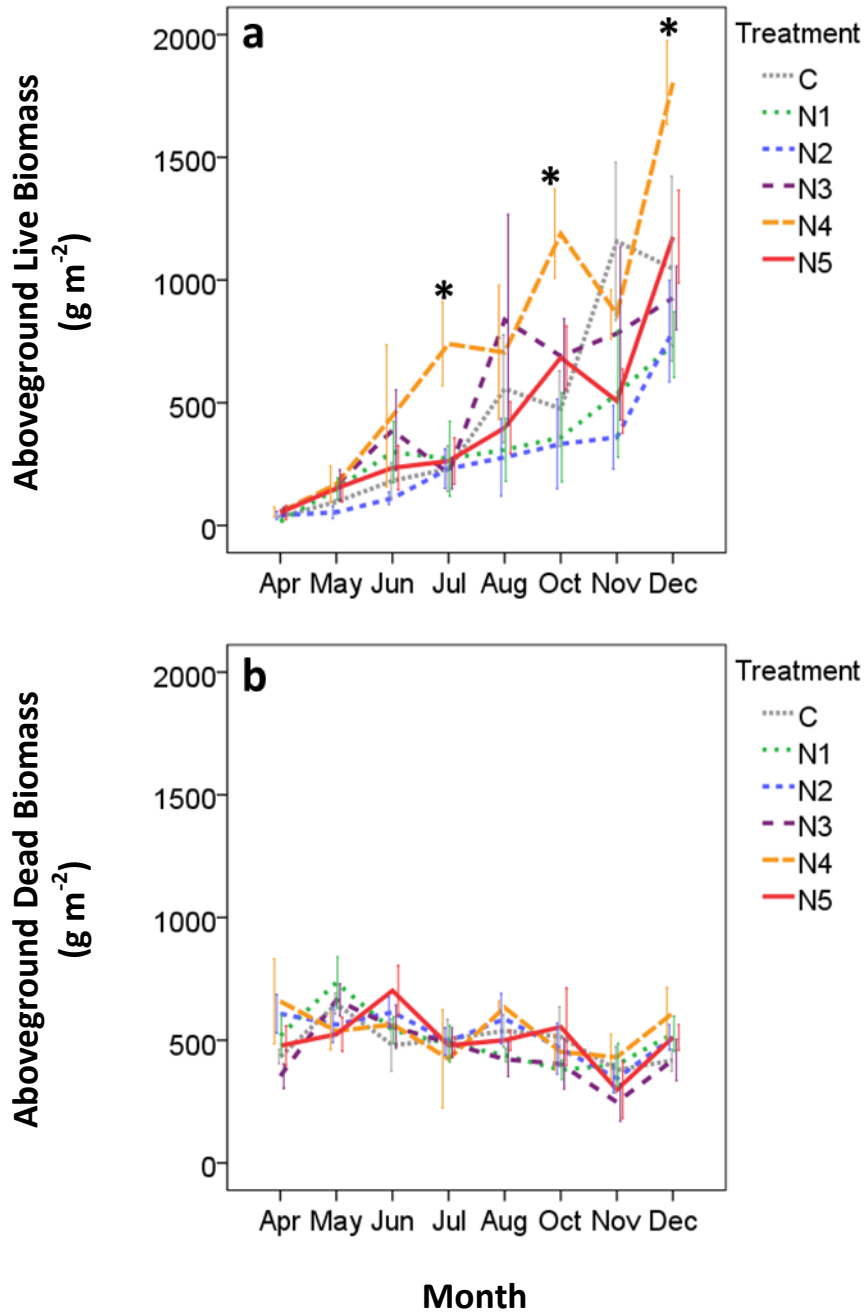
Figure 4. Mean aboveground net primary production (g m^{-2}; mean $\pm$ 1 SE) of three replicates of six N-fertilizer treatments. Means with the same letter are not statistically different from each other based on a one-way ANOVA.
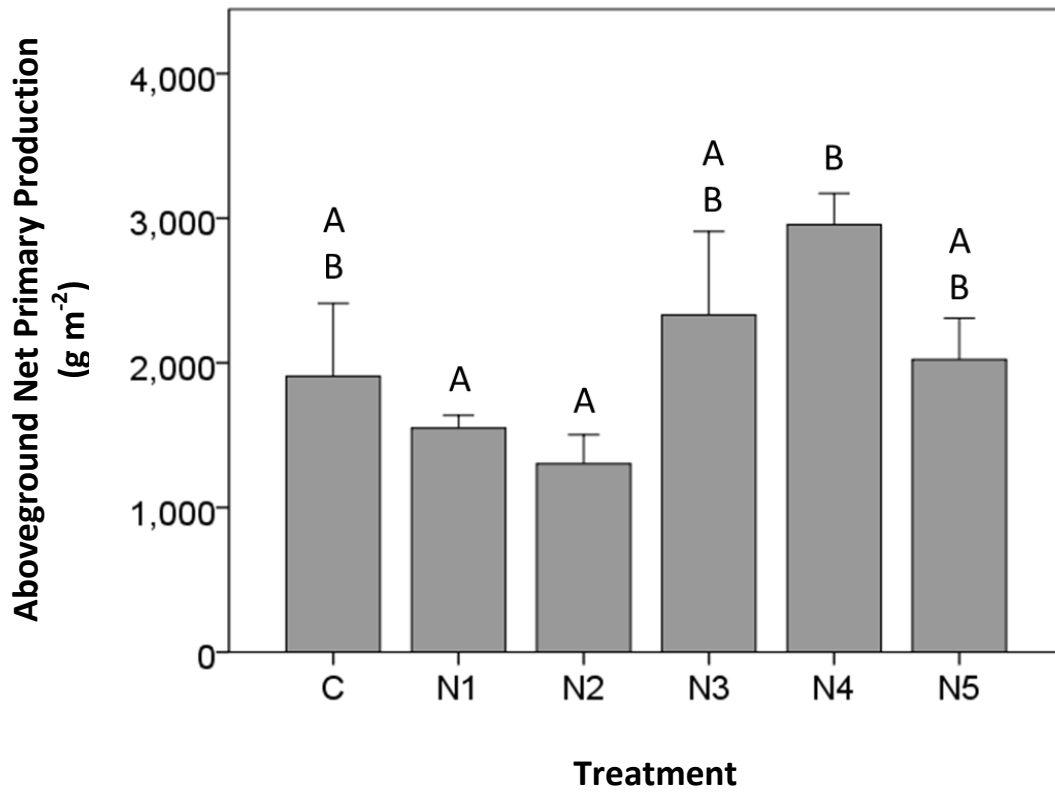
Figure 5. Aboveground net primary production (g m^{-2}) across three replicate blocks of six N-fertilizer treatment levels.
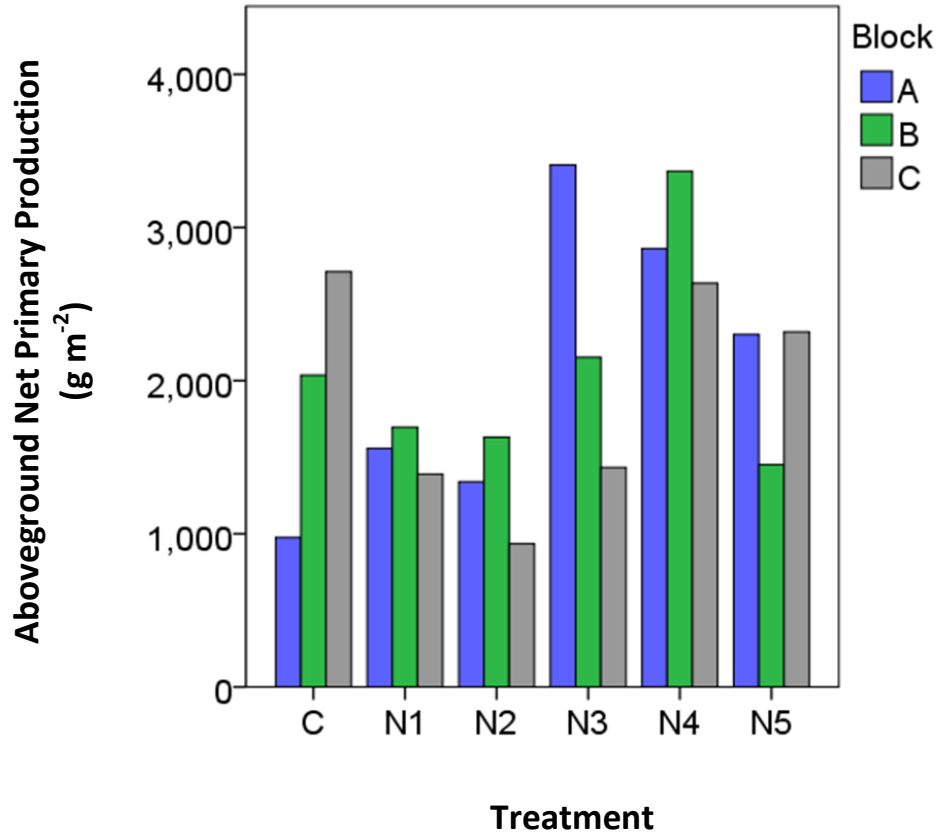
Figure 6. Mean aboveground tissue (**a**) nitrogen and (**b**) organic matter content (%; mean ± 1 SE) of six N-treatment levels.
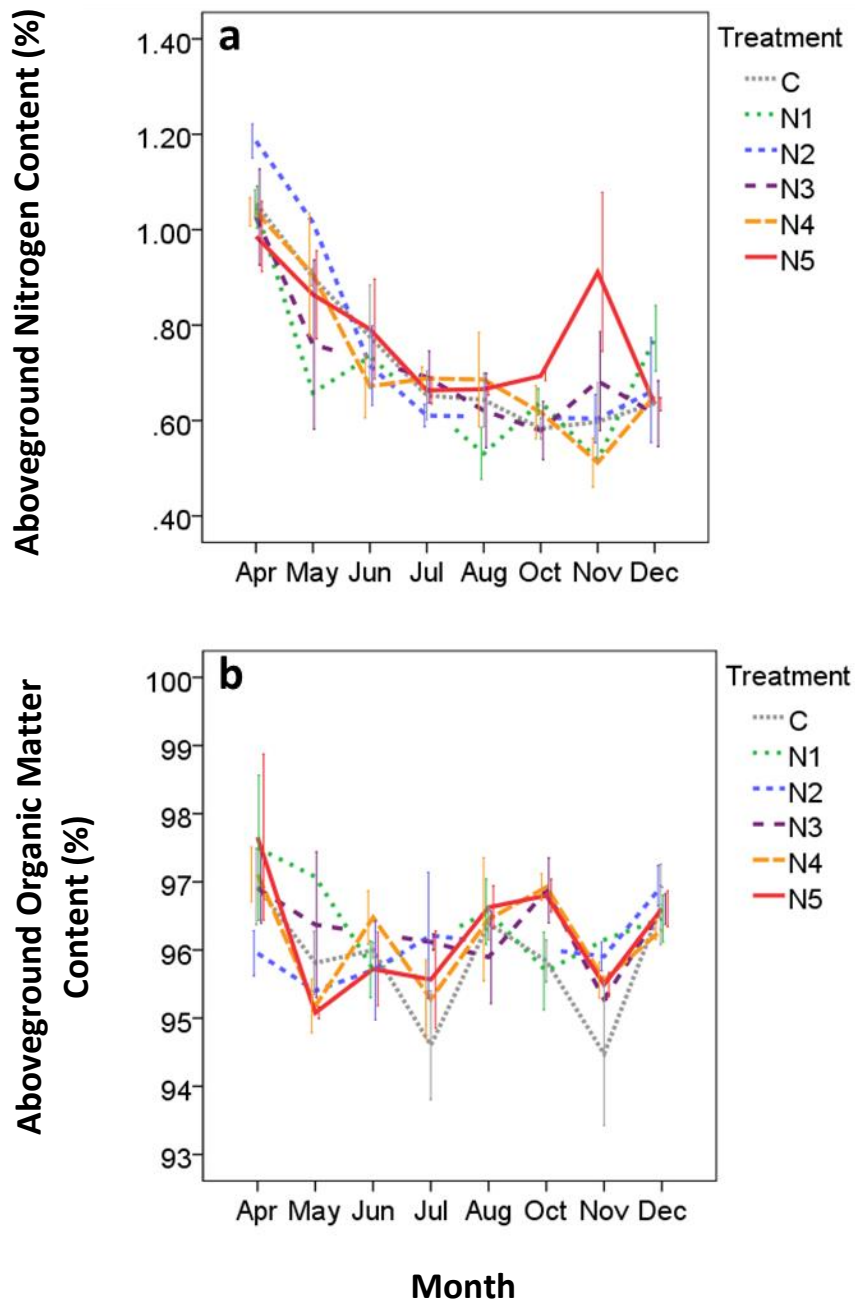
Figure 7. Linear relationship between the aboveground tissue organic and nitrogen content for the (**a**) C, (**b**) N1, (**c**) N2, (**d**) N3, (**e**) N4, and (**f**) N5 treatment plots. Each data point represents the organic and nitrogen tissue content (%) of an individual sample. Regression lines show the relationship of tissue components over the entire sampling period and across all blocks.
*One sample from the N5 treatment displayed a 100% loss in mass upon combustion, which is likely due to sampling error.
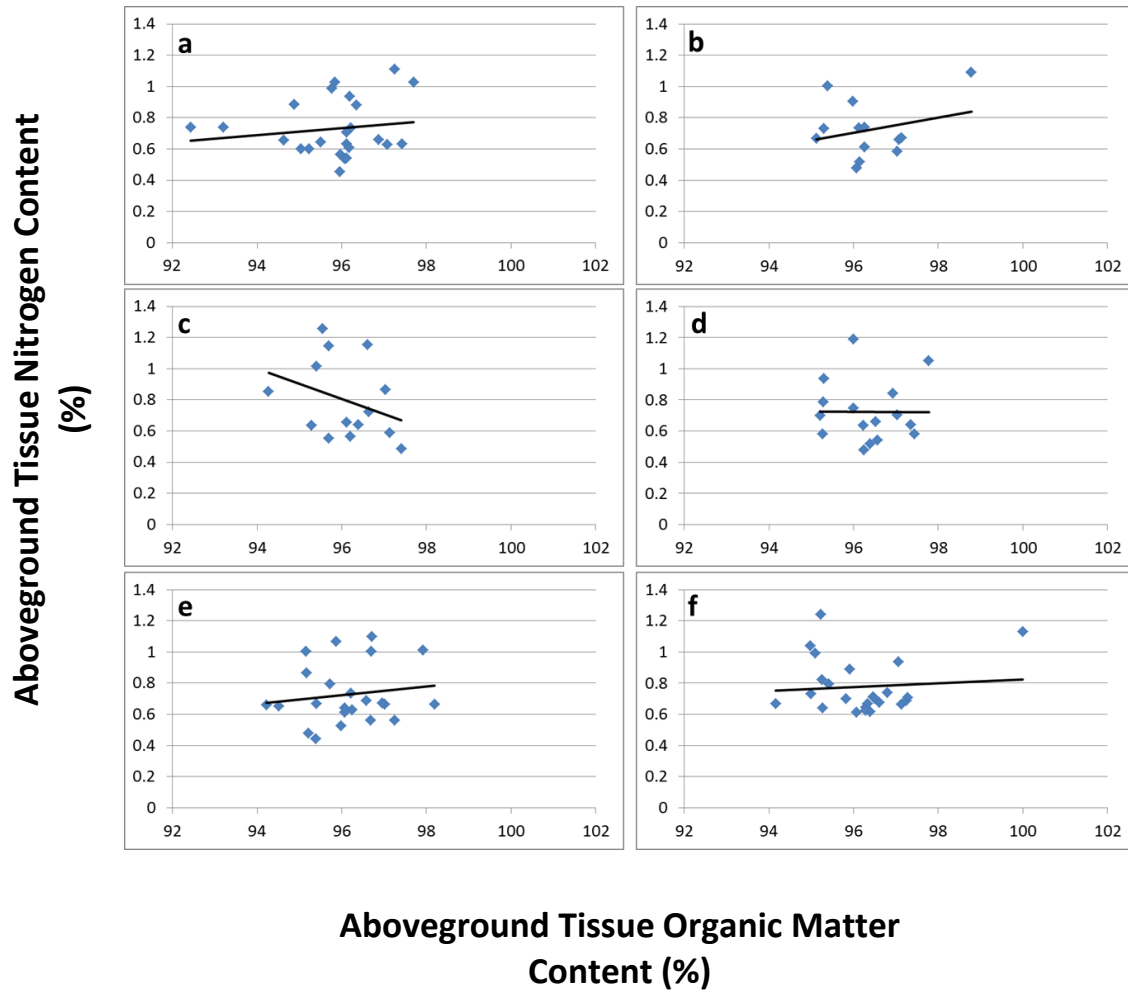
Figure 8. Mean belowground (**a**) total biomass, (**b**) macro organic matter, and (**c**) particulate organic matter (g m^{-2}; mean ± 1 SE) collected from sediment cores at five different sampling intervals throughout the growing season. Cores were collected from three different replicates of three N-fertilizer treatments (C, N2, and N5).
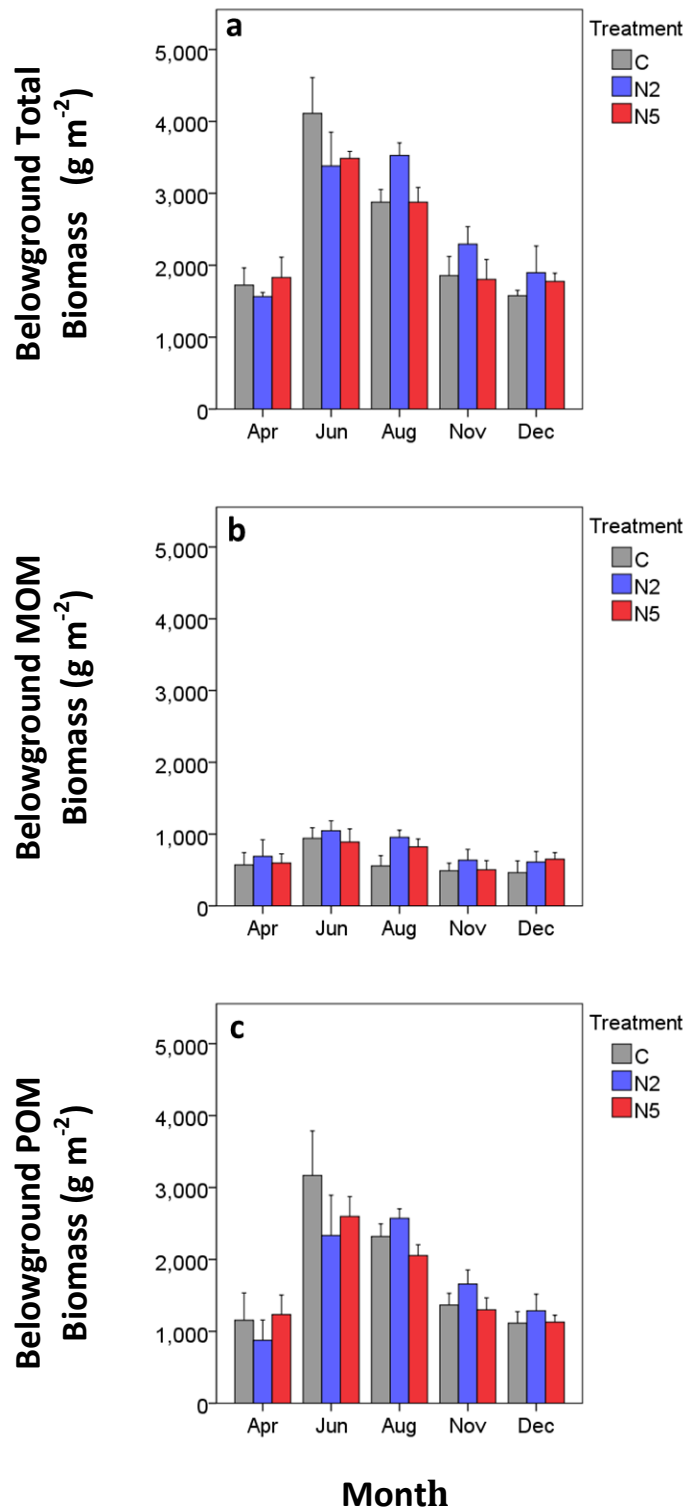
Figure 9. Mean belowground tissue nitrogen content of (**a**) macro organic matter and (**b**) particulate organic matter and organic content of (**c**) macro organic matter and (**d**) particulate organic matter (%; mean ± 1 SE) collected from sediment cores at five different sampling intervals throughout the growing season. Cores were collected from three different replicates of three N-fertilizer treatments (C, N2, and N5).
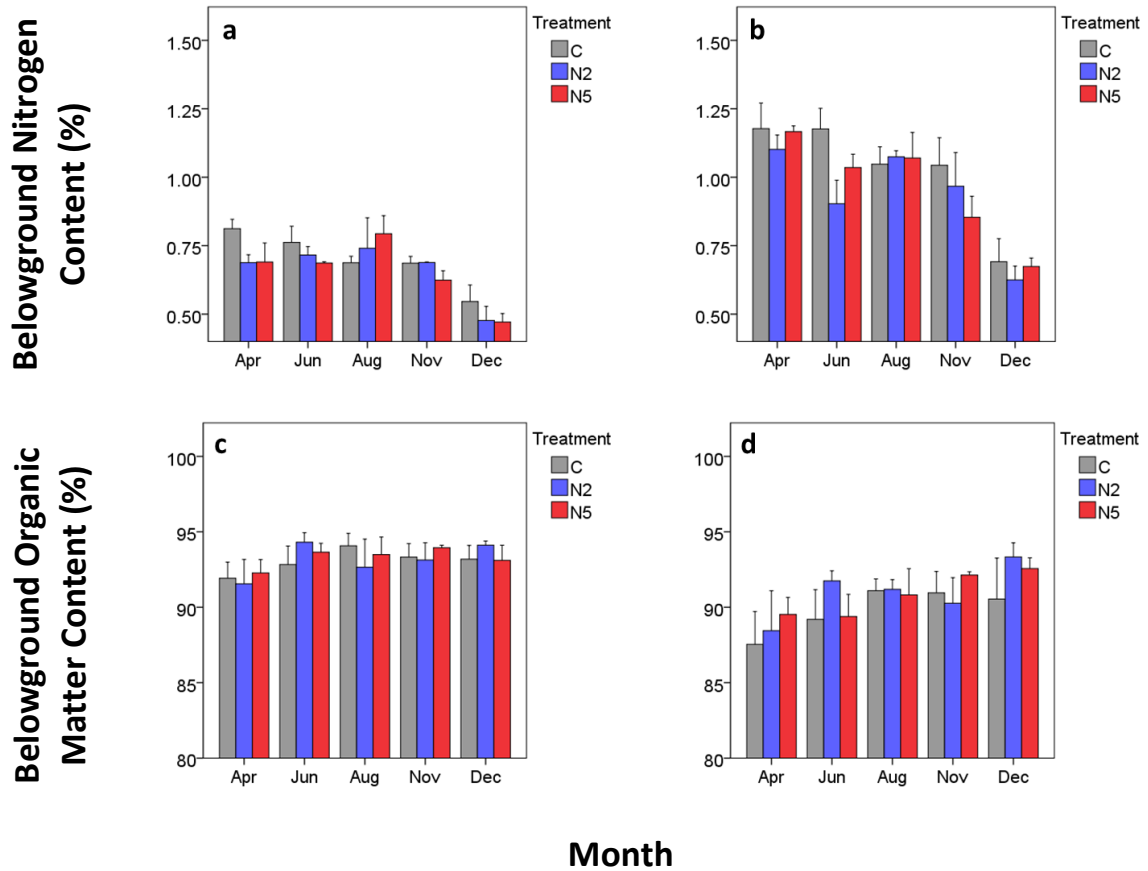
Figure 10a. Mass loss from litter bags (% change in mass) across fertilizer treatments in (**a**, **b**) Block A and (**c**) Block B. The legend displays the day the bags were deployed, retrieved, and the total number of days in the field. Regression lines display the relationship between treatment and decomposition for individual sets of bags.
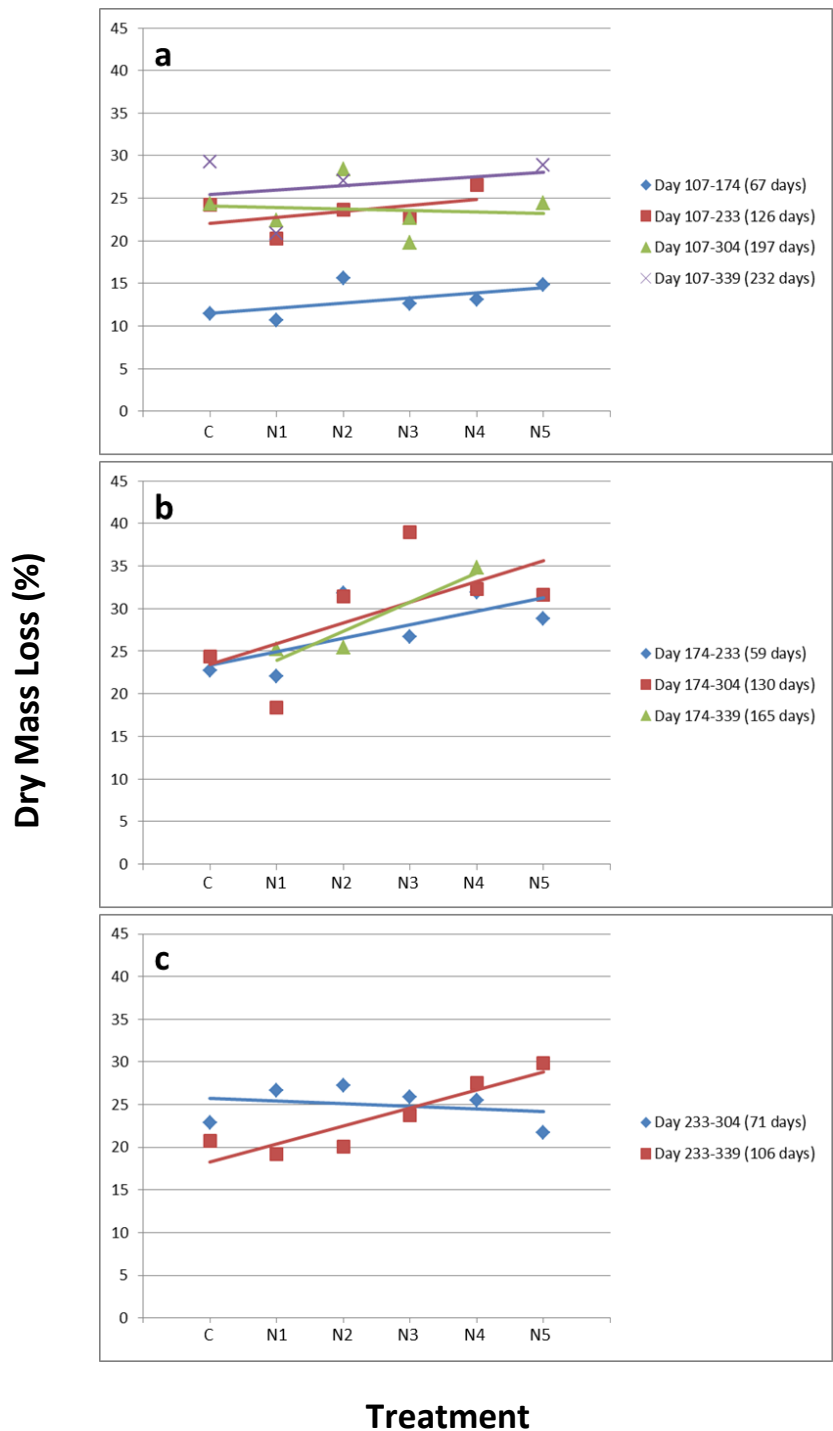
Figure 10b. Mass loss from litter bags (% change in mass) across fertilizer treatments in (**a**, **b**) Block C. The legend displays the day the bags were deployed, retrieved, and the total number of days in the field. Regression lines display the relationship between treatment and decomposition for individual sets of bags.
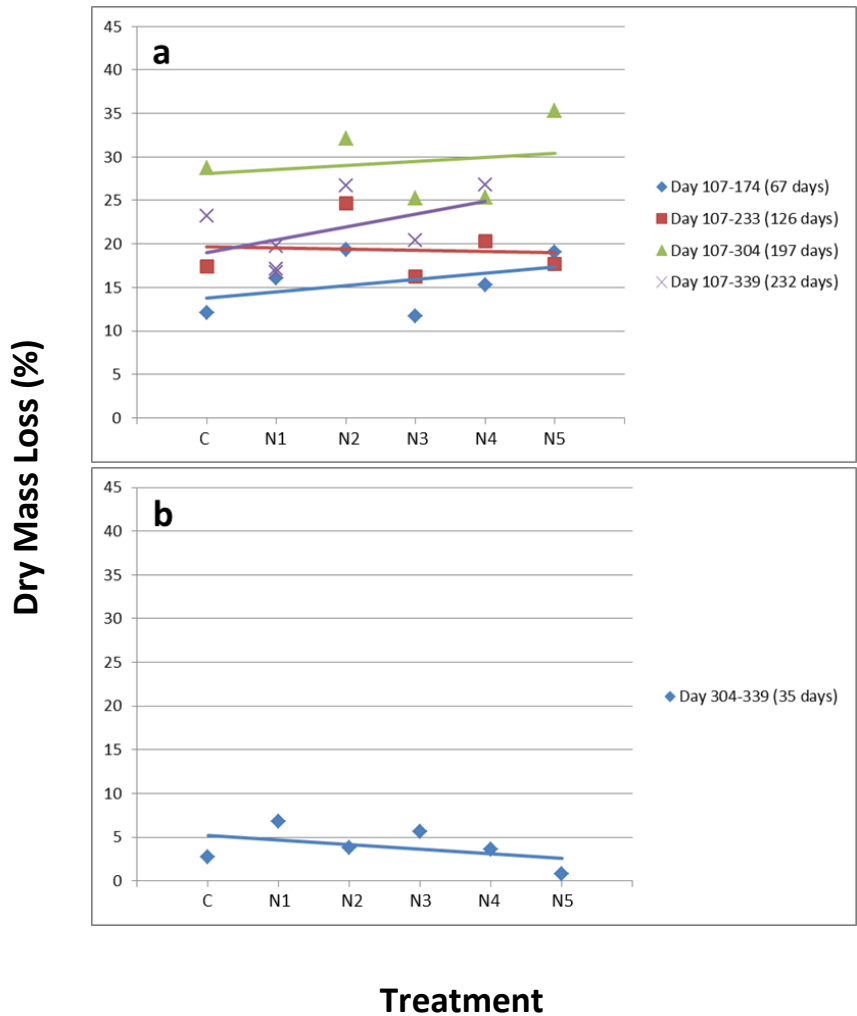
Figure 11a. Mass remaining (% dry mass) in litter bags left in (**a**) treatment plot C and (**b**) treatment plot N1 in block A for a given number of days. The legend displays the month in which the bags were deployed. The points showing 100% mass remaining correspond to the day the bags were deployed in the field.

Day of the Year
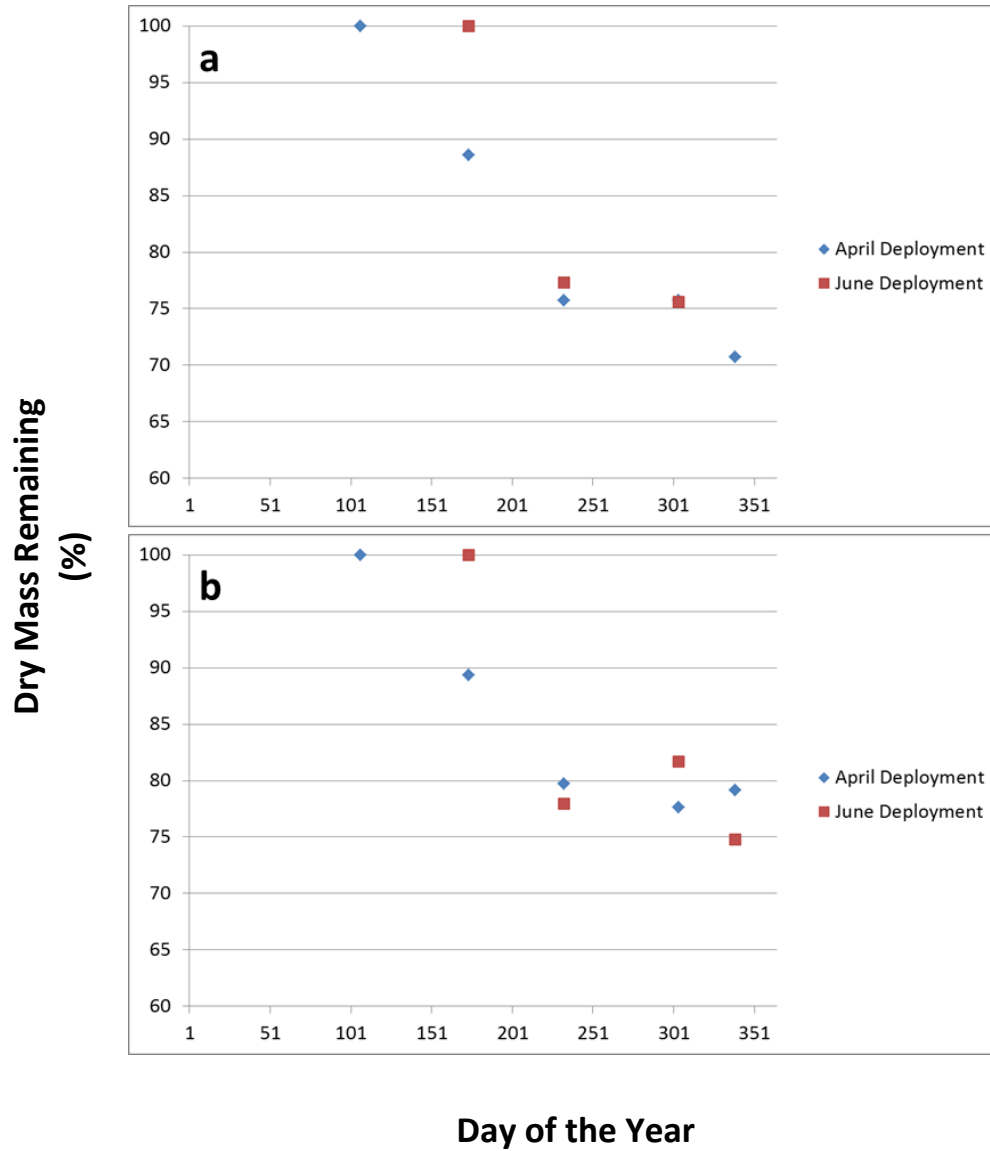
Figure 11b. . Mass remaining (% dry mass) in litter bags left in (**a**) treatment plot N4 and (**b**) treatment plot N5 in block A for a given number of days. The legend displays the month in which the bags were deployed. The points showing 100% mass remaining correspond to the day the bags were deployed in the field.
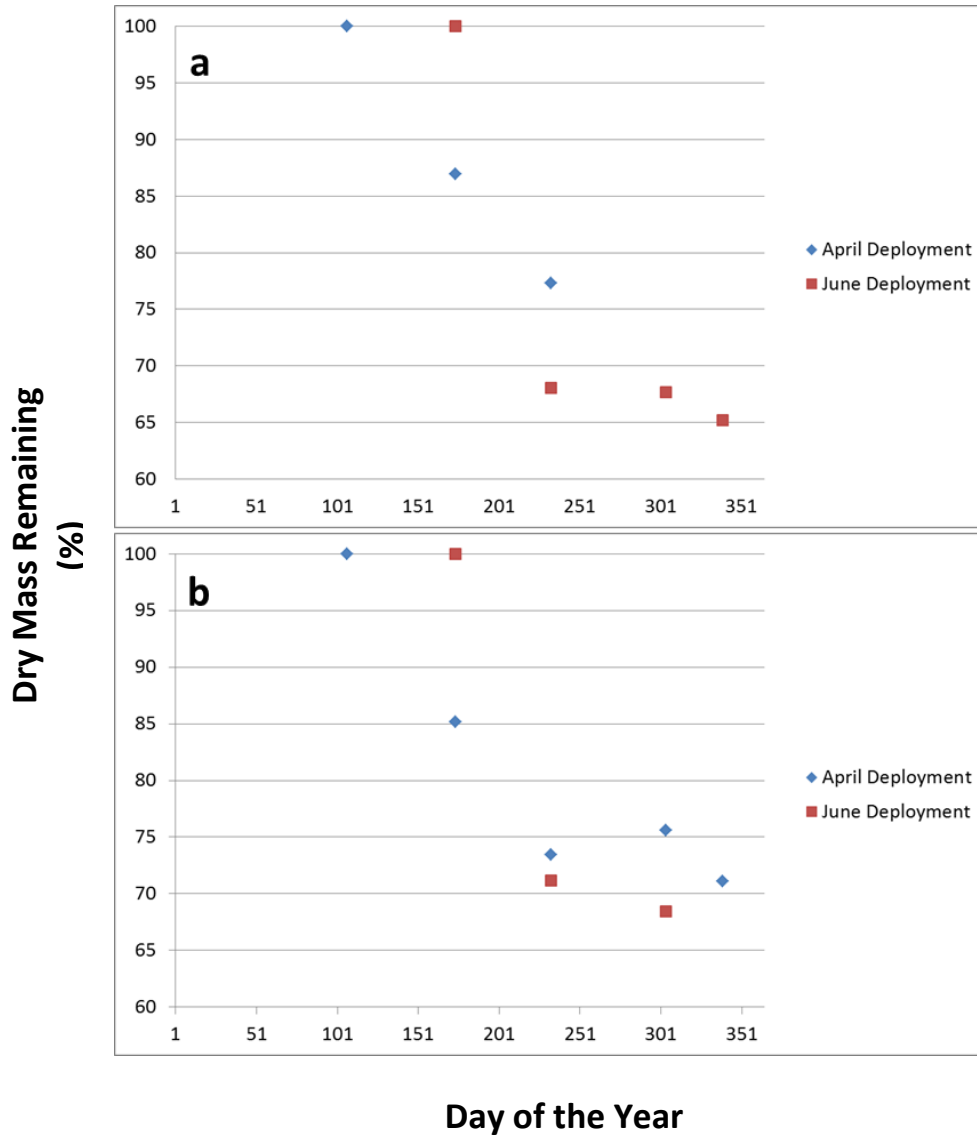
Table 1. Total concentrations (g N m^{-2}) of urea-nitrogen fertilizer applied to the treatment plots throughout the study. The total concentrations shown were applied to the plots in 7 doses. Treatment labels correspond to the concentration applied to the respective plot.

Label	Total Nitrogen Applied (g N m^{-2})
C	0
N1	3.45
N2	6.90
N3	13.80
N4	27.60
N5	46.00

Table 2. Climate data (mean daily temperature and total precipitation) for each month of the sampling period. Salinity readings were collected from pools of standing water within each block on July 23, 2012 and December 10, 2012 using a YSI Handheld Multiparameter Instrument. Water samples were collected from a drainage canal abutting the study site in July and December and analyzed for NH_3-N and PO_4-P in East Carolina University's Central Environmental Laboratory.

| Month	Mean Daily Temperature (°C)*	Total Precipitation (cm)*	Salinity (ppt ± SD)			NH_4^+-N (mg L^{-1})	PO_4^{3-}-P (mg L^{-1})
			Block A	Block B	Block C		
April	14.6	6.10		-		-	-
May	20.9	10.31		-		-	-
June	24.2	1.80		-		-	-
July	28.3	16.51	1.82 (0.12)	1.73 (0.22)	1.90	2.08	0.079
August	25.9	4.85		-		-	-
September	23.8	7.77		-		-	-
October	18.7	18.57**		-		-	-
November	10.9	1.65		-		-	-
December	10.0	15.88	9.50‡ (0.70)	8.50‡ (0.82)	10.0‡ (0.20)	2.94	0.175

*Climate data retrieved from NOAA National Climatic Data Center (http://www.ncdc.noaa.gov)

**Hurricane Sandy passed North Carolina on October 29, 2012

‡Hurricane conditions followed by low precipitation in November likely caused unusually high salinities in December

Table 3. Concentrations of nitrogen-fertilizer applied and corresponding biomass responses in similar *Spartina patens* studies conducted throughout the United States.

Study	Species	Location	Applied Fertilizer Concentration (g N m^{-2})	Nitrogen Source	Biomass Response	
					Above-ground	Below-ground
Wigand et al. (2004)	*Spartina patens*	Prudence Island, RI, USA	32	Calcium nitrate	+	N/A
This Study	*Spartina patens*	Point Peter Rd., NC, USA	0, 3.45, 6.9, 13.8, 27.6, 46	Urea	+/-	+/-
Ket et al. (2011)	*Zizaniopsis millacea*	Carrs Island, GA, USA	50	Ammonium chloride/ urea	+	-
Darby and Turner (2008a)	*Spartina alterniflora*	Cocodrie, LA, USA	4.6, 9.3, 18.6, 37.2, 74.4	Ammonium sulfate	+	-
Etheridge et al. (2012)	*Spartina patens*	Virginia Coastal Reserve, Delmarva Peninsula, USA	0, 7.5, 15, 30, 100	Urea	+/-	N/A
Valiela et al. (1976)	*Spartina alterniflora /patens*	Great Sippewisset Marsh, MA, USA	134	Urea	N/A	-
Crain (2007)	*Spartina patens*	Scarborough Marsh, ME, USA	163	Ammonium nitrate	+	N/A
Olcott (2011)	*Spartina alterniflora*	Virginia Coastal Reserve, Delmarva Peninsula, USA	30, 100, 300	Urea	+	+/-
Anisfeld and Hill (2012)	*Spartina alterniflora*	Hadley Creek Marsh, VA, USA	105, 210, 420	Ammonium nitrate/ Sodium nitrate	N/A	+/-

Table 4. Field sampling dates and respective monthly labels. Asterisks denote the dates in which soil cores were extracted for belowground analyses.

Month	Sampling Date
April	4/16/12*
May	5/22/12
June	6/29/12*
	7/02/12
July	7/23/12
August	8/27/12*
October	10/05/12
November	11/05/12*
December	12/10/12*
*Soil cores extracted	

Table 5. Date decomposition bags were placed in each treatment plot of block A (**a**), block B (**b**), and block C (**c**). Asterisks (*) correspond to the initial placement of bags within the given block and plus symbols (+) correspond to the second placement of bags within a block. Each individual asterisk or plus sign corresponds to six total decomposition bags (one bag placed in each treatment plot).

a

Month	April	May	June	July	August	October	November	December
Placement	****		+++					
Retrieval			*		*+		*+	*+

b

Month	April	May	June	July	August	October	November	December
Placement	****						+	
Retrieval			*		*		*	*+

c

Month	April	May	June	July	August	October	November	December
Placement					**			
Retrieval							*	*

Table 6. Aboveground biomass production of *Spartina patens* from similar studies calculated using various methods throughout the United States.

Study	Location	Production (g m^{-2} year^{-1})	Measurement Method
Gosselink et al. (1977)	Bayou Lafourche, LA, USA	4200	Wiegert-Evans (1964)
Hopkinson et al. (1978)	Bayou Lafourche, LA, USA	6043	Wiegert-Evans (1964)
Linthurst and Reimold (1978)	Bar Harbor, ME, USA	912/2523/3523/5833	Peak Biomass/Valiela et al. (1975)/Smalley (1959)/Wiegert-Evans (1964)
	Lewes, DE, USA	807/1241/980/2753	
	Sapelo Island, GA, USA	946/1028/1674/3925	
Hopkinson et al. (1980)	Bayou Lafourche, LA, USA	1200/2500/2000/5800	Peak Biomass/Valiela et al. (1975)/Smalley (1959)/Wiegert-Evans (1964)
Roman and Daiber (1984)	Canary Creek and Blackbird Creek, DE, USA	669-727/1089-1147	Peak Biomass/Smalley (1959)
Pezeshki and DeLaune (1991)	Barataria Basin, LA, USA	3677/5702	Smalley (1959)/Gas Exchange
Connor and Chmura (2000)	Point Lepreau, New Brunswick, CA	379	Peak Biomass
Wigand et al. (2004)	Prudence Island, RI, USA	584-1009	Peak Biomass
Elsey-Quirk et al. (2011)	Little Assawoman Bay, DE, USA	1336	N/A
This Study	Alligator River National Wildlife Refuge, NC, USA	2011	Smalley (1959)

Table 7. Results of linear regression analyses performed on the live aboveground biomass values for each treatment. Equations and R-square values describe the relationship between live aboveground biomass and time.

Treatment	Equation of Regression Line	R-square
C	Y=10.10x-15.92	0.5642*
N1	Y=5.35x-3.18	0.4424*
N2	Y=5.57x-7.88	0.5032*
N3	Y=7.97x-4.18	0.4302*
N4	Y=13.32x-13.35	0.6901*
N5	Y=8.28x-10.18	0.6587*
*All regressions were highly significant $(p < 0.01)$.		

Table 8. Total decomposition (% mass loss ± SD) and rate of decomposition (% loss day^{-1} ± SD) averaged over all treatments. Batches of bags are grouped based on their placement within a given block, their date of placement, and date of retrieval. Where the sample size (n) ≠ 6, bags were either lost or collected at a later date.

Batch #	Block	Deployment Date	Retrieval Date	Days in Field	Mass Loss (% ± SD)	Loss Rate (% day^{-1} ± SD)	Sample Size (n)
1	A	4/16/12	6/29/12	67	13.02 (1.90)	0.19 (0.03)	6
			8/27/12	126	23.49 (2.28)	0.19 (0.02)	5*
			11/05/12	197	23.66 (2.87)	0.12 (0.01)	6
			12/10/12	232	26.52 (3.91)	0.11 (0.02)	4*
	C	4/16/12	6/29/12	67	15.58 (3.28)	0.23 (0.05)	6
			8/27/12	126	19.26 (3.39)	0.15 (0.03)	5*
			11/05/12	197	29.36 (4.37)	0.15 (0.02)	5*
			12/10/12	232	21.54 (4.17)	0.09 (0.02)	7**
2	A	6/29/12	8/27/12	59	27.33 (4.31)	0.46 (0.07)	6
			11/05/12	130	29.50 (7.17)	0.23 (0.06)	6
			12/10/12	165	28.47 (5.48)	0.17 (0.03)	3*
3	B	8/27/12	11/05/12	71	24.98 (2.17)	0.35 (0.03)	6
			12/10/12	106	23.53 (4.35)	0.22 (0.04)	6
4	C	11/05/12	12/10/12	35	3.88 (2.12)	0.11 (0.06)	6

*Bags were either lost or collected at a later date
**Previously missed bag collected

Table 9. Species and frequency of vegetation collected during this study.

Species	Frequency	Percent of total sample size (%)
Baccharis halimifolia	3/314	1%
Cladium mariscus jamaicense	5/314	1%
Distichlis spicata	15/314	5%
Phragmites australis	3/314	1%
Spartina patens	288/314	92%

Table 10. Comparison table of above- and belowground *Spartina patens* tissue nitrogen content observed in previous studies.

Study	Location	Tissue Nitrogen Content (%)	Aboveground/Belowground Biomass
Roman and Daiber (1984)	Canary Creek and Blackbird Creek, DE, USA	0.50-2.08	Aboveground
		1.06-1.14	Belowground
Curtis et al. (1990)	Rhode River, MD, USA	0.70-0.80	Belowground
Windham and Ehrenfeld (2003)	Hog Island, NJ, USA	1.22-1.57	Aboveground
Elsey-Quirk et al. (2011)	Little Assawoman Bay, DE, USA	0.50-1.50	Aboveground
		0.50-1.20	Belowground
This Study	Alligator River National Wildlife Refuge, NC, USA	0.37-1.63	Aboveground
		0.50-1.09	Belowground

Table 11. Belowground biomass production values of *Spartina patens* plants from studies conducted throughout the United States

Study	Location	Production (g m^{-2} year^{-1})
Valiela et al. (1976)	Great Sippewisset Marsh, MA, USA	2520-4979
Roman and Daiber (1984)	Canary Creek and Blackbird Creek, DE, USA	3300-5900
Wigand et al. (2004)	Prudence Island, RI, USA	4783-6961
Saunders et al. (2006)	Rhode River, MD, USA	4119
Elsey-Quirk et al. (2011)	Little Assawoman Bay, DE, USA	2026
This Study	Alligator River National Wildlife Refuge, NC, USA	2121

References

Anderson CE. 1973. A review of structure in several North Carolina salt marsh plants. Pages 307-344 in R.J.Reimold and W.H. Queeen, editors. *Ecology of Halophytes*. Academic Press, New York, New York, USA.

Anisfeld SC, Hill TD. 2012. Fertilization effects on elevation change and belowground carbon balance in a Long Island Sound tidal marsh. *Estuaries and Coasts* 35:201-211.

Bertness MD, Ewanchuck PJ, Silliman BR. 2002. Anthropogenic modification of New England salt marsh landscapes. *Proceedings of the National Academy of Sciences* 99:1395-1398.

Blum LK. 1993. *Spartina alterniflora* root dynamics in a Virginia salt marsh. *Marine Ecology Progress Series* 102:169-178.

Bridgham SD, Megonigal JP, Keller JK, Bliss NB, Trettin C. 2006. The carbon balance of North American wetlands. *Wetlands* 26:889-916.

Chmura GL, Anisfeld SC, Cahoon DR, Lynch JC. 2003. Global carbon sequestration in tidal, saline wetland soils. *Global Biogeochemical Cycles* 17:22(1-12).

Chmura GL. 2011. What do we need to assess the sustainability of the tidal salt marsh carbon sink? *Ocean & Coastal Management* doi:10.1016/j.ocecoaman. 2011.09.006.

Connor R, Chmura GL. 2000. Dynamics of above- and belowground organic matter in a high latitude macrotidal saltmarsh. *Marine Ecology Progress Series* 204:101-110.

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

Curtis PS, Balduman LM, Drake BG, Whigham DF. 1990. Elevated atmospheric CO_2 effects on belowground processes in C3 and C4 estuarine marsh communities. *Ecology* 71:2001-2006.

Darby FA, Turner RE. 2008a. Below- and aboveground *Spartina alterniflora* production in a Louisiana salt marsh. *Estuaries and Coasts* 31:223-231.

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

Darby FA, Turner RE. 2008c. Effects of eutrophication on salt marsh root and rhizome biomass accumulation. *Marine Ecology Progress Series* 363:63-70.

Deegan LA, Johnson DS, Warren RS, Peterson BJ, Fleeger JW, Fagherazzzi S, Wollheim WM. 2012. Coastal eutrophication as a driver of salt marsh loss. *Nature* 490: 388-394.

Duarte CM, Middelburg JJ, Caraco N. 2005. Major role of marine vegetation on the oceanic carbon cycle. *Biogeosciences* 2:1-8.

Eastin EF. 1977. Total Nitrogen Determination for Plant Material Containing Nitrate. *Analytical Biochemistry* 85:591-594.

Elsey-Quirk T, Seliskar DM, Gallagher JL. 2011. Nitrogen pools of macrophyte species in a coastal lagoon salt marsh: Implications for seasonal storage and dispersal. *Estuaries and Coasts* 34:470-482.

Enríquez S, Duarte CM, Sand-Jensen K. 1993. Patterns in decomposition rates among photosynthetic organisms: The importance of detritus C:N:P content. *Oecologia* 94:457-471.

Etheridge SB, Christian RR, Costanza B. 2012. Effects of Nitrogen Addition on Salt Marsh Plants. Poster presentation at East Carolina University Research Week 2012.

Foote AL, Reynolds KA. 1997. Decomposition of saltmeadow cordgrass (*Spartina patens*) in Louisiana coastal marshes. *Estuaries* 20:579-588.

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. *Royal Swedish Academy of Sciences* 31:64-71.Gilbert PM, Harrison J, Heil C, Seitzinger S. 2006. Escalating worldwide use of urea – a global change contributing to coastal eutrophication. *Biogeochemistry* 77:441-463.

Gosselink JG, Hopkinson CS, Parrondo RT. 1977. *Common marsh plant species of the Gulf Coast area*. Vol. I: Productivity. U.S. Army Engineer Waterways Exp. Sta. Tech. Rep. No. 0-77-44, Vicksburg, MS.

Gotelli NJ, Ellison AM. 2004. A primer of ecological statistics. Sinauer Associates, Inc.

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.

Gruber N, Galloway JN. 2008. An Earth-system perspective of the global nitrogen cycle. *Nature* 451:293-296.

Hodson RE, Christian RR, Maccubbin AE. 1984. Lignocellulose and lignin in the salt marsh grass *Spartina alterniflora*: initial concentrations and short-term, post-depositional changes in detrital matter. *Marine Biology* 81:1-7.

Hopkinson CS, Gosselink JG, Parrando RT. 1978. Aboveground production of seven marsh plant species in coastal Louisiana. *Ecology* 59:760-769.

Hopkinson CS, Gosselink JG, Parrando RT. 1980. Production of coastal Louisiana marsh plants calculated from phenometric techniques. *Ecology* 61:1091-1098.

IPCC (Intergovernmental Panel on Climate Change). 2007. Working group III report: "Mitigation of climate change." Cambridge, UK: Cambridge University Press.

Keefe CW. 1972. Marsh production: A summary of the literature. *Contributions in Marine Science* 16:163-181

Kennish MJ. 2002. Environmental threats and environmental future of estuaries. *Environmental Conservation* 29:78-107.

Ket WA, Schubauer-Berigan JP, Craft CB. 2011. Effects of five years of nitrogen and phosphorus additions on a Zizaniopsis milacea tidal freshwater marsh. *Aquatic Botany* 95:17-23.

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

Laffoley D, Grimsditch G. 2009. The management of natural coastal carbon sinks. International Union for Conservation of Nature and Natural Resources 53pp.

Lee SY, Dunn RJK, Young RA, Connolly RM, Dale PER, Dehayer R, Lemckert CJ, McKinnon S, Powell B, Teasdale PR, Welsh DT. 2006. Impact of urbanization on coastal wetland structure and function. *Austral Ecology* 31:149-163.

Linthurst RA, Reimold RJ. 1978. An evaluation of methods for estimating the net aerial primary productivity of estuarine angiosperms. *Journal of Applied Ecology* 15:919-931.

McGranahan G, Balk D, Anderson B. 2007. The rising tide: Assessing the risks of climate change and human settlements in low elevation coastal zones. *Environment and Urbanization* 19:17-37.

Mcleod E, Chmura GL, Bouillon S, Salm R, Björk M, Duerte 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. *Frontiers in Ecology and the Environment* doi:10.1890/110004.

Mitsch WJ, Gosselink JG. 2007. Wetlands. 4th ed. John Wiley & Sons, Hoboken, NJ.

Nicholls RJ, Wong PP, Burkett V, Woodroffe CD, Hay J. 2008. Climate change and coastal vulnerability assessment: Scenarios for integrated assessment. *Sustainability Science* 3:89-102.

Odum WE, Odum EP, Odum HT. 1995. Nature's pulsing paradigm. *Estuaries* 18: 547-555.

Olcott CA. 2011. Impacts of nitrogen addition on the monthly above- and belowground production of *Spartina alterniflora* in a Virginia marsh. B.S. and Distinguished Majors Program Thesis. Department of Environmental Sciences, University of Virginia, Charlottesville, VA. pp. 44.

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.

Pezeshki SR, DeLaune RD. 1991. A comparative study of above-ground productivity of dominant U.S. Gulf Coast marsh species. Journal of Vegetation Science 2:331-338.

Raveh A, Avnimelech Y. 1979. Total nitrogen analysis in water, soil and plant material with persulphate oxidation. *Water Research* 13:911-912.

Reddy KR, DeLaune RD. 2008. Biogeochemistry of wetlands: Science and applications. Taylor and Francis Group, LLC.

Rejmánková E, Houdková K. 2006. Wetland plant decomposition under different nutrient conditions: What is more important, litter quality or site quality? *Biogeochemistry* 80:245-262.

Rejmánková E, Sirová D. 2007. Wetland macrophyte decomposition under different nutrient conditions: Relationships between decomposition rate, enzyme activities and microbial biomass. *Soil Biology & Biochemistry* 39:526-538.

Richardson JL, Vepraskas MJ. 2001. Wetland soils: Genesis, hydrology, landscapes, and classification. Lewis Publishers. Boca Raton, FLA.

Roberts SW. 2000. Primary production of *Distichlis spicata* and *Spartina patens* and effects of increased inundation on a salt marsh. Master's Thesis. Department of Biology, East Carolina University, Greenville, NC.

Roman CT, Daiber FC. 1984. Aboveground and belowground primary production dynamics of two Delaware Bay tidal marshes. *Bulletin of the Torrey Botanical Club* 111:34-41.

Saunders CJ, Megonigal JP, Reynolds JF. Comparison of belowground biomass in C3- and C4-dominated mixed communities in a Chesapeake Bay brackish marsh. *Plant and Soil* 280:305-322.

Smalley AE. 1959. The role of two invertebrate populations, *Littorina irrorata* and *Orchelimum fidicinium* in the Energy Flow of a Georgia Salt Marsh Ecosystem, Ph.D. dissertation. Athens. University of Georgia.

Smeets EMW, Bouwman LF, Stehfest E, van Vuuren DP, Posthuma A. 2009. Contribution of N_2O to the greenhouse gas balance of first-generation biofuels. *Global Change Biology* 15:1-23.

Sundareshwar PV, Morris JT, Koepfler EK, Fornwalt B. 2003. Phosphorus limitation of coastal ecosystem processes. *Science* 5606:563-565.

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

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, Etten RV, Goehringer D, Volkmann S. 1985. Decomposition in salt marsh ecosystems: The phases and major factors affecting disappearance of above-ground organic matter. *Journal of Experimental Marine Biology and Ecology* 89:29-54.

Valiela I, Teal JM, Persson NY. 1976. Production and dynamics of experimentally enriched salt marsh vegetation: Belowground biomass. *Limnology and Oceanography* 21:245-252.

Valiela I, Teal JM, Sass WJ. 1975. Production and dynamics of salt marsh vegetation and the effects of experimental treatment with sewage sludge. *Journal of Applied Ecology* 12:973-982.

VanZomeren CM, White JR, DeLaune RD. 2011. Fate of nitrate in vegetated brackish coastal marsh. *Wetland Soils* 76:1919-1927.

Vernberg FJ. 1993. Salt-marsh processes: A review. *Environmental Toxicology and Chemistry* 12: 2167-2195.

Wiegert RG, Evans FC. 1964. Primary production and the disappearance of dead vegetation on an old field in southeastern Michigan. *Ecology* 45:49-62

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. *Journal of Coastal Research* 45:134-149.

Windham L, Ehrenfeld JG. 2003. Net impact of a plant invasion on nitrogen-cycling processes within a brackish tidal marsh. *Ecological Applications* 13:883-896.