Impacts of Nitrogen Addition on the Monthly Above- and Belowground Production of *Spartina alterniflora* in a Virginia Marsh

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Abstract

Faced with a rising sea level, salt marshes must either accrete sediment vertically or move inland in order to persist. In the absence of mineral sediment input, vertical accretion occurs via the production of organic sediments; this creation of organic sediment is directly related to belowground production of marsh plants. These highly productive coastal systems are currently experiencing eutrophication or are likely to in the future as a consequence of the extensive use of nitrogen fertilizer in inland regions. Nitrogen has been shown to increase aboveground productivity in plants, but it may serve to decrease belowground production and therefore hinder sediment accretion. I used a root in-growth core method to assess the monthly belowground response of *Spartina alterniflora* to three levels of added nitrogen. I also examined monthly aboveground productivity in order to recognize any alterations in biomass allocation that could result from additional levels of nitrogen. I found that aboveground production was 47% greater in fertilized plots compared to controls, whereas belowground production into root ingrowth cores was not affected by added nitrogen. I did not find a significant influence of increased available nitrogen on root production in *S. alterniflora*. However, measures of C:N ratios of belowground tissues showed a significantly lower C:N ratio in plots that had received additional nitrogen. This shows that root production from fertilized plots had a significantly higher tissue nitrogen content, making this organic matter susceptible to faster rates of decay than the production from control plots. Though root production remained constant, it is possible that additional nitrogen from eutrophication can reduce organic matter accumulation in salt marshes by increasing rates of decomposition, hindering sediment accretion and subsequent increases in marsh elevation.

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Introduction

Salt marshes are important ecosystems that perform a variety of ecosystem services useful to humans. These highly productive environments are significant in their ability to assimilate and store carbon, support coastal fisheries and biodiversity, trap and transform contaminants, as well as protect developed areas from potentially damaging coastal storms (Mitsch, 2000; Zedler & Kercher, 2005). The extent of marshes, and other coastal wetlands, is decreasing worldwide at a rate of about 1% per year. This decrease in area is mainly driven by human population growth and direct destruction of habitat for development in coastal zones (Zedler & Kercher, 2005). In addition to these losses, anthropogenically-induced reductions in the water quality of coastal rivers and bays, in the form of nutrient pollution, could act synergistically with rising sea levels to effect the loss of more marsh habitat (Nicholls et al., 1999).

A significant pollution problem affecting water quality across the world is nutrient pollution, or eutrophication. Coastal waterways are being loaded with increasingly large quantities of nutrients, namely nitrogen and phosphorus, from widespread fossil fuel burning, substantial inorganic fertilizer use, and from the waste of growing coastal populations (Howarth et al. 2002). These alterations in the historical nutrient budgets of estuarine communities lead to diminished water quality as well as anoxic conditions, which can hinder the growth and survival of economically valuable aquatic species (Deegan et al., 2002; Howarth, 2008). Eutrophication can alter marsh plant community structure (Rogers et al., 1998), but alone may not have a dramatic effect on the persistence of marsh ecosystems.

Global sea level rise could pose a threat to the long-term survival of salt marsh communities especially along the USA mid-Atlantic coast (Reed et al., 2008). For a given marsh community to remain viable and able to perform valuable ecosystem services while sea level increases it must either transgress inland with rising waters, or the marsh platform must accrete sediment and rise vertically to avoid being overtaken by rising water (Ward et al., 1998). A marsh would likely retain its properties and ability to provide services as it transgressed inland until it hit a barrier, be it natural or artificial, that prevented additional horizontal movement (Brinson et al., 1995). Any vertical increases in marsh platform elevation are driven by sediment accumulation and accretion. Inorganic sediment, derived from terrestrial sources, can accumulate in sufficient quantity to match rising sea level in marshes that are located in areas of sediment deposition (Ward et al., 1998). Other marshes do not have adequate sources of terrestrial alluvium and derive sediments from organic matter production. These marshes rely on organic matter accumulation, notably from belowground production by macrophytes (Blum, 1993) to rise in elevation. For a marsh that lacks a requisite source of inorganic sediment to persist and continue to adequately perform ecosystem services in the face of rising sea level, its plant community must maintain strong belowground production.

The synergistic impacts of eutrophication and global sea level rise on the elevation of temperate salt marshes are less clear. Temperate salt marsh environments dominated by *S. alterniflora* are mainly nitrogen-limited (Dai & Wiegert, 1997; Howarth, 2008), and experience demonstrable increases in aboveground biomass in response to nutrient additions (Darby & Turner, 2008b; Haines & Dunn, 1976). Fewer studies have focused on the effects of supplementary nitrogen, simulating coastal eutrophication, on

belowground growth in *S. alterniflora*; growth that influences organic matter accumulation and sediment accretion (Blum, 1993). Short-term studies have shown no significant differences in belowground production between fertilized and unfertilized marsh areas (Darby & Turner, 2008b) while longer-term studies have noted no increase in sediment accumulation and a reduction in carbon storage in fertilized areas compared to controls (Turner et al., 2009). Increased nitrogen availability, due to eutrophication, could lead to reduced or stagnant belowground production and sediment accumulation, which could hinder the ability of certain marshes to effectively respond to sea level rise.

Other studies have found that root production in *S. alterniflora*, the dominant species in low elevation temperate salt marsh communities, varies throughout the growing season (Blum, 1993; Darby & Turner, 2008a; Darby & Turner, 2008b) but none measured these growing season dynamics under increased levels of nitrogen. I examined the monthly root production of *S. alterniflora* and assessed the monthly root to shoot allocation under four levels of nitrogen addition to determine if any reductions in belowground biomass occur when nitrogen is in adequate supply. I added nitrogen in amounts of 30, 100, and 300 g N m^{-2} in total over six consecutive monthly fertilizations from March to August. These amounts were selected to be consistent with other fertilization studies involving *S. alterniflora* and to encompass the range of fertilization rates used in other studies (Table 1).

Table 1. Amount of nitrogen added, in g m^{-2}, to *S. alterniflora* in similar studies on salt marshes along the east coast of the United States.

Study	Location	N added (g m^{-2} growing $season^{-1}$)
Darby and Turner (2008)	Cocodrie, LA USA	4.6, 9.3, 18.6, 37.2, 74.4
Levine et al. (1998)	Barrington, RI USA	394.4
Emery et al. (2001)	Prudence Island, RI USA	386.4
Brewer et al. (2003)	Ocean Springs, MS USA	168
John Haywood (Personal Correspondence)	Nassawadox, VA USA	100

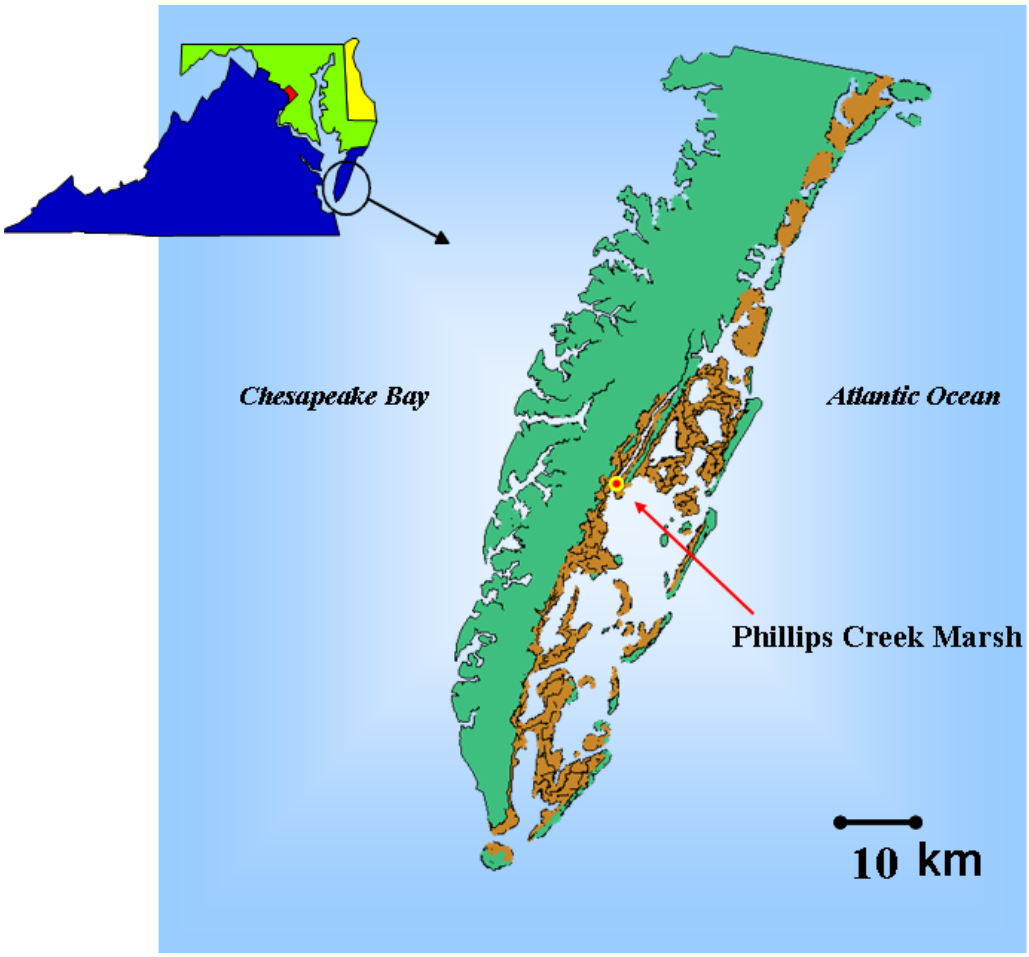
I chose Brownsville Marsh, on the Eastern Shore of Virginia (USA) as a study site because it is in an area of low human population density, it has no significant sources of terrestrial sediment, and currently it is not experiencing anthropogenic eutrophication of the coastal waters. My data allow better insight into the response of salt marsh ecosystems to eutrophication and their potential to respond to sea-level rise.

Materials and Methods

Study Site

This study was carried out in upper Phillips Creek marsh (PCM) on the Brownsville Plantation, located near the town of Nassawadox on the Eastern Shore of Virginia in the United States (Fig 1). This marsh is located within the Nassawadox, Virginia, 7.5 minute quadrangle at approximately latitude 37° 27’ 50” N and longitude 75° 50’ 04.99 W”. PCM is classified as a valley marsh and is typical of 67% of the marshes along the Virginia portion of the eastern the side of the Delmarva Peninsula (Oertel and Woo, 1994).

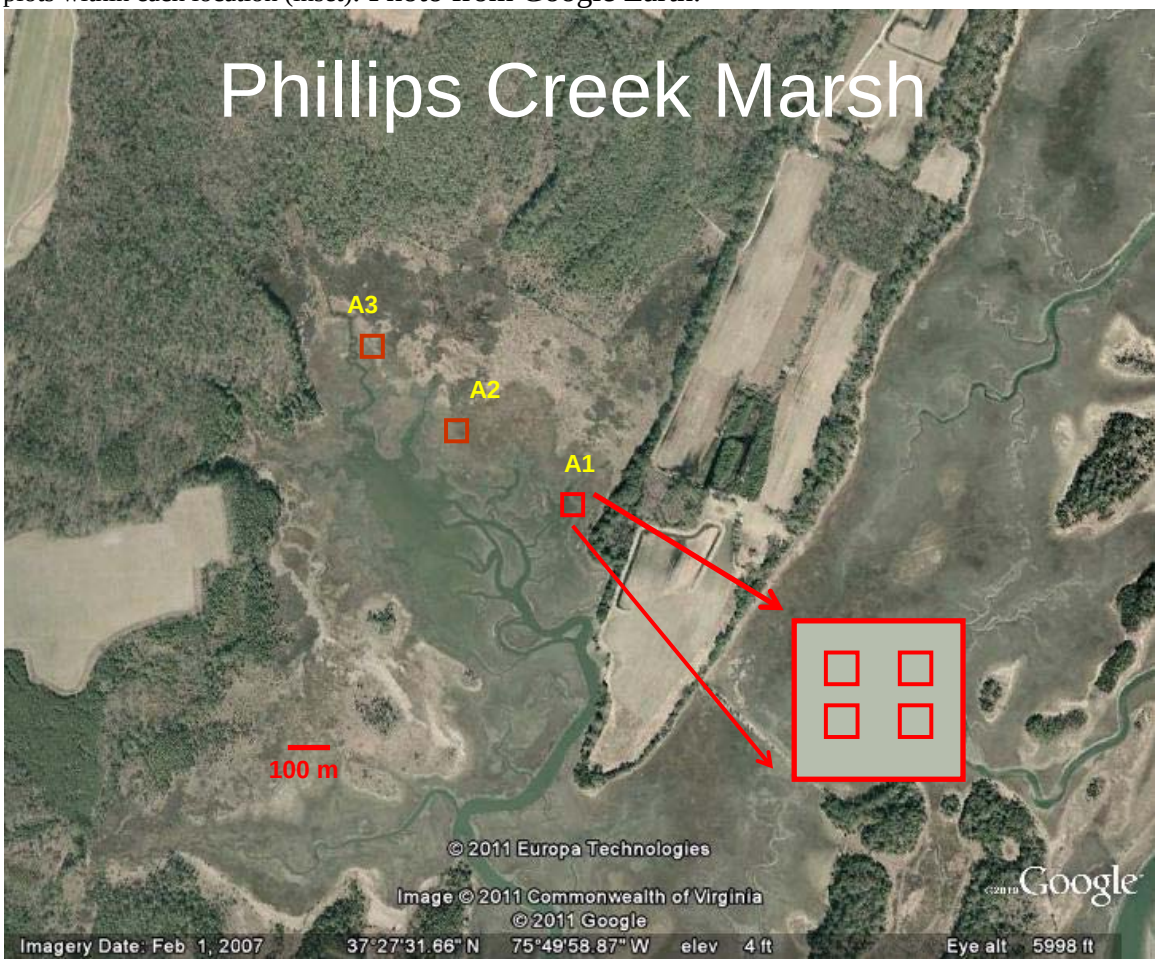
Figure 1. Upper Phillips Creek marsh is located on the Atlantic side of Eastern Shore of Virginia in the mid-Atlantic region of the USA.



Experimental Design

Three replicate locations, each containing four 3 x 3 m monoculture plots of *Spartina alterniflora*, were set up in distinct regions of PCM (Fig. 2) to facilitate the use of a one-way ANOVA for comparing the effects of treatments. The plots were established in March, 2010. A stratified random approach was used to assign treatments; within each replicate, the four plots received one of four randomly assigned levels of nitrogen addition. Plots were enriched with either a total of 0 g m^{-2}, 30 g m^{-2}, 100 g m^{-2}, or 300 g m^{-2} of N as urea ($CO(NH_2)_2$) divided evenly among 6 monthly additions from March to August 2010. The urea (Harvest Brand Fertilizer® The Valley Fertilizer Chemical Company, Inc. Mt. Jackson, VA), in amounts appropriate for each treatment, was dissolved in seawater from the nearby creeks and applied to the surface of each plot during low tide to allow infiltration of the fertilizer into the soil. An equal volume of unamended seawater was applied to the plots that were designated to receive 0 g m^{-2} during each of the fertilizations. Fertilizer was applied, in amounts consistent with individual treatments, to an area extending 0.5 m outside the perimeter of each plot to minimize edge effects, resulting in a total fertilized area of 16 m^2 per plot. Monthly fertilizations were intended to reproduce amounts of nitrogen added in other studies involving *S. alterniflora*. All of the plots were generally inundated by tides only during monthly spring tides (personal observation) which is consistent with plot elevations established during summer 2010 (Table 2 – see results section).

Figure 2. Location of replicates in Upper Phillips Creek marsh (designated A1, A2, and A3) and lay out of plots within each location (inset). Photo from Google Earth.



Soil Characterization

Two cores were removed from each plot in March, 2010 for soil characterization. The cores, 8.89 cm (diam) and 20 cm (depth), were extruded, cut into 10-cm sections in the field, and frozen for later analysis. Prior to laboratory analysis, the 10-cm sections were thawed and cut into 5-cm sections. These 5-cm sections were weighed, dried to a constant mass at 60 ° C, and weighed again to determine soil moisture content. The dry weight of each core was used to calculate bulk density. Additionally, approximately 5 g of dry soil was taken from each of the 5-cm sections, ground in a Wiley® Mini-Mill (Model 3379L10, Thomas Scientific, Swedesboro, NJ) with a size 40 mesh screen to

produce a homogenous sample and a known mass placed in a 450º C furnace for 12 hours. Samples were removed from the furnace, cooled in a desiccator, and re-weighed. Soil organic matter content was expressed as percent of dry mass.

To perform textural analysis on the samples, five-cm sections were recombined, to form the original 10-cm section of each core taken from the field initially, and crushed. Recombination of the core sections was necessary as there was insufficient soil remaining after determining organic matter content to perform a textural analysis on the 5-cm sections. Samples of the soil from these 10-cm sections were combusted at 450º C for 12 hours and 4 g of combusted soil was used for soil texture analysis. The relative percentages of sand, silt, and clay were determined using the hydrometer method of Liu and Evett (1984).

The samples were combusted before textural analysis because each core contained so much organic matter, mainly plant roots and rhizomes, that it was impossible to read the hydrometer even after the soils were treated with Chlorox to remove organic materials as recommended by Liu and Evett (1984). The plant matter floated to the surface of the liquid in the hydrometer jar and made readings impossible. Other research has shown that heating soils to 500º C increased sand content while decreasing clay content (Terefe et al., 2008), though it is not clear if combusting the soil samples affected the values for texture found in this study. These data may not be useful for comparisons of texture to studies that used other methods but should still provide a valuable relative comparison of soil texture across plots and replicates for this research.

Belowground Productivity by Root In-growth Cores

Eight root in-growth bags were placed within each plot; located at least 0.5 m away from the boundary of the plot to avoid edge effects. The bags were made of 30.5 x 63.5 cm squares of Nitex (Memphis Net and Twine Co, Memphis, TN) with 1 x 2 mm mesh apertures that were sewn into cylinders. The bags were knotted at the bottom and inserted into cored holes that were 8.89 cm (diam.) by 20 cm (deep). Each bag was filled with an equal volume of sand, and that was sufficient to fill the core hole level with the soil surface. Previously, the sand was freed of roots and other visible pieces of organic matter and shelly debris. The source of the sand used to fill the bags was the Butler's Bluff formation that is exposed in a borrow pit near Oyster, VA. The sand has been classified as predominantly quartz sand with small amounts of plagioclase and potassic feldspars. The sediment grains from the pit were described to have ferric-oxyhydroxide coatings (DeFlaun et al. 1997). The root in-growth bags were left open at the top to allow plant growth from seeds or vegetative materials deposited on the soil surface. Approximately a meter or so of nylon string was tied to the top of each bag and to a nearby PVC stake to allow easy retrieval of the in-growth bags.

A bag was randomly selected and removed from each plot once every month from April until November and frozen for later analysis. The root in-growth bags were thawed and all plant root material within the mesh bags was removed by hand. For each bag, roots and rhizomes were gently washed free of sediment with de-ionized water, dried at 60 º C for 12 hrs, and weighed. Production was determined from collected belowground biomass using a method devised by Smalley (1959). In this method, the weight of dried

biomass collected from each month was subtracted from the weight of biomass collected in the previous month. All positive differences during the entire growing season, i.e. an increase in biomass in a month, were added together. All negative differences, corresponding to less collected biomass in one month compared to the previous, were ignored. This produced an estimation of production for the entire growing season. Dried root material from each plot and sampling date was sealed in a 20-mL scintillation vial for later analysis of carbon and nitrogen content.

Aboveground Biomass, Productivity, and Production by Clip Plots

Each month, from April to November, a 25 x 25 cm quadrat of *S. alterniflora* shoots was clipped from within each plot. Care was taken to place the quadrat in areas of the plot that were at least 0.5 m away from the edge as well as in areas that were not adjacent to any in-growth core. The aboveground biomass was bagged and frozen for later analysis.

Live aboveground biomass was separated from the dead. Only leaves that were fully senesced along the entire length were considered to be dead in accordance with VCR LTER end-of-year-biomass protocols (http://amazon.evsc.virginia.edu/cgi-bin/w3e/msql/data/query/datasets/show_data.html?QDATA_ID=VCR09159). The sorted plant material was dried at 60 º C for 12 hours and weighed. Production was determined from collected aboveground biomass using a method devised by Smalley (1959). Dried plant material from each plot and sampling date was sealed in a 20 mL scintillation vial for later analysis for carbon and nitrogen content. Live and dead materials were stored separately.

Porewater Collection and Analysis

Porewater was collected monthly using equilibrators placed in the center of each plot (Huang and Morris, 2003). The equilibrator consisted of a PVC pipe with four holes, sized to fit 20 mL scintillation vials, drilled into it at even intervals (Fig. 3). Vials were filled with de-ionized water, capped with a 0.2-µm Versapore® membrane (Gelman Sciences, Inc., Ann Arbor, MI) cut to fit inside the vial cap, and autoclaved to prevent microbial activity within the vial. The vials were inserted into the PVC pipe and installed into the ground, with their respective openings at depths of 4, 7, 11, and 15 cm below the marsh surface. These were allowed to sit under the marsh surface for 4 weeks to allow for full equilibration with the surrounding marsh (Bertolin et al., 1995).

The equilibrators were placed in the marsh beginning in March of 2010 and porewater samples were collected monthly from April to November. Each time the porewater was sampled, the old vials were replaced with ones that had been freshly filled, capped, and autoclaved. In the field, 5.0 mL of equilibrated water was removed from an equilibrator vial by pipette and placed into 5 mL of 10 mM zinc acetate ($Zn(O_2CCH_3)_2(H_2O)_2$) in order to preserve any sulfide in the sample (Otte and Morris, 1994). The remaining 15.0 mL of porewater from the vial was transferred to a clean 20 mL scintillation vial. All porewater samples were placed on ice for transport to the lab where they were frozen within several hours of being removed from the soil. Samples were kept frozen until analysis for total dissolved sulfides (hereafter referred to as HS^-), NH_4^+, and total dissolved phosphates (hereafter referred to as PO_4^{3-}).

Porewater sulfide concentrations were determined by colorimetric analysis (Otte and Morris, 1994). Porewater NH_4^+ and PO_4^{3-} concentrations were also determined with a colorimetric assay using VCR LTER protocols described in the water quality monitoring methods manual (http://www.vcrlter.virginia.edu/monitoring/h2oqual.htm).

Figure 3. Photograph of PVC equilibrator alongside capped vials. The vials were inserted into the holes in the PVC pipe, which was then placed in the ground to equilibrate for four weeks.

C:N Ratio Analysis

Plant tissue carbon and nitrogen content was determined using the dried and preserved above- and belowground plant materials for samples colleted in August and September. Live and dead leaves were handled separately. Live aboveground materials included stems and leaves, analyzed together. Dead aboveground material primarily

consisted of only leaves as clip-plot samples contained very few entirely dead plants. Roots and rhizomes were handled together; they were not segregated into live and dead fractions. All plant materials were ground to a fine powder using a size 40 mesh screen in a Wiley® Mini-Mill (Model 3379L10, Thomas Scientific, Swedesboro, NJ). Approximately 2-5 mg of dry, ground plant materials in duplicate was placed into 5 x 9 mm pressed tin capsules, the capsules were sealed, and the %C, %N by dry mass, and C:N ratio were determine on a Carlo-Erba C-H-N analyzer model NA2500 (Rodano, Milan, Italy).

Plot Elevations

Measures of elevation were taken from all four corners and the center of each plot using a Topcon® model RL-50A rotating-laser level system. These laser level readings were referenced to the Hayden benchmark (Virginia Coast Reserve benchmark HAYD, N 372732.021 W 754958.036).

Statistical Analysis

Data were analyzed in SSPS Statistics 19 (SPSS, Inc.). A general linear model procedure for repeated measures ANOVA based on Pillai's Trace test was used to assess responses to fertilization rate for below- and aboveground biomass which were measured monthly during the experiment. For all variables measured once during the experiment or for which a single value was calculated (below- and aboveground productivity, whole plant productivity, root-to-shoot ratio, C-to-N ratio, average NH_4^+, HS^-, PO_4^{3-}, plot elevation, and soil characteristics), a one-way ANOVA and post-hoc Tukey HSD were

used to determine differences among treatments. For all analyses, an a priori α-level of 0.05 was chosen to be significant.

Results

Site Characterization: Soil properties and elevations

Measured soil variables confirmed qualitative observations that soil properties differed among the three locations where the experimental plots were established (Table 2; Appendices A and B). Silt content, which ranged from 7% - 10% of the mineral material, was the only variable that was not significantly different among the locations. Other properties of the soil reflected the textural categories. For example, the sand soil at location A1 had the highest bulk density (0.17 g cm^{-3}) and lowest organic matter content (36%), while the sandy loam at location A2 contained the most organic matter (56%) and had the lowest bulk density (0.10 g cm^{-3}). At all locations, there was a clear depth effect (Appendices A and B); sand content and bulk density increased with depth, and organic matter content decreased. Because experimental treatments were assigned using a stratified random approach, no significant differences in soil properties were detected among plots receiving different fertilization levels.

Table 2. Soil characteristics of three locations where experimental plots were established. Differing letter superscripts indicate statistically significant differences among locations based on a one-way ANOVA when $\alpha = 0.05$ for n = 4.

	Location in Marsh		
Variable	A1 (mean ± SD)	A2 (mean ± SD)	A3 (mean ± SD)
Sand (%)	85 (1)[a]	70 (3)[b]	80 (3)[a]
Silt (%)	7 (1)	10 (1)	9 (3)
Clay (%)	8 (1)[a]	20 (2)[b]	12 (1)[c]
Texture	Sand	Sandy loam	Loamy sand
Organic matter (%)	36 (5)[a]	56 (1)[b]	49 (3)[c]
Bulk Density (g cm^{-3})	0.17 (0.02)[a]	0.10 (0.01)[b]	0.13 (0.02)[b]
Elevation (m above msl)	0.92 (0.018)[a]	1.04 (0.004)[b]	1.00 (0.004)[c]

Although elevation differed among the locations by as much as 12 cm (Table 2, compare A1 to A2) and differences of this magnitude can affect tidal flooding frequency at this marsh (Christian et al., 2000), these differences had no apparent effect on plant community composition (100% short-form *Spartina alterniflora*), plant biomass, or stem density and height in previous years (VCR LTER end-of-year-biomass data at *http://www.vcrlter.virginia.edu/cgi-bin/w3-msql2/data/query/datasets/show_data.html?QDATA_ID=VCR09159*).

Aboveground Biomass, Productivity, and Production

Measures of live aboveground biomass from clipped plots demonstrated that standing stock was significantly greater in plots receiving nitrogen additions versus the controls (Fig. 4). Biomass in the plots receiving any of the three levels of nitrogen did not differ significantly from one another, but all fertilized plots had significantly greater biomass than the controls from July to November (Fig. 4). Dead aboveground biomass from clipped plots did not vary significantly between treatments (Fig. 5).

Live aboveground biomass peaked in June for all treatments and remained stable for the remainder of the summer (Fig. 4). The dry weight of live material decreased into October and November as the plants began to senesce. Dead aboveground biomass remained constant throughout the entire growing season, even as the amount of live aboveground biomass diminished in October and November (Fig. 5). This might indicate that leaves were rapidly detached from stems after senescing and were exported from the plots.

Aboveground production, calculated using the Smalley method (Smalley 1959), was 47% greater in fertilized plots compared to the control plots. Fertilized plots had an average production of 622 g m^{-2} during the growing season, whereas control plot production was 424 g m^{-2} (Fig. 6). The plots that received N did not differ significantly from one another in aboveground production, regardless of the amount of N applied.

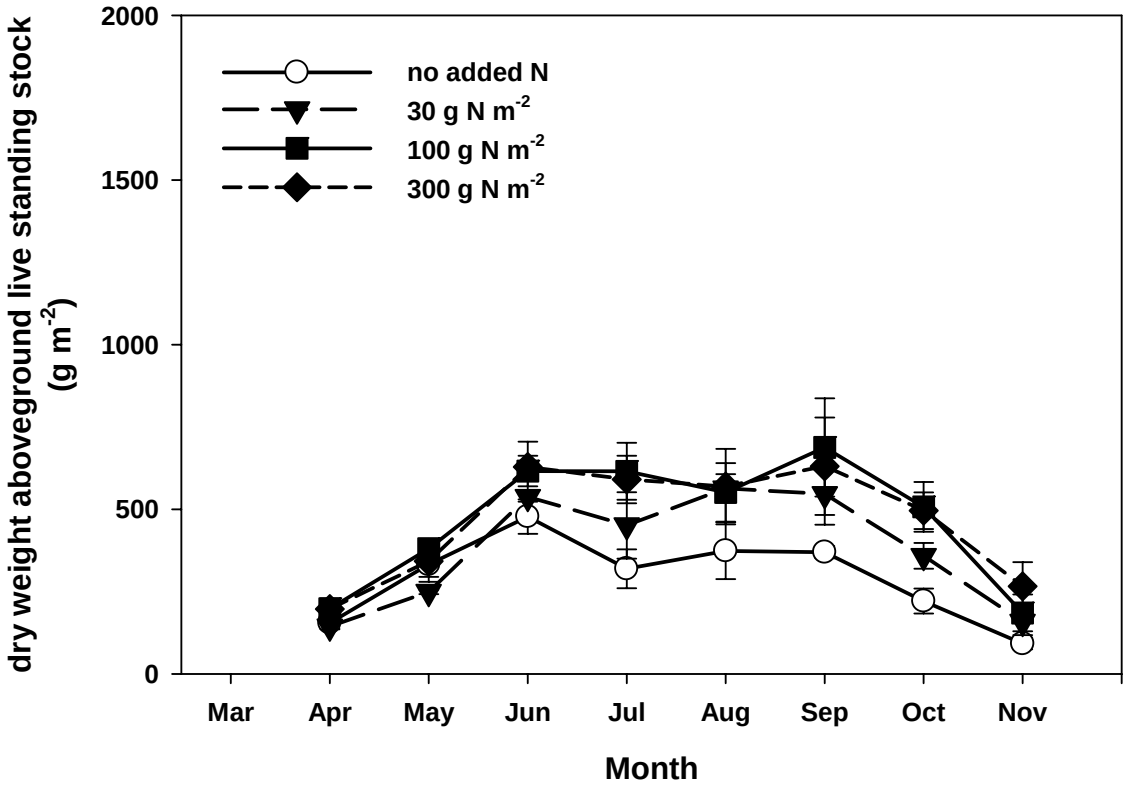


Figure 4. Aboveground live standing stock (g m^{-2}) sampled at monthly intervals in plots receiving four levels of fertilizer (0, 30, 100, 300 g N m^{-2} in six applications) for n = 3. All error bars are one standard error. No statistically significant differences were found among the plots receiving 30, 100, and 300 g N m^{-2} ($\alpha = 0.05$); however, all fertilized plots were significantly different from the plots receiving no fertilizer ($p=0.039$) based on a multivariate, repeated measures, general linear model analysis as estimated using Pillai's Trace using SPSS 19 (SPSS, Inc.). Temporal differences were significant ($p < 0.0001$) and a quadratic model described the data best.

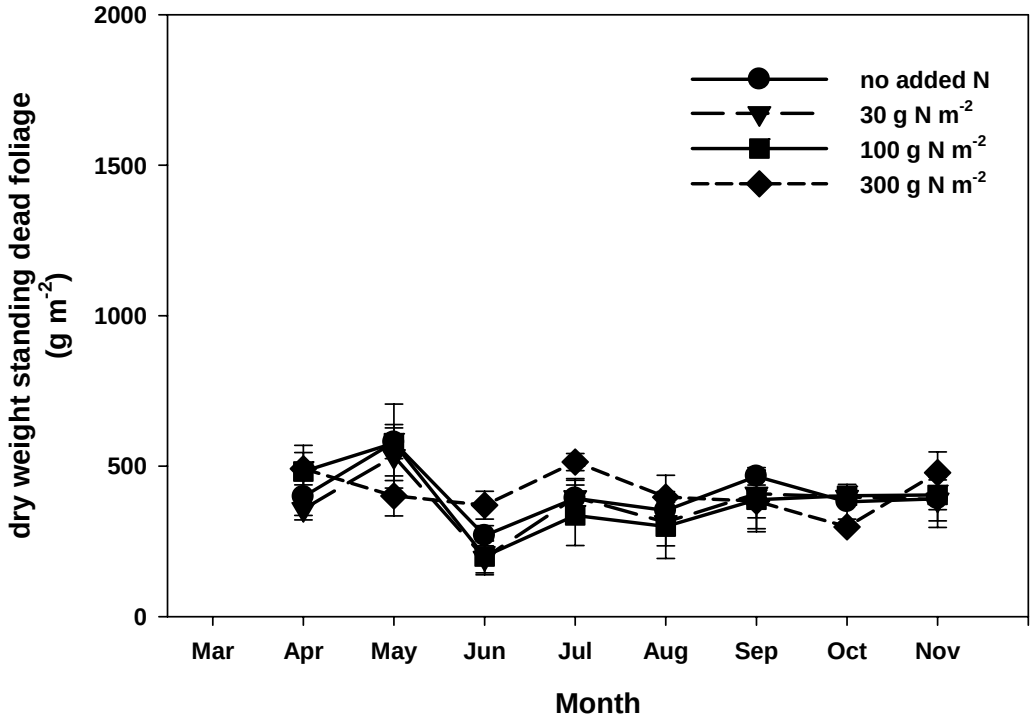


Figure 5. Standing dead foliage biomass core (g m^{-2}) sampled at monthly intervals in plots receiving four levels of fertilizer (0, 30, 100, 300 g N m^{-2} in six applications) for n = 3. All error bars are one standard error. No statistically significant differences were found among the fertilizer treatments at an $\alpha = 0.05$ based on a multivariate, repeated measures, general linear model analysis as estimated using Pillai's Trace using SPSS 19 (SPSS, Inc.). Temporal differences were not significant ($\alpha = 0.05$).

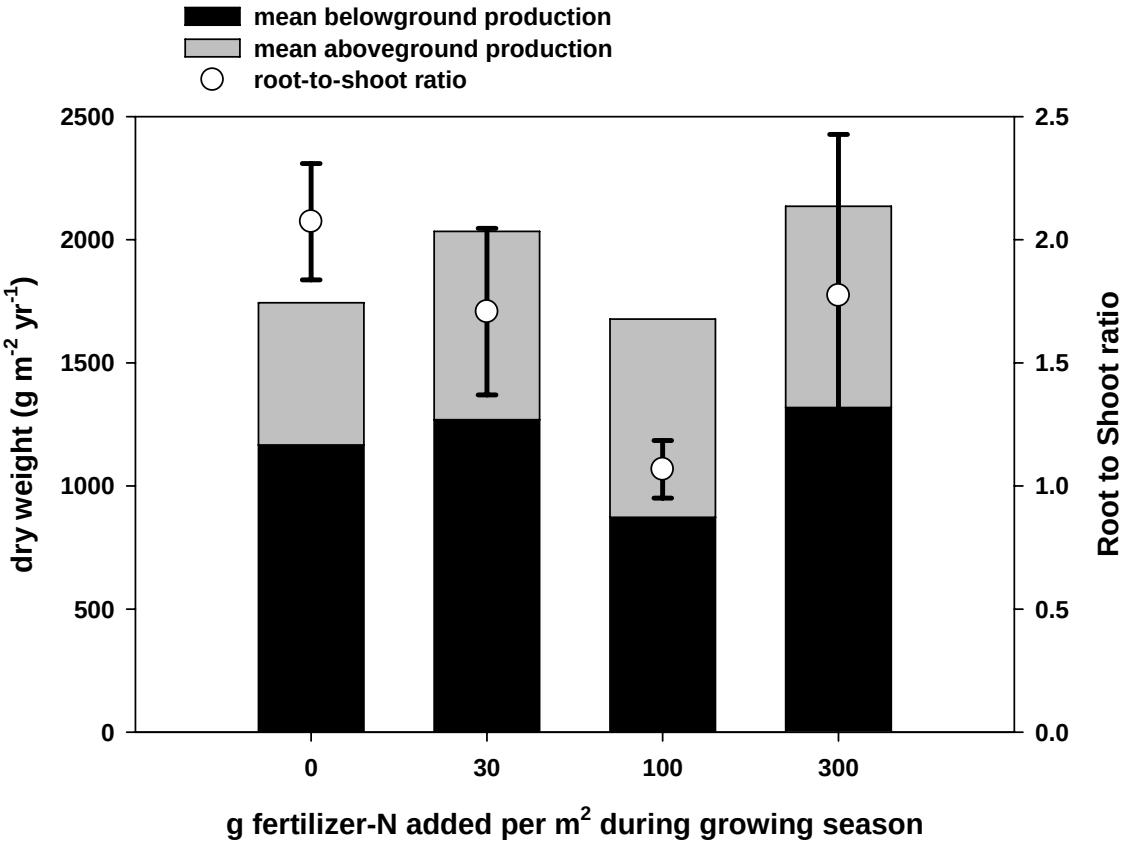


Figure 6. Aboveground (grey bars) and belowground (black bars) production (g m^{-2} yr^{-1}) and root-to-shoot ratios (circles) as a function of four fertilization levels (0, 30, 100, 300 g N m-2 in six applications) for n = 3. All error bars are one standard error. No statistically significant differences were found for the three variables shown at an $\alpha = 0.05$ based on a one-way ANOVA analysis using SPSS 19 (SPSS, Inc.).

Belowground Productivity and Production

Belowground productivity assessed by root in-growth cores did not differ significantly across treatments for any month of the growing season (Fig. 7). All treatments showed a similar significant trend of increasing biomass as the growing season continued. This was to be expected as root in-growth core measures reflect root and rhizome growth into un-colonized space and are not an estimate of current total root biomass belowground.

No significant differences in belowground production were associated with N fertilization (Fig. 6) calculated using either a Smalley approach as used for aboveground materials or using a maximum-minus-minimum approach. Root production (Smalley approach) was greatest for the 300 g N m^{-2} treatment (1336g m^{-2} growing $season^{-1}$), but was lowest for the 100 g N m^{-2} treatment (883 g m^{-2} growing $season^{-1}$) and not in the unfertilized plots.

Production-based (as opposed to standing stock) root-to-shoot ratios varied from just over 1 to more than 2 across treatments (Fig. 6). There was no meaningful variation in root to shoot ratio with nitrogen addition. The large error bars for the root-to-shoot ratio values for the 300 g N m^{-2} treatment are due to one belowground measurement in October that was significantly greater than others (Fig. 7). Although one of the three October replicates had an extremely high value, it was not considered to be an outlier because it did not meet the condition set a priori of exceeding 2 σ of the mean for the 300 g N m^{-2} treatments in October; nevertheless, this very high value still had a large skewing effect on the root-to-shoot ratio for the 300 g N m^{-2} treatment because justification to drop it from production calculations was lacking.

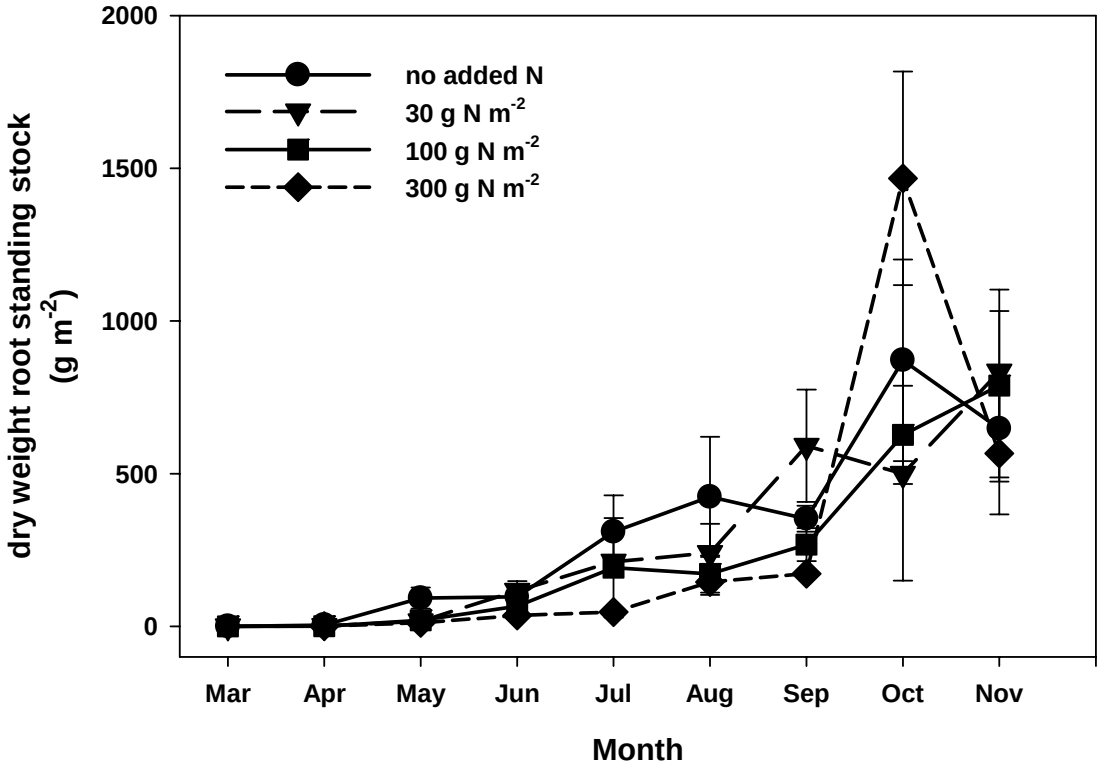


Figure 7. Belowground root growth into a sand-filled core (g m^{-2}) sampled at monthly intervals in plots receiving four levels of fertilizer (0, 30, 100, 300 g N m^{-2} in six applications) for n = 3. All error bars are one standard error. No statistically significant differences were found among the fertilizer treatments at an $\alpha = 0.05$ based on a multivariate, repeated measures, general linear model analysis as estimated using Pillai's Trace using SPSS 19 (SPSS, Inc.). Temporal differences were significant ($p < 0.0001$) and a linear model described the data best.

Porewater nutrients

Porewater concentrations of total dissolved PO_4^{3-}, depth-averaged for the months of April, September, October, and November, did not vary significantly across treatments(Fig 8). An increase in porewater available PO_4^{3-} was shown with depth for all treatments. Averages did not include some months as the marsh was unusually dry and insufficient porewater samples were obtained using equilibrators. Total rainfall during May, June, July, and August in 2010 was 30, 10, 21, and 77 mm, respectively. In a typical year, rainfall in these months is 41, 27, 71, and 53 mm, respectively. Precipitation measurements were obtained from the NOAA Climate Data Center (*http://cdo.ncdc.noaa.gov/ulcd/ULCD)* for the Accomack County Airport (MFV) Melfa, Virginia (latitude 37°39'N, longitude 75°46'W).

Porewater concentrations of NH_4^+, depth-averaged for the months of April, September, October, and November, were significantly greater in plots that received 300 g N m^{-2} (Fig. 8). Plots receiving 100 g N m^{-2} had significantly greater NH_4^+ concentrations only in the vials at 4 cm in depth, with concentrations at lower depths not varying significantly from the controls or the 30 g N m^{-2} treatment. The control plots and the 30 g N m^{-2} did not vary significantly in porewater NH_4^+ concentrations at any depth (Fig. 8). As for PO_4^{3-}, porewater data were not available for May, June, July, and August due to low rainfall during these months.

Porewater HS^- concentrations, depth-averaged for the months of April, September, October, and November, did not vary significantly across treatments. HS^- concentrations increased with depth for all samples (Fig. 8).

C:N Ratios

Although, belowground tissue of *S. alterniflora* showed a decrease in carbon-to-nitrogen ratios with increased added nitrogen, ratios for the 0 g N m^{-2} and 30 g N m^{-2} treatments did not differ significantly from one another and ratios for the 100 g N m^{-2} and 300 g N m^{-2} treatments were not significantly different from one another (Fig. 9). The control plots had an average C:N ratio of 51.5, while the plots with 300 g N m^{-2} added had an average C:N ratio of 18.2.

The C:N ratios for live aboveground tissues showed a similar decrease with increasing nitrogen addition (Fig. 9). The ratios for the 0 g N m^{-2}, 30 g N m^{-2}, and 100 g N m^{-2} treatments did not differ significantly from one another. Ratios for the 100 g N m^{-2} and 300 g N m^{-2} treatment did not differ significantly from one another as well. The 300 g N m^{-2} had the lowest C:N ratio.

The dead aboveground biomass also exhibited a decrease in C:N ratio with increasing nitrogen amendments (Fig. 9). However, the 0 g N m^{-2}, 30 g N m^{-2}, and 100 g N m^{-2} treatments did not differ significantly from one another and conversely the 30 g N m^{-2}, 100 g N m^{-2}, and 300 g N m^{-2} treatments were not significantly different. The dead matter collected from control plots had the highest C:N ratio while the plots treated with 300 g N m^{-2} had the lowest.

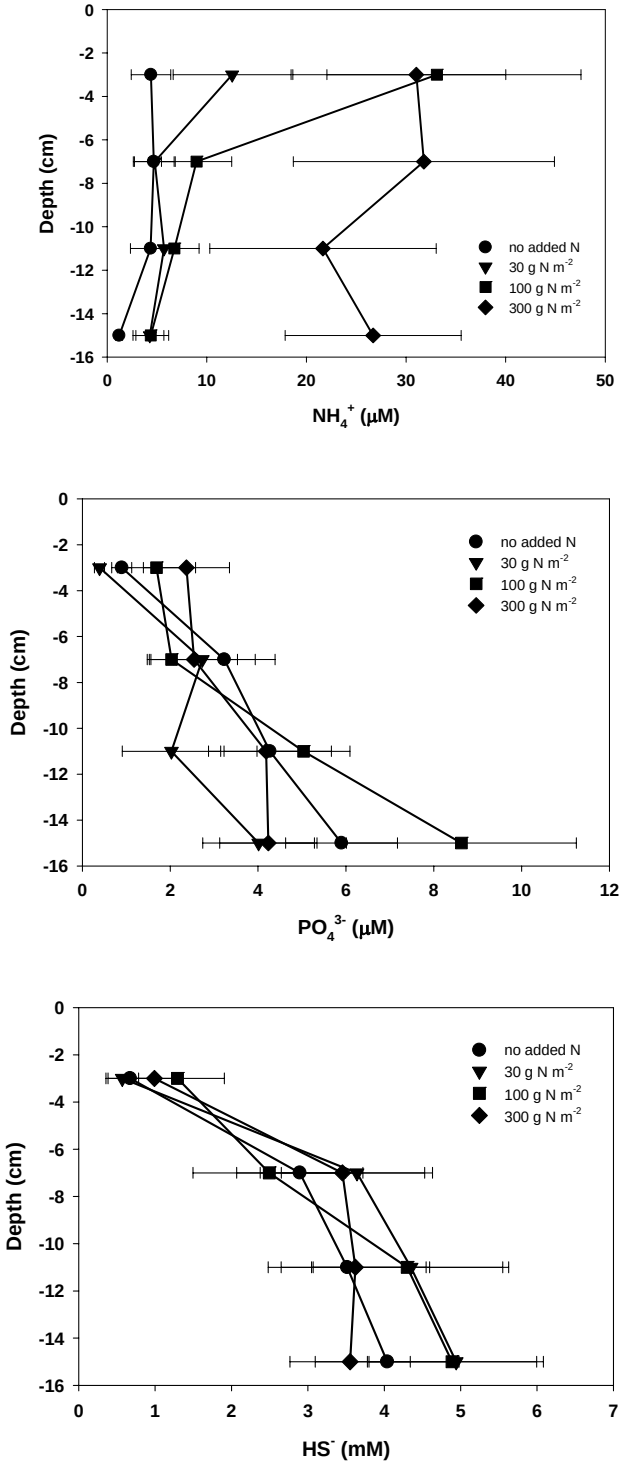


Figure 8. Time-averaged porewater concentrations of ammonium (top panel), phosphate (middle panel), and hydrogen sulfide (bottom panel). Note differences in concentration axis (abscissa) scales. Porewater concentrations of NH_4^+ in the plots fertilized with 300 g N m^{-2} were significantly higher than in plots receiving no added N and 30 gN m^{-2} ($p = 0.012$ and $p = 0.017$, respectively) based on depth- and time-averaged values analyzed by one-way ANOVA and Tukey's posthoc test in SPSS 19 (SPSS, Inc.). No significant differences were detected for depth- and time-averaged soluble PO_4^{3-} or HS^-.

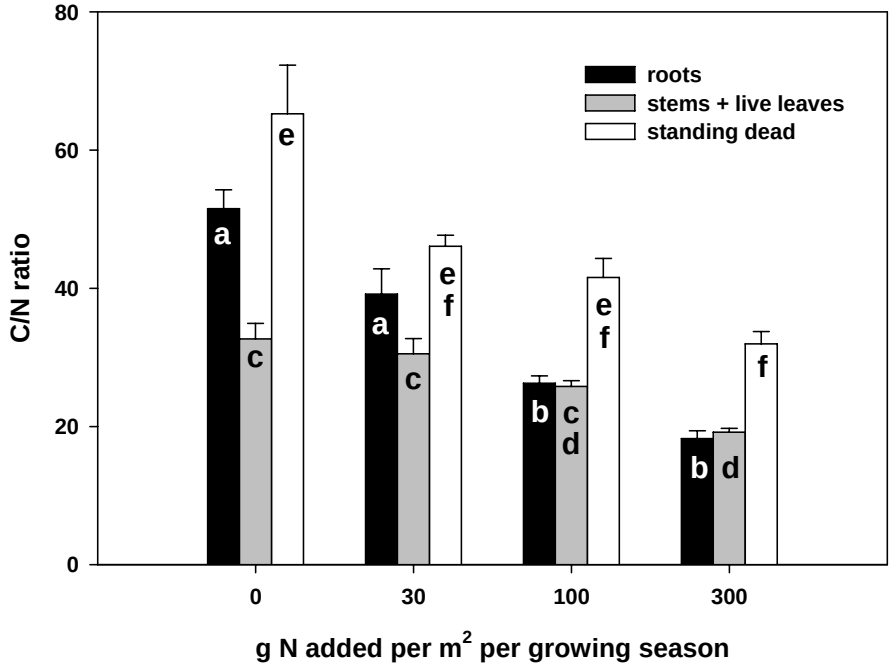


Figure 9. Carbon-to-nitrogen ratio of roots (black bars), live aboveground foliage and stems (grey bars), and standing dead foliage (white bars) as a function of four fertilization levels (0, 30, 100, 300 g N m^{-2}) for n = 3. Error bars are one standard error. Letters within similar colored bars indicate significant differences at $\alpha = 0.05$ based on a one-way ANOVA analysis and Tukey's posthoc test using SPSS 19 (SPSS, Inc.).

Discussion

These experiments provide no direct evidence that nitrogen fertilization alters *S. alterniflora* belowground productivity or production (Fig 7 and 6, respectively). Similar studies have shown no change in root in-growth production with added nitrogen (Valiela et al., 1976) while others have demonstrated no change in total belowground biomass following nitrogen enrichment (Darby & Turner, 2008b). Still others report an increase in belowground macroorganic matter (Buresh et al 1980, Gallagher 1975, Haines 1979) with the addition of fertilizer. Differences among studies, including the results I report here, likely stem from the use of various methods of data collection, i.e. coring versus root in-growth cores, as well as the use of different forms of nitrogen fertilizer (urea, sewage sludge, Milorganite®, or various ammonium salts) and varying application methods when fertilizing (pelletized, dissolved, slow-release, buried or surface application, etc.). For my experiment, the focus was to ascertain if there was a dose response in root productivity and production to different levels of additional nitrogen, the nutrient considered to be most limiting at most east coast US salt marshes (Dai & Wiegert, 1997; R. W. Howarth, 2008). Nitrogen was applied across a broad range of concentrations to determine if below- and aboveground plant growth responds differently to N-fertilization at differing application rates. For reference, the average cornfield in Virginia receives about 3 to 8 g N m^{-2} annually (http://pubs.ext.vt.edu/424/424-027/424-027.html), 4-10x less than the lowest concentration that was added in this experiment. Even with this large amount of added nitrogen, and under the controlled conditions of my experimental design, there were no detectable differences in belowground productivity or production among fertilized and control plots (Fig. 7 and 6, respectively).

Environmental factors other than N have been shown to diminish root production. These factors include low redox potential (Eh) (Pezeshki, 1997) and high salinity conditions combined with drought (Brown et al., 2006). HS^- is directly toxic to plant roots and can hinder nitrogen uptake at concentrations as low as 2.0 mM (Bradley & Morris, 1990). On average, the HS^- concentrations in this study actually exceeded 2.0 mM at depths greater than 4 cm (Fig. 8), and could have potentially had a deleterious effect on nitrogen uptake. While high HS^- concentrations have been shown to limit nitrogen uptake in the laboratory, HS^- concentrations greater than 2.0 mM are regularly found in natural marsh sites with minimal impacts on macrophyte survival (Bradley & Morris, 1990). In this study, root growth was notably concentrated in the shallower depths of the in-growth cores, depths that have lower average HS^- concentrations (Fig. 8). Decreasing C:N ratios with increasing levels of fertilization (Fig. 9) clearly demonstrated that nitrogen uptake was not differentially inhibited by porewater HS^- concentrations among the treatments. *S. alterniflora* was able to take in greater amounts of nitrogen as availability increased. There was no meaningful variation in porewater HS^- concentrations with differences in treatment (Fig. 8), showing that additional nitrogen did not directly affect porewater HS^- concentrations.

Root production has also been shown to be altered by differences in available phosphorus. Fertilized plots of *S. alterniflora* that received nitrogen and phosphorus have exhibited decreased belowground growth (Darby & Turner, 2008b). My porewater data indicate that available phosphate did not vary significantly between treatments (Fig. 8). Porewater phosphate concentration was constant with increasing nitrogen addition, indicating that nitrogen amendments did not produce a phosphorus limitation as would be

indicated by a drawdown of porewater phosphate. Thus, it is unlikely that fertilized plots differentially increased allocation to belowground tissues in order to acquire phosphorus and masked the effect of nitrogen treatments.

Additional evidence that nitrogen, not phosphate or sulfide, limits plant growth in Upper Phillips Creek (UPC) marsh comes from the clear increase in aboveground biomass in response to N additions (Fig. 4). All fertilized treatments had significantly greater live aboveground biomass than the control plots that received no additional nitrogen (Fig. 4). There was no dose response to varying levels of nitrogen addition; live biomass measures for the 30, 100, and 300 g N m^{-2} treatments did not differ significantly from one another in any of the months of the study. This finding demonstrates that adding 30 g N m^{-2}, and possibly less than that, is adequate to relieve the stress of nitrogen limitation in salt marshes similar to UPC.

The magnitude of UPC *S. alterniflora* biomass response to N fertilization is similar to that observed in other studies summarized by Morris (1991) where he compared the relationship between control plot biomass and the increase in biomass as an indicator of nitrogen availability (Fig 10). Peak biomass in control plots at UPC during the summer was 477 g m^{-2} and averaged 375 g m^{-2} between May and September. Based on Morris's analysis, the biomass of fertilized *S. alterniflora* at UPC should increase by approximately 75%. Increases observed were 125%, 152%, and 147% for plots receiving 30, 100, and 300 g N m^{-2}, respectively, but are well within the variation associated with the inflection point of the prediction.

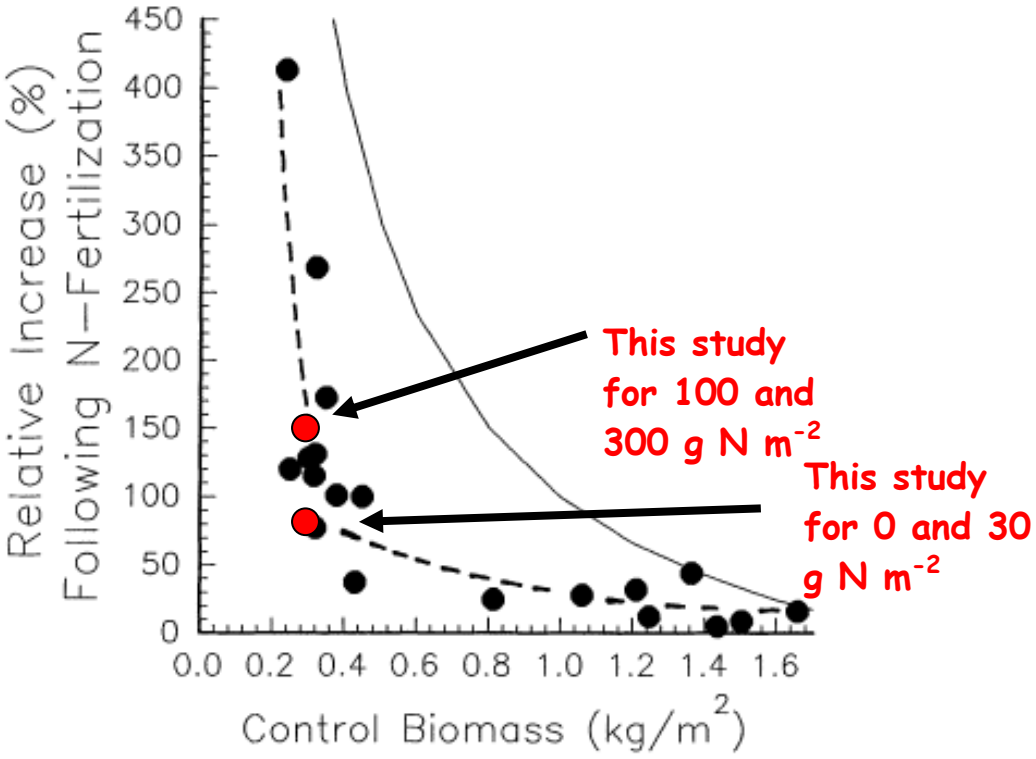


Figure 10. Relationship between control biomass and the relative increase in standing, dry biomass of the salt marsh grass *Spartina alterniflora* that was achieved after 1 to 3 years of N-fertilization. The original figure appeared in Morris (1991). Control biomass represents the maximum standing biomass on non-fertilized sites that was observed during the growing season. Also plotted is the relative response (solid line) that would be expected if control biomass from all sites were increased to a limit of 2 kg m^{-2}. The red dots show how my plots at Upper Phillips Creek marsh compare to the studies examined by Morris (1991). The response at Upper Phillips Creek is comparable to other similar studies. Studies include in Morris's analysis were from Massachuesttes, Delaware, South Carolina, Georgia, and Louisiana.

Values determined for production, calculated using the Smalley method (Smalley, 1959), did not differ significantly for any of the plots in this study. Despite significant differences in aboveground biomass for the fertilized plots versus the controls, there were no statistically significant differences in aboveground production for the growing season (Figure 6). This result is striking, but is most likely due to the lack of sensitivity of the Smalley method in estimating productivity (Morris and Haskins, 1990). The collected samples also showed substantial variation in biomass overall, which likely played a role

in the lack of significant differences in productivity and production. This reinforces the need for replication in highly variable salt marsh environments.

Measures of standing dead mass did not vary significantly with any of the treatments (Fig. 5). Standing dead mass stayed relatively constant throughout the growing season and was similar in the controls and the fertilized plots. There were some small changes in dead mass over time, but those changes were not found to be statistically significant. It would be expected that plots with significantly greater live biomass, i.e. the fertilized plots, would also have greater amounts of dead tissue, but this was not the case. Standing dead mass was a measure of the dry weight of fully senesced leaves removed from live shoots as well as fully dead shoots, presumably from the previous growing season. If dead plant material from the previous growing season made up a large portion of the standing dead samples, then newly dead leaves from the growing season under investigation would not be able to exert a significant influence on the overall dead mass despite the fact that leaves from fertilized plots were greater in size and biomass.

It is also important to note that grasshoppers, most likely *Orchelium fidicinium*, were observed grazing on some of the plots in this study during the end of June and beginning of July, and were centralized on fertilized plots. It has been shown that herbivorous insects favor marsh plants that have been fertilized (McFarlin et al., 2008). The insects are able to identify plants with higher shoot nitrogen contents and will preferably graze upon them. The impact of this nearly month-long grazing event on aboveground biomass and production is not clear; grazer density and type were not quantified, and the damage was apparent but not widespread. A study performed at the same site in 2004 reported a slight, but not significant, reduction in aboveground

production in *S. alterniflora* with heavy grazing (McGoff, 2004). As I did not determine the density of grazers, it is difficult to compare my results to those noted above. Although grazing may have reduced the biomass in plots where it occurred for this study causing production to be underestimated, it is likely that it did not strongly influence the overall results of this research. Other studies have found that grasshopper grazing decreases total biomass in a manner that is not statistically significant, and that grasshoppers do not exert a top-down control on *S. alterniflora* production, even when fertilizer is applied (Bertness et al. 2008).

Another consideration is the reported effects of grasshopper grazing on *S. alterniflora* biomass allocation to roots. It has been shown that heavy grazing yields greater belowground production in plants compared to those experiencing typical grazing in the same marsh as this study (McGoff, 2004). This finding is of interest, as the decreased belowground production response that I hypothesized would occur with greater nitrogen addition could have been offset by increased belowground biomass accumulation under grazing stress. Adding nitrogen could decrease belowground production while increasing aboveground growth, inviting selective and intense herbivory that would then stimulate increases in belowground production. The combination of those two factors could potentially account for the lack of differences in belowground production among treatments that I found (Fig. 6). Comparisons between the aforementioned study and this study should be made with caution, however, as grazing continued into August in the 2004 study, while it ceased in July for this project.

Carbon-to-nitrogen ratios measured for belowground tissues demonstrated a decrease with increasing nitrogen addition. Ratios in the plots that received 0 and 30 g N

m^{-2} were not significantly from one another, but values for those treatments did differ significantly from the 100 and 300 g N m^{-2} treatments (Fig. 9). The plots that received the highest levels of added nitrogen, 100 and 300 g N m^{-2}, had significantly more nitrogen incorporated into belowground tissues than the control and 30 g N m^{-2} treatments while maintaining similar amounts of belowground biomass production. This indicates luxury consumption and storage of excess nitrogen in belowground structures. Luxury uptake of nitrogen is likely an adaptation to environments, such as this salt marsh, that are generally nitrogen limited. The stands of *S. alterniflora* in the fertilized plots stored excess nitrogen in root structures and can be expected to utilize this nitrogen in the next growing season in shoot growth, increasing aboveground productivity and giving those stands a longer-term competitive advantage. Nitrogen concentrations in all tissues remained remarkably constant within plots and treatments for the months of August and September, indicating that there was no net movement of nitrogen between various plant structures during that time.

Aboveground structures, live and dead, demonstrated a decrease in carbon-to-nitrogen ratios with increasing levels of nitrogen addition, though the differences were less pronounced. C:N ratios in standing dead tissues decreased, but the significance of this decrease varied (Fig. 9). The live aboveground tissues also showed an overall decrease in C:N ratio with greater nitrogen amendments, but the difference between the ratios in the 0 g N m^{-2} plots and the 300 g N m^{-2}, though statistically significant (Fig. 9), is less extreme in the live tissues. The live shoots and leaves for the 0 and 30 g N m^{-2} treatments have C:N ratios that are nearly indistinguishable and neither of those ratios are significantly different from those of the 100 g N m^{-2} treatments. It is possible that this

result is due to the analysis techniques used in the laboratory. Samples of aboveground tissue that were saved were a mixture of stems and leaves and nitrogen content in stems may be different than the content of leaves. Thus, some of the samples may have contained different amounts of stems and leaves that were ground for later C:N analysis, which could impact the overall findings.

Another possible explanation for the relatively minor differences in C:N ratios of live aboveground tissues with differences in fertilization relates to herbivory. As mentioned earlier, grazers prefer plant tissues with higher nitrogen content (McFarlin et al., 2008). It is possible that, as available nitrogen increases, it is not advantageous for *S. alterniflora* to allocate significantly more nitrogen to shoots, as this can dramatically increase biomass loss to selective herbivory (Tripler et al., 2002). Once a certain amount of shoot nitrogen is reached, it may be ideal to retain excess nitrogen in belowground structures, safe from herbivores, to be used in subsequent growing seasons.

Although nitrogen addition did not produce any measurable changes in root production in this marsh in one growing season, added nitrogen has been shown to decrease marsh sediment accretion and elevation on decadal time scales (Turner et al., 2009). The results of my studied suggest that excess nitrogen does not impact the production of *S. alterniflora* roots in this marsh, but that the increased N-content of root and foliage materials could affect rates of organic matter decomposition once the roots and foliage die. Faster rates of organic matter decay would negatively influence the ability of a salt marsh platform to accumulate organic matter and increase in elevation, making marshes susceptible to deterioration as global sea level rises (Day et al., 2011).

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Appendices

Appendix A. Soil Texture across each replicate measured from each of two characterization cores taken from every plot in March, 2010. Texture was calculated in intervals of 10-cm in depth. Missing data are a result of laboratory errors- all samples processed incorrectly are not reported.

Replicate	N-added (gm^{-2})	Core No.	Depth (cm)	Sand (%)	Silt (%)	Clay (%)	Texture
1	30	1	10-20	90.3781	4.9738	4.6481	Sand
1	30	2	10-20	82.1233	8.185	9.6917	Loamy Sand
1	0	1	0-10	77.3822	10.3723	12.2455	Loamy Sand
1	0	2	0-10	79.8284	9.7295	10.4421	Loamy Sand
1	0	2	10-20	88.5544	5.9657	5.4799	Sand
1	100	1	0-10	83.63	8.7755	7.5945	Loamy Sand
1	100	1	10-20	87.5102	5.1658	7.324	Sand
1	100	2	10-20	89.4328	4.7673	5.7999	Sand
1	300	1	0-10	83.1123	5.6021	11.2856	Loamy Sand
1	300	1	10-20	87.897	6.8034	5.2996	Sand
1	300	2	10-20	88.2373	5.9279	5.8348	Sand
2	300	1	0-10	71.751	10.1018	18.1472	Sandy Loam
2	300	1	10-20	65.7272	13.1239	21.1489	Sandy Clay Loam
2	300	2	0-10	68.7522	12.3328	18.9151	Sandy Loam
2	300	2	10-20	68.9151	7.2106	23.8743	Sandy Clay Loam
2	100	1	0-10	73.4468	10.5265	16.0268	Sandy Loam
2	100	1	10-20	75.7097	7.7429	16.5474	Sandy Loam
2	100	2	0-10	69.5521	10.2647	20.1832	Sandy Loam
2	0	1	0-10	65.6137	12.2688	22.1175	Sandy Loam
2	0	1	10-20	68.4148	10.0291	21.5561	Sandy Loam
2	0	2	0-10	64.9389	11.7161	23.345	Sandy Loam
2	0	2	10-20	73.0919	7.0797	19.8284	Sandy Loam
2	30	1	0-10	65.0349	7.1611	27.804	Sandy clay Loam
2	30	1	10-20	66.8354	12.929	20.2356	Sandy Clay Loam
2	30	2	10-20	71.2653	8.0192	20.7155	Sandy Clay Loam
3	0	1	0-10	64.8197	27.7458	7.4346	Sandy Loam
3	0	1	10-20	89.0111	4.9651	6.0239	Sand
3	0	2	0-10	66.5532	12.2222	21.2245	Sandy Clay Loam
3	0	2	10-20	83.3682	8.1268	8.5049	Loamy Sand
3	100	1	0-10	78.5428	9.3601	12.0971	Loamy Sand
3	100	1	10-20	87.0361	6.6405	6.3234	Sand
3	100	2	0-10	74.0343	8.0628	17.9029	Sandy Loam
3	100	2	10-20	90.9744	2.4578	6.5678	Sand
3	30	1	0-10	76.5154	7.6963	15.7882	Sandy Loam
3	30	1	10-20	82.3008	7.1175	10.5817	Loamy Sand
3	30	2	0-10	75.1571	9.7964	15.0465	Sandy Loam
3	30	2	10-20	83.3682	5.8697	10.7621	Loamy Sand
3	300	1	0-10	75.5236	7.0593	17.4171	Sandy Loam
3	300	2	0-10	78.1094	9.171	12.7196	Sandy Loam
3	300	2	10-20	85.5788	6.4543	7.9668	Loamy Sand

Appendix B. Soil Organic Matter Content (%) shown for each of two soil characterization cores sampled from every plot in March, 2010. Organic matter content was determined for 10-cm depth intervals.

		Soil Organic Matter Content (%)		
Area	N-added (gm^{-2})	Depth (cm)	Core 1	Core 2
1	30	0-10	28.02	29.27
		10-20	28.16	35.8
1	0	0-10	46.7	45.19
		10-20	33.17	30.91
1	100	0-10	49.82	50.06
		10-20	30.48	33.49
1	300	0-10	45.07	31.82
		10-20	35.56	19.29
2	300	0-10	56.77	56.87
		10-20	56.86	52.04
2	100	0-10	62.52	63.26
		10-20	54.07	48.56
2	0	0-10	61.24	60.35
		10-20	49.93	52.61
2	30	0-10	54.33	56.87
		10-20	61.21	55.28
3	0	0-10	64.85	61.63
		10-20	36.26	37.68
3	100	0-10	53.33	59.88
		10-20	39.36	37.87
3	30	0-10	62.27	59.73
		10-20	41.09	43.04
3	300	0-10	56.77	55.79
		10-20	37.32	32.5