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Short-term Effects of Nutrients on a Barrier Island Grassland Community

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Short-term Effects of Nutrients on a Barrier Island Grassland Community

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science at Virginia Commonwealth University

By

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B. A. Virginia Commonwealth University 2013

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Abstract

SHORT-TERM EFFECTS OF NUTRIENTS ON A BARRIER ISLAND GRASSLAND COMMUNITY

By Ashley Moulton, Bachelor of Science

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Biology at Virginia Commonwealth University

Virginia Commonwealth University, 2017

Director: Julie Zinnert, PhD, Department of Biology

Increased nutrient availability globally has the potential to affect community functional composition of plants in nutrient limited environments, such as coastal grassland systems. Stability of these systems are threatened worldwide by urbanization, as well as effects of sea level rise and increased frequency and intensity of storms, and atmospheric N deposition, associated with climate change. Annual net primary productivity (ANPP), species composition, and functional traits (community weighted specific leaf area (CWSLA), leaf area index (LAI), growth form and photosynthetic pathway) were measured across four treatments to assess multiple resource limitation of nitrogen (N) and phosphorus (P) and functional community response in a coastal grassland on Hog Island, VA within the Virginia Coast Reserve, Long Term Ecological Research Network (LTER) applied at a rate of 10 g m⁻² yr⁻¹ Nutrient enrichment did not alter species diversity or richness. ANPP was highest in plots receiving any type of nitrogen enrichment, and was higher than expected of low nutrient systems. CWSLA was significantly higher in NP plots, and was lower than other grasslands. P treatments
were not significantly different from controls. Graminoid species, specifically C₄ species responded with higher ANPP than C₃ forbs or graminoids within treatments. Evidence of synergistic NP effects were seen on community level resource allocation and leaf construction, but no significant species changes occurred over a 1-year time span. These results have expanded the knowledge of functional response to increased nutrient availability in an understudied, coastal grassland, which are at high risk to being lost to sea level rise and anthropogenic development and inform community assembly processes in stressful environments.
Introduction

Atmospheric inputs of nitrogen (N) from anthropogenic activity coupled with increasing development through urbanization has dramatically increased nutrient availability in terrestrial systems (Vitousek et al. 1997). Anthropogenic induced alterations of the N cycle have implications to plant communities which globally are found to be N limited across terrestrial systems (LeBauer and Treseder 2008). Additions of nitrogen in to ecosystems, a primary element of net primary production, alters plant growth at the individual level that scale up to affect community functional composition. Broadly speaking, biological responses to nutrient additions result in increased productivity of dominant species and lower diversity through negative effects of competitive exclusion (Tilman 1993; Day et al. 2007; Isbell et al. 2013; Avolio et al. 2014). Increased availability of nitrogen switches the limitation from belowground competition for nutrients to aboveground competition for light, altering community composition favoring faster growing species (Tilman 1987). Alternatively, increases in species richness have been seen in terrestrial grassland systems following the loss of a dominant species following nutrient enrichment (Dias et al. 2014).

Recently, scientists have recognized that plant community responses to resource additions may be mediated by other mechanisms including plant functional traits (McGill et al. 2006; Suding et al. 2008). Plant functional traits are specific measurable strategies that underlie the success of plant species in various environments (McGill et al. 2006; La Pierre and Smith 2015). Functional community response is influenced by the abundance and types of functional traits that are dominant in a community (Laliberte et al. 2012) Functional traits can be scaled up to important ecosystem services (i.e.
pollination, primary productivity, carbon sequestration, etc.). Understanding functional trait relationships is becoming more important for assisting land managers and informing policy decisions (Lavorel 2013).

Many functional traits characterize plant responses to changes in the environment including increased nutrient availability. In a long-term nutrient study with nitrogen and phosphorus treatments in a tallgrass prairie, functional traits such as changes in type of photosynthetic pathway and growth strategy were associated with resulting community composition changes from nutrient enrichment rather than changes in species diversity within plots (Avolio et al. 2014). Increases in both nitrogen and phosphorus lead to decreased abundance of C_4 photosynthetic grasses and increased abundance of C_3 annual and perennial forbs and grass species (Avolio et al. 2014, La Pierre and Smith 2015). Initially plant communities consisted of primarily C_4 grass species, and after enrichment, more forbs and C_3 grasses dominated (La Pierre and Smith 2015).

Specific leaf area (SLA) is a standard leaf functional trait associated with faster growth rates inferring greater allocation of resources to more photosynthetic area; SLA is calculated as leaf area/ leaf dry mass (Luo et al. 2014). SLA is a fundamental functional trait that can be used to better understand how a plant functions in a community given that it is positively correlated with relative growth rate, photosynthetic rate, leaf nitrogen, and negatively correlates with leaf life span (Westoby et al. 2002). In general, plants with low SLA have lower growth rates but longer leaf life spans, where plants with high SLA have faster growth rates and shorter leaf life spans (Dwyler et al. 2014). In a previous studies, plants with higher SLA values dominated enriched plots in
comparison to the control sites that did not receive any nutrient additions (La Pierre and Smith 2015; Te Beest et al. 2015). Leaf Area Index (LAI) is another plant functional trait, related to SLA, and is measured as the leaf area per unit ground surface area (Niinemets 2010). LAI is an index that measures light availability that is able to intercept a canopy, and is used to determine to what degree shading occurs from neighboring plants (Keilliher et al. 1995). Together, high values of LAI and SLA are related to high transpiration rates, which convert soil moisture into annual net primary productivity (ANPP) (Keilliher et al. 1995; Niinemets 2010).

Coastal barrier island systems are highly nutrient limited due to a combination of edaphic and physical processes that result in leaching and removing nutrients from the system (Van Der Valk 1947; Art et al. 1974; Dilustro and Day 1997). Barrier island soils consist of highly weathered sand, accumulated by small scale sand deposition through aeolian and fluvial processes (Ehrenfeld 1990). Meteorological input in the form of sea spray supplies a minimal amount of potassium, calcium, and sulfur in most coastal barrier island systems (Art et al. 1974). Nitrogen, an essential component of photosynthesis, is considered one of the most limiting nutrients on barrier islands as nitrates and ammonium are easily leached through the root zone (Ehrenfeld 1990). Phosphorus in the form of phosphates, helps plant root development, and also has low retention in coastal systems due to increased mobility on during erosion events (Lambers et al. 2008; Chakraborty et al. 2012).

In coastal systems, studies of nutrient availability have been limited and do not include changes in the composition of functional traits (Dougherty et al. 1991; Lammerts et al. 1999; Day et al. 2004). Increased nitrogen alters plant composition with increased
productivity of the dominant species. The Virginia barrier islands present a unique opportunity to study the effects of changes in community and functional composition due to nutrient enrichment. These islands are low in nutrients and experience high precipitation rates, and low water holding capacity of sandy grasslands prevents long residence times of both water and nutrients (Day et al. 2004; Brantley and Young 2010). The Virginia islands have also recently experienced an increase in the nitrogen-fixing shrub, *Morella cerifera* (Zinnert et al. 2016), resulting in a substantial input of N into an otherwise N-limited system (Brantley and Young 2010). The increased availability of nitrogen through natural or anthropogenic alteration is an increasing threat to biodiversity, especially within nutrient limited environments (Isbell et al. 2013; Dias et al. 2014). Ecosystem functioning may be altered through nutrient limitation, altering vegetation composition, successional trajectories, and potentially altering overall stability of barrier islands (Ehrenfeld 1990; Zinnert et al. 2017).

The objective of my project was to determine how barrier island grasslands respond to increased nutrient enrichment of N and P. I expect to see increased annual primary productivity of aboveground biomass in response to N treatments. Experimentally adding nutrients gives insight into the degree and type of limitation found in nutrient limiting environments and the effects on species interactions, species diversity, productivity, and functional composition (Day et al. 2004; Isbell et al. 2013). I hypothesized that increased nutrients will shift plant species composition and diversity, and plants in enriched plots will exhibit high SLA. C₃ photosynthetic pathways and broad-leaved forbs will dominate enriched plots and displace species. I expect nitrogen to have a stronger effect relative to phosphorus. By quantifying functional trait
composition and ANPP response to nutrient enrichment in a predominantly nutrient limited coastal swale community, I will have a more robust understanding of patterns that dictate plant community composition in coastal systems.

**Materials and Methods**

*Study Site:*

This study area is located on the southern end of Hog Island, Northampton Co., VA which is one of the 14 Virginia barrier islands off of the Delmarva Peninsula that make up the Virginia Coastal Reserve (VCR), owned and managed by the Nature Conservancy (VCR) (Figure 1). Hog Island is also part of the Long Term Ecological Research (LTER) network that is funded by the National Science Foundation. Hog Island (37.417 N, 75.686 W) is approximately 8 km from the Eastern Shore peninsula of Virginia, USA, and is approximately 1170 ha, 10 km long and 2 km across at the widest point (Semones and Young 1995). Island soils have been dated over 200 years old in the northern end (Hayden et al. 1991). The southern end contains much younger soils typical of many barrier islands, due to previous disturbances from overwash and storms.

*Experimental Design*

To determine the role of nutrient alteration in the plant community within a coastal swale, a 1000 m² plot was established in a relatively uniform and homogeneous grassland between the fore dune of the beach and the salt marsh/primary dune. Samples sites include 20 experimental plots that followed a modified experimental
design from the Nutrient Network Experimental Protocol (http://www.nutnet.umn.edu/exp_protocol).

To control for potential environmental heterogeneity, a completely randomized block design was utilized with five blocks (R1-R5, Figure 1). There were four treatments per block: control (C), nitrogen (N), phosphorus (P), and nitrogen and phosphorus (NP) (n=5) (Figure 2). The grassland plot was divided into 20 3 x 3 m plots (referred to as A, B, C, D) and randomly assigned within each block. Each block was in a grid formation separated by a 1-m walkway. Corners of the experiment were designated with permanent PVC stakes and colored flags. The plot marking and designation occurred in March 2015. Each of the 3 X 3 m plots were subdivided again into 1 x 1 m subplots (referred to as 1, 2, 3, 4). Three of the 1 x 1 m subplots were designated for annual sampling and one of the four was for destructive sampling at the end of the growing season.

**Nutrient Applications**

Nutrient applications occurred in May of 2015 at the onset of the growing season. A second application occurred in late June of 2015. Multiple resource limitation was assessed in three nutrient addition treatments (N, P, and NP) compared to control plots, for a total of 8 treatment combinations. N, P, and NP were all applied in two applications, split by 30 days apart, at a rate of 10 g m\(^{-2}\) yr\(^{-1}\). N was added in the form of slow release urea (46-0-0) and P in the form of triple phosphate (0-45-0).

**Measurements in the Field**
**Sampling Methodology**

One of these permanent 1 m² subplots was designated for destructive sampling and the others were used for annual sampling. Sampling included ANPP (sorted by forbs and graminoids), percent cover of all plant species, and leaf area measurements.

**Species Composition**

In order to determine the relationship between plant community composition and response to treatment, species cover was measured. At the end of the growing season, percent cover was estimated in one permanently marked 1 m² subplots within the sampling subplot. Percent living cover was estimated for each plant species separately using a modified Daubenmire method (Daubenmire 1959), in which cover was estimated to the nearest 1% for each species rooted within the plot. Percent cover was also estimated for three cover types, living, dead/dormant vegetation, and bare sand present on each plot.

**Functional Traits: Leaf Area Index (LAI), Functional Composition and Specific Leaf Area (SLA)**

Leaf Area Index (LAI) was calculated in September of 2015 in constant sunlight using a plant canopy analyzer (Model LAI-2000, LI-COR Lincoln, Nebraska, USA). LAI was measured at the end of growing season in the same 1 m² subplot used for the species composition measurements on a cloudless day between 1100 and 1400 hours.

To examine responses of functional groups to treatments, each species present was classified by dominant photosynthetic pathway (C₃/C₄) and growth type (forb/graminoid).
Species percent cover then was summed by functional type in each replicate. Following harvest, ANPP was sorted into two functional groups by growth type (forbs/graminoid) in each plot in a lab setting. Specific leaf area (SLA) was calculated using the ratio of leaf area to dry leaf mass for all species within treatments. SLA values were converted into community weighted trait values using the following formula:

\[ CWSLA = \sum_{i=1}^{n} P_i \times SLA_i \]

SLA was weighted by species contribution \((p_i)\) within each plot. Converting specific leaf area into community weighted specific leaf area (CWSLA) incorporates the weight by the species contribution \((p_i)\) to the community. This allows comparison of how various traits respond to environmental factors and are able shape species abundance within a community (Garnier 2007; Laliberte et al. 2012).

**Annual Net Primary Productivity (ANPP)**

At the end of the 2015 growing season in mid-September, ANPP was estimated destructively by clipping at ground level all biomass of individual plants rooted within a 1 m\(^2\) sub plot. Biomass was clipped from within the 1m\(^2\) subplots designated for destructive sampling within the sampling subplot. Previous year's non-living biomass was not included in estimates of aboveground productivity. Location of the quadrants were permanently marked to prevent resampling during the duration of the study. After clipping and sorting, biomass was oven dried at 80°C for 48 hours prior to weighing to the nearest 0.01 g.
Soil Sampling

Prior to initiation of the experiment, soil cores were collected to a depth of 20 cm, during the growing season from all of the plots. All soil samples were double bagged in paper and allowed to air dry. Each bag was labeled with the following information: date of collection, name of collector, name of sampling site, and block/plot/treatment identification.

Statistical Analysis

Cover estimates for each species were averaged across the 5 replicates for the 4 treatments and were used to calculate a Shannon Weiner Diversity Index for each plot. A one-way ANOVA (α =0.05) was used to test for differences in measured response variables (e.g. ANPP, SLA, LAI) among treatments. If significant differences were detected, post-hoc comparisons (Tukey) tests were conducted in order to determine which treatment groups differed (Tukey 1949). Community weighted specific leaf area (CWSLA) was calculated by multiplying relative cover (converted from percent cover of each species estimates per plot) by SLA values (Garnier 2007). Block effect was tested for and was not significant for any variable (P > 0.10).

Results

Species Composition:

Species Diversity:

A total of 23 plant species from 9 families were found within experimental plots that were established in the coastal swale on the southern end of Hog Island (Table 1).

Biodiversity as determined by the Shannon Index, differed significantly across the four treatments (ANOVA, F=3.45, df=3, P=0.04, Tukey HSD, P < 0.05; Table 2). Biodiversity was low for the coastal grassland, ranging from 1.63 to 2.01, typical of plant communities in barrier island systems. Plots receiving the P treatment had significantly higher biodiversity compared to plots that received the NP treatment. Species richness (F=0.42, P=0.74), Forb richness (F=0.48, P=0.7), C3 graminoid richness (F=2.17, P=0.13), and C4 graminoid richness (F=1.31, P=0.30) did not differ significantly among treatments (Table 2).

**Percent Cover:**

Percent cover was estimated for three cover types: living, dead/dormant vegetation, and bare sand present on each plot. Percent cover by living vegetation varied significantly across treatments (ANOVA, F=44.8, df=3, P<.0001; Tukey HSD, P < 0.05; Table 3). N additions significantly increased the percent living vegetation cover, with the highest living cover found in NP treatment (91.2 ± 2.9 %). Treatments that did not receive any form of N addition had significantly less living cover (F=25.3, P<.001; Table 3).
Density Factors:

Leaf Area Index (LAI) was significantly different among nutrient addition treatments (ANOVA, F=13.8, df = 3, p=0.0001; Tukey HSD, P < 0.05; Figure 3). LAI in P plots did not differ from control treatments with values ranging from 1.35 ± 0.14 and 1.11 ± 0.21 in control and P plots, respectively. LAI was significantly higher in NP (3.83 ± 0.42) and N (2.87 ± 0.42) plots.

ANPP was significantly different among nutrient addition treatments (ANOVA, F=8.14, df=3, p=0.0019; Tukey HSD, P < 0.05; Figure 4) and was highest in plots that received the N (905 ± 99 m$^{-2}$) and NP treatment (798.9 ± 108.5 m$^{-2}$). ANPP was not significantly higher in P plots when compared to control plots. All plots that did not receive any N amendments had significantly lower ANPP than plots that received any type of N.

Functional Traits:

**Graminoid ANPP:**

Plots that received the N amendment had the highest graminoid ANPP (804 ± 79 m$^{-2}$), and were significantly higher than any of the plots that did not receive any N (ANOVA, F=5.83, df=3, P=0.008; Tukey HSD, P <0.05; Figure 5). Graminoid ANPP from plots receiving NP (625 ± 80 m$^{-2}$) was not significantly different from any other treatment. Graminoid ANPP was lowest in P plots (398 ± 53 m$^{-2}$), and statistically similar to control plots (411 ± 46 m$^{-2}$). ANPP of forb species did not vary significantly across treatments (F=2.2, P=0.1359).
**Functional Growth Form:**

Graminoid species were the most abundant and dominant functional type across treatments (Figure 6). Percent cover of graminoids \((F=0.86, P=0.48)\), forbs \((F=1.8, P=0.19)\), and \(C_3\) species \((F=2.09, P=0.15)\) did not vary significantly across treatments. However, N and NP treatments had higher variability in graminoid and forb cover (Figure 6).

**Percent Cover \(C_4\) Species:**

Percent cover was also estimated for each treatment by the photosynthetic pathway of each species. Both N and NP increased the percent cover of \(C_4\) plants present in plots (ANOVA, \(F=7.4, df=3, P=0.0029\); Tukey HSD, \(P<0.05\); Figure 6). NP treatment had significantly higher percent cover of \(C_4\) plants \((54.2 \pm 5.2)\), except when compared to plots receiving the N treatment, while control had the lowest cover of \(C_4\) plants.

**CWSLA:**

Community Weighted Specific Leaf Area (CWSLA) was highest in plots that received the NP treatment, and was significantly higher than all other treatments (ANOVA, \(F=6.29, df=3, P=0.0056\); Tukey HSD, \(P<0.05\); Figure 7). CWSLA was statistically similar between all other treatments.

**Discussion**

This study evaluated annual net primary productivity (ANPP) and plant functional group composition in response to additions of two historically limiting macronutrients (N and P) within the grassland coastal swale. My results indicate that nutrient availability is a key
component in developing coastal swale communities of barrier island systems, which is consistent with other studies (Art et al. 1974; Ehrenfeld 1990; Day et al. 2004; Brantley and Young 2010). Previous studies of functional composition are limited to other terrestrial systems and show the extent to which nutrient limitation alters the composition of functional traits, but none have determined if limitation has caused similar effects in coastal grassland systems. Specifically, N was responsible for aboveground changes including increased ANPP, whereas responses from P were minimal, but synergistic NP effects altered community level resource allocation and leaf construction.

ANPP response in this study is high for coastal grassland systems, showing ability of vegetation to be productive at this site, withstanding present nutrient limitations and other stressors. Regardless of treatment, swale plots had considerably higher ANPP (423 ± 43 g m⁻²) than other previously studied coastal systems, including dunes on the same island (Dilustro and Day 1997) and other coastal swales ranging from 58.9 to 274 g m⁻² (Dougherty et al. 1990; Lammerts et al. 1999). ANPP on young soils of Hog Island was surprisingly similar to 80-year-old chronosequence sites, as opposed to 2 and 6-year-old swale sites (Lammerts et al. 1999). Compared to non-coastal systems, the observed ANPP response was significant as it was comparable to rates seen in nutrient addition studies in temperate grasslands including tallgrass prairie (Avolio et al. 2014) and outperformed productivity in semi-arid shortgrass steppe (Ladwig et al. 2012).

As hypothesized, additions of N as well as NP increased ANPP in this system, whereas P did not significantly alter ANPP. Previous coastal swale studies saw similar
responses but at lower ANPP rates (Dougherty et al. 1990; Lammerts et al. 1999). My results indicate that P did not have an effect on ANPP without the presence of N, indicating a co-limitation. ANPP values in Dougherty et al. (1990) were primarily limited by N but at high levels of N, P limitation becomes apparent. Lammerts et al. (1999) stressed the importance of a co-limitations of N and P in early successional sites in coastal systems that have yet to form a significant soil organic layer. Other studies found that ANPP was limited by N only after drought stress was resolved (Lagwig et al. 2012). Terrestrial grassland studies have shown that P additions in combination with N can change belowground competition and alter community structure (Vitousek et al. 2010; Avolio et al. 2014). There were no differences of forb ANPP in response to treatments, contrary to my hypothesis that herbaceous forbs would become more abundant. This is different from other studies that observed an increase in short lived annual forbs in NP treatments (Avolio et al. 2014). Instead response to fertilization was driven by increased abundance and ANPP by graminoid species, showing that their productivity is limited by nutrient availability of N.

Coastal systems are inherently low diversity systems (Mccaffrey and Deuser 1990; Bissett et al. 2016). In this study diversity values ranged from 1.63 ± 0.11 to 2.01 ± 0.09 and species richness was approximately 10 across all treatments. Although differences in species composition among treatments were seen, species diversity and richness was not altered by addition of nutrients. Dominant coastal swale species consisted of two grasses: *Ammophila breviligulata* (C3 photosynthesis) and *Spartina patens*, (C4 photosynthesis). After one year of nutrient enrichment, differences in community composition were seen.
Unlike other grassland studies (Avolio et al. 2014 and La Pierre and Smith 2015), and contrary to my hypothesis, C₃ graminoids and forbs did not become the most dominant photosynthetic pathway in NP treatments. NP additions facilitated a community change in dominant species coverage from mostly C₃ species of forbs and grasses (Ammophila brevigulata, Conyza canidensis, and S. sempervirens) to include dominance by C₄ grass species including, Eragrostis spectabilis, Panicum amarum, and Setaria parviflora. This specific change in community composition with increased dominance of C₄ species was not anticipated, but has been seen in other studies in the first several years following initial fertilization (Avolio et al. 2014; La Pierre and Smith 2015). Future community composition changes seen in other studies (Avolio et al. 2014; La Pierre and Smith 2015) may result in increased dominance of C₃ forbs and grasses.

As expected, nutrient enrichment impacted community functioning. I observed a significant increase in functional traits (LAI, SLA). Increased N lead to changes in canopy density and LAI, potentially indicating that nutrient additions lead to higher above ground competition, rather than belowground competition (Tilman 1987). LAI is a functional trait that strongly influences ecosystem function, such as productivity and transpiration. LAI values recorded in this study indicated that ecosystem functioning in a coastal swale was limited by N availability. Although specific LAI of different functional groups were not studied, it is anticipated that C₄ species were more readily able to efficiently invest N into the canopy, given that they are more efficient at maintaining greater LAI per unit investment of N, which may have driven the increases in LAI values seen in this study (Kloedt et al. 2016).
SLA values were notably much lower for this herbaceous community relative to other studies of herbaceous species (Wohlfart et al. 1999; Dwyer et al. 2014). Low SLA values indicate thick, sturdy leaves which is a common leaf level adaption by plants selected to survive in stressful environments (Te Beest et al. 2014). Low SLA values reflect how resident vegetation in this system functionally have altered leaf composition to enable longer leaf life spans in response to high stress from salt spray, high winds, and high light experienced by plants in the coastal grassland environment (Laliberte 2012). SLA is an indicator of leaf nitrogen content in plants, and to my surprise, N enrichment alone did not alter leaf construction. Instead, our data supports that plants were more readily able to acquire and utilize available N when P limitation was also overcome. Plants responded with functional changes in leaf construction to make larger, thinner leaves in NP treatment. Specifically, C_4 plants were able to respond to NP additions with an increase in SLA values, which enabled C_4 plants to displace resident species. Previous studies have found that C_4 plants utilize available P when N is also available to increase belowground ANPP/root production, which enables higher SLA values (La Pierre and Smith 2015).

**Conclusion and Significance**

Nutrient availability studies that have taken place in coastal settings have historically been limited to sites including dune fields and bay side marshes and virtually nonexistent in areas between the dunes and the marsh, the interior of the island, where grassland swales are located. Utilizing a functional trait approach has many beneficial applications in furthering our understanding how coastal grassland systems have
adapted to tolerate limited NP availability. My study is the beginning of a long-term project to study the effect of N and P on coastal grassland community dynamics. This site is part of the Nutrient Network (www.nutnet.org), a grassroots research effort to address productivity-diversity relationships and colimitation within a coordinated research network comprised of more than 40 grassland sites worldwide.

My research demonstrates that grassland coastal communities respond to nutrient limitation by utilizing a more defensive functional leaf building strategy which limits their productivity and community composition. Knowing to what extent coastal plant community composition is dictated by inherent nutrient limited conditions has many implications for management of these systems and how stewards of these systems can best sustain nutrient availability to maintain productivity, functionality and biodiversity of endemic plant communities. Given that responses were seen when NP were added at rates of 10 g m\(^{-2}\) yr\(^{-1}\), shows that this coastal grassland community is vulnerable to changing on short term time scales if nutrient limitation is overturned. Expansion of an N-fixing shrub species may utilize N enrichment to encroach into nutrient stressed grassland areas on Hog Island. This has important global and local implications for these systems are at risk to high rates of sea level rise and anthropogenic development which affects diversity and stability in this dynamic system (Isbell et al. 2013; Garnish 2013; Dias et al. 2014).


Observations based on 5 years’ manipulation of N Dose and Form in a Mediterranean Ecosystem. PLoS One, 9, 1-10.


Table 1. Mean (± standard error) species cover (%) and dominant photosynthetic pathway for all species and plots sampled on Hog Island, VA

<table>
<thead>
<tr>
<th>Species</th>
<th>C</th>
<th>N</th>
<th>P</th>
<th>NP</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Forbs</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Euphorbia maculata</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Conyza canadensis</td>
<td>5.8 ± 1.9</td>
<td>7 ± 2.1</td>
<td>4.4 ± 0.9</td>
<td>3.4 ± 1.0</td>
</tr>
<tr>
<td>Dysphania ambrosioides (C₄)</td>
<td>12.2 ± 12.2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eupatorium capillifolium</td>
<td>1 ± 1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gamochaeta purpurea</td>
<td>2 ± 0.9</td>
<td>1.8 ± 1.4</td>
<td>3 ± 1.4</td>
<td>4 ± 1.9</td>
</tr>
<tr>
<td>Krigia virginica</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td></td>
</tr>
<tr>
<td>Lepidium virginicum</td>
<td>1.4 ± 1.4</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Linum medium</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td></td>
</tr>
<tr>
<td>Physalis walteri</td>
<td>&lt;1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Solidago sempervirens</td>
<td>4.8 ± 1.0</td>
<td>14 ± 6.6</td>
<td>7.6 ± 2.2</td>
<td>14.2 ± 5.6</td>
</tr>
<tr>
<td>Strophostyles helvola</td>
<td>&lt;1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lactuca canadensis</td>
<td>&lt;1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Total % Forb cover</strong></td>
<td>14.6 ± 1.1</td>
<td>22.8 ± 8.9</td>
<td>16 ± 2.9</td>
<td>35.6 ± 9.9</td>
</tr>
<tr>
<td><strong>C₃ Graminoids</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ammophila breviliigulata</td>
<td>14.8 ± 3.5</td>
<td>24.8 ± 6.2</td>
<td>6.6 ± 2.3</td>
<td>13.2 ± 3.0</td>
</tr>
<tr>
<td>Festuca rubra</td>
<td>1 ± 0.6</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td></td>
</tr>
<tr>
<td>Fimbristylis castanea</td>
<td>&lt;1</td>
<td>4.2 ± 4.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carex species</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>1.6 ± 1.0</td>
<td>&lt;1</td>
</tr>
<tr>
<td><strong>C₄ Graminoids</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Andropogon virginicus</td>
<td>1.4 ± 1.0</td>
<td>10 ± 5.7</td>
<td>1.2 ± 0.7</td>
<td>1 ± 1</td>
</tr>
<tr>
<td>Digitaria sanguinalis</td>
<td>&lt;1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eragrostis spectabilis</td>
<td>&lt;1</td>
<td>10.2 ± 5.7</td>
<td>9 ± 2.8</td>
<td>3.6 ± 1.9</td>
</tr>
<tr>
<td>Setaria parviflora</td>
<td>1.4 ± 0.9</td>
<td>4 ± 2.9</td>
<td>7.6 ± 3.9</td>
<td>17.8 ± 10.1</td>
</tr>
<tr>
<td>Panicum amarum</td>
<td>5 ± 0.9</td>
<td>11 ± 1.9</td>
<td>6.6 ± 1.4</td>
<td>12 ± 5.3</td>
</tr>
<tr>
<td>Panicum dichotomiflorum</td>
<td>2.4 ± 1.5</td>
<td>1.8 ± 0.8</td>
<td>&lt;1</td>
<td>1.6 ± 1.0</td>
</tr>
<tr>
<td>Spartina patens</td>
<td>6.8 ± 2.3</td>
<td>3.4 ± 1.2</td>
<td>8.4 ± 2.2</td>
<td>5.4 ± 1.2</td>
</tr>
<tr>
<td><strong>Total % Graminoid cover</strong></td>
<td>33.4 ± 3.6</td>
<td>65.6 ± 8.6</td>
<td>45.6 ± 4.0</td>
<td>55.6 ± 10.9</td>
</tr>
</tbody>
</table>
Table 2. Shannon Weiner (SW) Index and species richness values across all plots on Hog Island, VA. Letter codes represent significance differences between treatments. Values represent mean ± one standard error.

<table>
<thead>
<tr>
<th>Metric</th>
<th>C</th>
<th>N</th>
<th>P</th>
<th>NP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diversity</td>
<td>1.9 ± 0.1&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>1.8 ± 0.1&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>2.0 ± 0.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.6 ± 0.1&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Species richness</td>
<td>10.8 ± 1.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10.6 ± 0.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10.8 ± 0.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.6 ± 0.9&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Forb richness</td>
<td>4.3 ± 0.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.4 ± 0.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.2 ± 0.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.0 ± 0.5&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>C&lt;sub&gt;3&lt;/sub&gt; Graminoid richness</td>
<td>2.2 ± 0.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.2 ± 0.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.6 ± 0.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.6 ± 0.3&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>C&lt;sub&gt;4&lt;/sub&gt; Graminoid richness</td>
<td>4.3 ± 0.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.0 ± 0.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.0 ± 0.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4 ± 0.6&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>
Table 3. Mean (± standard error) % cover by treatment for all species and plots sampled on Hog Island, VA. Letter codes represent significant differences between treatments. Values represent mean ± one standard error.

<table>
<thead>
<tr>
<th>Cover Type</th>
<th>C</th>
<th>N</th>
<th>P</th>
<th>NP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dead</td>
<td>38 ± 4.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9 ± 0.8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>31 ± 3.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.6 ± 2.6&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Bare</td>
<td>14 ± 5.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.6 ± 0.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.4 ± 1.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.2 ± 1.3&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Alive</td>
<td>48 ± 3.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>88.4 ± 0.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>61.6 ± 3.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>91.2 ± 3.0&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>
Figure 1. The Eastern Shore of Virginia showing the barrier islands that consist of the Virginia Coastal Reserve (VCR). Field work for this study was conducted on Hog Island, VA.
Figure 2. Experimental Design. Completely Randomized Block design with 5 blocks, 4 treatments per block and five replicates per treatment (R1-R5). Plot direction is referenced in direction of north (N) and south (S) as well as marsh/bay side and ocean side. The 20 treatments are labeled and colored as follows. Control (C) sites are labeled in dark grey, nitrogen (N) treatments are labeled in light grey, phosphorus (P) treatments are labeled in orange, and nitrogen and phosphorus (NP) treatments are labeled in red.
Figure 3. Leaf Area Index across all plots on Hog Island, VA. Treatment refers to the type of treatment received where C = control, N = nitrogen, P = phosphorus and NP = nitrogen and phosphorus treatment. Letter codes denote significant differences between treatments. Bars represent mean ± one standard error.
Figure 4. Total ANPP (g) across all treatments on Hog Island, VA. Treatment refers to the type of treatment received where C = control, N = nitrogen, P = phosphorus and NP = nitrogen and phosphorus treatment. Letter codes denote significant differences between treatments. Bars represent mean ± one standard error.
Figure 5. Graminoid and forb ANPP across all plots on Hog Island, VA. Treatment refers to the type of treatment received where C = control, N = nitrogen, P = phosphorus and NP = nitrogen and phosphorus treatment. Letter codes denote significant differences between treatments. Bars represent mean ± one standard error.
Figure 6. Percent cover of forbs and C$_3$ and C$_4$ graminoid species present in all plots on Hog Island, VA. Treatment refers to the type of treatment received where C = control, N = nitrogen, P = phosphorus and NP = nitrogen and phosphorus treatment. Letters codes denote significant differences between treatments. Bars represent mean ± one standard error.
Figure 7. Community Weighted Specific Leaf Area (CWSLA) across all plots on Hog Island, VA. Treatment refers to the type of treatment received where C = control, N = nitrogen, P = phosphorus and NP = nitrogen and phosphorus treatment. Letter codes denote significant differences between treatments. Bars represent mean ± one standard error.
Vita

Ashley Moulton was born on May 14, 1991 in Fairfax, Virginia. Ashley’s fascination of the outdoors was engrained at early age from numerous family retreats to Maryland’s Eastern Shore and rural parts of Iowa to visit family. With the great help of her parents, Ashley graduated James Madison High School in 2009. She went on to study Environmental Studies at Virginia Commonwealth University, where she was recruited by her Ecology teacher, Dr. Edward Crawford, to assist Dr. James Deemy, a graduate student at the time, with his wetland seedbank study. In the following semester, Ashley began pursuing her own seedbank study under the guidance and encouragement of these two passionate mentors, and was awarded a VCU Rice Center Research Grant for her efforts. Before completing her undergraduate degree at VCU in 2013, Ashley developed her skills in plant ID under Dr. Dianne Jennings, and field work from assisting graduate student, Ben Dows, with his data collection on Hog Island. She also developed a passion for vegetation dynamics after taking Dr. Don Young’s Barrier Island Ecology class. At the time of her acceptance into the Coastal Plant Ecology Lab in 2014, Ashley had accepted a part time position as an Agricultural and Natural Resources Technician with the Chesterfield Cooperative Extension Office, where she worked part time while completing her Master’s degree coursework. During her last year of her Master’s degree coursework, Ashley accepted a full-time position coordinating the volunteer efforts of the county’s Master Gardener Program. Ashley plans to utilize her degree in pursuing work that helps further retain the functionality of natural ecosystems from local and global disturbances by pursuing future employment in natural resource conservation endeavors.