Fall 12-14-2018

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Marine Science

Submitted in Partial Fulfillment of the
Requirements for the Degree of Bachelor of Science
In the HTC Honors College at
Coastal Carolina University

Fall 2018

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Abstract

The aim of this study was to investigate the differences in nutrient limitation within the beach and marsh environments at Waties Island, SC. Conducting the experiment on Waties Island allowed most physical factors to be held as constant, meaning the marsh and beach environments would serve as the only variables. Experiments were performed in the winter, spring, summer and fall of 2018 to determine temporal changes in limitation to phytoplankton and cyanobacteria growth. Nutrient additions included dissolved inorganic nitrogen (nitrate, nitrite, and ammonium), dissolved organic nitrogen (urea), and phosphate. To test for co-limitation, a nitrate & phosphate treatment was also used. Triplicate treatments were incubated for 48 hours in a Thermo Scientific Precision Model 818 Incubator. Changes in phytoplankton and cyanobacteria biomass were determined by monitoring chlorophyll, phycocyanin, and phycoerythrin concentrations fluorometrically. What was found was typical of coastal marine environments in the southeastern United States; that nitrogen is the main limiting nutrient and each species of nitrogen exhibited a significant difference in growth from the control. Between the marsh and beach environments, the marsh exhibited higher control growth rates, while the beach was the more nutrient limited environment. Seasonally, the marsh became less nutrient limited as the seasons progressed from winter to fall, while the beach maintained constantly limitation year-round. Within the marsh, cyanobacteria were found to be non-nutrient limited however phosphorus caused a significant depression in cyanobacteria growth. In the beach, the primary cyanobacteria nutrient was phosphorus while nitrogen served as the secondary limiting nutrient.

Introduction

The most pervasive organism on this planet is autotrophic phytoplankton that resides in the world's marine environment. From open ocean, to coral reefs, to coastal marsh systems; phytoplankton are present in all ecosystems. Within each marine ecosystem phytoplankton play the most basic yet vital role; that of the producer. In most marine ecosystems photosynthetic plankton are the base of the food web and supply all upper levels with the energy needed for life. The overall biomass and productivity in an area of ocean is directly related to the amount of phytoplankton growth in that region. Phytoplankton are the source of the oceans productivity, as well as being a major contributor to atmospheric oxygen levels in the form of the waste O2 from photosynthesis.

While phytoplankton reside in every marine ecosystem with light available, due to varying environmental conditions around the globe the amount of phytoplankton productivity varies regionally in response to these environmental condition. The overwhelming control on phytoplankton growth is the bottom up control by resource availability (Lapionte, 1997). This lack of resource availability can be described as nutrient limitation, where the phytoplankton lack the necessary metabolic nutrients to grow and reproduce. Because of the nature of limitation, there will always be one nutrient at the "bottom" of the nutrient supply barrel that controls phytoplankton growth, even in the face of most other nutrients being abundant. Limited nutrients levels can vary from place to place in response to physical factors, and the limited nutrients themselves can be a wide range of chemical compounds. For example; in shallow coastal waters the limiting nutrients are often nitrogen and phosphorus while in open ocean pelagic waters the limiting nutrient is often iron.

Not only does the variation in nutrient levels change productivity around the world, it influences species diversity as different species of phytoplankton respond differently to varying nutrient levels (Leibold, 1999). These phytoplankton come in widely varying forms across a large number of genera; changing in basics such as body structure, composition, and nutrient uptake sources. Because of this wild variation in organisms, as various nutrient levels increase or decrease due to environmental factors; the concentrations and diversity of phytoplankton species will change accordingly. Beyond nutrient levels causing variation in phytoplankton, physical characteristic changes due to seasonal variations can influence phytoplankton diversity in an ecosystem (Huang, 2004).

The limitation of phytoplankton growth due to lack of nutrients is a topic that has important real world implications. Increasing open ocean productivity by decreasing limitation can be an important tool in the fight against global climate change, where higher ocean productivity causes an increase in carbon sequestration (Blain, 2007). Not only can nutrient limitation effect global carbon levels; understanding limitation can help to monitor coastal marine productivity, detail plankton species diversity, and prevent harmful algal blooms caused by a sudden saturation of a limiting nutrient.

In the southeast United States, the major coastal limiting nutrient has been found to be nitrogen (Reed, 2016). Extensive work on nitrogen limitation has been done in this part of the country, particularly in terms of anthropogenic effects on estuaries. It has been shown that not only does excess nitrogen and phosphorus entering an estuary effect phytoplankton (Altman and Paerl, 2012), this nutrient loading can cause large scale plankton blooms that eventually lead to anoxic water conditions. (Paerl, 1998).

Along the South Carolina coast, barrier islands and spits are highly prevalent geologic features. Because of the wave action and morphology of the coast of South Carolina, the dominant type of barrier island is the wave-dominated barrier island. A wave-dominated barrier island is a long, narrow, low-lying island with few inlets, small ebb deltas, and common over wash zones (Heron, 1984). It displays these features because the dominant water-moving force is waves, which generates a long shore current that smooths and elongates the islands. In addition to the features exhibited by wave-dominated barrier islands, the coastlines and beaches of South Carolina are for the most part low energy; a type of beach characterized by buildup of sand and a steep beach slope. While these are the physical characteristics of South Carolina barrier islands and beaches, the biological characteristics of the salt marsh present on the back-barrier of the island are also important to detail.

A quintessential part of the barrier island system is the inter-tidal salt marshes that form on the back-barrier of the island. The marsh is an environment of mostly mud and other fine sediments, with high amounts of organic matter prevalent in the soil from decaying vegetation. Salt marshes are extremely rich ecosystems; serving as an environment with high productivity, a nursery for juvenile oceanic animals, a filter for nutrients that run off the land, and a place for low trophic-level organisms to grow in order to sustain the high levels of the food chain (Adam, 1993). The salt marshes off the South Carolina coast are often referred to as *Spartina* salt marshes, because they are dominated by two species of *Spartina* plants (commonly referred to as cordgrass): *Spartina alternaflora* and *Spartina patens* (Adam, 1993). In the marsh, periwinkle snails can commonly be seen perched on the thick blades of the *S. alternaflora*, but most definitely live among the other two plants as well. In addition to the periwinkle snails, South Carolina salt marshes are inhabited by red fiddler crabs, sand fiddler crabs, heron, clams, as well

as a host of other organisms (Adam, 1993). These organisms are dependent on the continued health and productivity of the marsh.

For this study Waites Island South Carolina will be used as the experimentation site as it is a pristine example of the type of *Spartina* salt marsh found along the South Carolina coast as it exhibits all the features characteristic of a South Carolina barrier island system. Waites Island is a sandy, wave-dominated barrier island that portrays the classic back-barrier *Spartina* marsh environment opposite an ocean-side beach. These two sites will be where sampling takes place, providing data for both a marsh system and a beach system. By comparing the two systems present on the same barrier island, the bioassay serves as a way to fundamentally distinguish differences in *Spartina* salt marsh vs open beachfront nutrient levels as nearly all other variables are the same (climate, latitude, insolation, etc.).

Comparing the differences between the beach and the marsh will serve as a central objective within this study. It is expected that there will be a variation in nutrient limitation due to the natural differences between the two environments. Notably, the high concentration of organic matter as well as the higher residence time within the marsh will serve as a contrast to the much more open and dynamic ocean front. The high residence time within the marsh traps nutrients in the system longer, and allows more nutrient cycling to boost productivity. In contrast, the ocean is more free flowing with tides and currents acting on the water, preventing nutrients from being repeatedly cycled through the system. As a result it is expected not only productivity to change between the two environments but also the diversity of species present in response to the differing nutrient levels. Further, it is expected that for a given nutrient added the two environments will respond in different ways, due to the differences in the available ecosystem nutrients. This variation in nutrient limitation will not only give insight into how and

why productivity varies between the beach and marsh environments, but also how the diversity of species varies between each environment in response to the nutrients present. The change in diversity of species present as nutrient concentrations are changed will not only highlight how different genera are being controlled by the available ecosystem nutrients, but also give a prediction as to how the phytoplankton composition of the ecosystem will change in response to anthropogenic nutrient loading. The data found at the Waites Island site can be then used to serve as a proxy for the limitation and diversity of other South Carolina salt marshes.

This study will also observe how both systems progresses as the seasons change. Sampling will take place in each of the four seasons, and will help describe seasonal trends of nutrient limitation and species diversity within the beach-marsh system. As the seasons change most physical characteristics change as well: insolation, temperature, precipitation rates, wind/current speed, and salinity are only some of the major physical changes that occur as the season's progress. This study will look at whether beach-marsh nutrient limitation and diversity changes in a uniform manner, or if one system is affected by the change is seasons more than the other. This information can be used to infer several factors: such as seasonal productivity variations, changes in the rate of nutrient cycling, and what physical characteristics play a role in species diversity.

Cyanobacteria nutrient limitation and variation in cyanobacterial density between the two environments will also be investigated. Cyanobacteria play an essential role as the absolute base of the food chain by being involved in the microbial loop. The microbial loop is the system of bacteria that consume dissolved organic particles and nutrients in the water, who are then preyed upon by microzooplankton. In effect, the microbial loop allows nutrient recycling in an ecosystem by bacterial uptake (Azam, 1983). Looking at cyanobacterial density will give insight

into the nutrient efficiency of an ecosystem and the rate at which nutrient recycling occurs. Due to the differences in water mass residence time of the two ecosystems influencing the dissolved nutrient concentrations, the residence time will indirectly influence the cyanobacterial density and serve as a possible source of cyanobacterial limitation (Romo, 2013). If cyanobacterial nutrient limitation is occurring, the rate of nutrient recycling in an ecosystem will be lowered, lowering the overall productivity of the ecosystem (Azam, 1983).

This study has four major objectives: to determine what nutrient is limiting in both phytoplankton and cyanobacteria growth at Waties Island, to determine how nutrient limitation differs between the beach and the marsh at Waties Island, and detail how nutrient limitation at Waties Island change seasonally. In order to accomplish these goals, nutrient bioassays will be conducted for both the marsh and the beach environment in each of the four seasons, and phytoplankton and cyanobacteria growth rates will be analyzed to determine which nutrients cause significant growth.

Methods

Field Collection

Water samples were taken at Waties Island, South Carolina and were collected from the lagoon (Dunn Sound) behind the back barrier *Spartina* marsh as well as from the ocean-front beach. The water samples were stored and transported in a 20L acid washed carboy, in order to leave the samples uncontaminated. Water temperature, dissolved oxygen, dissolved oxygen %, conductivity, and salinity were taken with a Pro 2030 YSI. Turbidity were taken with a Hach 2100Q Turbidity meter. In-vivo chlorophyll measurements were taken on site with a Turner AquaFluor Handheld Fluorometer with optical units being used to determine chlorophyll and

cyanobacteria. Samples were taken in the winter (03/11/18), spring (4/30/18), summer (8/18/18), and fall (11/04/18) in order to measure seasonal changes in the system.

Experiment

Once the collected samples were transported back to the lab sample water was put into 250ml acid cleaned incubation bottles. Seven treatments were used to test limitation: control, nitrate, nitrite, ammonium, urea, phosphorus, and nitrate + phosphorus. Nitrogen treatment concentrations were 25uM, and phosphate treatment concentrations were 10uM. Nitrate, nitrite, ammonium, and nitrate + phosphorus represent DIN, or dissolved in organic nitrogen. After nutrient spiking the bottles were kept in a Thermo Scientific Precision Incubator set to ambient temperature and the correct day/night cycle. After 48 hours the bottles were taken out and subject to both in-vivo and in-vitro chlorophyll testing.

Chlorophyll and Pheophytin

Chlorophyll and Pheophytin analysis was done both In Vivo and In Vitro (Extracted). To determine extracted chlorophyll concentrations, samples were determined by the method outlined in Arar, 1997. Samples were filtered onto a GF/F filter and then stored frozen for at least 24 hours. 90% acetone was then added, and samples were stored in the freezer for an additional 24 hours. Samples were then centrifuged using a Horizontal Centrifuge, and then raw fluorescence was measured using a Turner Designs Trilogy Laboratory Fluorometer. Chlorophyll and Pheophytin was determined using the following formulas:

$$\text{Chlorophyll } (\mu g/L) = Fs * (r(r-1)) * (Rb - Ra) * (VE/Vs)$$

$$\text{Pheophytin } (\mu g/L) = Fs * (r(r-1)) * (rRa - Rb) * (VE/Vs)$$

Nutrient Composition

Upon collection sample water was frozen and stored for future analysis. Nutrients concentrations of the sample water were determined colorimetrically: nitrite and nitrate (Bendscheider, 1952), phosphate (Murphy, 1962), and ammonium (Holmes, 1999). The Nitrate and Nitrite concentrations were determined using Hach NitraVer 6 to reduce NO_3 to NO_2^-, following this, Hach Nitri Ver 3 was used to make NO_2^- quantifiable spectrophotometrically. The sample absorbance value measured by the spectrophotometer could then be compared to a standard curve to calculate the $NO_3^- + NO_2^-$ concentration in µmol/L. By running a sample under the same procedure but without use of the Hach Nitra Ver 6 to reduce NO_3^-, the concentration of NO_2^- can be calculated in µmol/L (Bendscheider, 1952). Finding the difference between [$NO_3^- + NO_2^-$] and [NO_2^-] will give the concentration of NO_3^-. Ammonium samples were mixed with a light-sensitive mixed reagent, stored for two hours in darkness in order for the reagent to make the Ammonium fluorometrically quantitative (Holmes, 1999) then run through a Fluorometer. The Fluorometer gives values in RFU that when compared against a standard gives NH_4^+ concentrations in µmol/L. For Phosphate the samples underwent the molybdenum blue complexation method (Murphy, 1962) to form a yellow complex with any PO_4^{3-} in the sample. The spectrophotometrically quantifiable complex was measured in a spectrometer, and the absorbance was then compared against a sample to obtain concentration in µmol/L.

Cyanobacteria

Concentrations of cyanobacteria were determined by placing sample water into a Turner AquaFluor Handheld Fluorometer with optical units for Phycocyanin (freshwater) and Phycoerythrin (marine). Testing of cyanobacteria was not only done *in-situ* at sample collection but also concurrently with chlorophyll testing. This additional cyanobacterial testing was done in

order to determine the nutrient limitation that cyanobacteria experience in each environment. In addition, by fluorometrically testing for Phycocyanin (freshwater) and Phycoerythrin (marine) the type of cyanobacteria (freshwater vs. marine) present at each environment could be examined.

Diversity Counts

To measure diversity samples of each nutrient concentration were preserved using Lugol's Iodine solution. From here samples from both the control and highest In-vitro chlorophyll treatment from a given sampling date were chosen to under-go diversity counts. Phytoplankton will be observed under a microscope and counted using a 15-section "spot" search. The individual species of phytoplankton were recorded and summed. The Simpson Diversity Index (Simpson, 1949), which gives the equation $D = 1 - \sum(\frac{n_i}{N_s})^2$ where n_i is the sum of one species of individuals and N_s is the sum of all individuals; was used to give a relative index to the diversity of phytoplankton seen.

Results

Physical Data

The temperature between the two environments did not differ much on any sample date (Table 1), which is to be expected as both environments are located on the same island and receive the same insolation and climate conditions. The salinity was higher in the beach than the marsh (Table 1) which is also to be expected as the marsh water is brackish. The chlorophyll was higher in the marsh on ¾ sample days with the exception of the Spring sample date (Table 1).

Chlorophyll concentrations being higher in the marsh suggest the marsh is a more productive environment than the beach. Both DO_2 and DO_2 % were larger in the beach than the marsh (Table 1), this is due to two possible mechanisms: the wave action of the beach stirs the water more and allows more O_2 to dissolve in, or the marsh is a more anaerobic environment due to higher rates of respiration of organic matter. Turbidity is also higher in the beach than the marsh (Table 1), which may be due to particulate sediments in the water column kicked up by the beach wave action.

In Vitro Chlorophyll

The Winter sample date demonstrated the highest individual chlorophyll concentration of any date within both the marsh (NH_4^+, 54.6 µg/L, Figure 1a) and the beach (NO_3^- + P, 38.9 µg/L, Figure 1b). In the marsh NH_4^+, NO_3^- + P, and Phosphorus were all found to be significantly different (Figure 1a, $p < 0.05$, t-test) from the control; while in the beach every nitrogen species used: NO_3^-, NO_2^-, NH_4^+, NO_3^- + P, and Urea was found to be significantly different than the control (Figure 1b, $p < 0.05$, t-test).

The Spring sampling date; within the marsh NH_4^+, NO_3^- + P, and Phosphorus were all found to be significantly different (Figure 2a, $p < 0.05$, t-test) from the control, while at the beach NH_4^+ and NO_3^- were found to be significantly different from the control (Figure 2b, $p < 0.05$, t-test). While the Phosphorus was significantly different compared to the control, Phosphorus had less average chlorophyll in µg/L (Figure 2a), indicating that rather than acting as a limiting agent, excess Phosphorus can repress phytoplankton growth. This was the sample date with the least amount of nutrients significantly different than the control with five treatments being significant, and when Phosphorus is considered to be significant in the negative direction it can be said that only four treatments between the two environments were growth limiting.

The Summer sample date exhibited the same trends of significance as the first two samples. In the marsh NH_4^+ and NO_2^- were significantly different than the control (Figure 3a, $p < 0.05$, t-test), while at the beach NO_3^-, NO_2^-, NH_4^+, and NO_3^- + P were all significantly different than the control (Figure 3b, $p < 0.05$, t-test). This was the first date in the marsh that NO_3^- + P was not significantly different than the control, where in both the Winter (Figure 1a) and Spring (Figure 2a) NO_3^- + P exhibited the second highest µg chlorophyll/L of any nutrient treatment (Figure 1a, 2a).

On the Fall sample date, less limitation was found in the marsh, while the beach was significantly limited. The only nutrient treatment from the marsh sample that was significantly different than the control was Phosphorus (Figure 4a, $p < 0.05$, t-test) and it should be noted that Phosphorus was significantly less than the control, meaning Phosphorus did not increase phytoplankton growth but rather served as a growth limiter. Although the beach also found Phosphorus to be significantly different than the control, similar to the marsh Phosphorus produced less average chlorophyll in µg/L (Figure 4b). Additionally the beach treatments NO_3^-, NO_2^-, NH_4^+, and NO_3^- + P were all significantly different than the control (Figure 4b, $p < 0.05$, t-test)

Environment Differences

Across all sample dates the control of the marsh exhibited higher µg chlorophyll/L than the beach did at that sample date (Figure 1, 2, 3, 4). Not only did the marsh controls have larger µg chlorophyll/L than the beach, on three dates (Winter, Spring, and Summer) (Figure 1, 2, 3) the marsh's chlorophyll was more than double that of the beach. As a control is untreated, these values reflect what can be thought of as the baseline productivity of each system. The samples indicate that the marsh has higher levels of primary productivity than the beach, and is thus less

nutrient limited. Evidence for the beach being more nutrient limited than the marsh is also indicated in the nutrient treatments. Summing the treatments that were significantly different than the control on each date, and omitting the treatments where Phosphorus was significant but < control µg chlorophyll/L; gives that the marsh had seven total treatments show significant growth from the control, while the beach had fifteen total (Figure 1, 2, 3, 4). This disparity in treatments that showed significant growth quantifies the beach exhibiting more nutrient limitation than the marsh.

Seasonal Trends

By looking at limitation on a seasonal basis, what was observed was that the marsh became less limited as the year went on before reaching a minimum of limitation in the Fall while the beach stayed limited throughout the year. Looking at treatments that were significantly different than the control on each date, and omitting the treatments where Phosphorus was significant but < control µg chlorophyll/L; shows that the marsh limiting treatments decrease from 3 (Winter, Figure 1a), to 2 (Spring, Figure 2a), remain at 2 (Summer, Figure 3a), before hitting a minimum of zero nutrient treatments showing limitation in the Fall (Figure 4a). Conversely when the same analysis is applied to the beach the number of limiting nutrients stays relatively constant: 5 (Winter, Figure 1b), 2 (Spring, Figure 2b), 4 (Summer, Figure 3b), and 4 (Fall, Figure 4b). The beach has already been shown to be more limited than the marsh, however the differences in limitation between the two environments implies that a physical aspect is changing in the marsh as the year goes on that is either not present or not changing in the beach.

Looking at how the marsh samples changes as the seasons progress shows that Phosphorus begins the year as the secondary limiting nutrient. In the marsh on the Winter and Spring treatments, not only is NO_3^- + P significantly different than the control (Figure 1a, 2a);

but NO_3^- + P is the treatment that exhibited the second highest µg chlorophyll/L, behind only NH_4^+ which is a reduced nitrogen source that phytoplankton will preferentially take up. Further, the NO_3^- and NO_2^- on these dates were not significant, indicating that Phosphorus acted as the secondary limiting agent and the Phosphorus in NO_3^- + P was able to cause enough excess growth that the treatment was significantly different from the control while NO_3^- and NO_2^- were not (Figure 1a, 2a).

Phytoplankton Community

The phytoplankton community counts indicate that the primary class of phytoplankton present in each environment was diatoms. Four separate counts of the preserved control sample from the Fall sample date from each environment was used to show the diversity of the sample as well as the species composition. In both the marsh and the beach, the top four species identified were diatoms. Not only were the dominant species diatoms, but diatoms were >50% of the community in both the marsh (69.5%) and the beach (59.1%). Diatom dominance of coastal South Carolina waters corroborates what has been found in other similar bioassays of the SC coast (Reed, 2016)

When the Simpson Diversity Index of the four counts were averaged, the marsh demonstrated an index of 0.68, and the beach demonstrated an index of 0.86 (Figure 9). Two counts were also done for the N + P treatment of the marsh and the beach on this sample date. These two counts averaged for each of the environments demonstrated an index of 0.80 in the beach, and a 0.70 in the marsh (Figure 9). For both nutrient treatments sampled from, the beach displayed higher diversity than the marsh. It should be noted that the Index ranges from 0.0 to 1.0, with a larger value indicating more diversity.

Cyanobacteria

Although concentrations of both phycoerythrin and phycocyanin were taken from both the marsh and the beach on the Fall sample date, phycoerythrin is predominantly found in marine cyanobacteria and phycocyanin is predominately found in freshwater cyanobacteria. In regards to this, phycoerythrin concentrations will only be displayed for the beach and phycocyanin concentrations will only be displayed for the marsh.

Within the marsh, phycocyanin concentrations were significantly different in two nutrient treatments when compared to the control; Phosphorus and NO_3^- + P (Figure 7). These two treatments did not experience significant growth compared to the control, but rather a significant depression in phycocyanin concentration; indicating that these two treatments served to inhibit cyanobacteria growth. Similarly, the Phosphorus treatment in the Fall marsh bioassay also exhibited chlorophyll concentrations that were significantly depressed compared to the control (Figure 4). No other nutrient treatment was significant from the phycocyanin control, suggesting that no other nutrient served as a limiting agent to cyanobacteria growth within the marsh on this sample day.

The phycoerythrin concentrations within the beach on this sample date displayed significant growth for three nutrient treatments; NO_2, Phosphorus, and NO_3^- + P (Figure 8). These three treatments causing significant growth indicate that both nitrogen and phosphorus was limiting to cyanobacteria growth in this environment on the sample date. Of the three, NO_3^- + P exhibited the greatest concentration, followed by Phosphorus, and lastly by NO_2 (Figure 9). This suggests that Phosphorus was the primary limiting agent, with nitrogen serving as the secondary limiting agent.

Nutrient Composition

The most direct indicator of what nutrients will limit as system is the nutrient concentrations within the environment. As this experiment conducted tests for nitrate, nitrite, ammonium, and phosphorus samples; the ratio of total DIN (dissolved organic nitrogen) to phosphorus could be determined, as well as the proportion of the DIN chemical species. Following the 16:1 N:P ratio (Redfield, 1934) indicates whether nitrogen or phosphorus will be limiting in a marine system: a ratio >16 indicates phosphorus limitation, a ratio <16 indicates nitrogen limitation. Figure 6 shows the molar ratio of nitrogen: phosphorus from each ecosystem sample on each date, and uses a diagonal line to indicate whether the samples displayed a ratio above or below 16. Each sample displayed an N:P ratio <16.

As DIN could be differentiated into nitrate, nitrite, and ammonium; it was possible to determine how not only total DIN varied between each date and season, but whether the component nutrients themselves varied between date and season. Figure 5 shows how the proportionality of DIN changed seasonality between the environments. While total DIN in each environment is about the same, and nitrite is primarily the dominant chemical species in both as well; two environmental differences in the secondary dominant chemical species are demonstrated. The marsh displays ammonium as its secondary dominant species, rather than nitrate (Figure 5a). Conversely, the beach displays nitrate as its secondary dominant species, rather than ammonium (Figure 5b).

Discussion

The overall objective of this study was to investigate the differences in nutrient limitation between a marsh and beach environment, and how nutrient limitation dynamics varied

seasonally. The limiting agent within the two coastal marine environments was consistently found to be nitrogen. For each environment on each sample date, a species of nitrogen demonstrated the highest amount of growth when treated. Of the four treatments: NO_3^-, NO_2^-, NH_4^+ and NO_3^- + P; the top growth values for each date and environment consisted of three of these four. NH_4^+ is especially noteworthy because, with the exception of the marsh on the Fall sample date (Figure 4a) it was significantly different from the control on each date in each environment. The effect of nitrogen limitation was pronounced in the beach; where on three of the four sample dates (Winter, Summer, Fall) all four species of DIN (NO_3^-, NO_2^-, NH_4^+ and NO_3^- + P) were shown to be significantly different than the control (Figure 1b, 3b, 4b). The fact that that all four nutrient species were taken up when in excess demonstrates that the phytoplankton did not preferentially uptake a nitrogen species on those dates, and any species of DIN (dissolved organic nitrogen) would have been uptake. The high uptake rates of DIN with low uptake rates of urea (DON) were unexpected as reduced forms of nitrogen such as urea have been shown to be preferentially uptaken over oxidized forms of nitrogen such as NO_3^-, NO_2^- (Fan, 2003).

The phytoplankton treatments demonstrating nitrogen limitation lines up with what would be expected out of the environment N:P ratio. Each environment on all of the sample days displayed an N:P ratio that would predict nitrogen limitation within the system (Figure 6). The N:P ratio being <8 at every sample station shows that nitrogen was consistently deficient within the environment, meaning the phytoplankton communities would be nitrogen limiting year round. The N:P ratio serves as a way to provide an environmental reason for why the nutrient treatments in this bioassay demonstrated nitrogen limitation within both environments throughout the year. Nitrogen serving as the limiting agent is not unexpected, similar bioassay

studies along the South Carolina coast have found nitrogen to be the primary limiting agent as well (Reed, 2016). This study helps to further confirm nitrogen as the limiting nutrient along the SC coast.

Although DIN was found to be the primary limiting agent, dissolved organic nitrogen (urea) only caused significant growth from the control within the beach on the Fall sample date (Figure 1b). Although urea serves is a nitrogen species, it is different from the other nitrogen species used as treatments as urea possesses carbon within the molecule. Further, urea is a reduced form of nitrogen, which is preferentially uptaken by phytoplankton, and high urea concentrations has been shown to correlate with phytoplankton blooms (Fan, 2003). In order for urea to be metabolized, an organism must produce Urease, the enzyme that breaks down urea. A possible reason that urea did not cause phytoplankton growth in the nitrogen limited systems of this study is that the phytoplankton community within these environments do not contain a species that is capable of producing Urease. Without a species that is capable of producing Urease, urea cannot be broken down and will not cause phytoplankton growth. As urea is a common animal metabolic waste; this study's demonstration that the phytoplankton communities in the environments samples are unable to break down urea would suggest that animal wastes is not broken down by phytoplankton, but is either deposited within the soils or is broken down by some other organism within the system.

When comparing the beach and marsh environments, the primary difference between the two environments is that the marsh exhibited greater growth of the control than the beach, and that the beach was the more limited environments (Figures 1, 2, 3, 4). These differences go hand in hand; an environment that has higher growth will be less limited, and an environment with high amounts of limitation will not have high limits of growth. This indicates that the marsh has

more nutrients within its system than the beach does, which would make it have more growth and less limitation as was demonstrated. A possible explanation for this is the high amount of organic material within the marsh. The marsh contains *Spartina* grass, oysters, crabs, birds, and a large host of other organisms that reside in the marsh. All these organisms contribute organic matter to the marsh in the form of either metabolic waste or detritus, which is then remineralized into nutrients such as DIN or phosphorus. The nutrients that are remineralized in the marsh are incorporated into the water column, which contributes to the higher growth and lower limitation as the increased nutrient supply increases phytoplankton growth. The beach, which is a much more open system with less organics to be remineralized into nutrients, experiences more limitation as less nutrients are incorporated into the system.

Another important difference between the marsh and beach is the presence of ammonium within the marsh. Within the marsh, ammonium composed >10% of DIN on all sample dates, while ammonium only was present >10% DIN on the Spring sample date (Figure 5). Overall, the marsh exhibited higher concentrations of ammonium than the beach. A possible cause for this is the large amount of organic matter in the marsh contributing to an anaerobic environment. When large amounts of carbon are decomposing, O_2 will be removed from the environment as bacteria use the O_2 to decompose the carbon via aerobic respiration. Once O_2 is depleted, anaerobic respiration will occur that will reduce NO_3^- in order to be able to oxidize organic carbon, a by-product of this reaction is NH_4^+ (Giblin, 2013).The large amounts of organic carbon within the marsh cause this reaction to occur, leading to the higher levels of ammonium seen in the marsh compared to the less carbon rich beach.

The main seasonal change of the limitation in the environments is that as the year progressed the marsh became less limited while the beach maintained constant limitation year-

round (Figure 1, 2, 3, 4). While it has already been established that the beach is the more limited environment due to the reduced presence of organic matter, there was some physical process that occurred during the year within the marsh to cause the environment to become less limited while the beach maintained constant limitation. Because nutrient limitation is a deficiency of nutrients for phytoplankton growth, the marsh becoming less limited suggests that excess nutrients were input into the marsh water to stimulate phytoplankton growth. While it is not impossible that the input of excess nutrients was anthropogenic, it is more likely that the input of nutrients was due to decaying plant matter as the seasons changed. The marsh had zero treatments cause significant growth on the Fall sample date (Figure 4a), which is when plant matter would be dying due to the change in seasons. As the terrestrial and marsh plants begin dying, they would enter the marsh waters as detritus which then can be broken down by bacteria into inorganic nutrients via remineralization. These inputs of nutrients would supply the excess nutrients to make the marsh biounlimited.

The Diversity Index of the two environments showed little variation from the control treatment and the NO_3^- + P treatment (Figure 9). The NO_3^- + P treatment exhibiting a similar DI to the control after the input of excess nutrients indicates that the phytoplankton community grew proportionally when under nutrient loading, rather than a community shift towards a single dominating species. Proportional growth from the control phytoplankton community implies that the ecosystem is not under threat of a Harmful Algal Bloom (HAB) should nutrient loading occur. HABs are single species dominant phytoplankton blooms that can occur under nutrient loading, and select HAB species can damage a marine ecosystem through toxin production (Anderson, 2002). Single species dominance that occurs when HABs form not only can harm the environment through toxins produced by the species, the dominant species may not be

consumable by the first trophic level consumers, causing environmental food chain disturbances. As both the marsh and beach phytoplankton communities at Waties Island grew proportionally under nutrient loading rather than a shift towards single species dominance, it suggests that Waties Island is not at risk from HAB formation.

In the marsh on the Fall sample date, cyanobacteria were not nutrient limited, with zero treatments causing significant growth; however cyanobacteria growth was found to be suppressed by excess phosphorus (Figure 7). This matches the nutrient limitation found in the phytoplankton community on this sample date, where no treatments experienced significant growth while the phosphorus caused a significant growth depression (Figure 4a). This implies two possibilities for the marsh system: either cyanobacteria growth is essential to phytoplankton growth through the microbial loop (Azar, 1983) and depressed cyanobacteria growth will limit phytoplankton productivity, or the species that comprise the cyanobacteria and phytoplankton communities are both depressed by excess phosphorus. Although it is not certain, as phosphorus was shown to be a limiting agent in the marsh on the Winter sample date (Figure 1a), and bacteria play an essential role in decomposing the high organic matter of the marsh; it seems likely that marsh phytoplankton productivity is related to cyanobacterial growth.

In the beach on the Fall sample date, both nitrogen and phosphorus were limiting to cyanobacterial growth (Figure 8). This lines up with the beach being the more limited environment due to the lack of organic matter within the system. Bacteria function as the decomposers within the system, remineralizing nutrients from decaying organic matter. Cyanobacteria being nutrient limited implies that the beach system is also then limited in the presence of organic matter to breakdown. This suggests that any organic matter available is

quickly remineralized before the organic matter can undergo sedimentation, and is quickly recycled back into the ecosystem.

Conclusion

Conducting a seasonal nutrient bioassay at the marsh and beach of Waties Island, South Carolina demonstrated that the primary limiting nutrient in both coastal marine environments was nitrogen. This determination from the nutrient bioassays was compounded by the measured nitrogen: phosphorus ratio that was consistently <16. Between the two systems, the marsh was the more productive environment while the beach experiences greater amounts of nutrient limitation. It is suspected that the reason for less limitation within the marsh is due to the high amounts of organic matter. Seasonally, it was found that the marsh hit a limitation minimum in the Fall season, while the beach was limited on a yearly basis. The possible cause for the marsh limitation minimum in the Fall is the input of excess organic matter as the seasons change. Additionally, cyanobacteria tests showed that within the marsh cyanobacteria are not nutrient limited, while in the beach cyanobacteria limitation exists. Further, it is suggested that marsh primary productivity is linked to cyanobacteria growth. Waties Island was also found to be not at risk from HABs as the phytoplankton community grows proportionally under nutrient loading.

This study has several implications when taken at a wider scope. Waties Island can serve as a model ecosystem for the type of barrier island that is prevalent along the southeastern United States' coast. When considering the growing threat of eutrophication from anthropogenic nutrient loading, this experiment details that a barrier island along the US southeast coast is primarily nitrogen limited, more limited within the beach than the marsh, and marsh limitation changes seasonally while beach limitation is constant.

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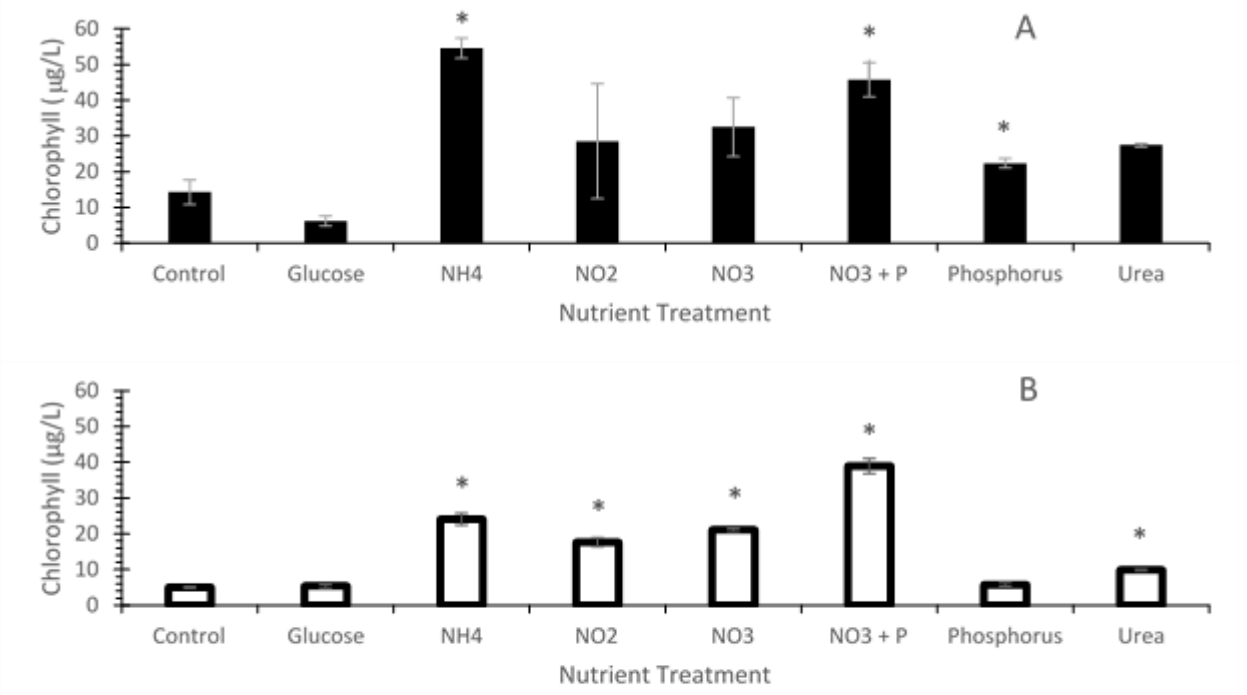

Figure 1 Average Chlorophyll measured on 03/11/18 (Winter) in the Marsh (A) and in the Beach (B). Error bars represent Standard Deviation

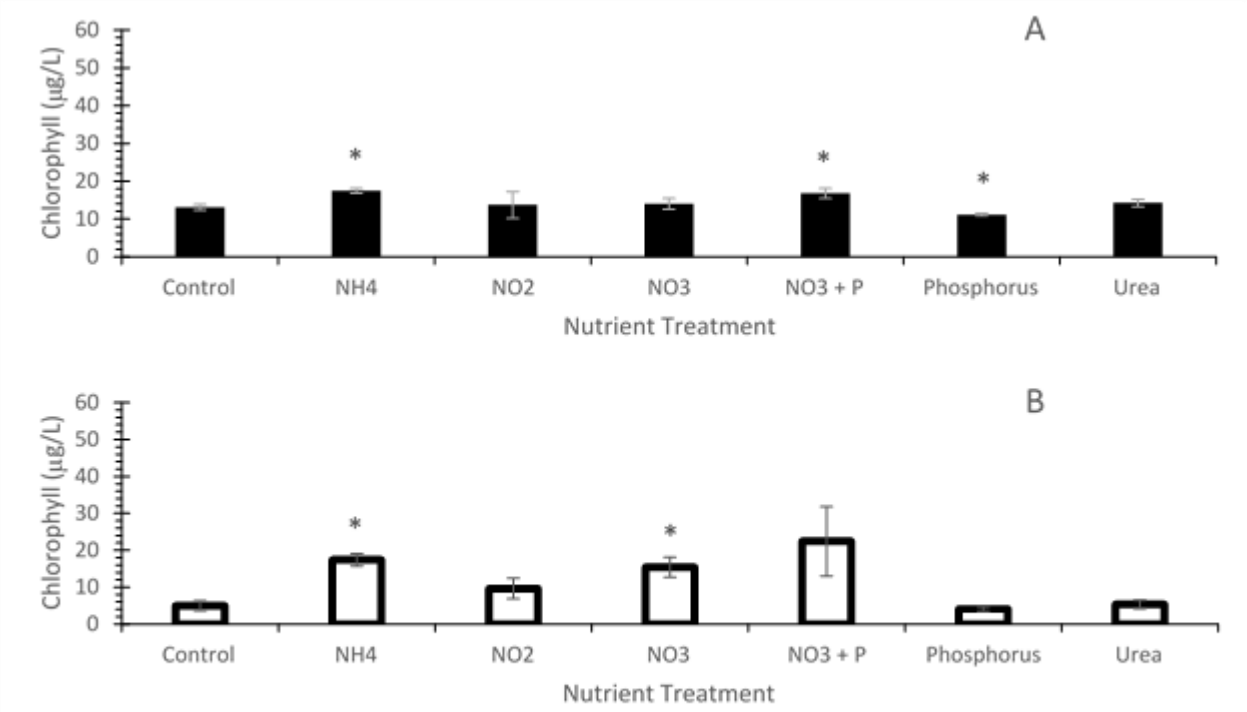

Figure 2 Average Chlorophyll measured on 04/30/18 (Spring) in the Marsh (A) and in the Beach (B). Error bars represent Standard Deviation

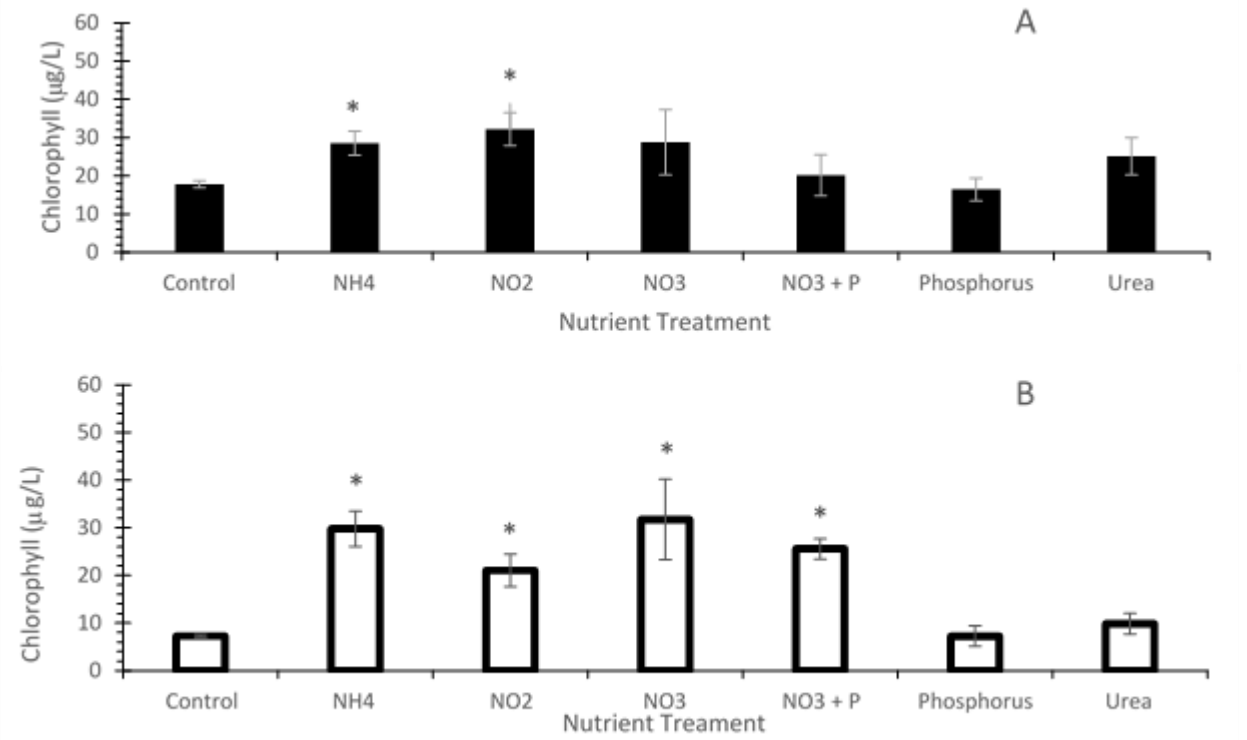

Figure 3 Average Chlorophyll measured on 08/18/18 (Summer) in the Marsh (A) and in the Beach (B). Error bars represent Standard Deviation

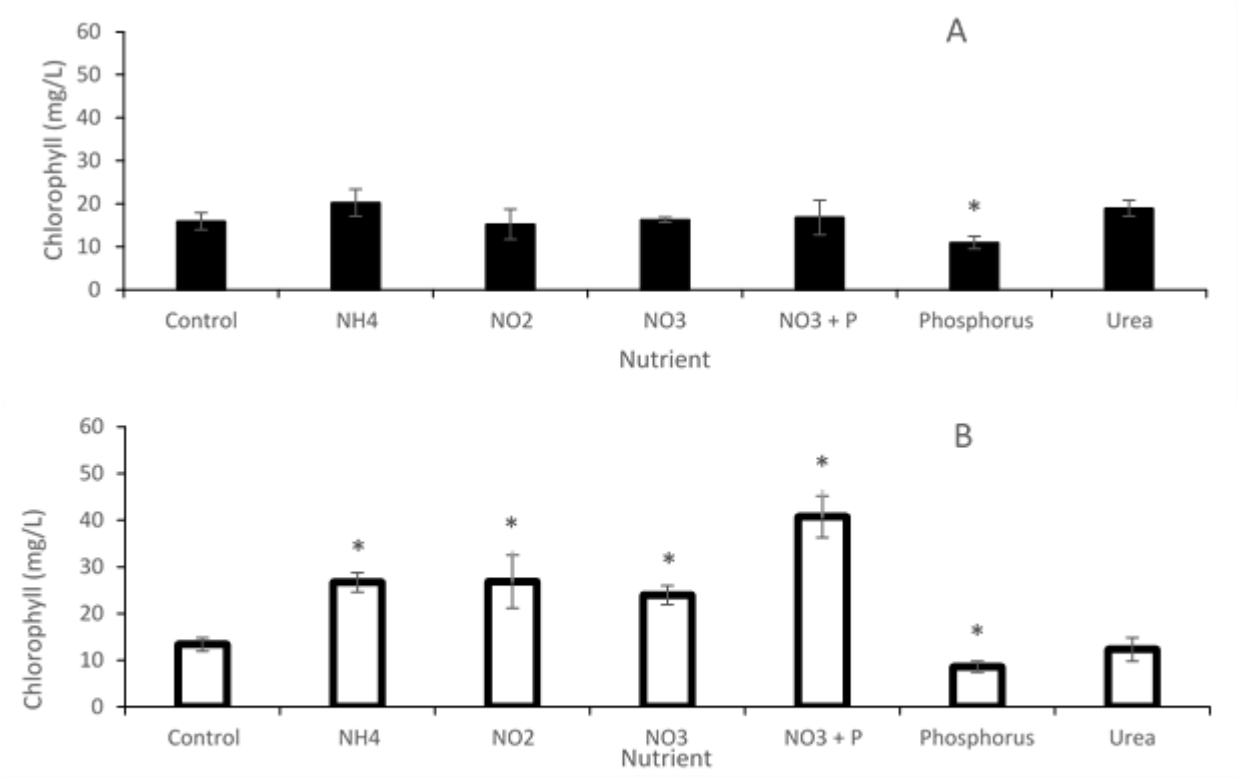

Figure 4 Average Chlorophyll measured on 11/04/18 (Fall) in the Marsh (A) and in the Beach (B). Error bars represent Standard Deviation

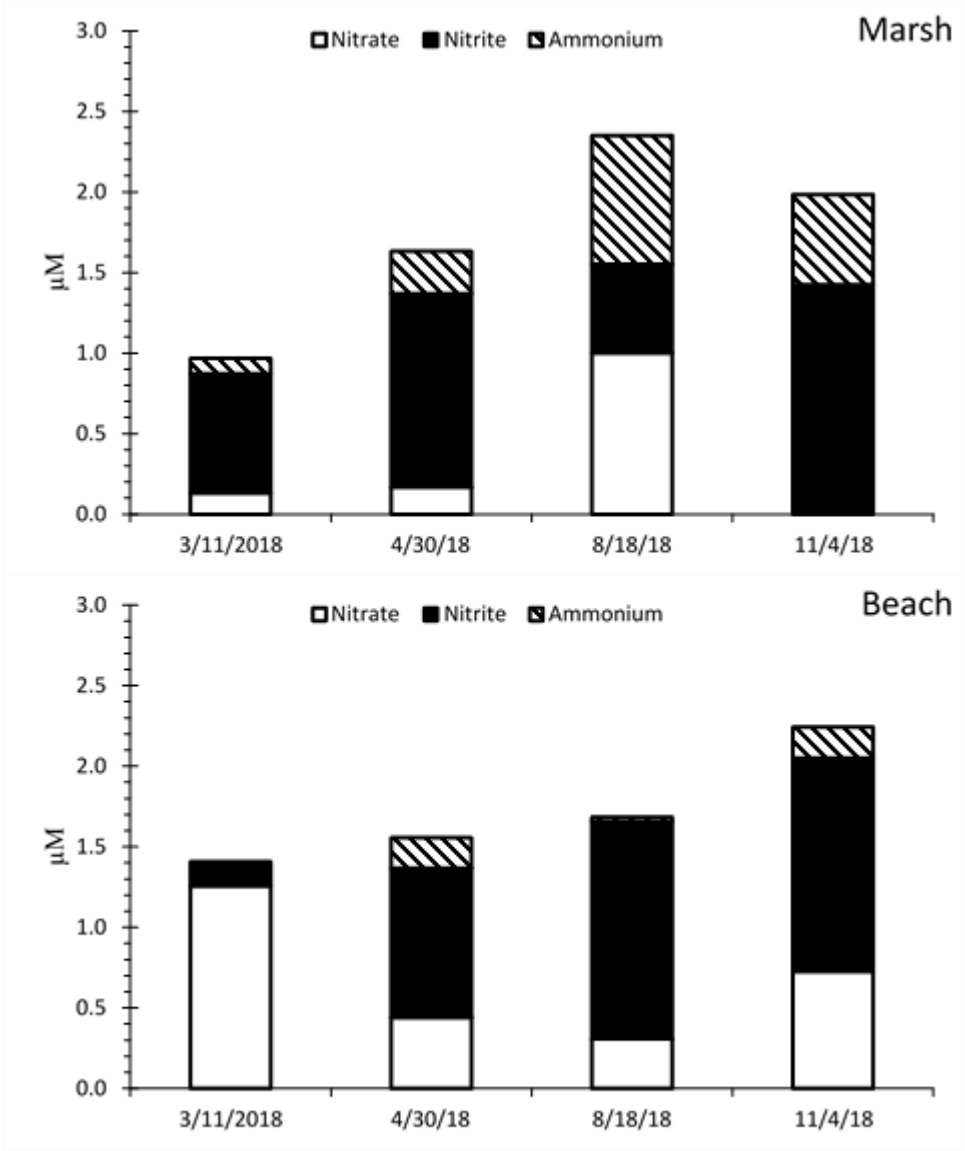

Figure 5 Speciation of the DIN (Dissolved Inorganic Nitrogen) in the Marsh (A) and Beach (B) on each sample date

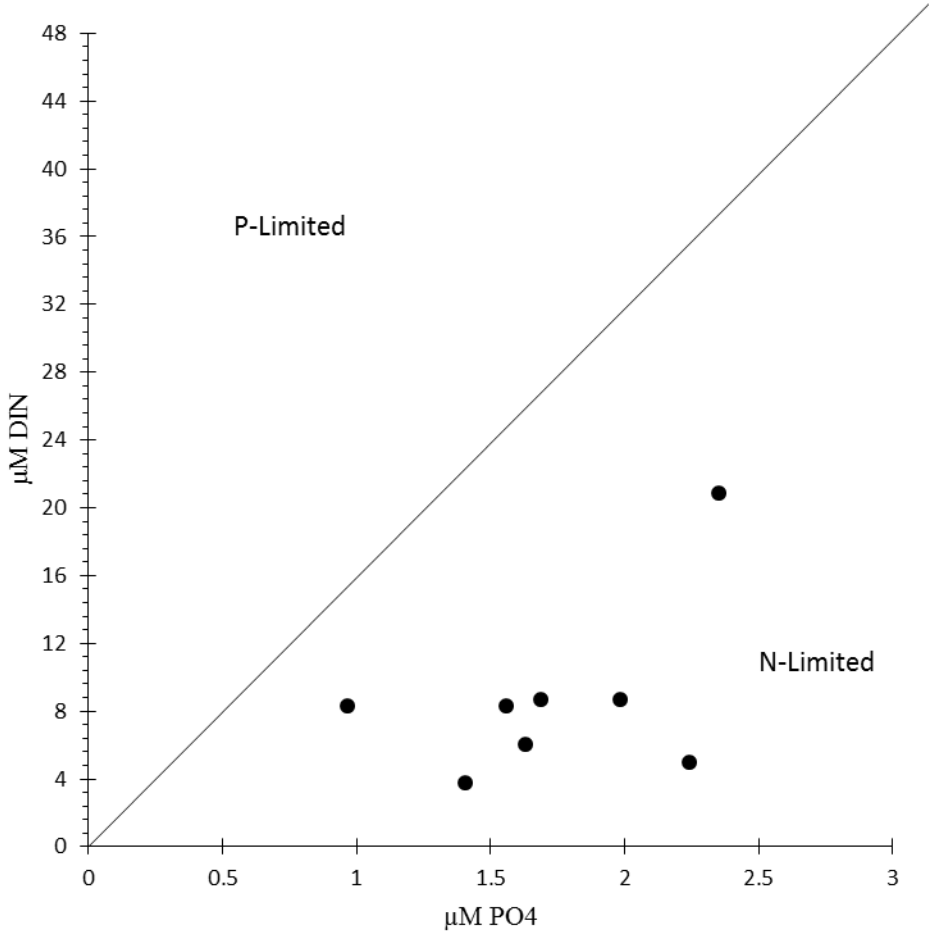

Figure 6 Graph shows the molar ratio between DIN and Phosphorus from each sample date and location. The line represents the 16:1 Redfield molar ratio of N:P

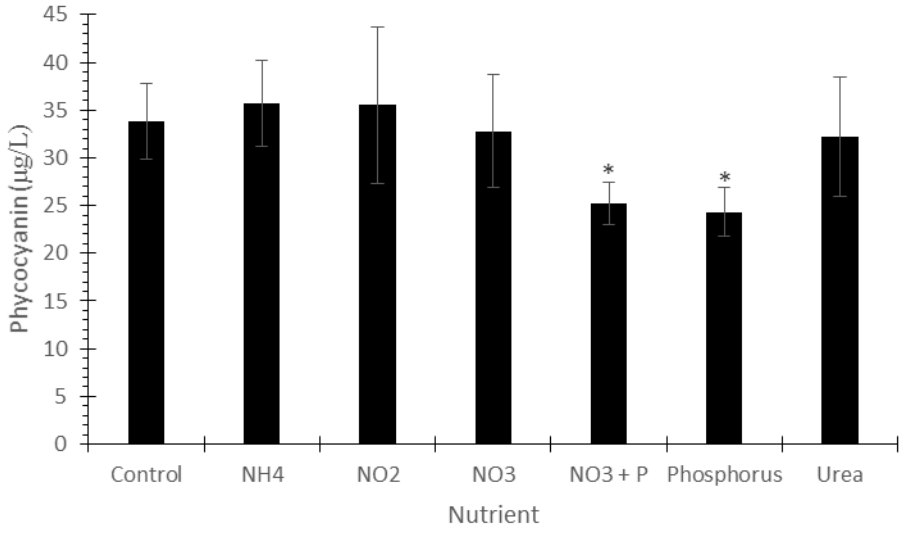

Figure 7 Average Phycocyanin measured in the marsh on 11/04/18 (Fall) Error bars represent standard deviation

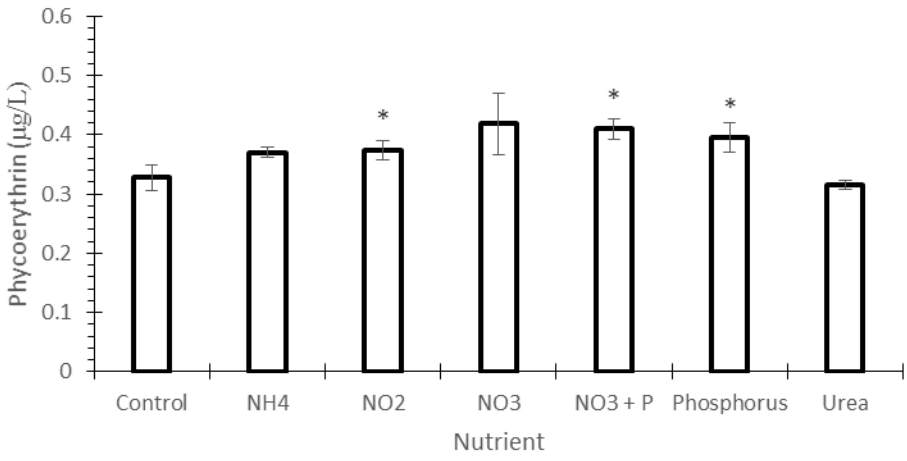

Figure 8 Average Phycoerythrin measured in the beach on 11/04/18 (Fall) Error bars represent standard deviation

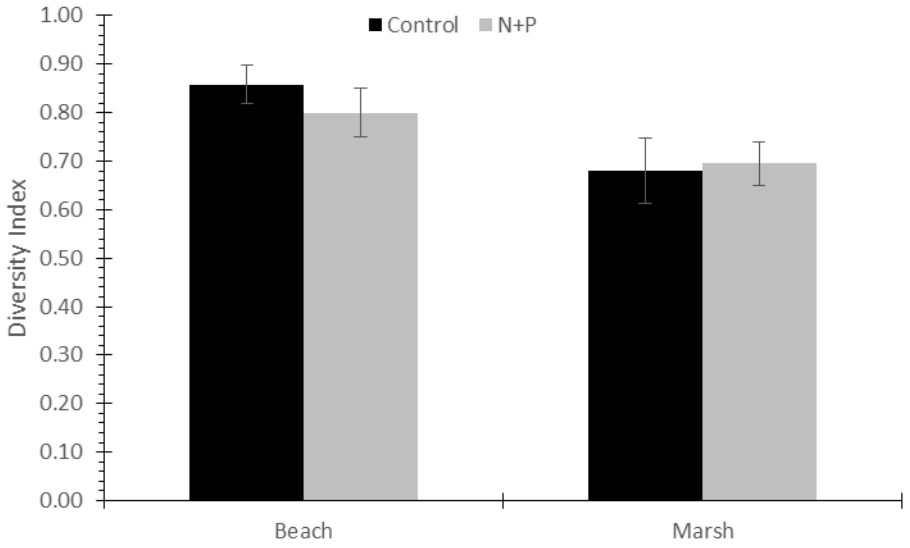

Figure 9 Diversity Index of the phytoplankton community taken of the Fall Control and N + P treatments. Error bars represent standard deviation

Table 1 Table showing the measured physical data on each date at each environment. Measurements were conducted in the field

Marsh	3/11/2018	4/30/2018	8/18/2018	11/4/2018
Temperature (°C)	12.3	19.4	28.1	15.1
Salinity (ppt)	27.5	22.5	27.1	21.4
Chlorophyll a (µg/ L)	12.54	11.78	40.16	16.2
DO mg/ L	6.2	6.4	3.17	6.15
DO %	68	78	46.2	70.9
Turbidity NTU	5.61	8.51	47.2	16.8

Beach	3/11/2018	4/30/2018	8/18/2018	11/4/2018
Temperature (°C)	12.7	17.7	27.9	15.1
Salinity (ppt)	34.4	35.2	34.4	32
Chlorophyll a (µg/ L)	8.51	23.29	38.04	13.76
DO mg/ L	10.1	7.3	6.16	7.35
DO %	115	93	95.1	89.5
Turbidity NTU	18.1	28.3	32.5	29.4