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Ecology-Scholtens-Summer 2008

Effects of nitrogen content on herbivory of *Ammophila brevigulata* by dune-dwelling herbivores

Abstract

We examined the effects of nitrogen levels in the dune grass, *Ammophila brevigulata*, on herbivory by the insects at Sturgeon Bay Dunes located on northern Lake Michigan. Samples of the grass were collected and analyzed for their nitrogen content as well as their level of herbivory. Additionally, the dunes were characterized by their soil temperature, wind velocity, humidity, and air temperature at various distances from the shore to show the environment which the insects and *Ammophila* are living in. We found no statistical correlation between herbivory and distance from shore, but increasing nitrogen levels were generally observed at distances located farther from the shore.

Introduction

Why have the insect populations at Sturgeon Bay Dunes have been found scouring the Lake Michigan shoreline to find water insect carcasses to consume? Could it be a taste preference or an unconscious need for more nitrogen in their diet? Is the plant life alone located on the dunes not sufficient to wholly sustain the herbivores living there? Our hypothesis is that the dune herbivores will choose more nitrogen rich foods (such as dead aquatic insects or higher nitrogen plants) and we will see higher levels of damage to these plants.

Because sandy soils are notoriously low in nutrients as well as water, this experiment focused on herbivory on the beach grass, *Ammophila brevigulata* (Valentine 2000). The nutrient levels in the sandy soil at Sturgeon Bay are much lower because of the lack of organic matter (Buckingham et al) and closer to the shore, the lower the nitrogen, carbon, and water levels (Lichter 1998). Therefore, the experiment was developed to focus on herbivory differences on *Ammophila* at varying distances from the shore. To provide further insight on data obtained, plot properties were observed and documented such as temperature, wind speed, and vegetative composition.

While nitrogen is the limiting nutrient for many insects, studies have shown contrasting information between the nitrogen levels of available food stuff and consumption by herbivores. Although in one study different insects were found in higher concentrations on corn plants containing higher nitrogen levels (Bastos et al. 2007), no correlation was observed between soil nitrogen levels of California tomato farms and their subsequent herbivory (Letourneau et al. 1996). On the other hand, beet armyworms were found to show a preference for plants of higher nitrogen content. Those that fed on the plants of lower nitrogen content ingested greater amounts of plant material (Chen et al. 2008).

Many of the insects living on the dunes are members of the order Orthoptera, which includes grasshoppers, locusts, crickets, and katydids. Because the diet of these dune dwelling insects is not yet thoroughly understood, it is hard to predict the extent of herbivory on the grasses in our study. Although many insects are generalists, some of the insects found at Sturgeon Bay dunes are specialists and therefore may prefer to eat only the *Ammophila*, no matter its nutrient levels (Rentz and Su 2003). Certain insects may prefer as well to only consume the plant matter located near their central home. Following the study by Chen and others in 2008, we would expect more of the food source (*Ammophila)* herbivorized to fulfill metabolic nitrogen requirements. Other insects localized at farther distances from the shore may have a preference for different vegetation even with the higher nitrogen levels in the dune grass.

Because of the high concentration of *Ammophila* on the dunes relative to the other plants present, any consumption observed of the beach grass could be reasoned by the fact that insects could have chosen to eat more of the available food source. This makes it a good subject for our study because it is abundant and a prevalent source of food for dune herbivores. We hypothesized that nitrogen is indeed the limiting nutrient on the dunes and larger extents of herbivory would occur in more nitrogen-rich areas. The fact that beach grass mostly dominates some of these areas should not have a negative effect on the data. The amount of plant material removed from the leaves by herbivory should relatively increase with increasing nutrient contents.

Because plant nitrogen levels change in relation with age of the plant and the type of species, composition of the soil may not be the only factor affecting differences in herbivory on the different plants because the insects are targeting nitrogen-rich plants. If dune-dwelling herbivore diets do depend on the nitrogen composition of the food being ingested, then herbivory should be the highest on younger leaves (Matson 1980). The ages of the plants used in this study were not determined however because the nitrogen of the plant clumps that were taken were analyzed to account for this. If a plant sample taken farther from the shore has lower nitrogen levels than individuals surrounding it or one closer from the shore, it would likely be an older plant.

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Insects are also shown to weigh the costs and benefits of eating a plant. If the secondary metabolites of ingested plant materials are toxic, then the insect will not eat it if the benefit of the plant nutrients is smaller than the extent of negative affects upon the individual (Behmer et al. 2002). If *Ammophila* does have toxic effects to different insects then no matter how satiated the herbivores become or the plant nitrogen content, the herbivores not consume more than their bodies allow them to.

Materials and Methods

Our study site was Sturgeon Bay Dunes in Emmet County, MI. The area, not heavily frequented by tourists, was relatively undisturbed and therefore ideal. At the bottom, top, and back of the first dune, as well as the crests of the two dunes located behind the first, data were collected on the environment at four random distances from one another. Soil temperature was recorded at 0, 5, and 40 centimeters located below the soil surface and air temperature and wind speed were measured at 0, 5, 40 and 100 centimeters above the ground. Vegetative composition was observed by laying out one-meter quadrat frames at each point and recording the percentage of ground cover by the leaves and stem of each plant species.

Four to seven leaves of *Ammophila* plant clumps were collected from five transects located ten meters from one another at distances of 0, 32, 50, 75, 100, and 125 meters from shore. The grass from each sampling site was immediately placed in a plastic Ziploc bag labeled with the transect number and the distance from the shore.

Once all samples had been collected, all of the leaves were observed for any signs of herbivory. Those leaves which had been eaten by dune-dwelling insects were laid on a pieces of white paper labeled with the corresponding transect numbers and distances from the shore. The leaves were held to the paper with scotch tape to prevent them from rolling up, placed next to a meter stick, and had their picture taken. The pictures were then uploaded onto a computer and overlain with a grid on Photoshop so the area removed from each leaf could be determined. The grid consisted of squares 0.1 cm^2 each. The squares were then counted where leaf matter had been removed.

After the herbivory on the samples had been determined, their nitrogen amounts were analyzed. To determine these nutrient levels, the separate samples were frozen at negative eighty degrees Celsius for one hour and then placed in a lyopholizer overnight. The samples were then ground in a Spex 8000D mixer mill for one minute, placed in individual vials, and weighed in a tin capsule. The nitrogen and carbon percentages were then determined by the Dumas dry combustion method in a Costech EA4010 analyzer.

Results

Herbivory was only observed on five plants out of the thirty samples collected. Six cm^2 total from the samples collected at fifty meters from shore was eaten, 18.2 cm^2 from 75m, and 10.4 cm^2 from 125m. As observed in figure one, a slight increase in herbivory with increasing distances from the shore was indicated. But with a p-value of

0.771, there was no statistically significant increase in herbivory on plants with increasing distances from shore.

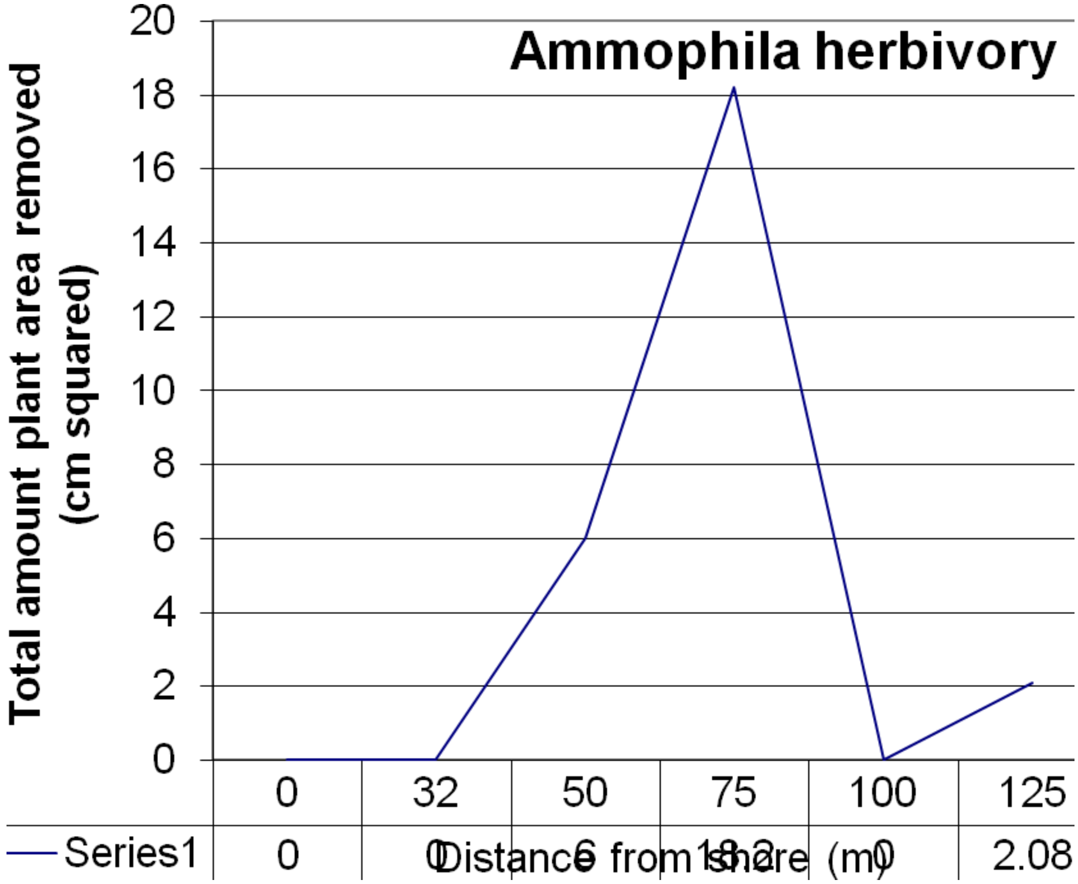


Figure 1: *Ammophila* herbivory with increasing distances from shore.

As observed in figures 2, a significant increase in wind velocity was observed with increasing height above the soil surface although no trend was observed with increasing distances from shore.

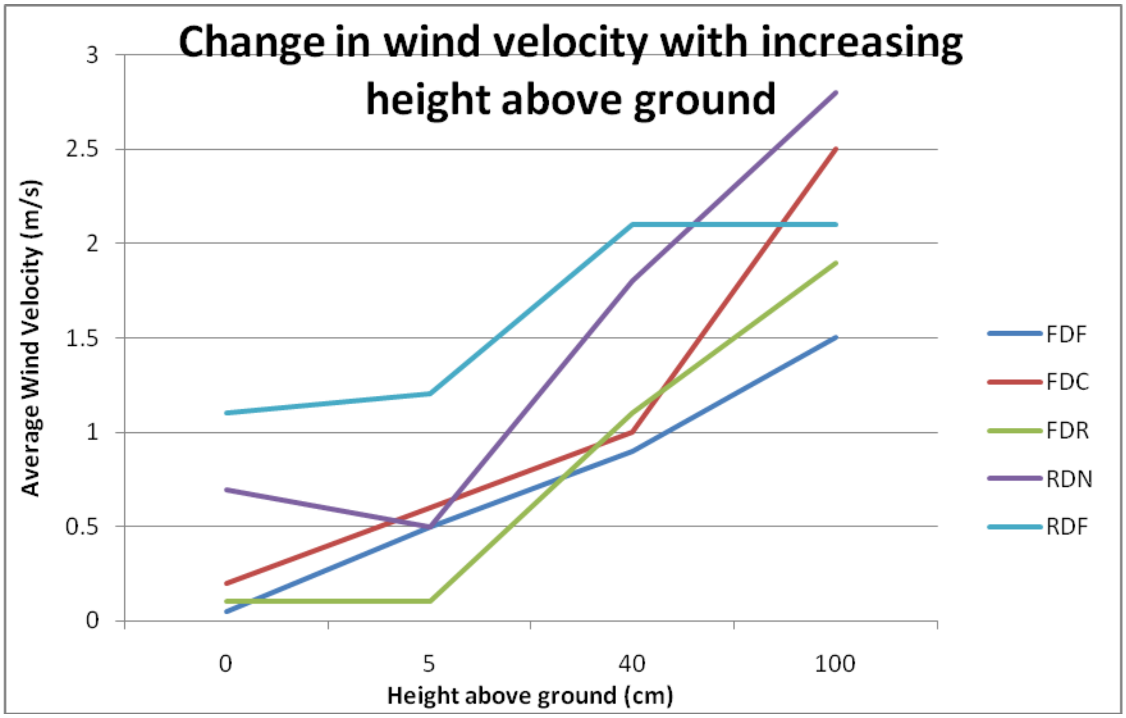


Figure 2: Wind velocity with increasing heights above the soil surface.

As can be seen from figure 3, no general trend was observed in humidity with increasing heights above ground or with increasing distances from shore.

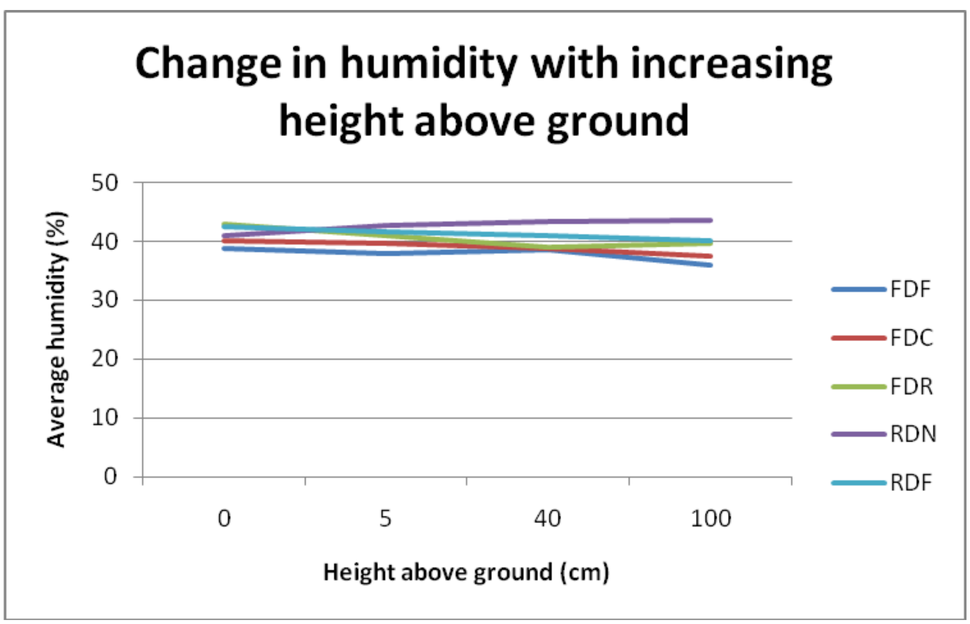


Figure 3: Changes in humidity observed over increasing heights above ground.

A significant increase for percent ground cover by *Ammophila* was observed at increasing distances from shore. This trend can be observed from the data plotted in figure 4.

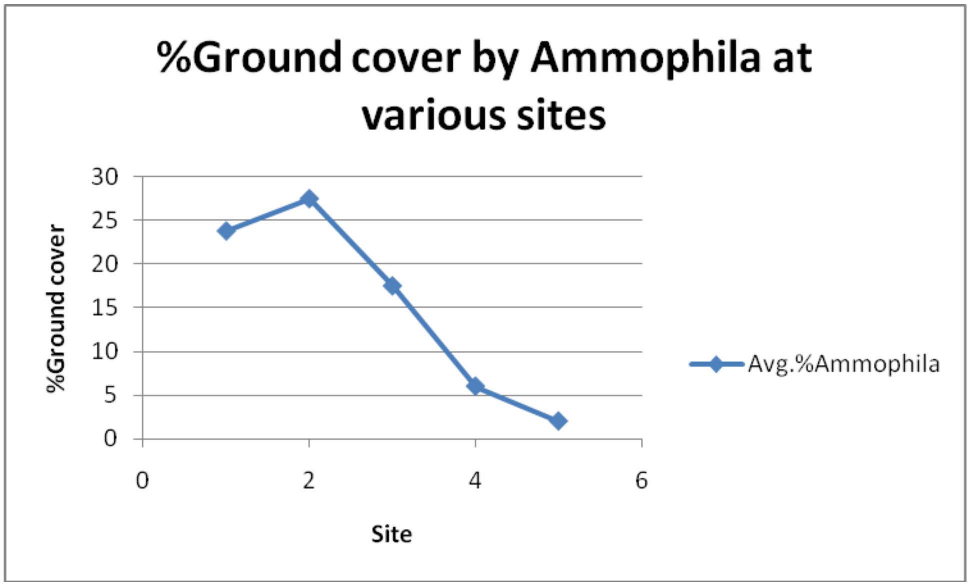


Figure 4: Site 1: FDF, 2: FDC, 3:FDR, 4:RDN, 5: RDF. Percentage ground cover by *Ammophila brevigulata at various distances from the shore.* (The higher the site number, the farther from shore the site).

A statistically significant increase of nitrogen composion was observed in *Ammophila* plants with increasing distance from shore as can be observed in figures six and seven, with an f-value of 4.8562, a p-value of 0.036, and an α-value of 0.05.

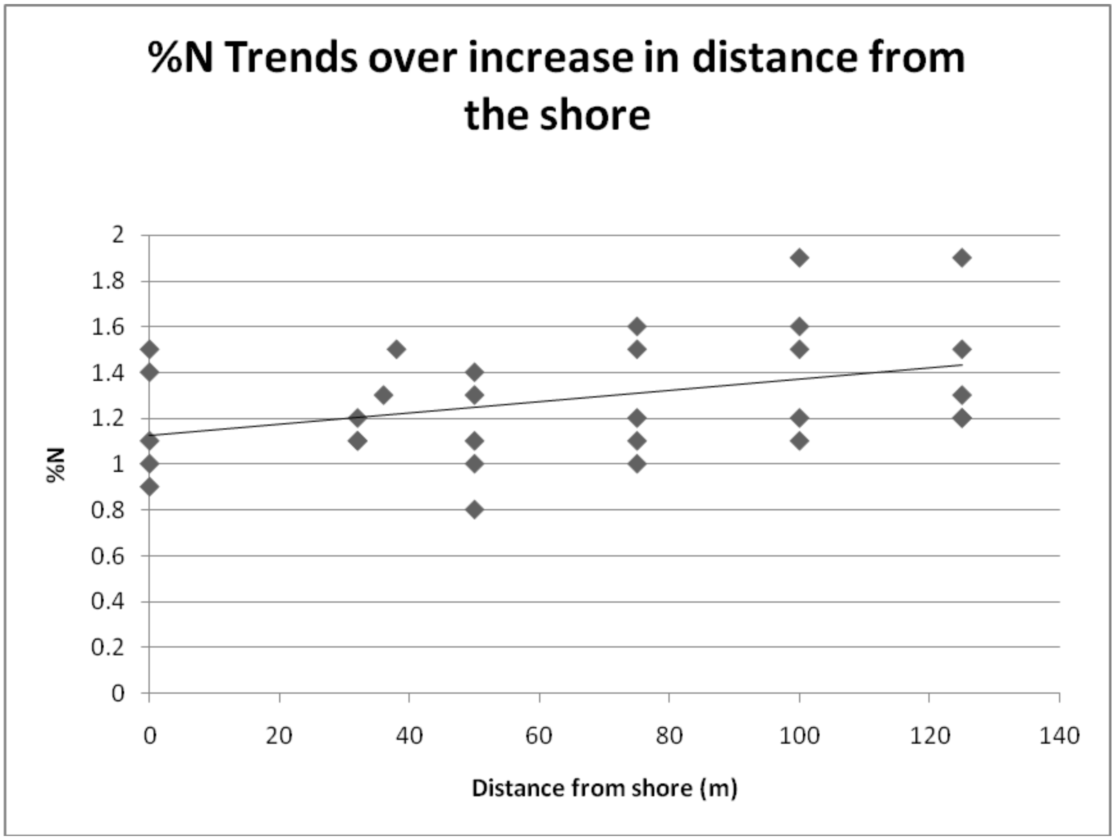
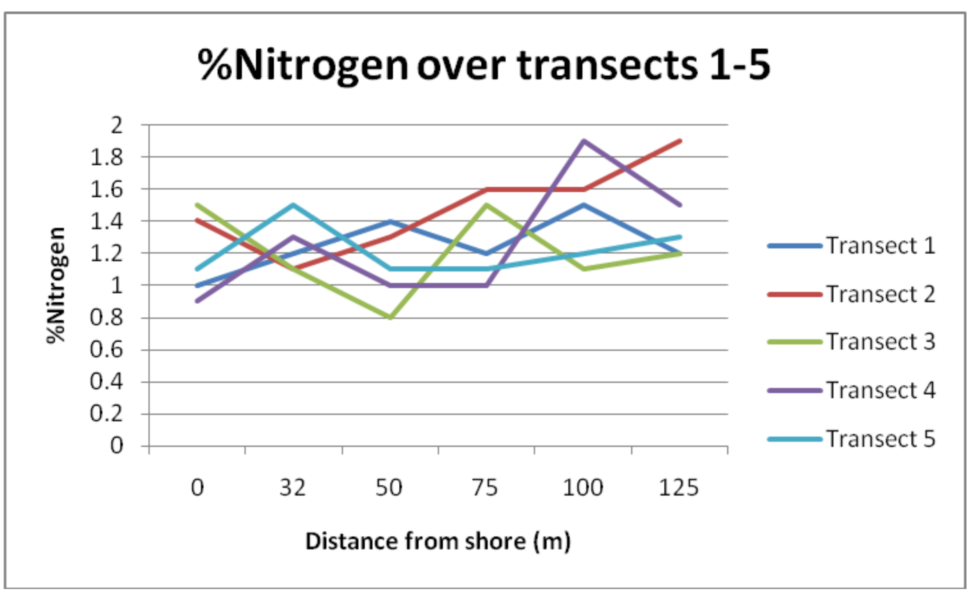


Figure 5(above) and Figure 6(below): A general increase in nitrogen percentages in *Ammophila brevigulata* is observed with increasing distances from the shore. R^2-value: 0.148; F-value: 4.8562; P-value: 0.036; df:28.



Discussion

With a p-value of 0.771, the data relating herbivory on the beach grass, *Ammophila brevigulata*, to distance from the shore showed no statistical difference. However, the general trend observed on the graph showed a clear increase in herbivory on beach grass from zero to seventy-five meters, suggesting that there is some relation between distance from shore and herbivory on beach grasses. Because only seven out of the thirty collected plants showed any signs of damage by herbivory, we would expect to see a statistical difference in herbivory at different distances from the shore if more samples were taken at more spots on different transects as well as more samples per transect at each site. Our hypothesis suggests that if the study were to be repeated but five samples were collected at each distance from the shore for each transect instead of only one, there would be a statistically significant increase in herbivory on those plants which were located farther from the shore. For this, we would expect to find more herbivory on those grasses located 100m from shore as well. No herbivory was observed for those samples collected 100m from the shore was likely due to random sampling area: a plant that had not yet been eaten was collected, and that if others had been taken, herbivory would have been observed. Whether that herbivory would have been greater or less than that observed at 75m cannot be predicted solely on the current herbivory data.

Knowing that younger plants tend to have higher nitrogen concentrations than their older counterparts grown under the same conditions, more spots resulting from herbivory would likely be seen on older plants. This is because they will have been eaten when they were young, growing shoots. If the majority of the leaves collected belonged to younger leaves, then perhaps the local insects had not yet taken the opportunity to eat the young leaves (Mattson 1980).

The different nitrogen levels that would have occurred because of age differences in the plants were accounted for however by finding the nitrogen percentages for each sample. Our data indicate a statistically significant increase in nitrogen percentage in *Ammophila brevigulata* over increasing distances from the shore, supporting the work of Lichter (1998). With increasing nitrogen composition, insects would, in theory, need to eat less of the plant to acquire their daily metabolic needs than they would of the same species closer to the shore. Because of this, insects staying in a certain area would be more likely to eat less of the plant closer to home that is richer than nitrogen than more of the plant further away less rich in nitrogen because moving not only takes time and energy, but also exposes the herbivore to more predation by other animals (Behmer et al. 2002).

Our data suggests that *Ammophila* is the most prevalent source of organic matter near the shore and that farther from the shore, other species are more prevalent. If *Ammophila* is the dominant source of vegetation, then herbivory would likely increase as its nitrogen composition increases. But, if it no longer is the most prevalent food source, then perhaps herbivores switch to other the other, more prevalent food sources located farther from the shore, as long as the nitrogen levels of those species are equal or greater to those had by *Ammophila*. It would be useful in the future to document the nitrogen percentages of those species other than *Ammophila* that dominate the older dunes and see if their nitrogen composition varies greatly from that observed in *Ammophila* at the same distance. Nitrogen percentages equal to or above those observed of the beach grass would support this, but percentages below would not support the proposed hypothesis. It is probable that the higher nutrient levels located farther from shore support plants which can out-compete *Ammophila* so it is therefore only dominant in the harsh sandy environment that cannot maintain many other plant species (Lichter 1998).

It is also possible that *Ammophila* contains such low nitrogen levels that any changes observed are insignificant because dune-herbivores are driven toward scavenging and feeding on the dead bodies of aquatic insects (Raubenheimer and Simpson 2003)

Although the data regarding temperature and air humidity were either inconclusive or not relevant to *Ammophila* presence, abundance, and herbivory, the increasing wind velocity over an increase in height above the ground likely influences the grazing height of insects, causing them to graze lower on the leaf. This could be supported if *Ammophila* plants that showed signs of herbivory were collected and the leaf area removed could be analyzed based upon how far up the leaf it was located.

Although our hypothesis that herbivory of the dune grass, *Ammophila brevigulata*, increases with increasing nitrogen concentrations remains to be statistically supported, the data collected shows promise. Future studies using larger samples and distances even more varied and far from the shore could provide more statistically significant data and further insight into the matter.

Literature Cited

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