ABSTRACT

The dune environment is harsh, lacking in many nutrients. One such nutrient is nitrogen. Insects can sense how much nitrogen is in a plant, and typically this plays a key role in determining herbivory of the plant. Our hypothesis was that plants growing farthest from the shoreline should not only have higher nitrogen content, but also have more herbivore damage because of these higher nitrogen levels. Nitrogen levels did, in fact, increase significantly with distance from the shoreline, but there was not a significant increase in herbivory with an increase in distance from the shoreline. There was, however, a trend towards increased herbivory as distance from shoreline increased, leading us to conclude that perhaps with more data points we would find a significant correlation between distance from shoreline and herbivory. It is probable that confounding variables besides nitrogen (for example increased plant diversity indexes) contributed to the non-significant results.

INTRODUCTION

The Huron Lake Locust (*Trimerotropis huroniana*), is a locust found in the dunes of Wilderness State Park's Sturgeon Bay, in the northwest corner of the lower peninsula of Michigan. This locust has been the subject of much research in the past few years, as it exhibits a characteristic not usually seen in locusts: scavenging (Scholtens, personal comment). After careful observation it was noticed that many dune insects scavenge, and this may contribute greatly to nutrient flow on the dunes.

The sandy soil of the dunes is not well suited for holding nutrients and water (Maun, 1993), due to the large particle size of the sand. One of the nutrients lacking in the dune environment is nitrogen (Buckingham et. al., 1994). Nitrogen is a key element for all living organisms, needed for the basic synthesis of amino acids and proteins. Moreover, it is well known that aquatic environments (and hence Lake Michigan) have higher nitrogen levels than their terrestrial counterparts (Grant, personal communication). Due to this knowledge, it was hypothesized that the low nitrogen levels on the dunes play a key role in scavenging behavior. So, when dune insects were seen eating mostly dead aquatic insects that have washed up on shore, researchers confirmed this hypothesis. The dune insects are supplementing their diet with the nitrogen rich carcasses of the aquatic insects (Scholtens, personal communication).

It is known that on the dunes, and in soils in general, the older the soil the more nutrient enriched it is (Olff et. al., 1993). This means that the nitrogen levels on dunes will increase as one travels farther away from the shore (and hence towards older dunes) (Lichter, 1998). It is also known that plant nutrient composition is an affect of the soil composition it is growing in. It has been shown, in fact, that plants growing in soil with higher nitrogen levels contain higher levels of nitrogen than plants growing in nitrogen poor soil (Valentine et. al., 2000). Because of this we should be able to conclude that plants growing farther back in the dunes will have higher nitrogen content than plants growing closer to the shoreline.

One plant that is found growing throughout open dunes is *Ammophila breviligulata*. This is also a plant that many insects include in their diet. It has been shown that insects can actually tell which plants are higher or lower in nitrogen, and that they prefer to eat plants with higher nitrogen levels (Bastos et al., 2007). Because of this our hypothesis is that plants growing farthest from the shoreline should not only have higher nitrogen content, but also have more herbivore damage because of these higher nitrogen levels.

METHODS

We first went to the dunes of Sturgeon Bay and found *A. breviligulata* plants that were closest to and farthest from the shoreline of Lake Michigan (going perpendicular to the shore). We recorded this maximum distance (125 meters), and the minimum distance (0 meters), subtracted the two giving us the total distance (125 meters), then divided by five to give us six data points (at 0, 25, 50, 75, 100, and 125 meters) along our sample transect. We did this across 5 transects, each 10 meters apart running perpendicular to the shoreline.

Next we took a sample of one *A. breviligulata* plant from each of the distances in each transect. However, the 25 meter spot was in a low, wet area that was completely void of *A. breviligulata*, so we took this measurement at the nearest point with a plant, which was anywhere from 32 to 38 meters (three transects were from 32 meters, one was from 36 meters, and one was from 38 meters). After we collected our samples from all 60 spots we brought them back to camp in plastic bags for analyses.

Upon return we went through all of the leaves and examined them for any signs of herbivory. Of all the leaves, only seven (all from different plants) showed signs of herbivory. With a digital camera we took photographs of each of these leaves, one at a time, on a blank background with a meter stick next to it for size comparison. These pictures were then uploaded to a computer, and overlaid with a graph. Later we counted how much area of the leaves was eaten.

After taking pictures of the plants with herbivory damage we cut four leaves from each plant into about two centimeter long strips and placed them in a wax bag. We took these samples and froze them for an hour in a -80°C, then transferred them to the lyophilizer for 24 hours. After this we used a grinding machine to pulverize them to a fine dust. We put this into glass vials labeled with the samples locations (which transect and what distance along the transect, and whether herbivory was observed on them or not), and gave them to Michael Grant, an analytical chemist as University of Michigan Biological Station, to perform nitrogen analysis. He did this via mass spectrometry using a Costech AE 4010 to prepare the samples, and then he placed them into a Thermo Finnigan Delta XP to take measurements of the samples. This instrument has a precision for stable isotope ratios of nitrogen of .3‰. He then analyzed the data on our samples via ISODAT 21, a computer program, and reported back to us the percent nitrogen of the samples. We used Microsoft Excel 2007 to run various tests on the data.

RESULTS

First we graphed the percent nitrogen levels as a function of distance from the shore, to see if the nitrogen levels increased as we got farther away from shore (Figure 1). The equation of this line was $y = 0.0025x + 1.123$, and the R^2 value was 0.151. The p-value for this was 0.037 (Table 3). The mean value and standard deviation of nitrogen levels for each location can also be found in Figure 3.

We also graphed the total and average amount of leaf missing for each distance from shore (Figure 2) and tested if there was a statistically significant increase in herbivory on plants as we got farther away from the shoreline (Table 3). The equation for the line of total amount missing was $y = 0.0729x + 1.1248$ with the R^2 value being 0.202. The equation for the line of average amount missing per plant was $y = 0.0146x + 0.225$, and the R^2 value was again 0.202. The p-value for increasing herbivory levels as we got farther from the shoreline was 0.372 (Table

4). The mean value and standard deviation of area missing for each location can also be found in Figure 4.

DISCUSSION

There was a significant increase in the amount of nitrogen found in *A. breviligulata* as distance from the shore line increases. However, we were surprised to notice a trend suggesting that the lowest point of nitrogen levels is not at the shoreline, as we had predicted, but around the 30 meter mark. Upon further investigation it was decided this could be due to a variety of elements, and would prove interesting to look at in a later study. The trend seems to be different than we expected because we did not factor in that there are two sources of nitrogen: aquatic and terrestrial. This may explain why there is more nitrogen near the front and back of the dune, with a dip in the middle. *A. breviligulata* near the shoreline (at 0 meters) is obtaining more nitrogen from the aquatic environment and hence has slightly more elevated levels than plants found immediately inland. On older dunes, the soil has been building up nitrogen over longer periods of time from a variety of sources, including old, dead plant matter. This explains the higher levels of nitrogen in the rear of the dunes (Olff, et. al., 1993; Lichter, 1998). Since we know nutrient levels in plants are directly related to the nutrient levels in the soil they are growing in (Valentine, et. al., 2000), this explains why the levels of nitrogen seem to steadily increase as we go farther back after that middle “dip” where the dunes are too new to have accumulated much nitrogen, but too far from the shoreline to be obtaining it from the lake (Buckingham, et. al., 1994).

The lack of significance in levels of herbivory is rather surprising, as we found that the levels of nitrogen did, indeed, increase, and it is known that insects can and do choose plants that they eat based on nitrogen levels (Bastos et al., 2007). Since there was a trend in our results that looked like herbivory *did* increase with distance from shoreline, we decided to go back over our methods and realized that the low number of plants with herbivore damage (seven total) was a likely cause for these insignificant results. If we had sampled more points we may have found significant results. Some ways we could have improved this include taking more leaves at each point along the transect; measuring more transects and stretching our study out across more of the beach, making them more frequent; and taking the whole plant at each point along our transect instead of just a few leaves.

We also know, from a class wide lab, some other trends on the dunes that could have contributed to the lack of significant herbivory results. It seemed the trend was as we went farther away from the shoreline on the dunes the percent of ground covered increased (however slightly) as well as the diversity indexes increased. One would think that with increasing ground cover you would find increasing herbivory (due simply to the fact that there is more opportunities for herbivory with more ground cover). However, since the ground cover increased *and* the diversity indexes increased, perhaps this is why we saw less herbivory on *A. breviligulata*. There is more opportunities for herbivory, but on multiple different plants, not just on the one we sampled. This could quite possibly be the reason we did not see significant results.

There are many opportunities for future studies on this subject. One is to try growing *A. breviligulata* in a controlled environment where we can manipulate the nitrogen levels in the soil, and test herbivore damage on those. That would eliminate the problems of other insects eating other plants, as well as inconsistent nitrogen levels and other factors that could be influencing the lack of herbivory. Another idea is, as stated before, to take more samples from each transect, take more transects by making them spaced closer together, and/or space them farther apart to have more transects. It might also be interesting to look into other nutrient levels and whether they affect herbivory levels.

LITERATURE CITED

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Figure 1. Percent nitrogen levels in *Ammophila breviligulata* as a function of distance from the shoreline.

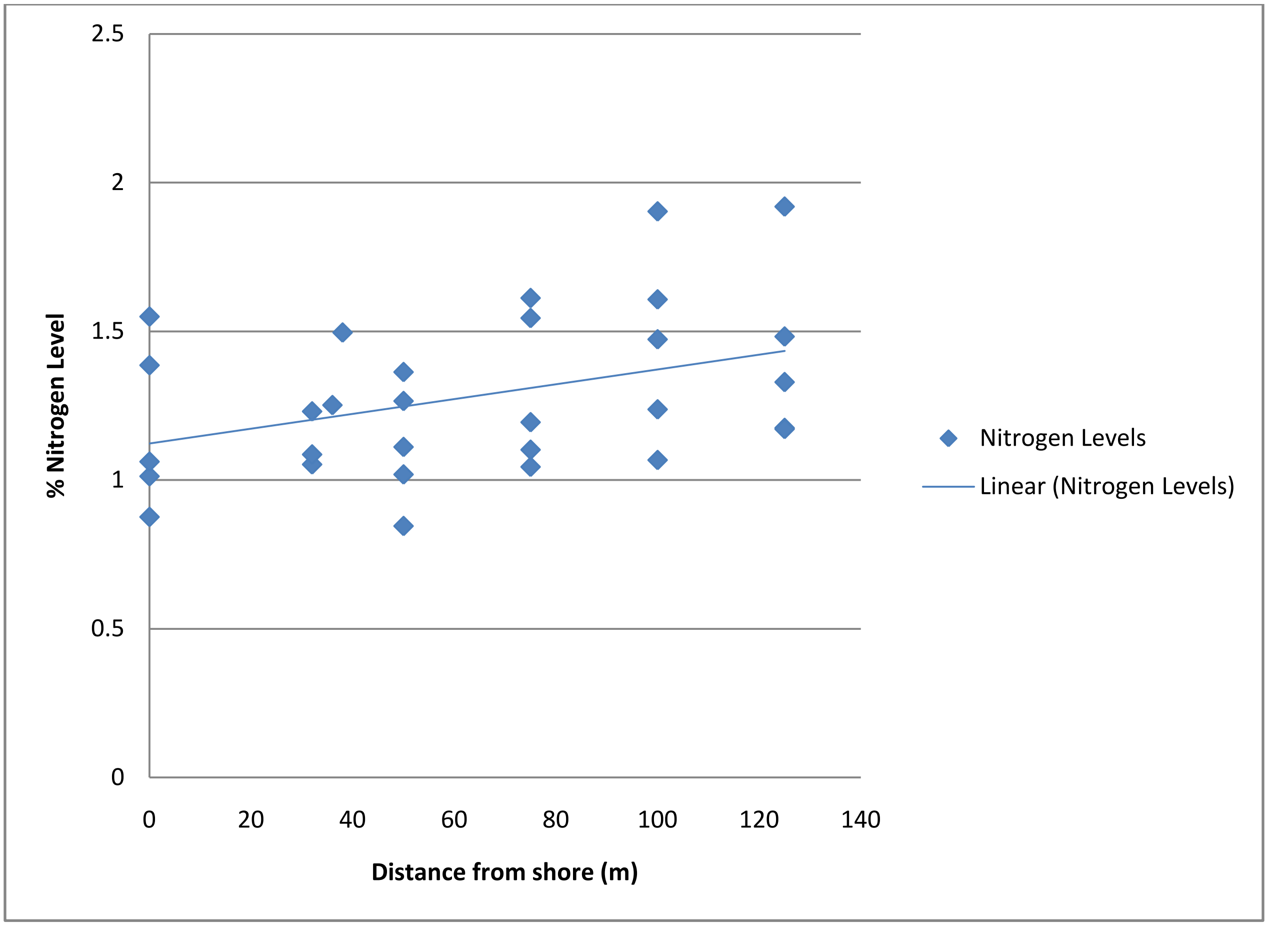

Figure 2. Herbivory levels in *Ammophila breviligulata* as a function of distance from the shoreline.

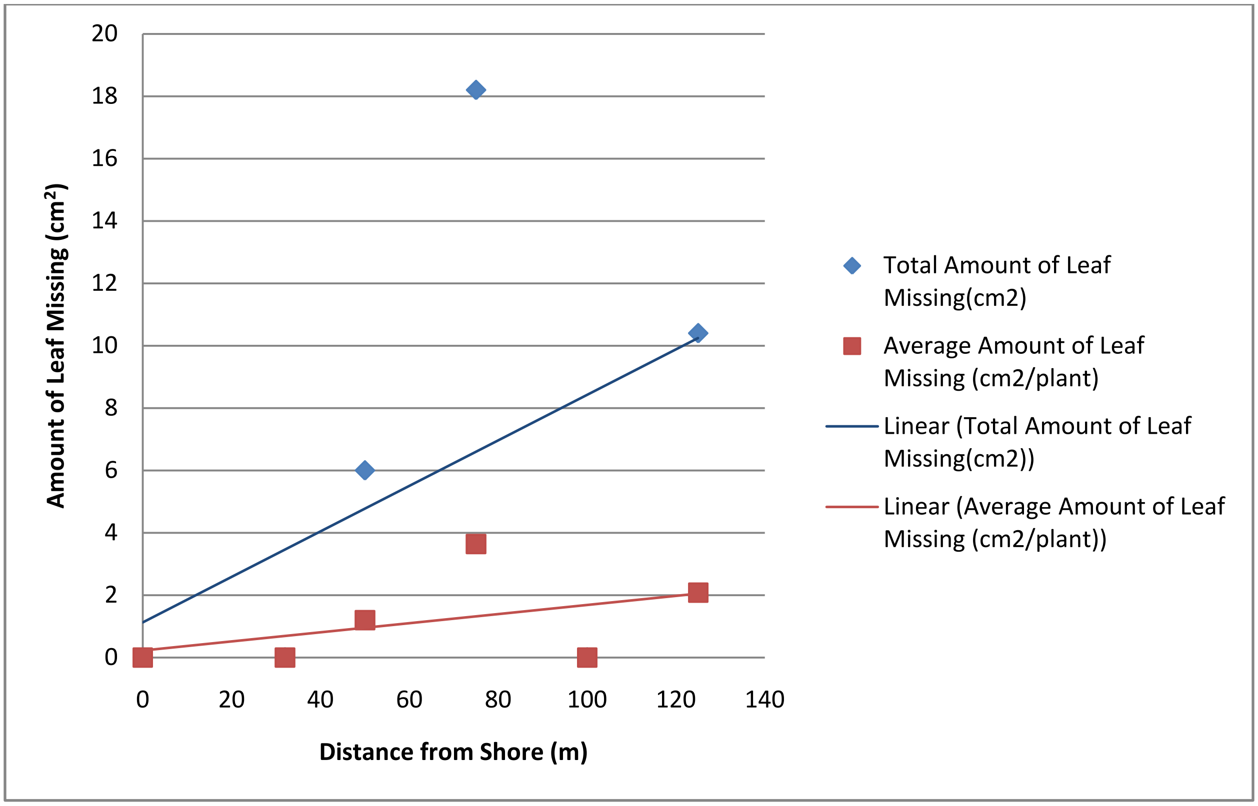

Table 3. Table of mean and standard deviation of nitrogen levels at each distance from the shoreline, as well as R^2 values and p-values for each condition.

Distance from Shoreline (m)	Mean Nitrogen Level (%)	Standard Deviation
0	1.177	0.280
34	1.223	0.175
50	1.121	0.204
75	1.299	0.261
100	1.457	0.325
125	1.415	0.309

P-value: 0.037

R^2 value: 0.151

Table 4. Table of mean and standard deviation of herbivory damage at each distance from the shoreline, as well as R^2 values and p-values for each condition.

Distance from Shoreline (m)	Mean Herbivory Amount (cm^2/plant)	Standard Deviation
0	0	0
34	0	0
50	1.2	1.65
75	3.64	8.14
100	0	0
125	2.08	4.65

P-value: 0.372

R^2 value: 0.202