The effect of plant nitrogen levels on insect herbivory in Northern Michigan sand dunes

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I. Abstract

Nitrogen is a limiting factor for plant and animal growth. Some herbivores have illustrated dietary preferences in regard to nitrogen, choosing some foodstuffs over others in order to get the highest possible levels of nitrogen in their diet. Nitrogen is especially low in particular environments, including the sand dunes at Sturgeon Bay of Lake Michigan, which may make selective feeding of herbivores there more common. Nitrogen levels in plants on dunes have been shown to increase with distance from shore, as moisture and amount of vegetation increase. At Sturgeon Bay, we tested the ideas both of increasing nitrogen with increasing distance from shore and the idea that herbivory increases at higher nitrogen levels, with insects showing a preference for the more nutritious plants. We looked at the dune grass *Ammophila*, analyzing percent nitrogen and amount of herbivory at 6 distances from shore, ranging from 0 to 125 m. We found that nitrogen levels did increase at increasing distances from shore, but there was no significant relationship between herbivory and nitrogen levels. There was a slight trend which appeared in graphs of increasing herbivory on the dune grass at increasing distance from shore. Further research is suggested on this relationship.

II. Introduction

Nitrogen is often a limiting factor in the growth of both plants and herbivores (Chen et al, 2008; Mattson, 1980). With more nitrogen, plants can grow faster and reach larger sizes. The benefits of nitrogen extend to the insects that eat the plants as well: they will also grow faster, will take less time to mature, and will be more efficient. Without enough nitrogen in their diets, herbivorous insects will be subject to higher mortality rates and numerous other growth problems (Chen et al, 2008). Although nitrogen is prevalent in our atmosphere, forms that are available for use by plants and animals are much less common, making it a limiting nutrient. Many herbivores have adaptations specific to solving the nitrogen-deficiency problem, such as generalist diets or specialized physical structures (Mattson, 1980).

While plants are limited in their ability to control their own nitrogen levels, dependent on how many nutrients they receive from the local soil, rainfall and any fertilizers that might be applied in the area, herbivores can make decisions that affect the amount of nitrogen in their diets. There have been many studies that show that herbivorous insects choose nitrogen-rich plants over nitrogen-poor plants, illustrating that insects can discern such differences and prefer higher nitrogen levels (Chen et al, 2008; Letourneau et al, 1996; Jauset et al, 1998; Valentine, 2000). Other groups of insects might switch to even more drastic methods if no nitrogen-rich samples of their preferred foodstuff is available; some insects may resort to carnivory or switch to another new type of food (Mattson, 1980).

The sand dunes along the northeastern shore of Lake Michigan in the state of Michigan are a prime example of an environment with nutrient-poor plants. Sand is a poor soil from which most water and nutrients leach, leaving nothing for the plant roots. The specific environment that we studied was the dunes at Sturgeon Bay in Wilderness State Park in northern Michigan. The sands are virtually universally nutrient-poor, which plants seem to have adapted to, but which has continuing consequences up the food chain. In the first two dunes, nitrogen levels are especially low because of a lack of decomposing organic matter; most usable nitrogen comes from the work of nitrogen-fixing bacteria (Buckingham et. al., 1994).

Nitrogen levels do increase as one moves away from the beach, moisture increases, and decaying vegetation accumulates. The youngest dunes, those closest to shore, will have the lowest nutrient levels, particularly levels of nitrogen and carbon. As dunes age, nutrients accumulate. This is due partially to increasing moisture and partially due to accumulating organic matter from decaying vegetation (Lichter, 1998; Gerlach et. al., 1994). This is not to say that nitrogen levels are still not quite low throughout the dune ecosystem, but they do increase from the especially low values right at the water's edge.

These dunes at Sturgeon Bay are home to a threatened species of locust, the Lake Huron Locust. The locust is limited to lakeshore dunes on Lakes Michigan, Huron, and Superior, with populations in Michigan, Wisconsin and Ontario (Scholtens et al, 2005). Recent observations have suggested that the locusts supplement their diets with scavenged aquatic insects (i.e. Ephemeroptera, mayflies) found washed-up on shore. The diets of locusts (order

Orthoptera, family Acrididae) have not been well-studied, but most species are complete herbivores, with a few predators and omnivores in the family (Rentz et al, 2003). Scavenging by normally herbivorous insects is never mentioned, suggesting that it's a rare method and an indication of an extreme need for nitrogen in their diets. (The aquatic insects will have a higher nitrogen level in their bodies than the plants do.)

We were curious about the dietary choices of the locusts and other dune insects that are presumably facing the same problem. Are the more nitrogen-rich plants further away from the shore eaten more frequently than the nitrogen-poor plants near the water? We weren't interested specifically in the dietary choices of the Lake Huron Locust, but rather of dune insects in general. An answer to this question could lead to more knowledge on how the insects make their dietary choices and how important nitrogen levels really are. In other habitats worldwide and with other herbivore species, experiments have shown that nitrogen level *does* sometimes affect food choices. For example, beet armyworms distinguished between nitrogen-rich and –poor plants and fed and laid eggs primarily on the more nutrient-rich plants (Chen et al, 2008; Showler et al, 2001). Whitefly adults were shown to preferentially lay eggs on tomatoes that had been fertilized with high nitrogen (Jauset et al, 1998). Even in the Gulf of Mexico, turtlegrass was grazed more frequently when it had higher nitrogen levels (Valentine, 2000).

The dominant grass species on the dunes of Sturgeon Bay is beach grass, *Ammophila breviligulata.* It grows using rhizomes, which extend sideways to collect as many nutrients as possible in the sand (Chown, 1986). It grows in open, often disturbed areas and helps stabilize the dunes with sets of deep roots (Cassin, 1988). This grass, growing in the dunes, has very low nitrogen levels near the water because of the lack of decomposing organic matter from which to gain nutrients.

Our study, then, was simple but two-fold: do nitrogen levels in plants at Sturgeon Bay Dunes follow the pattern studied by Lichter (1998) and Gerlach et. al. (1994) and increase moving away from the shoreline? Secondly, will the amount of herbivory reflect nitrogen levels in the plants and therefore increase as we move away from the shore? We hypothesized that both nitrogen levels and amount of herbivory on leaves *would* increase as we moved away from the lakeshore.

III. Methods and Materials

The study was conducted in the dunes along Sturgeon Bay (Lake Michigan) in Wilderness State Park. There are rows of dunes extending away from the beach; usually only the first two have not been covered by woody vegetation. The stretch of beach directly adjacent to the water is often partially flooded. The lake itself is normally cold and clear and shallow (below .5 m deep) for several tens of meters away from shore.

Experiment Set-Up: Taking Samples of Grasses

We wanted to compare herbivory levels between groups of *Ammophila* growing at various distances from the shore. At the dunes, we measured the distance farthest from the shoreline at which *Ammophila* still grew; this was our maximum distance (125 m, in our case.) We also measured the distance to the closest *Ammophila* plants; this was our minimum distance (0 m – there were *Ammophila* plants right on the water's edge.) We divided the total range of *Ammophila* plants by five to get six points at which to take data: very near shoreline, very far away from shoreline, and four points in between. If no *Ammophila* was found at a certain distance from shore, we changed the length of that interval so that we were still taking samples from the same distance on each transect. For example, we couldn't find any *Ammophila* at 25 m from shore on some of the transects, so we changed that sampling point to 32 m for each transect. Our final distances to sample from were 0 m, 32 m, 50 m, 75 m, 100 m, and 125 m.

At each of these distances, we took a sample of *Ammophila* along a straight transect. We selected the clump of grass that was nearest to the actual distance point along the transect – if two were equally close, we randomly chose one of them. Each clump of grass was uprooted and placed into a labeled Ziploc bag.

We then moved down 10 m and repeated the sampling procedure, again taking grass samples at each of the six distances. We repeated this three more times (making a total of five transects), moving down 10 m each time, until we had 30 labeled samples of grass.

Comparing Nitrogen Levels of the Grasses

We compared the actual nitrogen levels of samples of grass from each distance mark to test for significant differences. Each sample (approximately 4-6 blades of grass) was frozen for an hour at -80º Celsius, and then freeze-dried in a Lyopholizer. After this, we ground the samples for a minute in a Spex 8000 D Mixer Mill, weighed the ground samples into tin capsules, and analyzed them for percent nitrogen with the Costech Elemental Analyzer using the Dumas dry combustion method. We calculated the average level of percent total nitrogen for all samples from each distance group.

Analyzing Herbivory Levels on Blades of Grass

From each sample, we photographed each leaf and arranged the photograph on a grid on a computer screen. Then we counted how many squares of leaf were missing as a result of herbivory and how big each square was. This gave us a total amount of leaf missing from each plant. (We measured total area missing instead of percent area missing since the leaves all had greatly varying sizes.) We could then take the average for each group from each distance from shore to analyze if herbivory on *Ammophila* increased as we moved away from the water.

Dune Succession Data Collection

To learn more about how different factors change with dune succession, we also measured soil temperature, humidity in the air, and percent cover by vegetation at increasing distances from shore. We took each of these three measures at the front of the first dune (FDF), the crest of the first dune (FDC), the back of the first dune (FDR), and then the crest of two further dunes (RDN and RDF). Then we repeated this three more times, moving 5-10 m down the beach for each sequence of measurements.

We measured soil temperature at 5 cm below ground with a soil thermometer with a probe on one end which we inserted into the sand. Humidity was taken with a hygrometer at 5 cm above ground. To determine percent cover by vegetation, we randomly laid down a one square meter quadrat in each location and calculated what percentage of the area was covered (physically or via shading) by any type of vegetation.

Statistical Analysis

We did a regression on the data for percent nitrogen at each distance from shore to see if there was a significant positive slope as plants grew further from the shoreline. We also did t-tests comparing 0 m to 32 m, 32 m to 50 m, 50 m to 75 m, 75 m to 100 m, 100 m to 125 m, and 0 m to 125 m to see if there were any significant differences between subsequent values. We calculated average for percent nitrogen at each distance from shore and compared results graphically, both for averages of all transects and for transects individually.

We also did a regression for the average amount of leaf missing/plant moving away from the shore and graphed this comparison.

All p-criticals were 0.05 and all statistical analysis and graphing was done using MS Excel software.

IV. Results

There was a significant increase in percent nitrogen levels of the *Ammophila* with distance away from the shoreline (r^2=0.146, df=28, F=4.794, p=0.037) (Fig. 1). The trend was for nitrogen levels to be slightly high right along the shore (at 0 m and 32 m), then to drop (lowest at 50 m) and then progressively rise again. Average percent nitrogen was 1.2 at the shoreline, 1.2 at 32 m, 1.1 at 50 m, 1.3 at 75 m, 1.5 at 100 m, and 1.4 at 125 m (Fig. 2 and Fig. 3).

While there was a significant overall increase in nitrogen levels at increasing distance from shore, p-values for comparison of pairs were all insignificant. There was no significant difference in nitrogen levels between 0 m and 32 m (t=-0.313, df=7, p=0.763), between 32 m and 50 m (t=0.853, df=8, p=0.418), between 50 m and 75 m (t=-1.206, df=8, p=0.262), between 75 m and 100 m (t=-0.849, df=8, p=0.421), or between 100 m and 125 m (t=0.210, df=8, p=0.839.) There was also no significant difference between 0 m and 125 m (t=-1.277, df=8, p=0.237.)

There was no significant change in herbivory on *Ammophila* at different distances from shore (r^2=0.201, df=4, F=1.009, p=0.372.) A graph of total amount of leaf missing for every plant at one distance and average amount of leaf missing per plant at each distance does reveal perhaps a slight trend towards increasing herbivory at increasing distance from shore (Fig. 4 and Fig. 5). Very few of our sample plants, however, showed any herbivory at all; only 5 of 30 plants had been partially eaten. None of the plants taken from 0 m, 32 m, or 100 m had been eaten; two of the plants from 50 m had been eaten; two of the plants taken from 75 m had been eaten; and one of the plants from 125 m had been eaten.

A graph comparing average percent nitrogen and average amount of herbivory per plant at each distance from shore does not appear to show a trend in any direction, implying a lack of relationship between the two variables (Fig. 6).

Our data on soil temperature, humidity, and vegetation were, for the most part, inconclusive. Soil temperature remained relatively constant as we moved away from the shore; it rose slightly from 21.5°C to 24.7°C, but varied in the interval (Fig. 7). Average humidity rose evenly from the front of the first dune to the crest of the first rear dune, but it peaked there and dropped down again by the crest of the second rear dune. It didn’t vary greatly, with results ranging from 38% to 42.75% (Fig. 8). Results on average percent cover of vegetation do not appear to follow any particular pattern. Vegetation decreased from the front of the first dune to its rear, then jumped sharply at the crest of the first rear dune, before decreasing again. Average percent cover went from 41% to 30% to 20% to 62% to 49% (Fig. 9).

Although the pattern was slightly different, percent cover by vegetation does roughly correspond to nitrogen levels, although the comparison isn't perfect (Fig. 10).

V. Discussion

Our study did show that nitrogen levels in *Ammophila* rise at increasing distances from shore. Amounts of herbivory on the *Ammophila* did not correspondingly increase, however: there was no significant trend in herbivory as distance from shore changed.

The pattern in terms of percent nitrogen of first dropping slightly before increasing seems unusual at first, but can probably be explained based on observation of the habitat. The first 20-35 m of the beach are low-lying ground, extremely wet, and rich in wetland vegetation. These conditions could likely lead to higher levels of nitrogen. Once ground level began to rise and a more typical sand dune accumulates, after about 35 m from shore, the typical pattern of nitrogen increasing away from shore appears.

The higher levels of nitrogen at the shoreline could also be explained by an input there of aquatic nitrogen. Just as the Lake Huron locusts supplemented their nitrogen levels by eating aquatic insects, deposition of aquatic plants and insects on the shore could decompose and add nitrogen to the sand there.

Our nitrogen level data backs up other studies that found that nutrients in sand dunes increase further away from the shoreline. Lichter's study (1998) also looked at sand dunes surrounding Lake Michigan, and found similar results in terms of nitrogen.

Our herbivory data are less conclusive. Based on other studies and an understanding that nitrogen is necessary to insects' diet, we expected that since nitrogen *did* increase in *Ammophila* at increasing distance from shore, herbivory on the *Ammophila* would increase as well, with insects showing a preference for plants with higher nitrogen content (Chen et. al, 2008; Jauset et. al., 1998; Letourneau et. al., 1996; Mattson Jr., 1980; Showler, 2001; Valentine, 2000). No such pattern existed, however. Our data would suggest that herbivores showed no preference for higher nitrogen plants and chose *Ammophila* plants to eat either randomly or based on factors other than nitrogen levels (perhaps location, size, or density of plants.) We suggest further studies on the factors that most greatly affect herbivorous insects' dietary choices in these dunes.

However, there was a slight trend that appeared in our graphs that suggested that herbivory did increase at increasing distance from shore – it simply wasn't a large enough trend to be significant. This study could be improved and repeated to attempt to get a significant p-value. If we collected more samples of *Ammophila*, the study would be greatly improved. There is so much *Ammophila* on the dunes and we collected such a small fraction of it that our chances of collecting the plants that had been eaten were very small. If repeated, we'd also complete the study at the very end of the growing season, so as to have the accumulated results of herbivory from the entire season present. In this way, we could maximize our sample size and increase our chances of making that possible trend more obvious and significant.

It is also possible that nitrogen was so low throughout the dunes that even the slightly higher levels at greater distance were not tempting enough to provoke higher herbivory levels. The insects could be completely adapted to getting their nitrogen from scavenging, at this point, with nitrogen from plants being so inconsequential as to drive preference out of the equation.

Our data on soil temperature, humidity, and percent cover by vegetation were collected simultaneously out of curiosity, but they do not appear to correlate to our other results. The fact that soil temperature remained relatively constant at increasing distance from shore means that it was not likely to be a variable affecting nitrogen levels or herbivory. The fact that humidity tended to increase as we moved away from shore could reflect a corresponding increase in moisture, which would make sense, given the rise in nitrogen levels (we know that nutrient and moisture levels are related) (Gerlach et. al., 1994; Lichter, J., 1998.)

Since nitrogen levels did increase, it seems that percent cover by vegetation would have increased correspondingly, since a source for nitrogen is decaying vegetation. In fact, a relationship does seem to exist: nitrogen levels dropped initially after a higher level at shoreline, before rising suddenly and thereafter. The semi-wetland condition that exists near the shore in these dunes could explain the higher levels of vegetation near the shore as well as the higher nitrogen levels there. Percent cover of vegetation then dropped after the rising ground level initially cut off moisture supply, but when dune vegetation and transition vegetation were added, percent cover rose again.

In conclusion, our study supports other dune succession studies by illustrating that nutrient levels increase with dune age. The first part of our hypothesis was supported by our findings. The second part of our hypothesis, however, was rejected: there was no significant difference in herbivory in relationship to distance from shore. This contradicts most other studies on the relationship between herbivory and nutrient-levels. This lack of significance could be result of internal problems in the study, and further research is suggested.

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