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Comparison of herbivore damage to *Ammophila breviligulata* and plant nitrogen content on the Sturgeon Bay dunes along Lake Michigan

ABSTRACT

The dunes along Lake Michigan are especially nitrogen poor, and this presents a problem for all organisms that live there. Both plants and herbivores have aquired adaptations that help them obtain adequate amounts of nitrogen. The purpose of this study was to examine the amount of nitrogen in *Ammophila breviligulata* and its relationship to insect herbivory. *A. breviligulata* plants were collected at different distances from the shore and their nitrogen content and amount of herbivory were measured. Herbivory did not vary significantly with either distance from the shore ($p = 0.569$) or plant nitrogen content ($p = 0.894$). However nitrogen content did increase in *A. breviligulata* as the distance of the plant from the shore increased ($p = 0.037$) despite a decrease in the occurrence of *A. breviligulata* farther from the shore ($p < 0.01$). Increased nitrogen content of *A. breviligulata* at greater distances from the shore was consistent with previous results and with expected results based on patterns of primary succession on the dunes. Herbivory may not have shown significant variation with distance from the shore because the sample size was too small or the costs of travel to further plants did not allow optimal foraging.

INTRODUCTION

The dune habitat along Lake Michigan has been shown to be an especially nitrogen poor environment, particularly in younger dunes near the shore (Lichter, 1998). This presents a challenge for both plants and animals to obtain the nitrogen they need to build essential proteins and nucleic acids. Younger dunes lack organic nutrients, so organisms on these dunes depend on nitrogen fixing bacteria and their symbionts for most of their nitrogen (Buckingham et al., no date).

The beach grass, *Ammophila breviligulata*, is one such organism near the shore. Its rhizomes and long roots allow it to grow more vigorously (Disraeli, 1984), and also help stabilize it as the sand around it moves. Lateral roots allow *A. breviligulata* to take advantage of the few surface nutrients available (Buckingham et al., no date; Chown 1986).

Valentine et al. (2000) showed that plants grown in nitrogen rich soils have higher nitrogen contents than plants grown in nitrogen-poor soil. Because soil nitrogen content in sand dunes increases as the dune ages, young dunes near the shore have lower nitrogen content than old dunes located further from the shore (Lichter, 1998). Therefore *A. breviligulata* growing on younger dunes near the shore should have lower nitrogen content than *A. breviligulata* growing on older dunes further from the shore.

Several factors besides soil nitrogen content can affect the nitrogen content of plants. First, younger plants and plant parts tend to have higher nitrogen levels to support high rates of cell division (Mattson, 1980). Second, water-stressed and diseased plants release more of some free amino acids into the xylem and phloem (Seitz and Hochster, 1964; Jensen, 1969; Lodh et al., 197; Labanauskas et al., 1981; Shen et al., 1989; Gzik, 1996 in Showler, 2001), although this may not increase the overall nitrogen content of the plant. Nitrogen fertilization, and consequently higher nitrogen levels in the plant tissue, induces higher water content in tomato plants (Jauset et al., 1998). Other species-specific factors determine the number and identity of free amino acids in the tissue and the amount of nitrogen contained in secondary plant chemicals, some of which may be used for defense against herbivory (Showler, 2001).

On the dunes, insects are the primary herbivores. Several studies have shown that insects prefer to feed on plants with higher nitrogen content (Jauset et al., 1998; Showler, 2001; Bastos et al. 2007; Chen et al., 2008). This preference may be due to the decreased amount of lignin, a structural polymer, in nitrogen rich plants which makes plant tissue softer and easier to digest (Bastos et al., 2007). Previous positive experiences with high nitrogen plants may also play a role in this preference in older insects (Jauset et al., 1998).

Nitrogen-containing secondary plant chemicals, however, may influence insect preference (Showler, 2001) and interfere with the general correlation between increased nitrogen content and increased insect herbivory. Additionally, a study by Letourneau et al. (1996) found that there was no correlation between nitrogen content and the amount of herbivore damage to tomato plants on organic and conventional farms, suggesting that insect communities may not respond to the high nitrogen plants by eating greater quantities of them or that other factors may have greater influence on their feeding choices. In general, insects regulate their diet such that they achieve a good balance of nutrients while ingesting few toxins (Behmer et al., 2002). Recent observations of the dune-dwelling Lake Huron locust, *Trimerotropis huroniana*, suggest that this particular insect supplements its diet by scavenging insect carcasses that wash up along the lakeshore (Scholtens, personal communication). This is probably due to the low levels of nitrogen that they can obtain from the native dune plants. If higher nitrogen plants were available, this behavior might not be observed.

This study compares the amount of herbivore damage to *A. breviligulata* and its overall nitrogen content on the Sturgeon Bay dunes along Lake Michigan. Our hypothesis is that insects will prefer to feed on higher nitrogen blades of *A. breviligulata* as indicated by greater leaf damage to these plants.

MATERIALS AND METHODS

A. breviligulata plants used in this study were collected from the Sturgeon Bay dunes in Emmet County, Michigan. Five transects were set up 10 meters apart along the shoreline. The *A. breviligulata* plants closest to each transect were removed at 0, 32, 50, 75, 100 and 125 meters from the shore for a total of 30 plant samples. Samples were removed at 32 rather than 25 meters because a pond occurred at 25 meters and no *A. breviligulata* grew there.

Each *A. breviligulata* leaf that showed signs of herbivory was photographed using a digital camera. A ruler was included in the photograph. The photographs were loaded to Adobe Photoshop and a grid of known size was superimposed on the image using the Vanishing Point tool. The consumed surface area of the leaves was measured by counting the number of empty grid squares within the boundaries of each leaf.

The nitrogen content of each plant was analyzed. The leaves were cut into 2cm segments and frozen at -80°C for one hour. Then the samples were freeze-dried in a Lyopholizer for 24 hours and ground for one minute in a Spex 8000D mixer mill. Samples were weighed into a tin capsule and analyzed for percent nitrogen in a Costech EA4010 elemental analyzer using the Dumas dry combustion method.

Additional measurements were taken to better characterize the dune environment. Plant coverage, air temperature, wind velocity, humidity and soil temperature were measured at sites on the front, crest and rear of the second sand dune (foredune) from the shore, the crest of the third dune (near rear dune) and the crest of the fifth dune (far rear dune). To measure plant coverage, rough estimates of the percentage of a 1 x 1 m quadrat occupied by each plant species and the names of the species were recorded. Temperature, wind velocity and humidity were measured at 0, 5, 40 and 100 cm above the ground using a digital air thermometer, a digital anemometer and a digital hygrometer, respectively. Soil temperature was measured at 0, 5 and 40 cm below ground using a digital soil thermometer.

All statistical analysis was performed using Microsoft Office Excel 2007. Correlations between herbivory, nitrogen content and distance from the shore were examined using a least-squares linear regression model. To determine changes in plant coverage, t tests were used to compare averages at each site with the site closest to the shore. Air temperature, wind velocity, humidity and soil temperature were analyzed in the same manner and each height or depth was analyzed separately.

RESULTS

A total of five plants showed signs of herbivory; two occurred at 50 m and 75 m and one occurred at 125 m. The average area missing on these five plants was 6.92 cm^2, while the average area missing over all plants was 1.153 cm^2. Table 1 shows the amount of herbivory observed at each distance from the shore. Regression analysis indicated no significant difference in herbivory as distance from the shore increased ($r^2 = 0.0428$, $p = 0.569$, Figure 1).

The average nitrogen content of all plants was 1.3%. The average nitrogen content of the plants displaying herbivory was also 1.3%. Regression analysis indicated that nitrogen content does increase with distance from the shore ($r^2 = 0.1506$, $p = 0.037$, Figure 2). Similar analysis suggested that herbivory does not vary significantly with nitrogen content ($r^2 = 0.0006$, $p = 0.894$, Figure 3).

Total plant coverage (Figure 4) did not show a significant increase with greater distance from the shore ($p > 0.3$). However the percentage of cover attributed to *A. breviligulata* (Figure 5) showed a significant decrease between the shore (0 m) and 100 and 125 m from the shore ($t = 4.1955$, $p = 0.006$ and $t = 5.0297$, $p = 0.002$, respectively).

Air temperature, wind velocity and humidity did not show statistically significant changes with increased distance from the shore. Air temperature showed no recognizable trends (Figure 6), however the other environmental characteristics did exhibit some trends with height above ground and distance from the shore. Wind velocity tended to increase with both height above ground and distance from shore (Figure 7). Humidity showed no trend associated with height above ground, but did increase with distance from the shore (Figure 8).

Soil temperature tended to decrease with depth below the ground (Figure 9) and showed some significant differences with distance from the shore. At the surface of the foredune rear, soil temperature was significantly higher than it was at the foredune front ($t = 2.9427$, $p = 0.026$). Five cm below the surface, significant increases in soil temperature in comparison with the foredune front were observed at the foredune crest ($t = 3.1142$, $p = 0.021$), near rear dune ($t = 5.0952$, $p = 0.002$) and far rear dune ($t = 4.1967$, $p = 0.006$). Forty cm below the surface, significant increases in soil temperature compared to the foredune front were observed for all of the other four sampling sites (in order of increasing distance from the shore, $t = 3.8480$ and $p = 0.008$; $t = 4.3649$ and $p = 0.005$; $t = 4.7924$ and $p = 0.003$; $t = 5.1248$ and $p = 0.002$).

DISCUSSION

The significant increase in nitrogen content of *A. breviligulata* with increased distance from the shore observed here was consistent with previous results in the literature (Lichter, 1998). A decrease in nitrogen content of *A. breviligulata* plants observed at 50 m (Figure 2) probably marked a point in the dunes where nitrogen from aquatic sources was no longer transferred to the terrestrial environment. Such nitrogen transfer comes from many sources including bird guano, algal and aquatic insect detritus, terrestrial adult forms of aquatic insects and windblown spray. The effects of these nutrients decrease with increased distance from the contributing aquatic environment (Polis et al., 2004). The subsequent increase in nitrogen content as distance from the shore increases is probably due to the accumulation of fixed nitrogen by early colonizing plants as succession proceeds. Nitrogen contributions from decomposing plant detritus do not seem to make a significant contribution to the observed changes in nitrogen levels because there was no significant change in overall plant cover over the sample area. If the sampling area had extended farther from the shore into the trees, an even greater increase in nitrogen levels may have been observed as a consequence of increased plant coverage.

In addition to nitrogen increases due to successional processes, increases in soil temperature and humidity with increased distance from the shore may help explain the higher nitrogen content of *A. breviligulata* further from the shore. Warmer temperatures may increase the rate of diffusion of nitrogen-containing nutrient molecules into the root tissue of plants on the rear dunes, increasing their nitrogen content. Increased humidity may help the plants further from the shore transpire less than those plants closer to the shore. Because there is relatively more water vapor in the air, the concentration gradient of water across the surface of the leaves is reduced. Decreased rates of transpiration could allow the plants to make better use of nitrogen as it flows through the plant dissolved in water.

No significant correlation between herbivory and distance from the shore or nitrogen content was observed in this study. This may be due to the small number of plants in the sample that showed any signs of herbivory. A much larger sample of plants would have included more damaged plants. More species of plants could have been examined to better assess the levels of herbivory in different areas; the statistical methods used here do not account for the decreased incidence of *A. breviligulata* further from the shore. It is possible that herbivores do not show increased consumption of *A. breviligulata* with increased distance from the shore simply because it becomes less common and is less often encountered by the herbivores.

However, assuming there are no flaws in the sampling method, several ecological factors may also explain the lack of correlation between herbivory and distance from the shore. It is possible that herbivore choice in this habitat is not displayed by increased herbivory, but rather decreased herbivory as nutrient needs are met more quickly by eating higher nitrogen plants. A similar explanation has been offered for observations of lower herbivory rates on higher nitrogen tomato crops (Letourneau, 1996). However based on the results of the present study, this explanation is unlikely because no herbivory at all was observed closer than 50 m to the shore (Table 1).

It is also possible that *A. breviligulata* manufactures more defensive secondary plant chemicals when more nitrogen is available for their synthesis, however the overall nitrogen content observed here was probably not large enough to cause a significant difference in such production (Figure 2).

Observed trends in wind velocity (Figure 7), though not statistically significant, may affect the flight capabilities of herbivorous insects enough that they cannot feed as efficiently on *A. breviligulata* further from the shore. Negative impacts on flight capabilities may also make insects more vulnerable to predation where wind velocity is higher. If this is the case, it would be safer and more efficient for insects like the Lake Huron locust to stay nearer to the shore to feed on detritus from the lake to supplement the nitrogen in their diet. Similarly, changes in nitrogen levels across the dune habitat may be so insignificant to such herbivores that the cost of travel outweighs the benefits of increased nitrogen content in plants further from the shore.

Future studies could clarify some of the issues discussed here. One such study could expand the range of the dune habitat studied. By using a plant species that grows in the forest and which has an overlapping range with *A. breviligulata*, herbivory levels could be determined in their area of co-occurrence and extended to the end of the range of the second species. This may clarify issues related to scavenging behaviors caused by wind velocity in the calmer conditions of the forest. It may also contribute to the understanding of how the homogeneity of nitrogen levels may affect herbivore behavior.

Other experiments could be conducted in strictly controlled greenhouse or laboratory conditions to study how dune insect herbivores respond to increased plant nitrogen levels. *A. breviligulata* could be raised with different amounts of nitrogen fertilizers and then presented to dune insects in choice tests similar to those used by Jauset et al. (1998), Showler (2001) or Chen et al. (2008). Such simplifications are necessary to clearly understand all of the factors affecting herbivory on the Sturgeon Bay dunes. After these controlled studies have been completed, it may be possible to extrapolate the results back to the natural community.

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Table 1. Herbivory at different distances from the shore.

Distance from shore	Total Area Missing (cm^2)	Avg. Area missing/plant (cm^2)
0	0	0
32	0	0
50	6	1.2
75	18.2	3.64
100	0	0
125	10.4	2.08

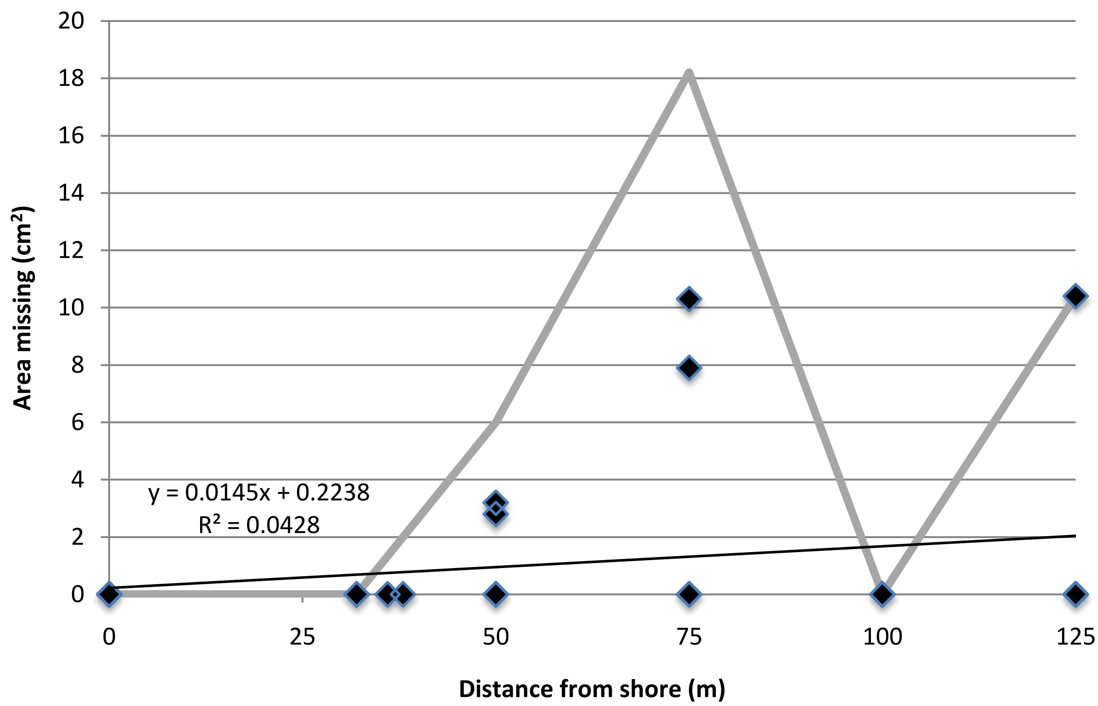

Figure 1. Herbivory, measured by leaf area missing, and distance from the shore. The grey line shows total herbivory at each distance, while the black line shows a best fit line for the individual data points.

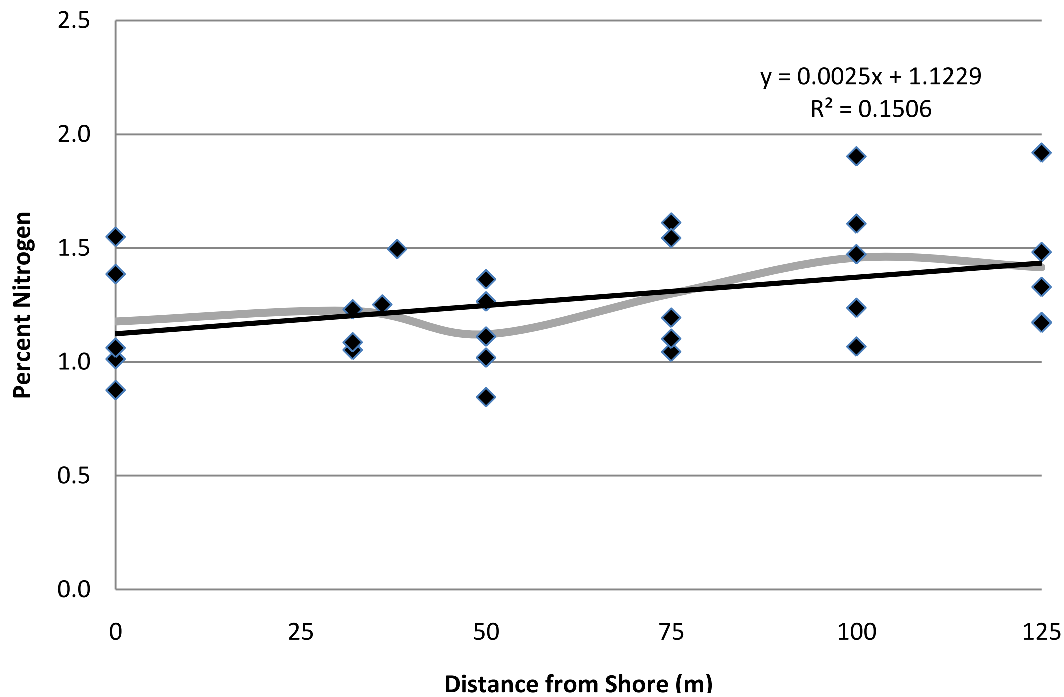

Figure 2. Nitrogen content, measured as percent nitrogen, and distance from the shore. The grey line shows average nitrogen content at each distance, while the black line is a best fit line of the individual data points.

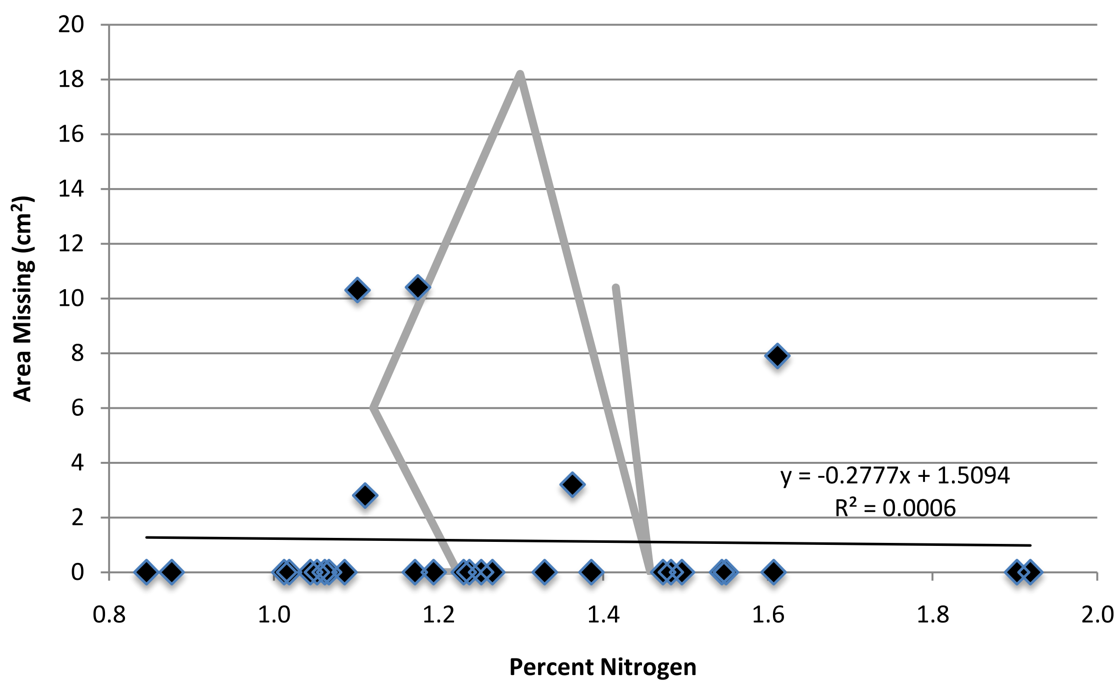

Figure 3. Herbivory, measured as leaf area missing, and nitrogen content, measured as percent nitrogen. The grey line shows total area missing vs. average nitrogen content at each distance, while the black line is a best fit line of the individual data points.

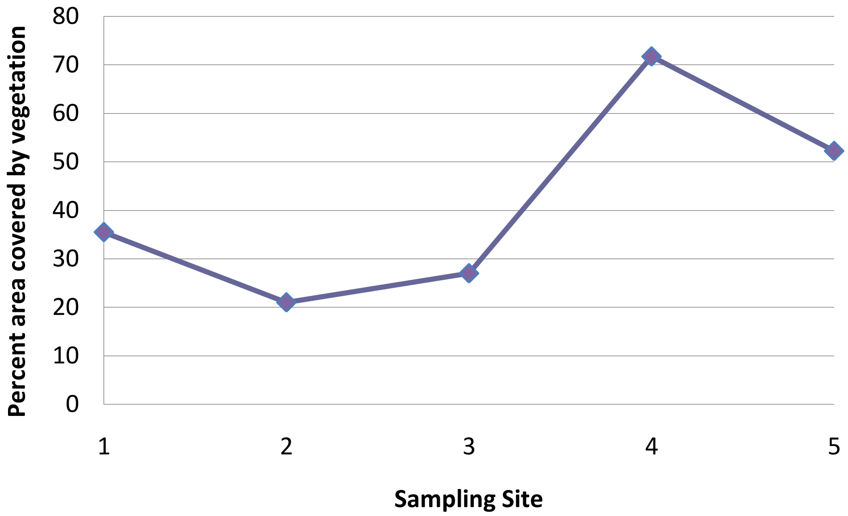

Figure 4. Total plant coverage and sampling site. Distance from shore increases as the site number increases. (1 = foredune front, 2 = foredune crest, 3 = foredune rear, 4 = near rear dune, 5 = far rear dune)

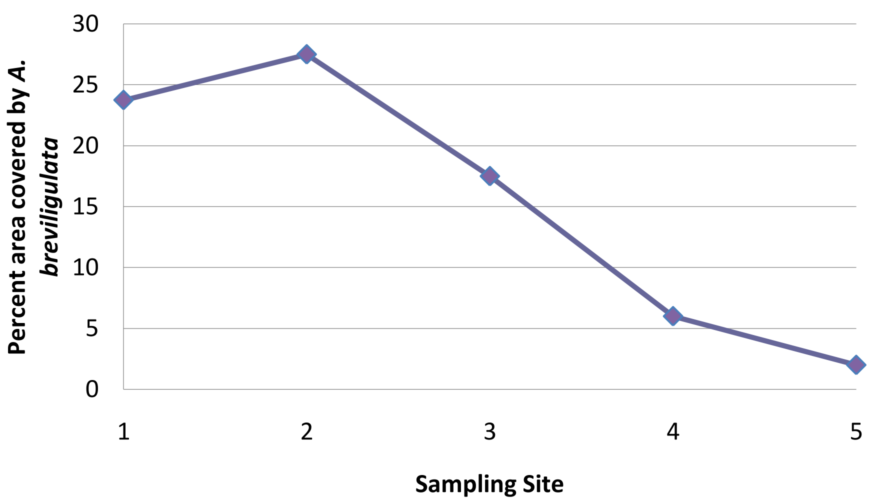

Figure 5. Area covered by *A. breviligulata* and sampling site. Distance from shore increases as the site number increases. (1 = foredune front, 2 = foredune crest, 3 = foredune rear, 4 = near rear dune, 5 = far rear dune)

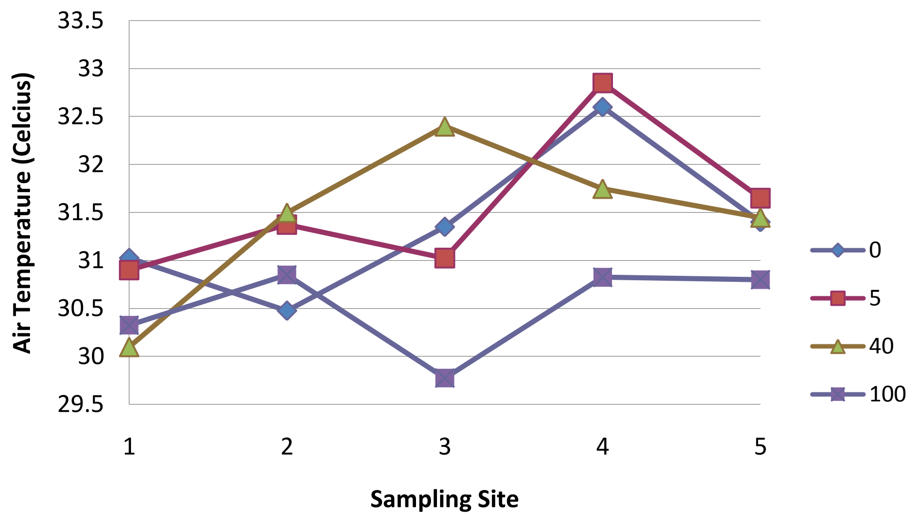

Figure 6. Air temperature and sampling site. Distance from shore increases as the site number increases (1 = foredune front, 2 = foredune crest, 3 = foredune rear, 4 = near rear dune, 5 = far rear dune). Heights of 0, 5, 40 and 100 cm are shown as separate lines on the graph.

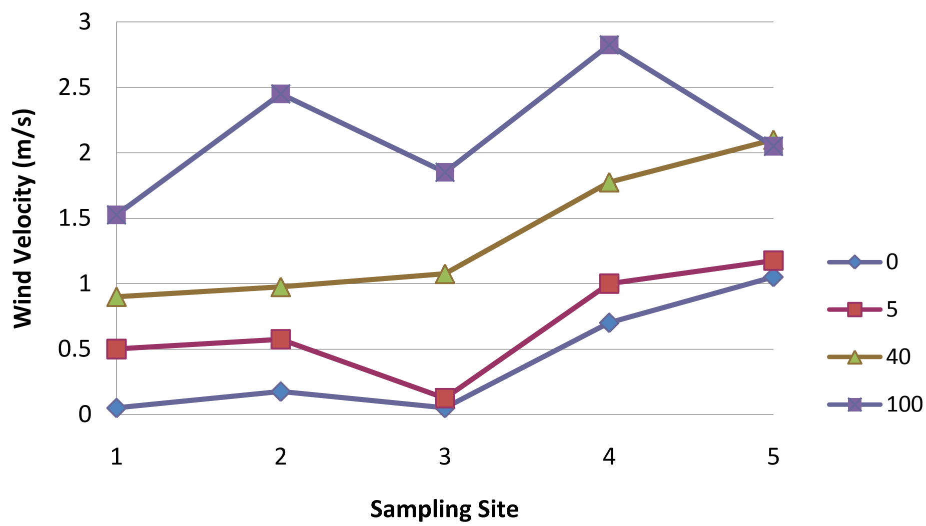

Figure 7. Wind velocity and sampling site. Distance from shore increases as the site number increases (1 = foredune front, 2 = foredune crest, 3 = foredune rear, 4 = near rear dune, 5 = far rear dune). Heights of 0, 5, 40 and 100 cm are shown as separate lines on the graph.

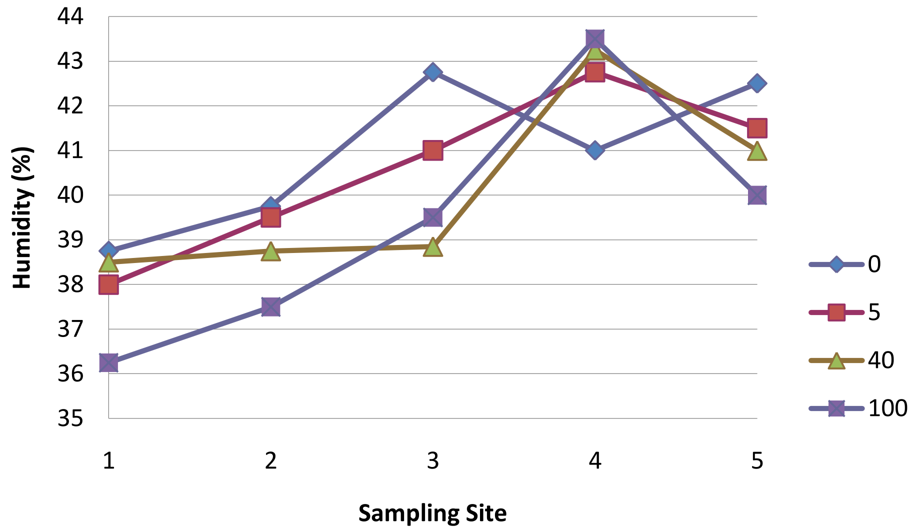

Figure 8. Humidity and sampling site. Distance from shore increases as the site number increases (1 = foredune front, 2 = foredune crest, 3 = foredune rear, 4 = near rear dune, 5 = far rear dune). Heights of 0, 5, 40 and 100 cm are shown as separate lines on the graph.

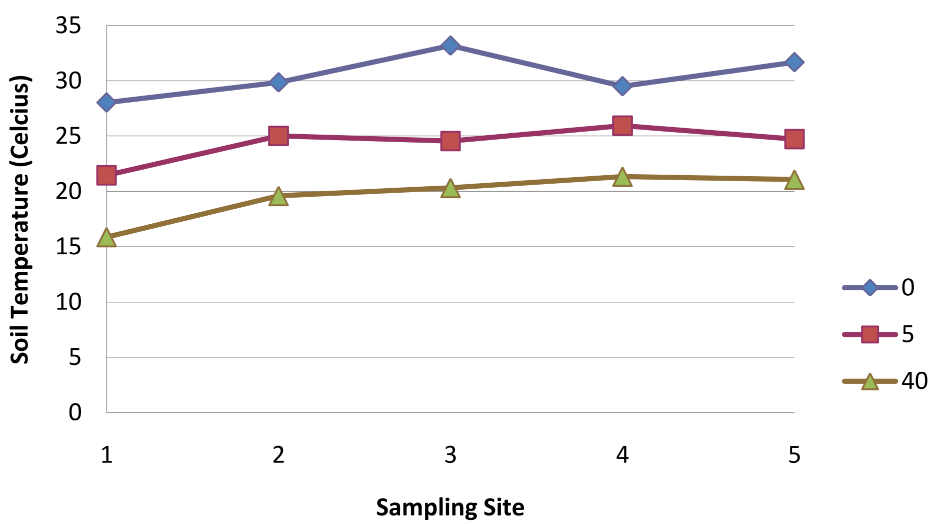

Figure 9. Soil temperature and sampling site. Distance from shore increases as the site number increases (1 = foredune front, 2 = foredune crest, 3 = foredune rear, 4 = near rear dune, 5 = far rear dune). Depths of 0, 5 and 40 cm are shown as separate lines on the graph.