

# **AQUATIC NITROGEN PREVELACE AMONG *AMMOPHILA BREVILIGULATA* IN THE DUNE ENVIRONMENT**

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*General Ecology, University of Michigan Biological Station 2008*

Sources of nitrogen, often a limiting factor to growth and development, can be very important to an ecosystem. Allochthonous inputs into a system can stabilize a low-nutrient system. Using stable isotope analysis, it is now possible to determine nitrogen sources as aquatic or terrestrial. The limited nutrient dune system at Sturgeon Bay in northern Michigan was observed by examining  $^{15}\text{N}/^{14}\text{N}$  ratios in beach grass, *Ammophila breviligulata* both at the shoreline and at distances 10m, 20m, and 30m from the shoreline. Algae were collected as an aquatic control; the 30m samples were used as a terrestrial control. Assuming only two nitrogen sources, aquatic (algae) and terrestrial (30m),  $^{15}\text{N}/^{14}\text{N}$  ratios were determined. While  $\delta^{15}\text{N}$  was depleted compared to the standard in all *A. breviligulata* samples, there was significantly more  $\delta^{15}\text{N}$  in samples taken from the shore. Samples at 10m and 20m were composed entirely of terrestrial nitrogen. Shoreline *A. breviligulata* was composed of 23.3% aquatic nitrogen. Primary succession modeling dune systems suggest the presence of high nutrients and terrestrial influence in older dunes far from the shore. The significant increase in total nitrogen (% in the plant) from 10m to 30m and from 20m to 30m supports this. There was a significantly greater amount of nitrogen at the shoreline than from plants at 10m, suggesting the aquatic source supplements nitrogen in *A. breviligulata* at the shore. It can then be assumed Lake Michigan also provides other allochthonous inputs into the shoreline of the dune system.

key words: aquatic nitrogen, stable isotopes, terrestrial nitrogen, *Ammophila breviligulata*, beach grass, sand dune

## INTRODUCTION

Nitrogen, being a vital component to growth and development in all organisms, is of great importance to an ecosystem. Because nitrogen can limit growth or, in abundance, help an ecosystem to thrive, an ecosystem's source of nitrogen is important. Allochthonous inputs into a system, for example, are often very beneficial to areas of low nutrients; allochthonous nutrients can help stabilize a food web, replenish limited nutrients, and ultimately increase productivity in a system (Huxel and McCann, 1998). Recent use of stable isotope analysis has allowed for determining nitrogen sources in an

organism through the  $^{15}\text{N}/^{14}\text{N}$  ratio (Polis et al., 2004). This ratio indicates how much influence a local body of water has on nitrogen levels in terrestrial environments nearby. This aquatic nitrogen could ultimately affect the entire ecosystem as it flows through trophic levels (Polis et al., 1997).

At the transition point of aquatic and terrestrial systems, nitrogen is transferred to the terrestrial system through various means. In addition to organic material wash-up from typical tidal action, scavengers also bring aquatic nitrogen in the form of invertebrate carcasses (Likens and Bormann, 1974; Polis et al., 2004). The guano of these scavengers is also a rich source of nitrogen (Polis et al., 1997). This aquatic nitrogen seeps into the ground and is used by plants. What has not been heavily studied is the quantity of aquatic nitrogen used by plants on the shoreline. Also of interest is how far from the shore this aquatic nitrogen has an influence. This study addressed these questions.

The dune environment, in particular, is relatively low in nutrients, having strong winds, high sand movement, and high evaporation rates (Lichter, 1998). The dune system along Lake Michigan in Wilderness State Park in northern Michigan is a prime example of this environment. Its low nutrient supply and proximity to the lake may suggest a high allochthonous input of nutrients, such as nitrogen, into the dune system. To study the inflow of aquatic nitrogen into the system, this study focused on the stable nitrogen isotope ratios in beach grass, *Ammophila breviligulata*. *Ammophila breviligulata* is prominent both on the shoreline and back into the dunes. Its rhizomes extend both vertically and horizontally in the sand, allowing it to survive in the dune environment (Voss, 1972). Addition of nitrogen to *A. breviligulata* has been shown to

significantly increase growth of the grass (Jagschitz and Bell, 1966). Because *A. breviligulata* significantly responds to nitrogen and because of its prominent location along both the shoreline and in the dune system, it was an ideal subject for this study. The affect of aquatic nitrogen in the primary producer *A. breviligulata* was predicted to decrease at further distances from the shoreline.

## MATERIALS AND METHODS

*Study area.*— This study was conducted along the dunes and shoreline of Sturgeon Bay in Wilderness State Park in northern Michigan (45.744°N, 84.957°W). In mid July 2008, samples of beach grass, *Ammophila breviligulata*, were gathered at four distances from the shore—30m, 20m, 10m, and at the shoreline. To account for potential confounding variables (e.g. the sandiness of the site), samples were collected at five locations at each distance, 5 meters apart. Because *A. breviligulata* is not an aquatic species, washed up organic material—composed primarily of *chara* a primary producing alga—was collected from the shore as an aquatic control.

*Analyses.*— The samples of *A. breviligulata*, after being trimmed to the stems of the plant, were frozen to -80°F and dehumidified in a Lyophilizer. The dry plants were then crushed and weighed, prepared with the Cuestech EA 4010 elemental analyzer and processed through a stable isotope mass spectrometer (Thermo Finnigan Delta XP) to determine the stable nitrogen isotope ratio from each of the 20 sites. The algae samples were treated with a hydrogen chloride solution to remove calcium carbonate and were similarly crushed and processed to determine their stable nitrogen isotope ratios, given in δ%. The nitrogen samples were quantified by ISODAT 2.1. Assuming only two sources of nitrogen were present (aquatic as demonstrated by algae and terrestrial as shown by

samples from 30m), a mixed model analysis was used to determine the proportion of nitrogen from each source as implemented by IsoError, a software provided by the US Environmental Protection Agency. Precision for the standard N<sub>2</sub> was +/- .3<sup>0</sup>/00. Individual T-tests were also run in DDXL, a Microsoft Excel statistical add-on software, to analyze the data.

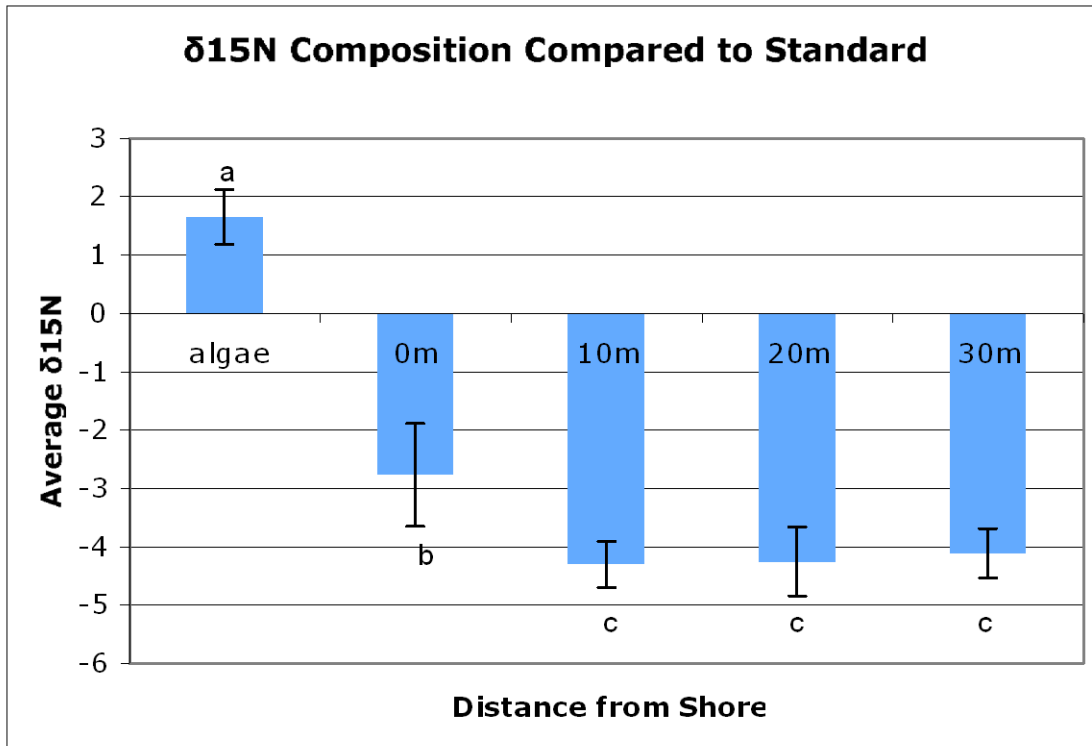
## RESULTS

The dunes at Sturgeon Bay stretched a few hundred meters from shoreline to the forested mature dunes. The first half-meter was devoid of all vegetation, although there was a relatively biodiverse population of insects present. After this short stretch, a few species of vegetation resided—mostly *Ammophila breviligulata* (this was considered 0m for sampling purposes). More vegetation was present with increasing distance from the shore, presumably with an older more stable local area with more nutrients. Percent cover of dune vegetation was 7% at the front of the first dune, while past the third dune it averaged 50%. *Ammophila breviligulata* was present from the shoreline and ceased where mature forested areas began, about 120m from the shore.

The aquatic control of algae was most enriched in <sup>15</sup>N compared to the standard of the samples taken (Table 1) and was significantly different to samples of *A. breviligulata* ( $p < 0.001$  in each case). *Ammophila breviligulata* at the shoreline was least depleted in <sup>15</sup>N, having the highest (least negative)  $\delta^{15}\text{N}$  of the plant samples, and was significantly different from samples taken at 10m ( $t=3.574, p=0.008$ ), 20m ( $t=3.127, p=0.008$ ), and the terrestrial control at 30m from the shoreline ( $t=3.089, p=0.014$ ; Figure 1). No significant difference in  $\delta^{15}\text{N}$  existed between 10m and 20m ( $t=-0.169, p=0.436$ ), 10m and 30m ( $t=-0.733, p=0.756$ ), or 20m and 30m ( $t=-0.419, p=0.344$ ).

**Table 1.**—Average  $\delta^{15}\text{N}$  content compared to the standard (air) and increasing distances from the shoreline of the dunes in Sturgeon Bay in northern Michigan.

	n	Average $\delta^{15}\text{N}$	Standard Deviation
Algae	3	1.650	0.468
0m	5	-2.768	0.874
10m	5	-4.306	0.403
20m	5	-4.252	0.602
30m	5	-4.113	0.430



**Fig 1.**—Average  $\delta^{15}\text{N}$  content in samples compared to the standard (air) at varying distances from the shore. Samples denoted a showed significant difference from b and c; b also showed significant difference from c. Samples denoted 'c' did not show significant variation within the group.

Figure 2 presents the composition of *A. breviligulata* at 0m, 10m, and 20m from the shore. At the shoreline, 23.3% of the total nitrogen was from an aquatic source (sd=0.073). 95% confidence intervals that include 100% of terrestrial nitrogen in the samples from 10m and 20m suggested all nitrogen in these samples was supplied from

the terrestrial source. These intervals were reported at over 100% because of small sample size.

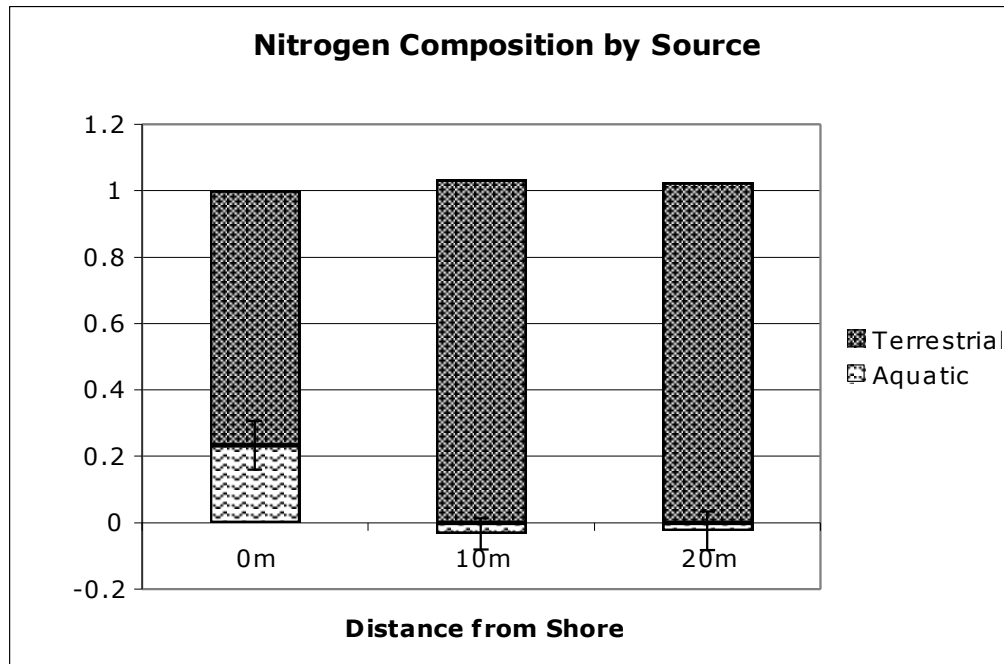


Fig 2.—Proportions of nitrogen from aquatic and terrestrial sources with confidence intervals.

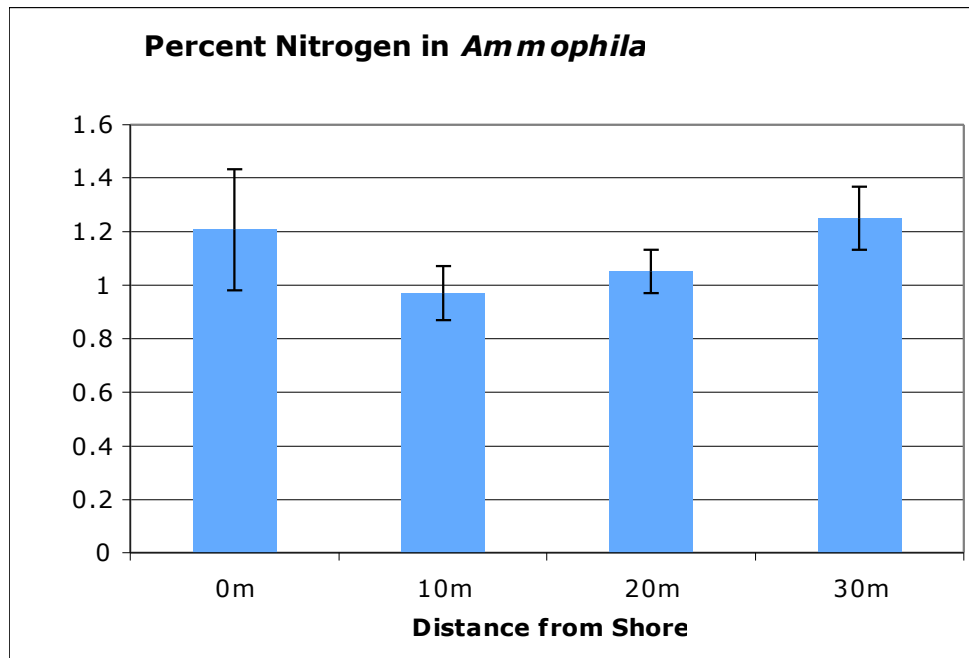


Fig 3.—Percent Nitrogen (compared to other compounds) in beach grass, *Ammophila breviligulata*, at varying distances from the shore.

Overall, nitrogen levels in *A. breviligulata* were very low. The highest total nitrogen content was present in samples from the shoreline and from 30m (Fig. 3). At 10m, there was a significant decrease in nitrogen content from the shoreline ( $t=2.143$ ,  $p=0.043$ ). Nitrogen content then gradually increased with distance from the shore; there was a significant difference in nitrogen content between 10m and 30m ( $t=-4.03$ ,  $p=0.003$ ), and 20m and 30m ( $t=-3.107$ ,  $p=0.009$ ).

## DISCUSSION

Although aquatic nitrogen influence was hypothesized to decrease with distance from the shore, it only had a significant influence in *Ammophila breviligulata* samples taken from the shoreline. The  $\delta^{15}\text{N}$  was depleted at the shoreline, but was significantly more depleted at samples 10m, 20m, and 30m from the shore. In fact, terrestrial nitrogen (30m) comprised all of the nitrogen in samples taken from 10m and 20m. At the shoreline, 23.3% of the nitrogen came from an aquatic source, Lake Michigan. Confidence intervals reveal this percentage could be, within error, from 4.5% to 42.2% aquatic nitrogen. Even with this error, it was clear that nitrogen from Lake Michigan had a significant influence in *A. breviligulata* at the shoreline.

This allochthonous input could have great importance for the dune system. In *A. breviligulata*, the total nitrogen content significantly increased from 10m to 30m and from 20m to 30m. This progression follows proposed dune chronology. The dune system at the shoreline is more exposed to wind and erosion, has fewer nutrients, and is younger (Lichter, 1998). Further back into the dunes, the system is older, has more nutrients available and has more stable plants (Lichter, 1998). This increase in nutrients and flora allows for greater biomass at higher trophic levels (Lichter, 1998), which results

in more terrestrial inflow of nutrients into the system (from these higher trophic levels). At 30m, *A. breviligulata* had significantly higher nitrogen content than at 10m; more terrestrial influences added nitrogen to the system. Older, more mature environments are resilient and can better maintain harsh external pressures; they are more able to retain vital nutrients (Likens and Bormann, 1972). The local vegetation at Sturgeon Bay flora reflects this nitrogen content. At further dunes, a higher diversity of plants was observed. Also, for all vegetation, a higher percent cover was observed at rear dunes; while vegetation cover at the first dunes was only 7%, back dunes showed up to 58% cover. Increased nutrient supply supported a higher biomass. Nitrogen content is expected to level off at some point past 30m, for terrestrial sources are themselves limited. With further study, this potential upper limit of nitrogen could be determined for the mature dune system.

Following the idea of primary succession represented by the dunes, it would seem the shoreline would have little organic material and be low in nitrogen, limiting plant growth (Peterson and Fry, 1987). In fact, the shore had significantly more nitrogen than at 10m. Because 23.3% of this nitrogen was from an aquatic source, it appeared Lake Michigan was supplementing life at the shoreline with nitrogenous inputs. Where the shoreline might have been nutrient-poor, Lake Michigan provided an important allochthonous input, benefiting the system in its replenishment of nutrients and encouragement of plant growth (Huxel and McCann, 1998). The proportion of aquatic nitrogen decreases to 0% by 10m, and further study is required to determine the gradation of aquatic influence between 0m and 10m. This study suggested nitrogen is brought into



the dune system, at least at the shoreline, from Lake Michigan; it was probable the lake also provided other sources of allochthonous nutrients.

Lake Michigan was only shown to have significant influence on plants at the shoreline, but the allochthonous inputs aided the area of the dunes that lacked the most nutrients. Although the lake did not influence the system beyond the shoreline directly, this shoreline influence of plant growth could have encouraged diversity at higher trophic levels at the shore. Although not sampled in this study, a higher biodiversity of invertebrates was observed on the shoreline. These nutrient-carrying higher-level organisms could easily traverse back into the dune system, influencing the system. The lake could have greater influences than providing an allochthonous nitrogen source, and further study is encouraged in this area.

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