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Analyses of Nitrogen Flow from Aquatic Environments to Terrestrial Environments: Ratio of $^{15}N/^{14}N$ ratios of *Ammophila breviligulata* in Dune Ecosystems

Abstract: With the possibility of nutrient flow from aquatic ecosystems to terrestrial environments, dune grass *Ammophila breviligulata* from the Sturgeon Bay, MI shoreline were analyzed for $^{15}N/^{14}N$ isotopic ratios to determine if there is a flow between these two ecosystems. Three transect lines were sampled 10 meters apart and parallel to the shoreline, along with a terrestrial and aquatic control samples. All samples were analyzed by Stable Isotope Mass Spectrometer, and $\delta^{15}N$, percentage of $^{15}N/^{14}N$ composition, and total nitrogen composition were measured for all samples. The preliminary results indicate that there is an input of ^{15}N from the aquatic ecosystem into the immediate terrestrial shoreline.

Introduction

The nutrient flow from aquatic systems to terrestrial systems has been shown to subsidize shoreline productivity along coastal regions, and the analysis of certain elements can determine the direction and quantity of this nutrient exchange across these ecosystem boundaries (Anderson et al., 1998). Elements such as carbon and nitrogen exist in two different elemental forms in nature, and the ratios of these stable isotopes can reflect certain environments since they accumulate in organismal tissue over time (Anderson et al., 1998). Nitrogen, which is the focus of this project, exists as both ^{14}N and ^{15}N. These isotopes exist in different ratios depending on the environment, and typically aquatic environments can maintain a higher ratio of ^{15}N to ^{14}N than terrestrial environments (Petersen et al., 1987). If there is, however, a fluctuation of nitrogen from an environment of higher ^{15}N concentration to that of lower concentration, the ratios should effectively track the flow of the nitrogen exchange between those environments.

In the region of northern Michigan, there is a possibility of nitrogen flow from the aquatic system of Lake Michigan to the dune ecosystem via washed-up, decomposing organic material from the lake. This project focuses on the flow of that nitrogen across the border between the lake and the dunes. Dunes are an ideal location for studying nitrogen flow since they are essentially bare and devoid of nutrients when they begin. The dunes closest to the shoreline are the most recently developed, and therefore are expected to have the lowest nutrients and biodiversity. One of the first organisms to inhabit new dunes is the dune grass *Ammophila breviligulata.* It is a dune stabilizer and sand binder found in the Great Lakes region, and it grows by rhizomes which make it well adapted to young dunes (Voss 1972). Since *A. breviligulata* is also well established across the open dunes regardless of dune age, it is an ideal organism to study any possible correlation between the ^{15}N /^{14}N ratios and the distance of these grasses from the shoreline, which directly tests the flow of nutrients from aquatic to terrestrial ecosystems. Dune grass further from the shoreline, having less exposure to the washed-up organic material, should have less intake of ^{15}N, which is more prominent in aquatic ecosystems. *A. breviligulata* samples collected should reflect this correlation of ^{15}N distribution, and in those grasses that grow further from the shoreline, the total amount of ^{15}N isotope should decrease, either gradually or dramatically. This transition, if any, will depend on the type of biotic vectors influencing the flow of nutrients onto the shoreline. High mobility factors, such as birds and turtles would influence the dunes much further inland than low mobility factors whose influence only encompasses the immediate shoreline (Fariña et al., 2003). If low mobility factors, such as wash-up and waves, are responsible for the input of nitrogen into the dune system, then there will be a dramatic transition in nitrogen content between the transects. It should show that the transect closest to the shoreline has ^{15}N levels most similar to the aquatic ecosystem, and there will be an immediate transition between the sampling line adjacent to the shoreline and those further back in the dune. If there is a gradual transition of ^{15}N due to high mobility factors or terrestrial input, the *A. breviligulata* samples should form a gradient of ^{15}N through the sampling lines which transition from aquatic levels to terrestrial levels.

Materials and Methods

To determine the influx of nitrogen into the dune ecosystem, *A. breviligulata* samples were taken at Sturgeon Bay along the northern Lake Michigan shoreline where organic debris, such as algae or aquatic insects, wash up on the shore. *A. breviligulata* samples were taken in four transects parallel to the shoreline. The first of these transects was measured at the grasses closest to the shore (0m). Every consecutive transect was 10m further than the previous one, making the furthest transect 30m from the shoreline. This last transect served as the terrestrial control. Within each transect, five 1m^2 quadrats were sampled with each quadrat being 20 paces apart along the individual transects. Five *A. breviligulata* individuals were randomly collected from each quadrat to create a composite sample for each quadrat which should show the overall $^{15}N/^{14}N$ composition for that quadrat. This sampling was repeated for all the transects, giving a total 20 samples of *A. breviligulata,* 5 from each transect. This method was used to help randomize the sample collected, but also to provide a broad range of shoreline to help represent the dune environment.

Algae was collected for sampling as the control for the aquatic environment. Since organic aquatic material only washes up on the shoreline, a composite sample of algae was taken from the immediate shoreline in the first transect. The ^{15}N content of this algae sample was compared against ^{15}N ratios in the *A. breviligulata* to determine if there is indeed a flux of nitrogen between the two environments.

The dune grass and algae samples were freeze-dried in a Lyophilizer which sublimated the water so as to maintain the nitrogen ratios without losing any to evaporation. Had these samples been left out, ^{14}N, being lighter and more volatile, would evaporate more quickly and the samples would then show a falsified increase of ^{15}N in the samples taken. The algae had an additional step of processing; to remove the structural wall of the algae, they were soaked in HCl before they were dried. After these samples were freeze dried, they were finely crushed and put into the Elemental Combustion System, which converts all organic matter into a measurable gas, N_2. The gaseous form of nitrogen was run through the Stable Isotope Mass Spectrometer, which was used to determine the $^{15}N/^{14}N$ ratio in *A. breviligulata* samples as well as in the algae samples. ISODAT 2.1 software was used to determine the $\delta^{15}N$ and to convert all measurements to the international standard.

Once the content of the samples was collected, each transect was complied to get the mean for each *A. breviligulata* sample as well as the algae. The standard deviations of each transect for the $\delta^{15}N$ were measured. Total percent nitrogen content of the *A. breviligulata* samples was determined by ISODAT 2.1. The mean and standard deviation were also taken of these. IsoError data analysis was used to perform statistical mixing of the terrestrial and aquatic to determine the source of ^{15}N in the 0m, 10m and 20m transects.

Results

The immediate results showed that there was a significant difference in the $^{15}N/^{14}N$ ratio among the dune grass transects (Fig. 1). The average of the algae sample showed a high $\delta^{15}N$ (mean≈1.65, sd≈0.468) compared to the accepted standard, which is 0. This was to be expected since ^{15}N concentration in aquatic systems is higher than terrestrial systems (Petersen et al., 1987). For the *A. breviligulata* samples, the sample taken at 0m from the shoreline shows the highest $\delta^{15}N$ (mean≈ -2.768, sd≈0.8737), which means that, of the grass samples taken, those at the 0m mark have the highest $^{15}N/^{14}N$ ratio. The 10m, 20m and 30m transects had means of -4.306 (sd≈0.403), -4.252 (sd≈0.602) and -4.113 (sd≈0.430), respectively. All the transects had the high standard deviation, most likely due to a very low number of samples taken. T-tests were performed between each of the transects, and the 0m mark was significantly different than the 10m transect ($t= 3.574$, $p= 0.00798$), as well as the 20m ($t=3.127$, $p=.00834$) and 30m ($t=3.089$, $p= 0.0136$) transects. The letters assigned to each bar in Figure 1 indicate levels of individual significance. Those that share the same letter have no statistical significance between themselves with regards to $\delta^{15}N$ values, but they differ significantly from those of other letters.

Figure 2 shows the composition of transects 0m, 10m and 20m as compared to the aquatic (algae) and terrestrial (30m) controls after undergoing statistical mixing test. As shown in the graph, the transect taken at the 0m mark is composed of 1.206% (sd=0.226) nitrogen from the aquatic source, which is a higher percentage than the other two transects. The 10m transect was composed of 0.0335% aquatic nitrogen. The 20m transect was composed of 0.0240% aquatic nitrogen.

The last set of data analyzed (Fig. 3) shows the total average nitrogen composition by percentage in the terrestrial samples taken from the dunes. The first transect at 0m (mean=1.2066, sd=0.226) has a higher total composition of N compared to the transects immediately inland of it. However, there is a trend of increasing nitrogen composition going from 10m to 30m. The 10m (mean=0.9702, sd=0.1001) transect is composed of significantly less nitrogen than the 0m, and the 20m (mean=1.0502, sd=0.0809) has higher nitrogen than the 10m transect. The terrestrial control 30m (mean=1.2486, sd=0.1176).

Discussion

The high algae $\delta^{15}N$ values correlates well with its ^{15}N enriched aquatic environment (Petersen et al., 1987). The next highest $\delta^{15}N$ value occurs at the terrestrial transect closest to the shoreline (Fig. 1). The data fits perfectly with the hypothesis proposed that there would be a *dramatic* transition, as opposed to a gradual or no transition, in ^{15}N between the transect closest to the shoreline and those further back in the dune. This trend probably occurs due to a high impact of low mobility factors, such as water and washed-up organic material, such as algae and aquatic insects. These low mobility factors only impact the area closest to the shoreline, while higher mobility organisms can impact a larger range further back in the dune. The influx of nutrients from aquatic to terrestrial follows a close correlation with the mobility of the vectors transporting the nutrients (Fariña et al., 2003). Our data reflects the extent to which the aquatic environment of Lake Michigan influences the terrestrial ecosystem coastline with aquatic nitrogen, where the extent of the influenced terrestrial habitats, in this case, is the immediate coastline (0m).

The most notable characteristic in Fig. 2, when analyzed by mixing, was the transect taken at the 0m mark. Compared to the other transects, the 0m transect had the largest composition of ^{15}N, which showed that the overall source of ^{15}N mostly likely came from the aquatic ecosystem. Transects at 10m and 20m seem to have very little influence from the aquatic environment, as their composition most closely resembles the terrestrial controls.

Dune systems in general are low in nitrogen, primarily due to their early stages of primary succession. Sand piles up along the shoreline, and then accumulates nutrients and organic material later. This idea contradicts what is seen in the 0m transect which shows increased levels of nitrogen. Intuitively, we think that the nitrogen content builds up in these dunes over time, and would therefore form a gradient of enrichment. Even Lichter (1998) found in his study of dunes that "the dune chronosequence represents a complex gradient of changing environmental constraints… Young dune ridges near the lake shore are characterized by… low availability of nitrogen and phosphorus." This implies that total nitrogen composition would start very low and would increase in the later successional dunes, and most of the enrichment of nitrogen in the dunes would come from a terrestrial source (Fig. 2). Although Fig. 3 shows an increasing enrichment of total nitrogen in the samples through the transects moving away from the shoreline, the 0m transect does not fit this trend. The aquatic ^{15}N input into that first transect (Fig. 1) may be enough to subsidize the entire shoreline with a higher total nitrogen composition (Fig. 3). An increased amount of nitrogen in that transect would possibly increase biodiversity and aid in dune stabilization. Though biodiversity was not quantified at any point in this project, there was an observed difference in plant species composition between the transects, and the 0m transect line did have a seemingly larger number of species than the 10m transect.

Though the data are still preliminary, there is a strong indication that there is nitrogen flow from the aquatic system to the immediate terrestrial system. Whether that flow is due to low mobility factors like dead organic material, is yet to be determined. Seeing as only the shoreline transect (0m) seemed to be strongly affected by aquatic ^{15}N, it may be a safe assumption that high mobility factors between these aquatic and terrestrial environments do not significantly affect the flow of nitrogen between the systems (Fariña et al., 2003).

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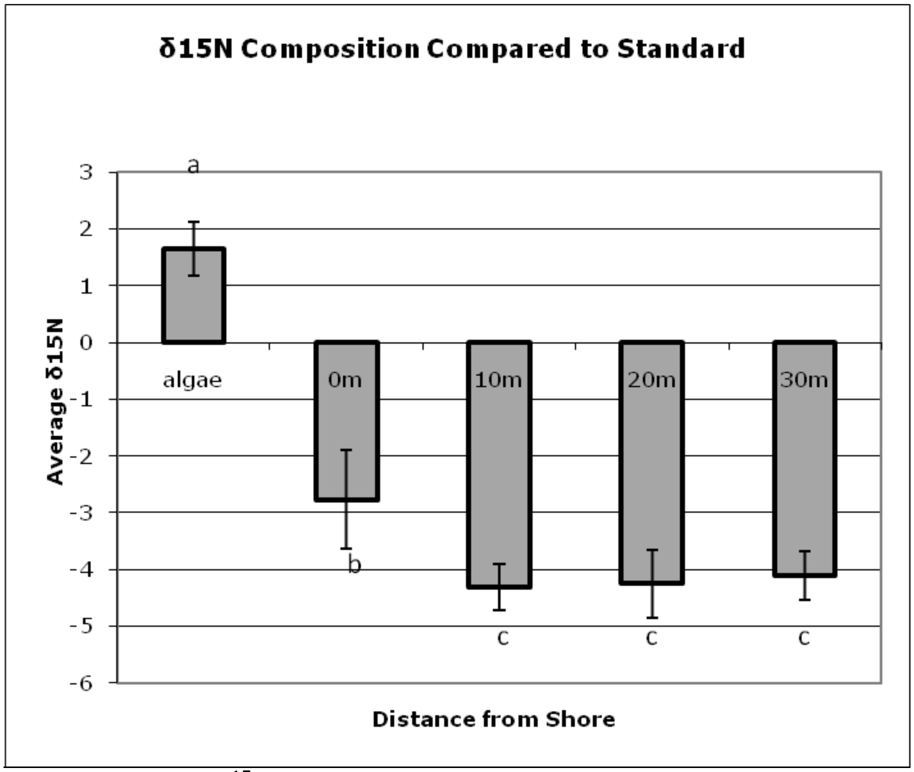

Fig. 1: Average $\delta^{15}N$ composition for each transect with standard error bars. Letters represent significant difference

Transect	# samples	Average $\delta^{15}N$	STD
Algae	3	1.650338947	0.468382825
0 m	5	-2.768063762	0.873769717
10m	5	-4.306302636	0.403145073
20m	5	-4.251662505	0.601832494
30m	5	-4.113121558	0.42962174

Table 1: Value chart to accompany Figure 1

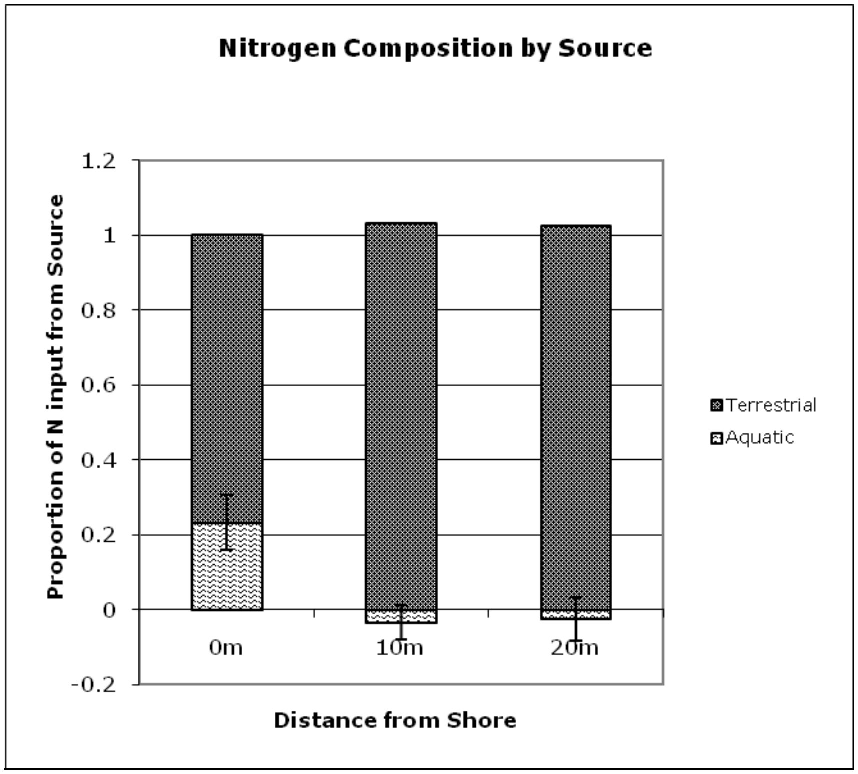

Fig. 2: Percent composition of nitrogen in each composite sample. Terrestrial represents ^{14}N, and aquatic represents ^{15}N.

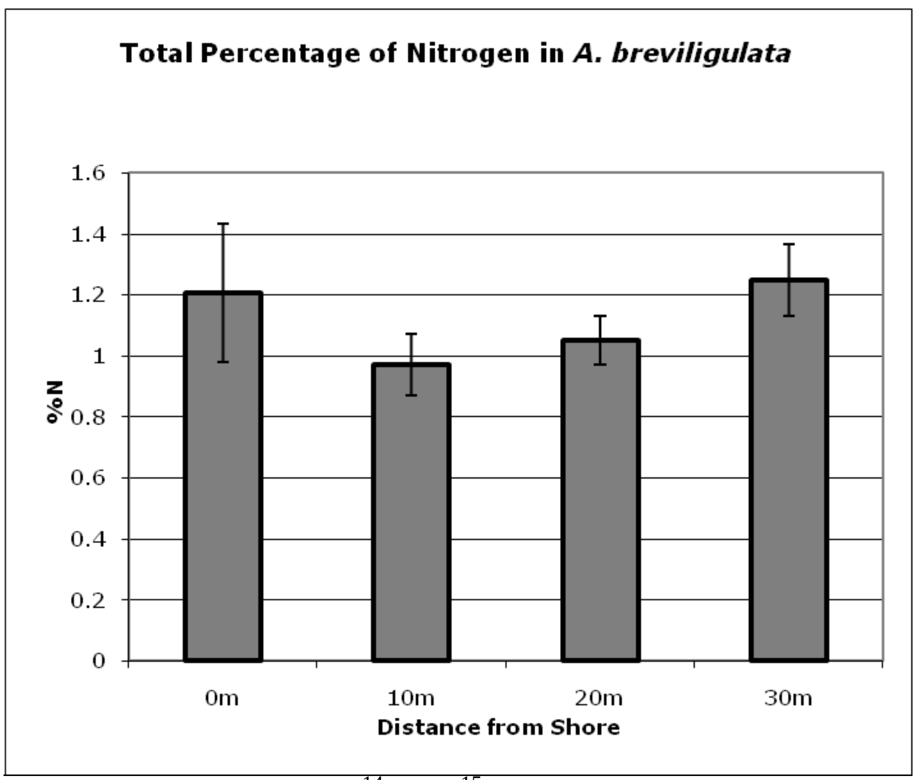

Fig. 3: Total nitrogen, both ^{14}N and ^{15}N, composition in the *A. breviligulata* samples