Nitrogen 15-N Uptake by *Typha* x *glauca* and *Scirpus lacustris* in Cheboygan Marsh and Cheboygan State Park Marsh

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ABSTRACT

Typha x *glauca* is an invasive plant species growing in Cheboygan Marsh, which is located downstream from the Cheboygan Wastewater Treatment Facility. Its establishment in the marsh has been attributed to the deposition of excess nutrients from the treated water from the treatment facility, specifically deposition of Nitroge15-N (15-N). Given that previous research has shown *T.* x *glauca*'s ability to displace native plants, we are interested in seeing if *T.* x *glauca* provides any ecosystem services, such as ameliorating the effects of eutrophication by taking up more 15-N than native plant species. We hypothesized that *T.* x *glauca* takes up more 15-N than *Scirpus lacustris*, a native bulrush. To test our hypothesis we collected soil and leaf samples of juvenile *T.* x *glauca* and *S. lacustris* from Cheboygan Marsh, our experimental site, and Cheboygan State Park, our control site. Stable isotope analysis was performed using a mass spectrometer to obtain 15-N to 14-N ratios; the results did not support our hypothesis and showed that *S. lacustris* took up more 15-N than *T.* x *glauca* in the Cheboygan Marsh. Our findings suggest that *S. lacustris* may be a better denitrifier than *T.* x *glauca*. Given that we faced some limitations to our study, we recommend that further research includes more plant and soil samples and that comparative research be done comparing lifetime uptake of 15-N between *T.* x *glauca* and *S. lacustris*.

Keywords: *Typha* x *glauca*, *Scirpus lacustris*, cattail, invasive species, Cheboygan Marsh.

INTRODUCTION

Wetlands are areas such as swamps, marshes or bogs saturated by surface or groundwater that support a variety of plant species adapted to these soil conditions. Wetlands serve as sinks for nutrients such as carbon and nitrogen. Marshes are wetlands with an abundance of nutrients and plant life, having a pH of 7 which is neutral (US EPA 2009). Marshes keep excess nutrients from draining into nearby bodies of water, thus preventing eutrophication. Algal blooms form when these excess nutrients are not absorbed by marsh vegetation (Angeloni, 2006). Algal blooms are accumulated masses of algae or cyanobacteria that can affect food web dynamics and harm surrounding plant and animal species (Anderson, 2002). Harmful algal blooms (HABs) produce toxins as they degrade, leading to foams and scums and oxygen depletion in the lake, and destruction of natural habitats. Algal blooms can lead to eutrophication, which is the accelerated process of ecosystem decay caused by excess nutrients and human activity.

Plants that can uptake excess nutrients may provide an ecosystem service by mitigating pollution in marshlands, and invasive plants are usually effective denitrifiers. Invasive species are those which have been accidentally or purposefully introduced to an area outside their origin, and they are competitive for resources (Tuchman 2009). Invasive plant species effectively exploit resources in marshes, so they provide a beneficial ecosystem service while harming their surroundings at the same time by decreasing biodiversity.

We studied the Cheboygan Marsh, which was invaded by cattail plants in the 1960s after the Cheboygan Wastewater Treatment Facility was built (City of Cheboygan Water Department [updated 2011]). The treatment plant treats approximately two million gallons per day for residential and commercial accounts, and treats sewage from Inverness Township. The facility expanded in 1978 to provide water treatment using Rotating Biological Contractors, which are disks that allow microorganisms to grow and remove nutrients in wastewater. The water that is discharged goes into the Cheboygan Marsh, which leads to Lake Huron. Pollution from the facility is a threat to Lake Huron, because it can cause algal blooms. The marsh begins growing next to the facility and extends to Lake Huron.

Vegetation in the area helps to uptake excess nitrogen and prevents the formation of algal blooms in Lake Huron. We chose to study two plant species in the Cheboygan Marsh and the Cheboygan State Park Marsh. The first species was an invasive cattail hybrid called *Typha* x *glauca*, which is a hybrid of native cattails (*Typha latifolia*) and invasive cattails (*Typha angustifolia*). *T.* x *glauca* can be identified by stout stems that are 3-6 feet in height, and cigar-shaped stalks with brown fibrous flowers at maturity. It is a perennial plant that forms monocultures in marshy habitats in Northern Michigan. The second plant species we studied was *Scirpus lacustris* (green bulrush), which was chosen because it is a native plant growing in both marshes. *S. lacustris* is a perennial plant that grows to about 5 feet in height and has brown, fibrous flowering that grows in bunches (USDA 2011). *S. lacustris* is found growing in freshwater marshes all over North America.

Previous research has been done on *T.* x *glauca* and its effects on species composition in the Cheboygan Marsh. *T.* x *glauca* is an aggressive and invasive species that appeared about 50 years ago in this marsh, outcompeting the native plants for space, nutrients and sunlight (Tuchman 2009). It has a clonal growth pattern, allowing it to spread quickly; it has greater biomass than surrounding species and dead *T.* x *glauca* litter builds up as mats (Larkin 2010). The litter keeps other plant species from growing in the same area because it reduces soil temperatures and seed germination ability (Farrer 2009). The invasiveness of *T.* x *glauca* and its ability to displace native plant species suggests that it negatively affects marsh ecosystems, but we suspect that *T.* x *glauca* benefits the area by acting as a denitrifier. Invasive plants should be better able to exploit excess nutrients than the plants that they displace (Tuchman 2009) and our goal is to see if this is true for *T.* x *glauca* compared with *S. lacustris.*

Stable isotope analysis is one method used to measure nitrogen levels in soil and plant samples. Mass spectrometers measure mass-to-charge ratio of ions, separate the ions according to this ratio, and detect these different ions. Ratios of different stable isotopes can be measured in a single plant or soil sample (Tissue 1996). 14-N is the naturally occurring stable isotope of nitrogen, and 15-N is the heavier stable isotope of mineral nitrogen compounds. Human disturbance and waste deposits cause unnaturally elevated nutrient levels in the area. Consequently, higher 15-N levels are expected in the Cheboygan Marsh than in the Cheboygan State Park Marsh because of the waste deposited from the Cheboygan Wastewater Treatment Plant. We predict that stable isotope analysis will show that *T.* x *glauca* has higher 15-N to 14-N ratios than *S. lacustris* because it is a better competitor for nitrogen, so it should be able to make better use of the nutrients in the environment and act as a denitrifier. The 15-N isotope is a tracer of enriched nitrogen and can be measured using a mass spectrometer.

METHODS AND MATERIALS

Study Sites

Two sites were used for this study: the first study site was located in Cheboygan Marsh and the second study site was located in Cheboygan State Park (Fig. 1). Two plots were set up in each of these sites.

Fig. 1. Map of Cheboygan Marsh and Cheboygan State Park.

Site Description. Site 1: Cheboygan Marsh (Experimental Site) (Fig. 2)

Cheboygan Marsh is a freshwater coastal marsh situated in the city of Cheboygan. The marsh is located approximately 205 meters downstream from the Cheboygan Wastewater Treatment Facility and feeds directly into Lake Huron. The marsh was heavily colonized by an invasive typha species in the 1950's, which later hybridized with the native typha to form *T.* x *glauca* (Tuchman 2009). We chose to use this as our experimental site because there has been an observable difference in plant species composition in the past 50 years (Tuchman 2009). This change in composition has been speculated to possibly be facilitated by the deposition of nutrients from the wastewater treatment facility, specifically of the Nitroge15-N isotope (15-N).

Fig. 2. Map of Cheboygan Marsh: Cheboygan Wastewater Treatment Facility indicated with black circle, plots 1 and 2 indicated with red circles. Plot 1 is 511 meters from the facility (25 m from Lake Huron shoreline) and plot 2 is 450 meters from the facility (86 m from the shoreline).

Plant Species Composition at Cheboygan Marsh

From what we observed, the area of the marsh closest to the wastewater treatment facility is substantially covered in thick senesced *T.* x *glauca* beds and there is relatively little other plant growth present. Thus overall observable plant composition in this area (~205 meters from facility) is comprised of juvenile *T.* x *glauca* plants and juvenile white cedar (*Thuja occidentalis).* As we continued further into the marsh (~450 meters from facility), plant species composition shifted to juvenile and senesced *T.* x *glauca* and juvenile *S. lacustris*, the latter of which we used in our study to compare its uptake of 15-N to *T.* x *glauca's* uptake of 15-N. Plant species composition remained the same between the 450 meter mark and the shoreline.

Site Description. Site 2: Cheboygan State Park (Control Site) (Fig. 3)

Cheboygan State Park is located on the Straits of Mackinaw and borders Lake Huron. The site we studied at the park is also a freshwater coastal marsh. We used this site as the control for our study because it is not dominated by invasive *T.* x *glauca*--though it is present there--and there is no wastewater treatment facility in the vicinity nor other evidence of excess 15-N deposition. We also chose Cheboygan State Park as our control site because we wanted to only look at plant uptake of 15-N by the same plant species as were present in Cheboygan Marsh: *T.* x *glauca* and *S. lacustris*.

Fig. 3. Cheboygan State Park: plots indicated with red circles. Plot 1 is 50 meters from shore and plot 2 is 97 meters from shore.

Plant Species Composition at Cheboygan State Park

Plant species composition at this site consisted predominantly of *S. lacustris* with patches of *T.* x *glauca* interspersed throughout the marsh. There was a noticeable difference in *T.* x *glauca* density at this site in comparison with it's density at the Cheboygan Marsh; the latter having much more *T.* x *glauca* biomass. We also found there to be no *Thuja occidentalis* at the park.

Materials

Plant samples. We used scissors to cut leaves of *T.* x *glauca* and *S. lacustris* at the sites. Samples were stored in plastic bags. A fifty meter transect was used to measure distance and select plots (plot selection process explained in “Sampling Design”). A meter stick was used to measure 1x1 meter plots from which we collected samples. Sample preparation required the use of an oven set at 60 degrees Celsius; samples were dried for two days and then ground. Prepared samples were then tested by stable isotope analysis via a mass spectrometer.

Soil Samples. A soil core was used to collect soil samples from each plot (plot selection process explained in “Sampling Design”). Soil samples were frozen at (-) 80 degrees Celsius for one day and dried for two days. A stable isotope test was also run on the soil samples using the mass spectrometer.

SAMPLING DESIGN

Cheboygan Marsh. Two 1x1 meter plots were set up in Cheboygan Marsh. The first 1x1 m plot was 511 meters from the facility (25 m from Lake Huron shoreline) and the second 1x1 m plot was 450 meters from the facility (86 m from the shoreline). We chose to set the second plot 450 m from the facility because there was no growth of *S. lacustris* closer to the facility past this distance. Fifty meter transect tapes were used to measure distance from shore. At each of the two plots, 10 total leaf samples were collected: 5 independent *T.* x *glauca* samples and 5 independent *S. lacustris* samples. All leaf samples were taken from juvenile plants to eliminate variability in age between *T.* x *glauca* and *S. lacustris*. Soil samples were also collected. Two soil samples were collected from each plot and three more were collected between the two plots for the purpose of having a gradient we could analyze.

Cheboygan State Park. Two plots were set up in Cheboygan State Park. The first 1x1 m plot was 50 meters from shore, as it was the first area in the marsh we found both *T.* x *glauca* and *S. lacustris*, and the second 1x1 m plot was 97 meters from shore. The 97 meter plot was selected at that distance because it closely mirrored the distance we selected for at the Cheboygan Marsh. Fifty meter transect tapes were used to measure distance from shore and 10 total samples were collected between the two plots: 5 independent *T.* x *glauca* samples and 5 independent *S. lacustris* samples. We also collected soil samples from the park- two at each plot and 3 in between plots for the gradient.

Data Analysis. To analyze our results of the stable isotope ratios, we used three SPSS tests: independent t-tests, one-way ANOVA and Tukey's. One-way ANOVA is used to compare the means of two or more samples. Assumptions of this test are: normal distribution of response variables, independent samples, and equal variances. Independent T-test compares the mean of two groups of a given variable. Assumptions of T-test are: equal variances and independent samples. Tukey's is used to find which means are significantly different from one another. Assumptions of Tukey's are: independent samples and equal variation across observations (samples).

RESULTS

Calculations. In order to compare levels of 15-N in *T.* x *glauca* and *S. lacustris* in the Cheboygan Marsh and Cheboygan State Park a value was calculated by taking the difference from a ratio of 15-N:14-N in sample to 15-N:14-N of universal constant (1). This value can be positive or negative; more positive numbers correlated to a higher presence of 15-N and a more negative number correlated to more of the lighter 14-N isotope.

15-N Levels in Soil Samples Between Cheboygan Marsh and Cheboygan State Park. Soil samples from Cheboygan Marsh contained, on average, higher levels of 15-N than soil samples from Cheboygan State Park: the mean difference was 1.54 with a p-value of .006, making it statistically significant at a 99% confidence level.

Total 15-N levels between all plant species at Site 1 (Cheboygan Marsh) and Site 2 (Cheboygan State Park). Cheboygan State Park contained higher levels of 15-N: the mean difference was -0.72 with a p-value of .02. Equal variances were assumed.

15-N Levels of T. x *glauca at Cheboygan Marsh versus T.* x *glauca at Cheboygan State Park.* 15-N levels were significantly lower at Cheboygan Marsh than at Cheboygan State Park; the mean difference was -1.253 with a p-value .000, making it statistically significant.

15-N Levels of S. lacustris at Cheboygan Marsh versus S. lacustris at Cheboygan State Park. 15-N levels were lower at Cheboygan Marsh with a mean difference of -.1922; p-value was 0.865.

Total 15-N Levels between Plot 1 at Cheboygan Marsh and Plot 1 at Cheboygan State Park. Difference in total 15-N levels of at Plot 1 at Cheboygan Marsh and Plot 1 at Cheboygan State Park was -0.45 with a p-value of .063. 15-N levels were higher in Plot 1 of Cheboygan State Park.

Total 15-N Levels between Plot 2 at Cheboygan Marsh and Plot 2 at Cheboygan State Park. Difference in total 15-N levels at Plot 2 at Cheboygan Marsh and Plot 2 at Cheboygan State Park was -1.092 with a p-value .044, making it statistically significant. 15-N levels were higher in Plot 2 of Cheboygan State Park.

DISCUSSION

Our predictions. We expected several items to hold true in our study: Cheboygan Marsh to have significantly higher levels of 15-N in the soil compared to Cheboygan State Park given that it's located downstream from the wastewater treatment facility, and for the 15-N levels to be higher in leaf samples from the *T.* x *glauca* compared to *S. lacustris*. The latter prediction was made because we wanted to see if the higher abundance of *T.* x *glauca* at the Cheboygan Marsh was providing an ecosystem service (denitrification) to the area or if *T.* x *glauca* is only negatively affecting the area by displacing the local flora, such as *S. lacustris*.

Soil comparisons. Given that the Cheboygan Marsh is located downstream from the Cheboygan Wastewater Treatment facility, we expected to see higher levels of 15-N in the soil samples we collected there. Our results supported this assumption--we found that the mean difference in levels of 15-N between Cheboygan Marsh and Cheboygan State Park was 1.54. This result was statistically significant at the 99% confidence level. Furthermore, this result was expected since the treatment facility is releasing 15-N into the marsh and thus there is more excess 15-N at the Cheboygan Marsh as compared to the Cheboygan State Park, which is not within close vicinity of a treatment facility.

Plant comparisons. Contrary to our prediction that 15-N levels would be higher at Cheboygan Marsh than at Cheboygan State Park, we found that 15-N levels were actually higher in both plants at the Cheboygan State Park. We found this results when we compared the average of total plant species at Cheboygan Marsh to total plant species at Cheboygan State Park. The mean difference was -0.72, which means that Cheboygan Marsh had lower total 15-N levels in all plant species combined (both *T.* x *glauca* and *S. lacustris*). This result was surprising in that we expected Cheboygan Marsh 15-N levels to be higher given that the 15-N level was higher in the soil there. We theorized that we got this unexpected result because we only tested juvenile leaf samples from both sites but did not test senesced *T.* x *glauca* or *S. lacustris.* A study done by Farrer (2009) mentions that senesced Typha litter typically contains high levels of 15-N, which contributes to soil nutrients and elevated 15-N levels. Thus had we measured senesced litter as well as juvenile plants we may have found higher levels of 15-N in the senesced *T.* x *glauca,* which could have shown a significant impact on total 15-N uptake of *T.* x *glauca* as compared to *S. lacustris*. Therefore, we may have seen higher 15-N levels at the Cheboygan Marsh than at the Cheboygan State Park, as we had expected to see.

In the same vein, we found that 15-N levels of *T.* x *glauca* and *S. lacustris* were significantly lower at Cheboygan Marsh compared to Cheboygan State Park: the mean difference was -1.253 (p-value = .000). Again this was contradictory to what we expected to find as we predicted that uptake of 15-N of both plant species would be higher at the Cheboygan Marsh than at the Cheboygan State Park since there is more excess 15-N available for uptake at the Cheboygan Marsh. We found that the total 15-N levels between Plot 1 (sum of both *T.* x *glauca* and *S. lacustris)* at Cheboygan Marsh and Plot 1 at Cheboygan State Park, as well as total 15-N levels between Plot 2 (sum of both *T.* x *glauca* and *S. lacustris)* at Cheboygan Marsh and Plot 2 at Cheboygan State Park, were also higher at the Cheboygan State Park. Given that we found lower concentrations of 15-N in the soil at the Cheboygan State Park than at the Cheboygan Marsh, it was unexpected that the 15-N levels in leaf samples were higher in the state park.

Implications of our results. These results suggest that perhaps the 15-N levels in the soil do not have a significant effect on plant uptake of 15-N. We state this because though we found higher levels of 15-N in the soil in Cheboygan Marsh compared to Cheboygan State Park, our results yielded that uptake of 15-N by both *S. lacustris* and *T.* x *glauca* was higher at Cheboygan State Park. Though our findings were contradictory to our prediction, it's still important to note that *S. lacustris* takes up more 15-N than *T.* x *glauca*. This suggests that *S. lacustris* may be a better denitrifier than *T.* x *glauca*, and that *T.* x *glauca* is not providing an ecosystem service. These results are not intuitive because *T.* x *glauca* seems to be a better competitor for space, as we've seen with the documentation of the *T.* x *glauca* front to moving towards Lake Huron (Tuchman 2009).

We originally predicted that plants which are better competitors for space are also better competitors for nutrients and will therefore take up more of the excess 15-N than the plants that they are displacing. However, it is possible that *T.* x *glauca* is not actually a better competitor, and that the reduction in ecosystem diversity in the marsh is due to some disturbance in the area or soil conditions rather than to the invasion (Larkin et al. 2010). Farrer (2009) found that the actual *T.* x *glauca* plant in the Cheboygan Marsh had little to no effect on the native plants and that it was actually the typha litter that caused a decrease in biodiversity of the marsh; the litter inhibited the growth of native plants and allowed typha to take over, creating a positive feedback loop in the typha's favor. Thus, the non-trophic effects of typha litter on native plants are greater than the actual competitive effects (Farrer 2009). Based on this research, we cannot conclusively say that *T.* x *glauca* is a better competitor for nutrients than native *S. lacustris*.

Life History Patterns. After analysis of our results, we found a study that was done by the Tennessee Valley Authority done on marshes in 1996. The results of this study showed that *Scirpus atrovirens* took up more 15-N than the *T.* x *glauca* that was growing alongside it. The researchers attributed their results to the rapid growth rate of *Scirpus atrovirens* (Behrends 1996). Because of it's shared genus with *S. atrovirens*, we inferred that *S. lacustris* also has a rapid growth rate; thus the growth rate, along with the dispersal patterns of the plants, may account for *S. lacustris*'s higher 15-N uptake. *S. lacustris* releases its fruits in August and September while *T.* x *glauca* disperses its seeds in the winter (Coops 1995) and both species are perennial. Therefore, the juvenile plants that we sampled from the marshes must have differed in age by a few months, giving *S. lacustris* more time to take up 15-N than the *T.* x *glauca.* This factor could account for the difference in 15-N levels that we observed between the two species.

Project Limitations. One of the biggest obstacles we encountered during our research process was time limitation. Given that we had four weeks to gather, prepare, and analyze samples, our sample size was small and was likely to have given us skewed results. Ideally we would have liked to gather a larger sample size (>100) in order to paint a more accurate picture of which plant species takes up more 15-N at the Cheboygan Marsh. Another obstacle we encountered was that the mass spectrometer available to us could only hold approximately thirty samples at a time, which further prevented us from having a larger sample size to analyze. It would have also been helpful if we had been able to collect more soil samples for stable isotope analysis; this includes setting up longer transects that span from the shoreline to the coastline. In doing so, we could have had a more inclusive and informative gradient of 15-N levels in the soil at both locations. We also would have liked to have collected more observational data regarding field patterns of plant species abundance and distribution both at the Cheboygan Marsh and at the Cheboygan State Park to see the effects *T.* x *glauca* has had on composition in recent years.

Future research. From our own research we saw that juvenile *S. lacustris* takes up more 15-N than *T.* x *glauca* at the Cheboygan Marsh, but we were limited to sampling only juveniles of both plant species as the adult plants from the previous year had already senesced. However, future research should be done on a comparison of lifetime uptake of 15-N between *S. lacustris* and *T.* x *glauca* because *T.* x *glauca* may have a higher overall (lifetime) rate of 15-N uptake compared to *S. lacustris*, which has only been shown to have a higher rate of 15-N uptake during early developmental stages. Further research should also be done to measure *S. lacustris* and *T.* x *glauca* density at both Cheboygan Marsh and Cheboygan State Park to assess interspecific competition for both space and nutrients. These findings can then be used to track recent changes in species composition compared to species composition recorded by Tuchman's research team.

ADDENDUM

Setting up a soil gradient. Halfway into our study we opted to collect several more soil samples from both the experimental and control sites in order to set up a soil gradient between Plot 1 and Plot 2 at each site. Our basis for setting up a soil gradient was to see if there would be a significant difference in 15-N levels within the soil at the Cheboygan Marsh in association with the soil's vicinity to the treatment facility. The soil gradient we set up between Plot 1 and Plot 2 at Cheboygan State Park was, again, used as a control for this portion of our study.

Role of previous knowledge. Based on the original prediction we made prior to any sampling we did, we assumed that the 15-N levels in soil would increase as the distance from the treatment facility decreased. However, after we collected the raw data from the stable isotope analysis of our original four samples--in which one soil sample was taken from each plot at each site--we saw that the 15-N level at Plot 1 in Cheboygan Marsh was 1.8 and Plot 2 was 1.7 (Table 1).

Table 1. Measurement of DN15 v Air. Table reflects the 15-N levels in each soil sample from Cheboygan State Park and Cheboygan Marsh.

Site	Plot 1	Plot 2
Cheboygan State Park	0.9 DN15 v Air	0.9 DN15 v Air
Cheboygan Marsh	1.8 DN15 v Air	1.7 DN15 v Air

Table shows that we essentially saw the opposite pattern of what we expected to find: the 15-N level at the plot furthest away from the facility (Plot 1; 511 meters from facility) had marginally higher levels of 15-N than the plot closer to the facility (Plot 2; 450 meters from facility). As previously stated, we were surprised to find that the 15-N levels were lower at the plot closer to the facility compared to the plot further away but that this finding may be due to there being more 15-N being stored in senesced *T.* x *glauca* litter, which was not measured for our study, than in the soil or in the juvenile *T.* x *glauca*.

Prediction for soil gradient. Thus bearing this confounding information in mind, our prediction in regards to the soil gradient was that there would be no discernible or significant gradient between Plot 1 and Plot 2 at the Cheboygan Marsh, nor at the control site, since the original 15-N levels we tested were nearly identical. Furthermore, we predicted that there may be no general pattern in 15-N levels in the collected soil samples when based solely on their vicinity to the facility, but rather that 15-N levels in the soil are more dependent on the amount of senesced *T.* x *glauca* litter present in the given area and thus 15-N levels in soil change as a function of litter amount. However, given our time constraints and the need to control for variables, we looked at 15-N levels within the soil gradient based only on vicinity to the treatment facility and did not take *T.* x *glauca* litter into consideration. Therefore, we highly recommend that further research is done to determine whether a gradient or pattern of 15-N levels in soil exists based on the amount of senesced *T.* x *glauca* litter is present in the area.

Methods and Materials. In total we collected nine more samples: one duplicate sample from each of the original four plots, two samples along the transect we originally set up between Plot 1 and Plot 2 at Cheboygan Marsh, and three samples along the original transect between Plot 1 and Plot 2 at the Cheboygan State Park. We repeated the same sampling and analysis process as with the original soil samples. To reiterate: a soil core was used to collect samples from each plot, samples were frozen at (-) 80 degrees Celsius for one day and dried for two days, and a stable isotope test was run on the soil samples using the mass spectrometer.

Data Analysis. We used two SPSS tests to analyze the results of our stable isotope ratios: independent t-test and linear regression model. Independent T-test compares the mean of two groups of a given variable; the assumptions of T-test are equal variances and independent samples. Linear regression model shows the strength of correlation between two variables using a line-of-best-fit; the assumptions of this test are normal distribution and independent samples.

Results. In the Cheboygan State Park 15-N levels increased as distance to land decreased, which can be seen by looking at the slope of the linear regression equation: the slope was 0.631 with p-value of 0.564 and the R-square value was 0.133 (Fig. 4).

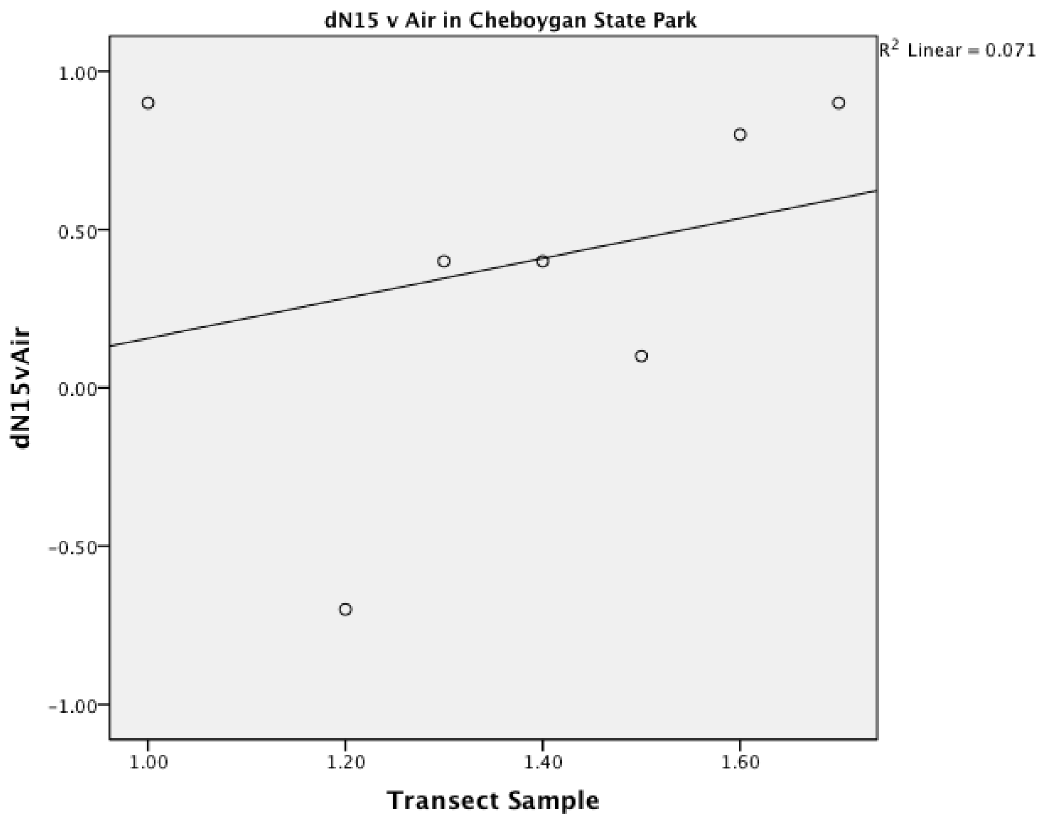

Fig. 4. Scatter plot of N-15 levels (dN15 v Air) in Cheboygan State Park. Transect sample numbers 1.0-1.7 (1.7 being closest to land) correlate with the seven samples taken. Linear regression line represents linear relationship between sample points. Regression analysis produced a p-value of 0.564 showing this relationship to be insignificant.

This result suggests that there is no significant relationship, and thus no strong correlation, between distance from land and levels of 15-N in the soil at the Cheboygan State Park. However in the Cheboygan Marsh 15-N levels decreased as distance to land decreased, which can be seen by looking at the slop of the linear regression equation: the slope was -1.314 with a p-value of 0.650 and the R-square value was 0.057 (Fig. 5).

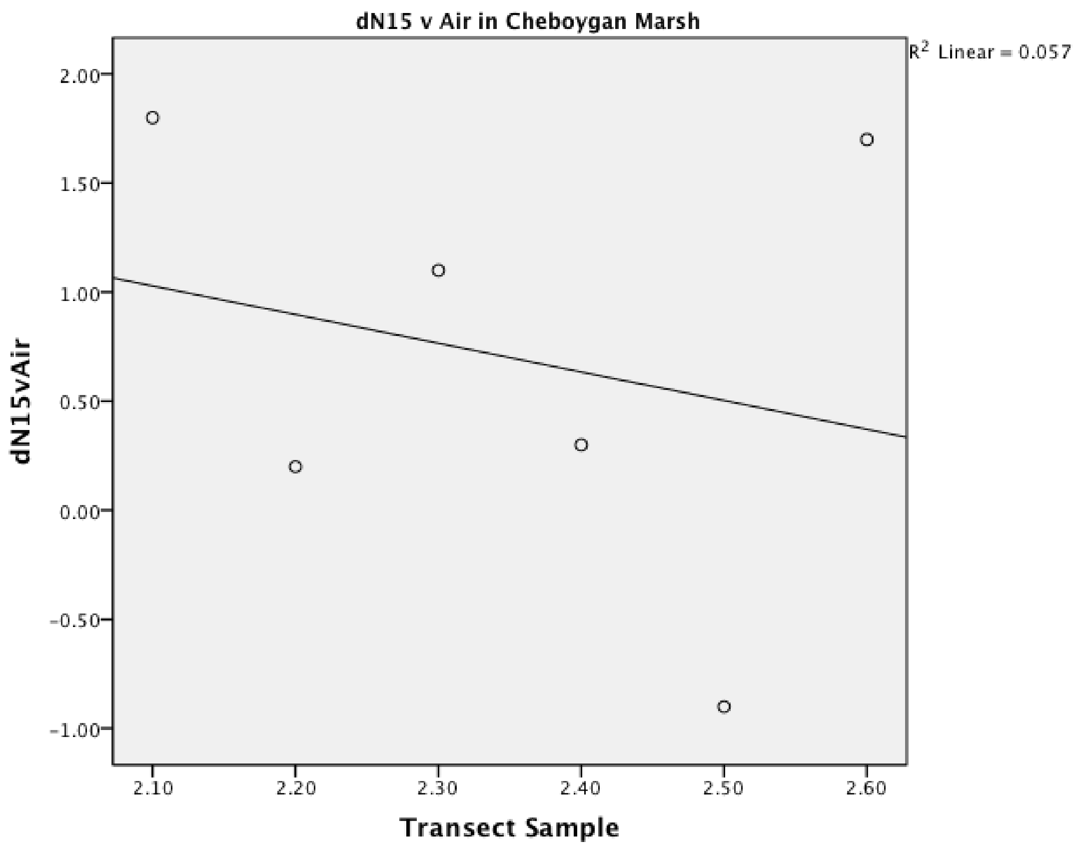

Fig. 5. Scatter plot of N-15 levels (dN15 v Air) in Cheboygan Marsh. Transect sample numbers 2.1-2.6 (2.6 being closest to land, or water treatment facility) correlate with the seven samples taken. Linear regression line represents linear relationship between sample points. Regression analysis produced a p-value of 0.650 showing this relationship to be insignificant.

This result also suggests that there is no significant relationship or strong correlation between the distance from land at which the soil was collected and it's distance from land.

Conclusions and discussion. Our results from both gradients show that there is no significant relationship between the levels of 15-N in soil samples relative to their distance from land.

Cheboygan State Park gradient. In the case of the two duplicates we sampled at Cheboygan State Park, the values were fairly consistent in relation to one another but differed with the values of the two original soil samples (Table 2).

Table 2. Measurement of DN15 v Air. Table reflects the 15-N levels of each soil sample collected from Cheboygan State Park. All soil samples collected from this site are included. Plot 1 denotes original plot, Gradient 1 denotes gradient sample taken on transect closest to Plot 1, as Gradient 3 denotes sample site closest to original Plot 2.

Cheboygan State Park Sample Type	15-N Value
Plot 1 (50 m from shore)	0.9
Plot 1 Duplicate	-0.7
Gradient 1	0.4
Gradient 2	0.4
Gradient 3	0.1
Plot 2 Duplicate	0.8
Plot 2 (97 m from shore)	0.9

Furthermore, the values of soil samples used as part of the gradient, which were labeled as Cheboygan State Park gradient 1 (SPG1), Cheboygan State Park gradient 2 (SPG2), and Cheboygan State Park gradient 3 (SPG3), were relatively consistent with one another (Table 2). Though there is a non-significant general trend of increase in 15-N levels as distance to land decreases (Fig. 4) the data points show that there is still no concrete trend in this relationship.

Cheboygan Marsh gradient. In the case of the two duplicates we sampled at Cheboygan Marsh, the values were also fairly consistent in relation to one another but again differed with the values of the two original soil samples (Table 3). The values of the soil samples used as part of the gradient, which we marked as Cheboygan Marsh gradient 1 (CMG1) and Cheboygan Marsh gradient 2 (CMG2), also showed large variability in relation to one another. CMG1 was the gradient point closest to Plot 1 and had a value of 1.1 while CMG2 was the gradient point closest to Plot 2 and had a value of 0.3 (Table 3).

Table 3. Measurement of DN15 v Air. Table reflects the 15-N levels of each soil sample collected from Cheboygan Marsh. All soil samples collected from this site are included. Plot 1 denotes original plot, Gradient 1 denotes gradient sample taken on transect closest to Plot 1, as Gradient 2 denotes sample site closest to original Plot 2.

Cheboygan Marsh Sample Type	15-N Value
Plot 1 (511 m from facility)	1.8
Plot 1 Duplicate	0.2
Gradient 1	1.1
Gradient 2	0.3
Plot 2 (450 m from facility)	-0.9
Plot 2 Duplicate	1.7

From these results we concluded that there is no gradient between Plot 1 and Plot 2 at Cheboygan Marsh. However it's important to note that our results are not absolute for both the control and experimental sites given that we needed a larger sample size and to have obtained larger quantities of the soil that we had sampled for stable isotope analysis purposes. A larger sample size will be more indicative of any patterns that may underlie 15-N levels in soil and proximity to a treatment facility. To conclude, despite the limitations in sample size we still found that our data supports our prediction of there being no significant or discernible gradient between Plot 1 and Plot 2 neither at Cheboygan Marsh nor at Cheboygan State Park. Thus we can infer that 15-N levels in soil is highly unlikely to be only dependent on proximity to a wastewater treatment facility, because if proximity had a greater effect 15-N levels we would have observed higher 15-N levels as we moved down the gradient towards land where the treatment facility is located. As previously mentioned, we would recommend that further research to be done on 15-N levels in soil based on the amount of senesced *T.* x *glauca* litter present in a given area. We would also like to note that from our personal observations we saw that senesced *T.* x *glauca* litter biomass was much larger the closer we got to the facility. Thus this relationship is perhaps more intricate than we anticipated in that 15-N levels in soil are lower as distance to the facility decreases, despite there being more nutrients directly deposited there, however senesced *T.* x *glauca* biomass increases as distance to the facility decreases.

Harkening back to our earlier discussion, senesced *T.* x *glauca* litter may have a greater impact not only on 15-N levels in the soil but also on the 15-N levels found in juvenile *T.* x *glauca* in comparison to native *S. lacustris*. We are more confident after completing the soil gradient analysis and finding no significance that our results for differences in 15-N uptake between *T.* x *glauca* and *S. lacustris* may be attributed to a combination of senesced *T.* x *glauca* litter acting as a storage pool for 15-N collected over the plant's lifetime and to the growth cycle of *S. lacustris*, as it has a more rapid growth rate and earlier seed dispersal time than the *T.* x *glauca* (Behrends 1996); which means that the *S. lacustris* we sampled is not necessarily a better denitrifier than *T.* x *glauca* but rather that we sampled older *S. lacustris* than we did *T.* x *glauca* and thus *S. lacustris* had a longer period of time during which to take up 15-N.

Recommendations and Future Research. We recommend that larger soil amounts be collected for running tests on, collecting a larger sample size, and measuring senesced *T.* x *glauca* litter, as it could be highly indicative of the fate of both excess 15-N and *T.* x *glauca* in wetland ecosystems. As we recommend prior to completing a soil gradient, we still recommend future research to be done on lifetime uptake of 15-N by *T.* x *glauca* in comparison with *S. lacustris,* which will also give more insight as to how much 15-N senesced *T.* x *glauca* stores and if it has significant effects on distribution of 15-N in soil and on 15-N uptake by non-senesced plants of both natives and the invasive *T.* x *glauca*. We also recommend that further research is done to determine whether a gradient or pattern of 15-N levels in soil exists based solely on the amount of senesced *T.* x *glauca* litter is present in the area as we think this may play a critical role in helping to map out an existing pattern of how 15-N is distributed in soils of areas within close vicinity of treatment facilities or in areas where excess nutrient deposition is present.

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