Observing the effects of increased availability of nitrogen on the salt marsh plant communities of the Plum Island Sound: A focused study on *spartina alterniflora*.

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   MBL – SES 2008
Abstract

Salt marsh ecosystems have been considered not susceptible to nitrogen overloading because early studies suggested that salt marshes removed excess nutrients in plant growth. However, the possible effect of nutrient loading on species composition, and the combined effects of nutrients and altered species composition on structure and function, was largely ignored. Failure to understand interactions between nutrient loading and species composition may lead to severe underestimates of the impacts of stresses (Deegan et al 2007). Through the TIDE project at the PIE-LTER research center at the Plum Island test sites (the right branches of Sweeney Creek: Fertilized and West Creek: Reference/Control) I was able to gain access to a whole ecosystem manipulation experiment, wherein the effects of a moderate amount of nitrogen enrichment (~70 μM NO3- across estimated creek area of 60,000 square meters) was being observed for a period of (at current) five years (See Appendix A).

From literature research I developed a method of testing and confirming what the impact of this level of nitrogen loading was on the herbivory of salt marsh plant communities. From the Spartina alterniflora region of the salt marsh I collected clip plot plants and soil cores to examine the root: shoot ratios as well as grazing intensity of individual plants. Also I was able to test C: N ratio and Carbon Fractionation (carbohydrates, phenolics and lignin). However due to the seasonality the plants I collected in October/November 2008 were considered to be already in the later stages of senescence and thus carbon fractionation tests were done on plants collected at the end of August 2008 (provided by TIDE).

The results from these test showed a higher amount of aboveground biomass with a lower belowground biomass resulting in a smaller root: shoot ratio in Sweeney Creek (fertilized). Sweeney plots had a greater amount of their belowground biomass devoted to root growth, whereas West Creek (control) plots had a significant proportion in favor of rhizomes. When CHN analysis was conducted on the end of summer plants it was found, strangely, that the C: N ratios were higher in the fertilized plots and showed (generally) a higher concentration of simple carbohydrates (sugars). While there were no phenolics observed, West Creek plants showed a higher amount of lignin than the fertilized plants of Sweeney.

Due to time limitations no protein tests were carried out.
Theoretical Framework

Influential Effects of Nitrogen on Herbivory in an Ecosystem

Species Composition
- Shift toward types of plants that are more susceptible to herbivory
  - Fast growing, soft, leafy green plants

Plant Morphology
- Change in Root: Shoot ratio
  - More Aboveground biomass available to be grazed

Plant Chemistry
- Increase in Nitrogen content
  - More susceptible to grazing due to a higher carbohydrate and protein concentration
  - Less susceptible to grazing due to an increase in anti-herbivory compounds: phenolics and lignin

Introduction

Previous studies have shown that anthropogenic inputs of nitrogen have increased levels of nitrogen concentrations in biogeochemical cycles, consequently causing a variety of environmental alterations. From these studies we also know that nitrogen enrichment to both aquatic and terrestrial ecosystems can particularly alter morphology, productivity and species composition. Among terrestrial ecosystems, salt marshes are considered highly ecologically important in that they serve as nursery areas for fishes, have plant communities that act as buffers for inland areas during storm surges and act as filters by trapping or absorbing the pollutant load to estuaries and other downstream ecosystems (Pennings et al, 2002).

Terrestrial net primary production is limited by nitrogen availability, thus any increase in the supply of nitrogen can dramatically alter local species composition and plant community structure and eventually have large scale effects (Pennings et al, 2005). In studying these effects, the basic mechanisms that influence community diversity, composition and dominance patterns may be revealed. Salt marsh ecosystems, however, have been thought of as not susceptible to nitrogen overloading, because early studies found added nitrogen increased marsh grass production (primarily Spartina spp., cord grass) and concluded that salt marshes can adsorb excess nutrients in plants and salt marsh plant-derived organic matter as peat. Detritus from Spartina is important in food
webs and in creating peat that forms the physical structure of the marsh platform. Nonetheless, the accumulation of peat and inputs of sediments, and the losses of peat through decomposition and sediment through erosion, may be altered under high nutrient regimes and threaten the long-term stability of marsh systems. Nitrogen addition may lead to either net gain or loss of the marsh depending on the balance between increased marsh plant production and increased decomposition (Deegan et al 2007).

Through the TIDE project at the Plum Island Sound, Massachusetts we can attempt to map the effects of an ecosystem-scale experiment of nitrogen loading in its entirety (Deegan et al 2007). In this experiment however I attempt to observe the effects of this form of nutrient enrichment on herbivory by comparing root: shoot ratios, grazing, C: N ratios and carbon fractionation data collected from soil cores and plants from the TIDE investigation sites of West (Control) and Sweeney (Fertilized) Creeks in the Plum Island Sound. I assume that an increase in nitrogen supply would increase the susceptibility of some plants to herbivory by lowering the C: N ratio, that is, I surmise that if a plant has more nitrogen, it is more nutritious and thus more palatable. Consequently this increased herbivory may cause the shift of dominance (in terms of cover) either to a less grazed or more competitive species. Or it may result in a plant species which is able to allocate the excess nitrogen to anti-herbivory compounds (which can be observed from carbon fractionation data). This alteration to herbivory can have a drastic effect because (when regarded as an absolute flux) herbivory reflects the levels of herbivore biomass and production maintained in the ecosystem. Herbivore production is a fraction of the amount of producer biomass ingested and the proportion is less variable than absolute consumption among diverse ecosystems. Therefore, systems supporting higher consumption tend to support larger herbivore standing stocks and production. Considering herbivory as the percentage of primary production removed has consequences for the role of herbivores in carbon and nutrient storage (as plant biomass) and recycling in the ecosystem. High rates of consumption leave only a small percentage of carbon and nutrients fixed by producers available for accumulation as producer biomass, and thus have the potential to act as significant controls of carbon and nutrient storage by producers (Cebrian & Lartigue 2004).
Study Area (Appendix A & B)

The Plum Island Sound estuary in New England, USA, where we conducted our experiments is a classic salt marsh estuary that is currently unaffected by nutrient loading. Tall *Spartina alterniflora* (200 cm in height; smooth cordgrass) is found in pure stands in low marsh and along creek banks that receive daily tidal inundation, while *Spartina patens* (20–60 cm) in height; saltmeadow cordgrass) is most abundant in higher elevation areas that are well drained and flood less frequently. *S. alterniflora* also occurs in a short form (20–60 cm in height) in the high marsh, often in pure stands in areas that are poorly drained (Deegan et al 2007). *Distichlis spicata*, a low-growing sodgrass, (10-40 cm in height) with tough, scaly rhizomes and rigid stems is also abundant in the high marsh vegetational zone.

![Vegetational Zonation in a Salt Marsh](image)

The lowest parts of the salt marsh are always covered by water and are called tidal channels. These are bordered by mudflats which are mostly devoid of rooted vegetation and are exposed to air only during the lowest tides. Adjacent to the mudflats and higher in elevation is the zone where salt marsh cordgrass grows. This is called the low marsh and its upper limit, as far as elevation is concerned, corresponds to the average height of the high tide (mean high water). Where the low marsh meets the mudflats is where the tallest *Spartina alterniflora* grows. Above the low marsh is the high marsh, which is bounded by the mean high water and spring high water lines. This area is characterized by a mixture of salt meadow grass (*Spartina patens*) and spike grass (*Distichlis spicata*) which are generally shorter and not as upright in appearance as the *Spartina alterniflora* which grows in the low marsh (Richard & Richard 2005).
Methods

Clip Plots (Plant Aboveground Biomass)

Eight sites were chosen at morphologically similar areas along the right branches of Sweeney and West Creek. At each site I placed 0.25 m\(^2\) quadrat in the tall *Spartina alterniflora* stand and collected all the plants from within the clip plot area. Care was taken to clip the plants whole and as close to the soil as possible, so that the total aboveground biomass could be accounted for. The plants from each plot were washed to remove any sediment and/or salt then dried for 48-60 hours, weighed and the dry weight values scaled up to be representative of a square meter.

Soil Cores (Plant Belowground Biomass)

Within the freshly cleared quadrat area, I used a 60cm (length) by ~6cm (diameter) soil corer (area: ~28.3 cm\(^2\)) to extract a core for rooting (belowground) biomass that would be scaled up to be representative of a square meter. The soil core was separated into a 0-10cm section and a 10-20cm section, each of which were halved and the roots and rhizomes from each section were placed in a drying oven for later weight measurements.

Grazing

In order to quantify grazing: the plants were separated into piles of grazed (plants with definite signs of grazing; discolorations that were not consistent with any form of disease/fungus or jagged edges that were not consistent with any post-collection physical damage) and non-grazed and then counted. Next, the grazed plants were individually examined and the grazed areas were quantified by a gridded square scale method.

Carbon: Nitrogen Ratio

Conglomerates of a few randomly selected plants (*Spartina alterniflora*, *Distichlis spicata* and *Spartina patens*) were ground, packed and stored in a desiccator for later CHN analysis.

Carbon Fractionation

Separation of ground plant material from the *Spartina alterniflora* conglomerates were subject to tests for simple carbohydrates, simple polyphenols and lignin (see Appendix B).
Results

The average aboveground biomass for both Sweeney and West Creek are of the same order of magnitude, however West Creek has approximately 90g more than Sweeney Creek (see Table 1, Fig 1) and the Root: Shoot ratio shows that for the investigated plots, West Creek has a slightly higher average (see Table 1). In the 0-10cm layer of soil at the Sweeney Creek plots, plants seem to allocate more to roots than rhizomes; the converse is true for West Creek. Below this level West Creek continues to show more activity, in that it has a higher proportion of live roots and rhizomes and a lower proportion of the same that are dead (see Table 1, Fig 2 & 3). The average root: shoot ratio (see Fig 4, Table 1) also shows that West Creek has a higher value, which is mirrored by the fact that there is a larger proportion of belowground biomass in comparison to Sweeney and that Sweeney Creek plants have more abundant in aboveground biomass. In general West Creek has more live and dead belowground biomass than Sweeney (see Fig 5, Table 1).

The grazing intensity (% per plant) seems to have a range of about 6.3-19.6% in Sweeney whereas in West Creek, the intensity of grazing ranges from 5.8-20.0% (see Fig 6). On average Sweeney Creek Spartina alterniflora plants have a higher grazing intensity percentage than the plant samples collected at West Creek (See Fig 7).

The Carbon: Nitrogen Ratio (see Fig 8.) shows that the plants at Sweeney have a higher value than those at West, however the lignin data (see Fig 10) indicates that despite the C: N ratio, plants at West Creek have a higher percentage of lignin and (generally) a lower concentration of sugar (see Fig 9). Neither of the investigated sites have any detectable amount of phenolics (see Table 3).

Discussion

The C: N ratio values for the three investigated species shows on average, that there is less nitrogen in the fertilized plots and relatively the same percentage of carbon as the control plots, resulting in a higher C: N ratio observed in Sweeney. This is contrary to my initial expectations of having a higher percentage of nitrogen in the fertilized due to increased uptake. However a concurrent study (Strebel 2008) shows that there is a significantly higher amount of N₂O production at Sweeney (i.e. more denitrification/nitrification processes). Therefore, I infer that in the recent past, the increased availability of nitrogen has shifted the ecosystem to the support of more diverse and numerous microbial communities which would result in making the nitrogen unavailable to the plants by microbial immobilization, thus destabilizing the whole community interaction. However due to the limitation of this study, this is only speculation. Only a few sources are available on the biomass of microorganisms in peat (which forms the majority of the high marsh top soil). However, it is difficult to apply data widely because the organic matter of peat soils in different climatic regions varies in composition due to variations in the initial substrate and in the conditions for microbial decomposition. Nevertheless, the turnover of the microbial biomass must have an enormous importance for the availability of plant nutrients, especially for P which is transferred in negligible amounts by rain to ecosystems in contrast to N (Brake et al, 1999). This may be supported by further data from Strebel (2008) which shows that there
is a lower bulk density at Sweeney Creek which may indicate the presence of more microbes consuming and immobilizing the organic material in the soil as an energy source.

In looking, specifically, at the C: N ratio values for tall *Spartina alterniflora* at Sweeney, we can see that they match closely to those reported by Gallagher (1975), (see Table 2). However the actual comparison to those in the control plot is not correlative to my initial expectations. That is, as stated before, given the input of nitrogen I expected that the C: N ratio would be lower in the fertilized (in comparison to the control). This may suggest that there is some emerging preferential allocation to a carbon compound that is being directly influenced by the increased nitrogen availability. It is my supposition that the plants at Sweeney Creek are diverting much of their production to simple carbohydrates as opposed to structural compounds such as lignin (see Fig 9 & 10). This preferential distribution to simple carbohydrate can be physically observed by the slightly higher percentage of average grazing intensity on the fertilized plot plants (see Fig 6 & 7). That is, these plants are slightly more palatable (being preferentially grazed) due to their higher sugar concentrations. I also make the inference that the percentage of acid insoluble lignin observed in West (which is higher than Sweeney) is actually the “normal” magnitude of lignin production and the Sweeney Creek *Spartina alterniflora* plants, due to the high availability of nitrogen, are keyed to growth and (in the near future) may become acclimated to this level of nitrogen input and we may see a change in the allocation of carbon compounds from less simple carbohydrate to more lignin in the fertilized plots.

At this stage, nitrogen is no longer the limiting element and the plants are able to produce without having to focus as much on anti-herbivory/structural compounds such as lignin, i.e. they are becoming “top-heavy”. Also the root: shoot ratio of these plants are consistent with expected results and confirm the initial expectation of having a large amount of aboveground biomass. However this “growth only” phase may result in creek bank instability. This is supported by the figures obtained for average aboveground and belowground biomass (Table 1). We see that there is more allocation of belowground biomass to roots as there is not need for the presence of large storage structures such as rhizomes. This is a stark contrast to West creek where there is a higher amount of rhizomes which accounts for a significant amount of the belowground biomass, both live and dead (Fig 2, 3 & 5).

In summary, the data concerning root: shoot, aboveground and belowground biomass (see Table 1, Fig 1, 2, 3 & 4) and simple carbohydrates (easily available energy source for growth: see Fig 9) indicate that the plants at Sweeney are producing at a higher level than West and are producing more *simple* carbon structures (see Fig 9). This may explain why we observe a greater degree of grazing intensity at Sweeney (see Fig 6 & 7). Thus one may infer that the increase in nitrogen is (slightly) increasing the susceptibility of these plants to herbivory. Also, the lignin data from West Creek may further substantiate this as the Sweeney Plots show a general trend of lower concentrations of this compound (which serves as a structural as well as an anti-herbivory compound: see Fig 10). This may be due to the fact that they are not under a considerable amount of ecosystem stress (primary nutrients are not limiting) in comparison to West and therefore simply do not need to devote resources to such compounds. However this may be also be due to a limitation of the data or methodology.
Conclusion

Given the aforementioned data and analyses, I conclude that as a general trend, the increased availability of nitrogen in the salt marsh ecosystem at Sweeney Creek is slightly increasing the susceptibility of *Spartina alterniflora* plants to herbivory. However due to the time and data limitations a definite conclusion cannot be determined as there are many unaccounted for factors involving the role of the microbial community and the need for more extensive research done on plant chemistry over longer periods of time.

Future Considerations
- If certain plants do produce anti-herbivory compounds, it does not, necessarily mean that it will deter feeding by all grazers. Thus there may be possible experimentation with feeding trials.
- Other factors such as the presence of silica and salt content may also act as a deterrent to herbivory.
- Estimation of the role of microbial community
- More extensive analyses on plant chemistry
Acknowledgements
I would like to express my deepest thanks to all who were pivotal to the conceptualization and completion of this project: Dr. Linda Deegan and the members of the TIDE project (PIE-LTER group), Richard McHorney, Amanda Keledjian, Jennifer Peters, Stefanie Strebel, Jessica Lambert, Paliza Shrestha, Andrea Coughlan, Jessica Kunke, Ian McPherson, Wayne Daniel and the faculty and staff of the Marine Biological Laboratory in Woods Hole, MA.
### Table 1. Above and Belowground Biomass Data Summary

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<td>Total Aboveground Biomass (g)</td>
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Fig 1. Aboveground Biomass of Sweeney Creek and West Creek
Fig 2. Belowground Biomass at Sweeney Creek

Belowground Biomass 0-10cm soil depth at Sweeney Creek

Belowground Biomass 10-20cm soil depth at Sweeney Creek
Fig 3. Belowground Biomass at West Creek
Fig 4. Root: Shoot Ratio

Fig 5. Total Live versus Total Dead Belowground Biomass
Fig 6. Grazing Intensity at Sweeney Creek and West Creek
Fig 7. Average Grazing Intensity
Fig 8. Carbon: Nitrogen Ratio of *Spartina alterniflora*, *Distichlis spicata* and *Spartina patens* at Sweeney and West Creek.
### Table 2. Carbon : Nitrogen Ratio

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Fig 9. Absorbencies and corresponding simple carbohydrate concentration values within samples
Table 3. Absorbencies and corresponding concentrations of phenolics within samples

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Fig 10. Percent Acid Insoluble Lignin per plot at Sweeney and West Creek
Appendix A

Yellow, green and blue lines indicate plant transects - indicated with colored PVC pipe. Brown circles indicate approximate location of distance markers (50, 100 m etc - brown pvc pipe). Distance is marked on the poles.
Appendix B

Yellow, green and blue lines indicate plant transects - indicated with colored PVC pipe. Brown circles indicate approximate location of distance markers (50, 100 m etc - brown pvc pipe). Distance is marked on the poles. ) mark is indicated by a brown and yellow striped pole on the left branch.
Appendix B
Carbon Fractionation Methods:
See Original: Written by – Andrea Ricca
Modified by – Chris Neill (15 May 1992)
Modified by – Timothy James Shankar Ramnarine (Nov 2008)

Extraction of water soluble constituents
1. Dry samples at 50 deg C.
2. Weigh out 0.5 g into Digestion Tube
3. Add ~80ml of Distilled H2O (20ml to wet sample, the remainder to wash the sides of tube)
4. Place tubes into block digestor preheated to 100 deg C. Adjust the temperature to allow the liquid to boil gently for 3 hours.
5. Filter directly into 250 ml digestion tubes using fritted glass filtering crucibles which have been pre-ashed, cooled and weighed. The fiber residues are washed with deionized water several times. The filtrates are brought to 250 ml with DI and saved for analysis of phenolics and sugars.

**Note for all filtration processes a vacuum pump filtration system (manual or mechanical) may be required**

Acid Insoluble Lignin
1. Approximately 200mg (0.2g) of the 50 deg C dry fiber is placed in to a 15ml round-bottom Pyrex test tube and 2ml of 72% H2SO4 is added. The tubes (TDN tubes) are placed in a 30 deg C water bath for 1 hr, during which they are mixed with a vortex several times.
2. 56ml of DI are added to each sample – 6ml to end the incubation and 50 ml to transfer the material to a 250 ml digestion tube.
3. Autoclave at 120 deg C for 1 hour
4. Filter through a pre-ashed, cooled and weighed Gooch filtering crucible. Wash residue several times with DI.
5. Store filtrate for acid soluble sugar test.
6. Collect residue, ash and weigh.

Phenol-Sugar Assay
1. Dilute suitable amounts of the dextrose primary standards to give a standard range of 0 – 50 ug/ml. A typical suite of standards contains 6 or 7 concentrations (evenly distributed).
2. Aliquots of the filtrate to be analyzed are diluted with DI to have sugar concentrations within the range of the standards. Extracts are diluted 20 to 1 using a pipette. Vortex after each addition.
3. 2ml of sugar solution is pipetted into a Pyrex tube. Samples and standards are prepared in duplicate.
4. The intensity of the color produced with a constant amount of phenol is proportional to the amount of sugar in the sample. 0.09ml of 90% (W/W) phenol
is pipetted into each tube with a pre-calibrated Eppendorf and the solution mixed with a vortex.

5. 5mls of conc. H2SO4 is added rapidly using a pipette with a large bore (good mixing)

6. Sit the tubes under the hood for 10 minutes at room temp and then vortex.

7. Place tubes in a 25—30 deg C water bath for 20 minutes.

8. Measure absorbance at 490nm.

### Soluble Polyphenol

1. Pipette 1—6 ml of the tannic acid primary standard (0.1mg tannic acid/ml) into 50ml volumetric flasks to give a standard range of 0—0.6mg tannic acid/50ml volumetric.

2. Using an Eppendorf, introduce an appropriate aliquot (0.1ml) of sample into a 50ml volumetric flask.

3. Add DI to each flask to about 2/3 full

4. Add 2.5ml Folin-Denis reagent to each volumetric flask and q.s. with DI. Shake 10 times and incubate for 20—30 minutes into a 25 deg C water bath.

5. If precipitate forms, centrifuge before reading.

6. Set spectrophotometer to 760nm against a water reference. Any sample with an optical density which lies outside the range of the standards must be rerun using a suitable sample aliquot.

### In Summary:

*Water-Soluble Constituents - Extraction*

- Residue
  - Water insoluble
  - Filter
    - Acid insoluble
    - Residue
  - Filtrate
    - Carbohydrates
    - Phenolics
References:


STREBEL, STEFANIE. EFFECT OF NITROGEN LOADING ON THE STRUCTURAL AND FUNCTIONAL STABILITY OF THE PLUM ISLAND SOUND SALT MARSH ECOSYSTEM. 2008. MBL – SES.