Effects of Nitrogen Loading on the Structural and Functional Stability of the Plum Island Sound Salt Marsh Ecosystem.

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ABSTRACT

Salt marshes are important transitional ecosystems that provide critical connections between terrestrial and oceanic ecosystems, channel substantial fluxes of materials and energy and provide a variety of other valuable ecosystem services: wildlife, nursery and stock habitats; shoreline stabilization; flood abatement; carbon sequestration; and improvement of water quality by interception and removal of land-derived nutrients and contaminants.

Salt marshes are especially valued for their nitrogen removal capabilities, and were not considered susceptible to nitrogen over-enrichment. Though nitrogen-loading is known to increase denitrification rate and has now been shown to change root to shoot ratio, species richness, competitive dynamics, plant productivity, and marsh susceptibility to erosion, few studies have investigated whether and to what extent nitrogen loading affects the capability of a salt marsh to carry out the functions we value so highly. Benthic macroinvertebrates are a ubiquitous component of salt marshes and represent a base component of various food webs, yet we have limited understanding of their impact on salt marsh function and of the environmental conditions that control their distributions and abundances.

In this investigation I examine the effects of nitrogen-loading on the structural and functional integrity of the Plum Island Sound salt marsh ecosystem. Specifically, I compare: the potential denitrification rate, C:N ratio, bulk density and % organic matter content of the marsh plateau (bank) and creek wall (creek); as well as the benthic macroinvertebrate community integrity, between an undisturbed marsh system and a fertilized marsh system subjected to five years of low to moderate nutrient-loading.

Though potential denitrification was almost twice as high in the fertilized site as in the reference, it also had a lower benthic integrity and a lower soil bulk density, suggesting that the overall integrity of the fertilized marsh is lower than the unfertilized reference. Additional preliminary data from a study by Timothy Ramnarine (SES 2008) suggests that root to shoot ratio is also lower in the fertilized marsh, which, together with a lower soil bulk density, suggests that fertilization adversely affects the structural stability of salt marsh soils.

Key words: denitrification, nitrogen-loading, salt marsh, benthic integrity, bulk density, slumping.

INTRODUCTION

Wetlands throughout the world have been conservatively estimated to contribute an average of US $5 trillion worth of ecosystem services annually, or US $15,000 worth of services per hectare per year (Costanza et al., 1997). Salt marsh systems form a dynamic interface between terrestrial and oceanic systems and as well as being one of the most productive ecosystems in the world (Deegan et al., 2007), they provide valuable ecosystem services including: the export of energy-rich material to deeper waters; wildlife and nursery and stock habitats; shoreline stabilization; flood abatement; carbon sequestration (Costanza et al. 1997, Weslawski et al. 2004, Zedler & Kercher 2005) and water quality improvement by interception and removal of land-derived nutrients (especially the removal of nitrate through denitrification to N\textsubscript{2}) and contaminants (Valiela et al., 2004). They thus form an important component of coastal areas, which are home to about 75% of the world’s human population (Deegan et al., 2007).
Today, as many as 80% of the marshes that once occurred in New England have been lost to human development (Bertness et al., 2002), and continuing loss of wetlands remains a global issue (Valiela, 2004).

Salt marsh ecosystems were originally considered not to be susceptible to nitrogen overloading because early studies suggested that salt marshes adsorbed excess nutrients in plant growth (Deegan et al., 2007) and then buried this organic matter in peat formation, or simply had high enough rates of denitrification to keep the system nitrogen (N) limited. However, recent research has shown that N over-enrichment will change salt marsh structure (e.g. root to shoot ratio, plant and animal species richness and competition) and function (e.g. plant productivity, trophic interactions and denitrification) which, together with marsh susceptibility to erosion, have been closely linked to the ecosystem services salt marshes provide (Wigand, 2008).

Nutrient enrichment has been shown to cause changes in the competitive dynamics and relative abundance patterns of marsh plants (Levine et al., 1998) and benthic organisms. Though biotic diversity in salt marshes is inherently low, each organism’s functional significance is great, so shifts in composition and diversity are likely to be particularly important. It is also unclear to what extent the ecological similarity of many benthic species will mitigate the loss of any one species, and whether a benthic habitat that loses biodiversity is still able to provide the same sorts of ecological services as one with a greater number of species (Austen et al. 2002). I investigated the effect of nitrogen loading on the benthic community of a salt marsh and attempted to determine whether the integrity of the benthic community was correlated to the integrity of the marsh ecosystem in terms of its denitrifying capabilities and sediment stability.

The accumulation of organic material in a salt marsh soil profile is the determining factor controlling vertical accretion in the upper decimeter. This organic matter is mostly from roots and not from detrital material settling on the marsh surface. The effect of a 10% change in root density distributed equally throughout a 1m sediment profile would change the vertical position of the marsh surface by 10 cm, a potentially significant amount for a plant living at the edge of its preferred tidal range (Turner et al., 2006). Bertness (1991) found that New England salt marsh species die when they are transplanted to marsh elevations lower than their typical range.

Nutrient additions tend to stimulate microbial respiration and decomposition (Deegan et al. 2007). Increased denitrification may lead to a more rapid consumption of belowground organic matter, which will reduce peat accumulation and adversely affect the marsh’s ability to maintain elevation in equilibrium with sea level (Roman and Daiber, 1984; Morris et al., 2002) even more. This, combined with the observation that N-loading may favor plant species with less belowground storage (Levine et al., 1998), could result in less dense sediment (organic matter is being consumed by denitrifiers and peat accumulation is decreased) with less stabilization from roots (lower root to shoot ratio). This would increase the susceptibility of the soil to erosion by tidal fluctuations, could lead to the structural collapse of creeks and could threaten the long-term stability and integrity of the salt marsh ecosystem, especially as the functionality of a marsh system can be difficult to restore once severely impacted (Zedler & Kercher, 2005).

I investigated the effects of nitrogen loading on salt marsh structure and function in the Plum Island Sound salt marsh ecosystem, focusing specifically on whether a) potential denitrification rates are higher in the fertilized site and whether this is reflected by a lower soil bulk density and organic matter content, b) the root to shoot ratio of vegetation correlates to the potential denitrification rate (since the aerobic microzone around Spartina alterniflora roots increases the surface area for coupled nitrification-denitrification) and/or the soil stability and
organic matter content, and c) the vegetation quality (C:N ratio) is correlated to the integrity of the benthic community and whether this can be linked to potential denitrification (as the activities of benthic communities have important ecological functions: bioturbation supplies O\textsubscript{2} to the anoxic sediments and creates a larger nitrification-denitrification surface area, while burrowing and shredding by benthic organisms causes the burial of organic matter and speeds up decomposition by breaking up organic material into smaller particles, respectively).

**METHODS**

*Site Description*

I collected my samples from the Plum Island Ecosystems (PIE) LTER sites in Rowley, MA, using West creek (WE) as the unfertilized reference and Sweeney creek (SW) as the fertilized comparison site. The Plum Island Sound estuary is fed by the Parker, Rowley and Ipswich River watersheds and flows into the coastal ocean, the Gulf of Maine. The WE and SW experimental sites were established in 2003, and were chosen for their similar hydrology and geomorphology. Each creek also has two branches which represent 2 “replicates” for each the reference and control site. For every incoming tide from May through October since 2003, liquid fertilizer has been added to SW creek in the form of liquid fertilizer to give a final concentration of 70 mm NO\textsubscript{3} in each branch. This results in a low to moderate loading rate to the SW marsh system and the yearly addition of about 1 ton of NaH\textsubscript{2}PO\textsubscript{4} and 22 tons of NaNO\textsubscript{3} to SW (Deegan et al. 2007).

*Potential denitrification*

Two sediment cores were collected using clear plastic coring tubes at 4 locations on the right branch of both the reference (WE) and fertilized (SW) site. One core (15cm by 45cm) was taken from the top of the bank within the *S. alterniflora* (WEb and SWb), and the other (6cm by 35cm) was taken horizontally from within the creek wall (WEc and SWc) below the rooting zone of the *S. alterniflora* above. Sediment cores were transported to the laboratory on ice, and stored at 4 °C in the dark until analyses could be performed.

Potential denitrification (PD) rates were measured by creating a sediment slurry and using the acetylene inhibition technique according to standard methods (Andersen, Jensen and Sørensen, 1984; Bernot et al., 2003; Golet and Ward, 2001; Joye et al., 1996; Martin et al., 1999; Tiedje et al., 1989). About 12 g of soil from the top 0-5 cm and 5-10 cm of the bank cores and the bottom (furthest within the creek) 0-10 cm of the creek cores were placed into separate Erlenmeyer flasks. Each soil sample was amended with 25 mL of “food solution” composed of 100 mg/L KNO\textsubscript{3}, 550 uM dextrose and unfiltered sea water, and then stirred to make a slurry. Flasks were sealed with septa and vacuum grease, and then alternately evacuated and then flushed 3 times with Ultrapure N\textsubscript{2} for 2 minutes. 25 mL of purified acetylene (C\textsubscript{2}H\textsubscript{2}) was added to each flask to give a final concentration of about 10 kPa (10%). C\textsubscript{2}H\textsubscript{2} distribution throughout the sediment was fostered by shaking and alternately reducing and increasing pressure in the soil pore space by pumping with a 20 cc syringe. This pumping will be done immediately after the acetylene is added and prior to each gas sampling. Sampling consisted of the removal of 15 mL of headspace using plastic gas-tight syringes at 30, 60, 90, and 120 minutes after C\textsubscript{2}H\textsubscript{2} addition. Directly after each sampling, 15 mL of zero-grade N\textsubscript{2} was added to the sample and mixed well in order to keep the volume of the headspace constant.

The samples were analyzed directly on a Shimadzu 14A gas chromatograph equipped with an electron capture detector and an ultra high purity carrier gas (N\textsubscript{2}). Silica could not be
used as a desiccant due to C$_2$H$_2$ buildup, so a reverse flow N$_2$ desiccator system was used to remove H$_2$O from the injected sample. Most of the cores were analyzed more than 48 hours after collection, and though Parkin et al’s (1985) cores were stored at 4 °C for up to 19 days without significant effects on the denitrification rate, it was necessary to measure the older cores at several time points after 120 minutes to allow time for the bacteria to reactivate. Known concentrations of N$_2$O were used to plot a standard curve and discern the N$_2$O concentration of each injected sample. The amount of N$_2$O within the flask at each time point was then calculated as:

$$\text{mmol of N}_2\text{O produced at time } i = \frac{\text{concentration of N}_2\text{O at time } i \text{ (ppm) } \times 1 \text{ atm}}{R(\text{L atm K}^{-1} \text{ mol}^{-1}) \times T(\text{K})} \times \left( \frac{\text{gas volume in flask (L)} + (\text{volume of slurry(L))}}{x \text{ Bunsen solubility coefficient} + \sum_{i=1}^{15} 0.015 \text{ L}} \right)$$

This calculation takes into account the volume of gas removed in sampling, the N$_2$O concentration within the slurry (using 0.67 as a rough Bunsen solubility constant). These average N$_2$O production values were then plotted over time, and the slope of the data was used as the average N$_2$O production rate in mmol N$_2$O per minute and converted to nmol N$_2$O produced per gram dry weight per hour.

**Sediment density and stability:**

Sediment bulk density was measured at each core site by collecting bulk density cores (5 x 4.8 cm) and weighing before and after drying in an oven for three days. Subsamples of soil were taken from each bulk density core after oven drying and weighing and were then analyzed for %C and %N using a CHN analyzer (5-6 mg soil per sample). Percent organic matter content of the soils was determined by loss on ignition at 450°C for 48 h.

**Benthic & Epibenthic Macroinvertebrate Sampling**

The epibenthic macroinvertebrate population was sampled using litter bags as described by Paul Fell et al. (2003). The bags measured 29 by 13.5 cm and were made of 5 mm Delta weave nylon mesh. Each bag was filled with about 20 g of dead *S. alterniflora* leaves and stems collected from the marsh. A litter bag was placed in a 1 m range from the creek bank edge at 12 sites at 12 m increments along the creek bank on both branches of WE and SW. The bags were placed beneath the grass and anchored in place with two-pronged metal stakes. The litter bags were retrieved two weeks later and swiftly placed into separate plastic whirlpack bags. At the laboratory, 50% ethanol and a dash of rose Bengal were added to each bag. Macroinvertebrates were separated from the litter, preserved in 95% ethanol and then identified and sorted according to taxon. The Shannon-Wiener diversity index was used as a measure of benthic and epibenthic integrity, and was calculated for the left and right branch of each site.
RESULTS

C:N Ratio

The C:N ratios (Fig 1) suggest a trend with a higher C:N ratio in WE than in SW, but they are all very close to each other and fall within the range of 14.5:1 to 15.5:1. The C:N ratios were not significantly different between sites (P=0.105), between site banks (P=0.067), between site creeks (P=0.442) or between creek and bank within a site (WE → P= 0.395, SW → P= 0.232).

Percent Organic Matter

Percent organic matter was at about 14-16% of the soil (Fig. 2), and was not significantly different between sites (P= 0.131), between site banks (P=0.307), between site creeks (P=0.175) or between creek and bank within a site (WE: P= 0.398, SW: P= 0.114).

Bulk Density

Bulk density (Fig. 3), however, was significantly different between sites. The bulk density was significantly higher in WE than SW for both the bank soil (P=0.024) and the creek soil (P=0.0034) by about 0.1 g/cm$^3$. Neither WE nor SW had a significant within site difference between bank and creek bulk density (WE: P=0.291, SW: P=0.326), making the bulk density in WE about 0.46 g/cm$^3$ compared to 0.56 g/cm$^3$ in SW.

Potential Denitrification

Potential denitrification was not significantly different between the top 0-5 cm and 5-10 cm of the bank soil cores (Fig. 4). It was almost two times higher on average (Fig. 5) in SWb (39.19 nmol N$_2$O · hr$^{-1}$ · (g dry wt.)$^{-1}$) than in WEl (73.15 nmol N$_2$O · hr$^{-1}$ · (g dry wt.)$^{-1}$), a very significant difference (P=0.008). Though potential denitrification was not significantly different between the creek soil (WEl: 9.49 nmol N$_2$O · hr$^{-1}$ · (g dry wt.)$^{-1}$; SWc: 8.70 nmol N$_2$O · hr$^{-1}$ · (g dry wt.)$^{-1}$) of the two sites (P=0.330), it was significantly higher in the bank soil than the in the creek soil for both sites (WE: P=0.007, SW: P=0.0000) – WEl PD was about 4 times higher than WEc, while SWb PD was about 7 times higher than SWc.

Benthic Macroinvertebrate Integrity

Five different benthic macroinvertebrate species were collected with the litter bags: the talitrid amphipods Uhlorschedia spartinophila, the isopod Philoscia vittata, the coffee bean snail Melampus bidentata, an unidentified species of annelid and an unidentified soft-bodied invertebrate. Benthic Integrity (Fig. 6 and Fig. 7) was estimated using the Shannon-Wiener Diversity Index (H'). WE-L shows the highest H', but both WE-R and WE-L had a higher H' than SW-R and SW-L. The average H’ for WE was significantly higher than that for SW (P=0.034).

DISCUSSION

C:N ratio

The C:N ratios suggest the expected trend with nitrogen loading, with a lower C:N ratio in SW where nitrogen availability is greater. Unfortunately the sample variance is too large to be
able to deduce any statistically significant trend. It is likely that with a larger sample size the C:N ratios would be better defined, as the difference between site banks was almost significant at P=0.067. Broome and Craft (1998) put the soil C:N of a natural marsh at about 18, so average C:N of about 15 is a bit lower than that, perhaps due to deposition of inorganic nitrogen in precipitation. Curiously, Tim Rammarine’s (2008) data on vegetation C:N ratios shows the exact opposite trend, with a significantly higher (P=0.006) C:N ratio in SW (~53) than in WE (~ 42). His data also showed a greater aboveground biomass in SW, and thus posits that the C:N ratio is so high because the SW plants are putting a large amount of resources into aboveground growth, thus producing higher amounts of simple sugars than the WE plants are. This does not appear to be reflected in the soil C:N ratio, potentially due to a high nitrogen immobilization by the bacterial communities.

**Percent Organic Matter**

The organic matter of peat soils in different climatic regions and ecosystems varies in composition due to variations in the initial substrate and in the conditions for microbial decomposition (Ivarson, 1977; Charman et al., 1994), so it is difficult to define a typical organic matter content of salt marsh soils. Broome and Craft (1998) give a value of 45% as the organic matter content of natural marsh soils, which is three times as high as the about 15% that I found for both the reference and the fertilized site. It is possible that because the soil bulk density cores that I used for % organic matter were from the surface of both the creek and the bank, they did not accurately depict the actual organic matter content of the marsh soil. It would be necessary to re-do this experiment with a larger sample size and at different depths within the soil profile. Besides increasing the holding capacity of soil water, nutrient storage and nutrient cycling, organic matter influences the number and kind of sediment-dwelling invertebrates (Levine et al. 1998). Soil organic matter content is thus a very important salt marsh ecosystem characteristic to take into account when investigating the physical, chemical and biological processes in salt marshes.

**Bulk Density**

The lower bulk density in SW does seem to suggest that SW soils are more waterlogged, and probably more prone to re-suspension and erosion than the denser, firmer WE soils. It is very surprising that the bulk density was not significantly different between bank and creek for either site, since most of the creek samples came from within the slumps. This evaluation would need to be carried out again with a much larger sample size, and at different depths within the soil profile and creek bank, in order to confirm that there really is no significant difference between creek and bank. It is possible that the creek soils were slightly compressed from the slumping, or that the surface samples that I took were a little dried-out from exposure to the air. However, even though compaction of sediments provides some resistance to erosion, it is likely of minor importance compared to other factors (Kneib et al, 2008). Erosion of the marsh substratum is determined to a greater extent by sediment composition (sediments with high organic content tend to erode more easily), the robustness of rooted vegetation, sediment re-suspension by rain (Torres et al., 2004), animal activities (e.g., crab burrows, Rudnick et al., 2005), or wind-driven waves or tidal flows (Wood and Widdows, 2002).

It would probably be more useful to find a measure other than bulk density for looking at sediment stability, potentially using a penetrometer to measure resistance to shear stress or a way to measure soil re-suspension that would allow for an estimate of how much of the creek wall
and slumps are washed away over time. It would also be useful to conduct a visual survey of slumping as a subjective comparison between sites.

**Potential Denitrification**

Potential denitrification was expected to be higher in SW than in WE due to the higher nitrogen availability in SW. The bank samples show this trend very clearly, but the creek samples do not and are both much lower than the bank samples and very similar to each other. I had not known what to expect for the bank samples, but the data seems to suggest that even though potential denitrification depends heavily on the available nitrogen, it is also significantly affected by a variety of other factors that influence the microbial communities within salt marsh soils. The results to the C:N analyses and the % organic matter were not statistically significant, so it is difficult to deduce to what extent these factors influence each other and the potential denitrification of a site. Nevertheless, it would appear that the potential denitrification is influenced by the position of the soil sample within the soil profile – potential denitrification seems to be higher in the rooting zone than below it. Potential denitrification is not significantly different between the top 0-5 cm or 5-10 cm of the bank samples, which suggests that the denitrifiers do not depend on algae (within the top 0-2 cm) as an energy source. Timothy Ramnarine (SES 2008) found that both average belowground biomass and the root to shoot ratio is higher in WE than in SW, which implies that the greater nitrification/denitrification surface area provided by a greater root density is not the determining factor in potential denitrification differences between the two sites. This does not quite fit the general belief that macrophyte productivity and microbial processes in marsh ecosystems are closely coupled (Howarth, 1993), but the stark difference in potential denitrification rate between bank samples within the rooting zone and creek samples below the rooting zone suggests that there is some factor linked to the roots that is determining this trend in potential denitrification. Ramnarine’s (SES 2008) data indicates that the vegetation C:N ratio is higher in SW than in WE, and also suggests that the SW plants are producing more simple sugars and less lignin than those in WE. The root exudates of the SW plants could thus be a very labile, rich energy source for denitrifying bacteria, and would allow microbial communities in SW to better take advantage of the higher nitrogen availability. Unfortunately, statistically significant soil organic matter content data would really have been very useful in providing an insight to the differences between WE and SW.

**Benthic macroinvertebrate integrity**

Benthic integrity was higher in the reference site WE, as was expected. This suggests that fertilization is negatively affecting the benthic community in SW, and thus probably the whole ecosystem. It would have been very interesting to compare the two marsh ecosystems using an overall ecological integrity index that takes into account the soil and vegetation C:N ratio, soil organic matter content, bulk density, potential denitrification rate and the benthic integrity, but this proved to be quite complicated could not, unfortunately, be carried out in the available time period.

**CONCLUSION**

Nitrogen-loading in SW appears to favor species with less belowground biomass (lower root to shoot ratio), resulting in decreased peat accumulation and less soil stabilization by roots. High nitrogen availability and greater sugar production by plants is probably spurring
denitrification rates in the rooting zone of SW soils, potentially reducing rates of peat accumulation. Together with a lower root biomass and a lower soil bulk density, nitrogen enrichment could adversely affect the marsh’s resistance to erosion and its ability to maintain elevation in equilibrium with sea level. This would increase the susceptibility of the soil to erosion by tidal fluctuations, could lead to the structural collapse of creeks. The benthic integrity of the fertilized salt marsh was significantly lower than that of the unaltered marsh, indicating that nitrogen loading may threaten both the long-term stability and integrity of the salt marsh ecosystem.

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REFERENCES


FIGURES & MAPS

Map 1. West creek experimental plot.

Map 2. Sweeney creek experimental plot.

Fig 1. Mean C:N ratios for each WE, SW, WE creek (WEc) and SW creek (SWc). P = 0.0669 for WE vs. SW, P = 0.442 for WEc vs. SWc.

Fig 2. % Organic matter content of the soil at each site for the bank (WEb and SWb) and the creek (WEc and SWc) soil samples.

Fig 3. Mean soil bulk density at each site for both bank (WEb and SWb) and creek (WEc and SWc).

Fig 4. Potential denitrification rates in the 0-5 cm and 5-10 cm range of both WE and SW bank samples.

Fig 5. Average potential denitrification at each site for both bank (WEb and SWb) and creek (WEc and SWc).

Fig 6. Benthic integrity for WE-R, WE-L, SW-R and SW-L.

Fig 7. Average benthic integrity for WE and SW.
Map 1. West creek (reference) experimental plot.

Map 2. Sweeney creek experimental plot.
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