The Role of Alternative Respiration Pathways and the Effect of Nutrient Loading on Peat Decomposition in Plum Island Marsh Sediment

David A. Dodge\textsuperscript{1, 2}
Collaborator: Austin Ritter\textsuperscript{1, 2}
Advisor: Anne Giblin\textsuperscript{3}

\textsuperscript{1}Middlebury College, Middlebury, VT, 05753
\textsuperscript{2}Semester in Environmental Science, Woods Hole, MA, 02543
\textsuperscript{3}The Ecosystems Center, MBL, Woods Hole, MA 02543
Abstract

The combined effects of sea level rise and eutrophication are responsible for the observed global decline in coastal wetland ecosystems. Nutrient loading is thought to stimulate rates of nitrate reduction in belowground marsh sediment, weakening soil structure and contributing to marsh subsidence. In this study, rates of aerobic respiration, nitrate reduction, and sulfate reduction were compared under pristine and eutrophic conditions. Nitrate reduction was also compared in pristine marsh sediment and sediment exposed to chronic fertilization over a seven-year period under the TIDE project at Plum Island LTER. Nitrate reduction had the lowest rates of respiration in sediment slurries over a fourteen day period, however, results showed evidence of increased rates of nitrate reduction in belowground sediment with nutrient loading. Comparison of TIDE and pristine marsh sediment showed evidence of shifts in the composition of the microbial community and organic carbon composition of the soil. Conservation efforts therefore should aim to decrease anthropogenic nitrogen loading to wetland ecosystems.

Key Words: Wetland, Eutrophication, Microbial Respiration Pathways, Plum Island LTER, TIDE

Introduction

The Intergovernmental Panel on Climate Change estimates that global sea levels have risen approximately 1.7-1.8 millimeters per year over the last 150 years (IPCC, 2010). Climate change models predict that sea level rise will accelerate in the 21st century as ice caps and glaciers continue to melt (Giblin, 2010). The implications of sea level rise are numerous and in some cases yet to be defined; however, one clear effect is the global
loss of wetland ecosystems. The IPCC predicts that by 2080, 33 percent of the world’s coastal wetlands will have been converted to open water (Giblin, 2010).

Wetland ecosystems provide habitat to many species, play important roles in nutrient uptake, and prevent flooding in many coastal communities (Giblin, 2010). In cities that are particularly vulnerable to storm flooding, like New Orleans, the consequences of wetland loss are especially significant.

Wetlands can keep pace with rising sea levels by decomposing trapped sediment from the land and ocean, thereby accumulating belowground organic matter (Giblin). Eutrophication disrupts this natural process by stimulating soil metabolism and increasing translocation of belowground resources to aboveground plant biomass via roots and rhizomes (Turner et al., 2007). Loss of belowground biomass can compromise soil structure in marsh sediment. A 2010 study by Turner found that high nitrogen and phosphorous loading to a Louisiana salt marsh caused a 35% decline in soil strength at a depth of 60-100 cm (Turner, 2010). Weakened soil may degrade and/or collapse during flooding events, contributing to marsh subsidence. Understanding the combined effects of eutrophication and sea level rise on soil processes in belowground marsh sediment is key to advancing long-term maintenance of salt marshes (Turner 2007).

This study focuses on the effects of eutrophication on three microbial respiration pathways in belowground marsh sediment: aerobic respiration, nitrate reduction (denitrification), and sulfate reduction. Aerobic respiration uses oxygen as an electron acceptor to oxidize organic carbon, releasing CO₂ in the process (table 1). Aerobic respiration generally occurs within the top centimeter of marsh sediment after which oxygen availability rapidly declines (figure 1 and 2). Nitrate reduction uses nitrate as an
electron acceptor to oxidize organic carbon, releasing CO₂ and nitrogen gas in the process (see table 1). Nitrate reduction generally occurs within a narrow depth range (around 2 centimeters) at which nitrate is present but oxygen is absent from the soil (figure 1 and 2). Sulfate reduction uses sulfate as an electron acceptor to decompose organic carbon and release CO₂ and hydrogen sulfide in the process (figure 1 and 2). Hydrogen sulfide produces a strong odor that is easily recognizable at low concentrations. In addition to reducing sulfate, some strains of sulfate reducing bacterium use nitrate as an electron acceptor producing ammonia in the process (Dalsgaard et al., 1994). Sulfate reduction generally occurs in deep anoxic, typically below 4 centimeters.

These heterotrophic respiration reactions have accompanying energetic efficiencies, expressed as delta G of the reaction and measured in kcal mol⁻¹ (table 2). A reaction with a negative delta G releases energy. Aerobic respiration has a delta G of -114 kcal mol⁻¹ and is the most energetically favorable of the three respiration pathways. With a delta G of -108 kcal mol⁻¹ nitrate reduction does not proceed as favorably as aerobic respiration. Sulfate reduction has a delta G of -20 kcal mol⁻¹ and is therefore the least energetically favorable of the three reactions. The delta Gs of the reactions determine the efficiency at which microbial populations are able to break down certain kinds organic carbon compounds via one of these three pathways.

Aerobic respiration and sulfate reduction account for all or nearly all carbon decomposition in marsh sediment. Nitrate reduction, although energetically favorable, only occurs within a narrow depth range. Aerobic respiration rates are not intrinsically higher than anaerobic rates, however, they are typically greater than aerobic decomposition rates at depth because most labile carbon has already been consumed
before it is buried in anoxic sediment (Henrichs et al., 1987). Figure 3, adapted from Westrich et al., 1984, makes the point that aerobic respiration, nitrate reduction, and sulfate reduction have equal decomposition rates in the G1 phase when most labile carbon is being consumed. Aerobic respiration and nitrate reduction, however, are able to decompose the full suite of organic compounds, while sulfate reduction, a typically energetically limited reaction, is less efficient at breaking down more recalcitrant forms of carbon like cellulose and lignin. We therefore see nitrate reduction and aerobic respiration rates pull-away in the G2 and G3 phases of soil decomposition.

As previously stated, eutrophication has proven to increase soil metabolism in marsh sediment leading to overall decreases in soil strength (Turner, 2010). One theory is that stimulated nitrate reduction, a typically nutrient limited reaction, is responsible for increasing decomposition rates in belowground sediment. In particular, stimulated nitrate reduction may increase decomposition of more recalcitrant carbon compounds, like lignin, that are structurally important components of the soil. This study therefore sought to address the following questions: 1) is nitrogen and phosphorous loading stimulating nitrate reduction in belowground marsh sediment to rates above those of sulfate reduction? 2) To what degree (if at all) is nutrient loading increasing rates of sulfate reduction and aerobic respiration? 3) What is the effect of long-term fertilization on rates of nitrate reduction in marsh sediment? I will be able to address these questions be preparing sediment slurries and separately stimulating aerobic respiration, nitrate reduction and sulfate reduction in 300 mL BOD bottles. To half of the bottles I will add phosphate and ammonium, mimicking eutrophication, and compare rates of respiration to
the without nutrient bottles. I will also compare rates of nitrate reduction between pristine sediment and sediment exposed to chronic fertilization (see table 3).

I expect aerobic respiration to have the highest respiration rate due to its more negative delta G (table 2). I predict that nitrate reduction will have a slightly lower respiration rate and sulfate reduction rates to be substantially lower than the other two respiration pathways (figure 5). I expect carbon decomposition to be higher in the chronically fertilized plot than in the control plot, owing perhaps to changes in the composition of the microbial community. Nitrogen loading over a seven-year period would likely favor nitrate reducers over sulfur reducing bacteria in the belowground sediment.

I expected ammonium and phosphate addition to have little to no effect on the rates of nitrate and sulfate reduction in the sediments because nitrate reduction is typically nitrate limited and sulfate reduction is typically energetically limited. In the aerobic sediment I expected nutrient addition to increase carbon decomposition because oxic respiration is generally a nutrient limited pathway (Jorgensen, 1980).

Methods

Six sediment cores were extracted from the Plum Island salt marshes on Nov. 15th, 2010 (figure 4). Four cores were extracted from a pristine reference site (“West”) and two cores from a site exposed to chronic fertilization over a seven-year period under the TIDE project. Addition of mineral nitrogen to the “TIDE” plot ended this past summer (2010). All six cores were extracted from areas of the marsh that are in the
process of falling into the river channel. Cores were sealed and brought back to Loeb lab for treatment and analysis.

In order to compare the relative efficiencies of different microbial respiration pathways in the belowground marsh sediment, I stimulated aerobic respiration, nitrate reduction, and sulfate reduction separately in 300 mL BOD bottles. In the TIDE sediment, I was most interested in the effects of long-term nitrogen fertilization on rates of nitrate reduction. I therefore did not stimulate aerobic respiration or sulfate reduction in the TIDE sediment. In order to test the effects of eutrophication on each of the three respiration pathways, I added low concentrations of ammonium and phosphate to half of the treatment bottles. For each treatment I prepared 3 replicates and 1 blank control, totaling 27 BOD bottles. The experimental design is presented in Table 3.

Anaerobic sediment between 5 and 10 cm was removed from the cores and homogenized separately by site in plastic bags. The bags were pumped with nitrogen gas to keep the sediment anaerobic. Two 25-30 g sediment samples from the TIDE plot and two samples from the West plot were weighed and placed in an oven to dry. The samples were reweighed at the end of the experiment and CHN analysis was performed on the dried samples. Approximately 2 grams of sediment went into each labeled BOD bottle, excluding the oxygen, nitrate and sulfate blank bottles (table 4). Stir bars were placed in the oxygen treatment bottles in order to measure dissolved oxygen over the course of the experiment. Sediment slurries were prepared by adding oxygen, sulfate, and nitrate artificial seawater solutions to the oxygen, sulfate, and nitrate treatment bottles.

The seawater solutions were prepared from 5-micron filtered Marine Resources Center seawater. The filtered seawater was diluted 10:1 in a carboy and NaCl was
subsequently added. The resulting stock solution had low nutrient background and standard seawater salinity of 7 ppt (175 mM C). This solution was added directly to the oxygen treatment bottles. For the sulfate seawater solution, MgSO$_4$ was added to make a 27.5 mM sulfate solution. For the nitrate seawater solution KNO$_3$ was added to the stock to make a 1 mM nitrate solution. The nitrate and sulfate solutions were bubbled with nitrogen gas until no oxygen was present in the water. These solutions were then added to the appropriate treatment bottles. Phosphate and ammonium were additionally added to the 12 nutrient bottles in order to obtain concentrations of 20 uM PO$_4$ and 100 uM NH$_4$. The bottles were then placed on a shaker table to mix over the next 14 days. A black plastic bag was wrapped around the apparatus to prevent autotrophic respiration in the bottles.

Water samples were extracted from the bottles on day 3, day 11, and day 14 of the 14-day experiment using 30 mL syringes. The bottles were quickly refilled with the appropriate seawater solution after sampling. Water samples were transferred to scintillation vials and stored in the freezer for subsequent analysis. I left 5 mL of sample in the syringe for subsequent DIC analysis.

I ran nitrate, sulfate, phosphate, ammonium and DIC on all of the water samples. Nitrate concentrations were measured according to the SES “Lachat Flow Injection Analysis for Measuring Nitrate” protocol 2010. Sulfate concentrations were measured according to the SES “Dionex for Sulfate Analysis” protocol 2010. Phosphate concentrations were measured with a spectrophotometer according to SES “Phosphate” protocol 2010. Ammonium concentrations were also measured using a spectrophotometer according to SES “Ammonium” protocol 2010. DIC was measured with a gas
chromatograph according to SES “Total Dissolved Inorganic Carbon in Aqueous Samples” protocol 2010. These measurements of concentrations over three time points were used to estimate rates of respiration in the oxygen, sulfate, nitrate, and TIDE nitrate treatment bottles.

**Results**

Sediment from the Plum Island West site had roughly the same percent water weight as sediment from the TIDE research site (see figure 6). Two samples from each site were weighed and dried for CHN analysis. TIDE sediment was approximately 36% water weight and West sediment was approximately 39% water weight. This difference is not statistically significant given the small sample set. The C:N ratio of the TIDE sediment was lower than the C:N ratio of the pristine West sediment (figure 7). The average C:N ratio of the two TIDE sediment samples was 12.29 vs. 13.88 in the two West samples. The TIDE plot is therefore more nitrogen rich. Mineral nitrogen was added to the TIDE research plot over a seven-year period that ended this past summer (2010).

In the oxygen treatment bottles D.O. decreased rapidly from the first time point on 11/19 to the second time point on 11/21 (see figure 8). Rates of decrease were equivalent in “with nutrient” and “without nutrient" bottles. The bottles were left on a shaker table with a 30 mL headspace to reoxygenate over a seven-day period (thanksgiving break). The D.O. in the nutrient bottles climbed to 8 mg/L vs. 5.5 in the w/o nutrient bottles over this period of time. There was greater variation in the w/out nutrient oxygen readings (see error bars). D.O. decreased faster in the nutrient bottles
between time points 3 and 5 than in the w/o nutrient bottles. D.O. fell from 5.5 mg/L to 1.6 mg/L in w/o nutrient bottles vs. from 8.1 mg/L to 1.2 mg/L in the nutrient bottles.

Nitrate concentrations dropped 100 uM between time points 1 and 2 the nitrate treatment bottles (figure 9). Nitrate concentrations were 15-20 uM higher in the nutrient bottles. In the TIDE treatment bottles, concentrations of nitrate fell only 2 uM in the w/out nutrient bottles and 14 uM in the nutrient bottles (figure 10). In the sulfate and oxygen treatment bottles no additional nitrate was added to the slurry and no nitrate appears to have been consumed (figure 11 and 12). There are low concentrations of nitrate present in the dilute standard ASW I prepared. In the oxygen treatment bottles nitrate concentrations go up over time in the nutrient bottles.

Sulfate concentrations dropped 1000 uM between time 1 and time 0 (figure 13). The blank bottle was used as a reference for time 0. Data from time points 2 and 3 was too variable to present in this report. The error bars show that sulfate concentrations were not significantly different in w/ nutrient and w/out bottles.

Ammonium was added to the nutrient bottles at time points 0, 1, and 2. If no ammonium uptake occurred, then we would expect to see 300 uM concentrations in the nutrient bottles. In all of the treatment bottles ammonium concentrations were below 80 uM. In the oxic treatment, ammonium was completely consumed by aerobic respiring microbes in the w/out nutrient bottles (figure 14). Excess ammonium accumulated in the nutrient bottles. Approximately 90 uM ammonium was consumed between each time point, leaving behind 10-20 uM ammonium at each time point. In the w/ nutrient sulfate treatment bottles, there was an excess of approximately 30 uM at time 1 (figure 15). The amount of excess ammonium decreased to a difference of 10 uM at time point 3,
indicating greater ammonium uptake over time in the nutrient bottles. In the nitrate and sulfate w/out nutrient bottles, ammonium concentrations went up slightly over time indicating mineralization (figure 15 and 16). Ammonium concentrations were lower in both nutrient and w/out nutrient TIDE sediment than in the West sediment (figures 16 and 17).

DIC and phosphate analysis did not yield usable results. DIC data showed substantial inconsistencies, even after accounting for outliers. As a result, DIC did not serve as an accurate tool for measuring respiration rates in this experiment. Phosphate data showed a complete set of blanks and there was no sample left to run another set.

Rates of oxygen and nutrient flux were used to estimate respiration rates in umol CO2 per gram sediment per day. In calculating respiration rates, I made sure to balance the reaction. For every mole of sulfate consumed, 2 moles of carbon dioxide are respired in sulfate reduction (table 1). For every mole of nitrate consumed, 1.25 moles of carbon dioxide are respired via nitrate reduction. For every mole of oxygen consumed, a mole of carbon dioxide is respired in aerobic respiration. These respiration rates were all converted to umol per gram of sediment per day by multiplying uM concentrations by the size of each bottle (0.3 liters) and dividing by the weight of each sediment sample added to each bottle at the start of the experiment.

Sulfate reduction rates were than10 times higher than aerobic respiration and nitrate reduction rates (see figure 18). Sulfate reduction rates in the nutrient and w/out nutrient were statistically the similar. Rates of aerobic respiration in the oxygen treatment bottles were higher than rates of nitrate reduction in the nitrate treatment bottles by approximately 4 umol CO2 g-1 (wet) day-1 (figure 19). Nitrate reduction in sediment
from the West plot was higher than in sediment from the TIDE plot. Denitrification in the TIDE plot was very low at 0.18 umol CO2 g-1 (wet) day-1. Addition of nutrients to the oxygen and nitrate treatment bottles significantly increased respiration rates.

**Discussion**

The TIDE sediment was more nitrogen rich than the West sediment (figure 7). This data indicates higher rates of immobilization by soil microbes in the TIDE plot after seven years of chronic fertilization. This conclusion is consistent with the findings of Deegan et al. (2010), who observed significant alterations in the composition of the microbial community in the TIDE plot over time. Rates of nitrate reduction, however, were higher in the West sediment than in the TIDE sediment (figure 19). This result is inconsistent with the CHN data, but may be explained by shifts in the organic carbon composition of the TIDE plot over time. If lignin analysis were run on sediments from the two sites, I would expect the TIDE sediment to have a significantly higher lignin to nitrogen ratio than West sediment. Chronic fertilization may have stimulated decomposition of labile carbon in the belowground sediment via nitrate and sulfate reduction leaving behind more recalcitrant carbon compounds, like lignin. The West sediment would therefore have higher rates of nitrate reduction because oxidation of labile carbon is more efficient than oxidation of lignin-like compounds.

Addition of ammonium and phosphate to the nitrate treatment bottles increased nitrate reduction in the West sediment. There was an excess of ammonium and nitrate in the nutrient and without nutrient bottles at each measured time point. Nitrate reduction is typically limited by the availability of nitrate, however, phosphate data may have
indicated a phosphate limited system. This would have been an interesting result. Overall, the data supports the conclusion that eutrophication will initially increase rates of nitrate reduction in belowground sediment, however, there is little evidence to support the theory that nutrient loading significantly increases oxidation of more recalcitrant carbon compounds, thereby weakening soil structure. I would need to perform lignin analysis in order to make conclusions about the kinds of organic compounds that were being decomposed in the nitrate bottles.

As predicted, nutrient loading significantly increased aerobic respiration in the oxygen treatment bottles. The dissolved oxygen data shows increased rates of oxygen consumption in the nutrient bottles and the ammonium data shows strong evidence of ammonium limitation in the without nutrient bottles. In addition, more ammonium was consumed in the oxygen treatment bottles than in the sulfate or nitrate treatment bottles. These results suggest that eutrophication will increase soil decomposition rates in the aerobic layer of the sediment and that aerobic respiring bacteria will immobilize large amounts of ammonium entering a system (i.e. the top centimeter of sediment).

As expected, there was no observed difference in the sulfate nutrient and without nutrient bottles and the ammonium data shows that ammonium concentrations did not change in the sulfate treatment bottles over time. This data supports the conclusion that sulfate reduction is an energetically limited process. Eutrophication would therefore have minimal effects on rates of sulfate reduction in belowground sediment.

The data shows that the rate of sulfate reduction was much higher than aerobic respiration and nitrate reduction rates in the sediment. This was an unexpected result because sulfate reduction has a less negative delta G and is therefore less efficient at
decomposing organic carbon than nitrate and aerobic respiration. Although some human and machine error may be involved, a plausible explanation is that the microbial population of sulfate reducers was already very high in the belowground soil from the sediment cores. Fourteen days may have been too short a time for populations of denitrifying bacteria and aerobic respiring bacteria to reach comparable levels. Excess ammonium decreased in the nutrient bottles over time, suggesting that microbial populations were in the process of growing. With more funding and time, I would ideally conduct this experiment over the course of year. Disregarding sulfate reduction, aerobic respiration rates were, as predicted from the delta Gs of the reactions, higher than nitrate reduction rates.

Although this study was hindered by time constraints and prone to methodological errors, we can draw several important conclusions from the data. Nutrient loading did in fact stimulate nitrate reduction in the belowground sediment and chronic fertilization of TIDE marsh sediment may have shifted the microbial composition towards nitrate reducers. Nutrient loading may initially increase rates of nitrate reduction, but then slow them over time as labile carbon is consumed, leaving behind more recalcitrant carbon compounds. Nutrient loading also stimulates rates of aerobic respiration, but have no observed effect on sulfate reduction rates. We thus conclude that eutrophication alters belowground soil processes in ways that may contribute to marsh subsidence. Conservation efforts should therefore seek to minimize anthropogenic loading of nutrients to these important ecosystems.
Acknowledgments

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Literature Cited

Ammonium Determination in Natural Waters. Methods. The Semester in Environmental Science. Woods Hole, MA.


Figures and Tables

<table>
<thead>
<tr>
<th>Microbial Respiration Pathway</th>
<th>Chemical Equation</th>
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</thead>
<tbody>
<tr>
<td>Aerobic Respiration</td>
<td>( \frac{1}{4} CH_4 O + \frac{1}{4} O_2 = \frac{1}{4} CO_2 + \frac{1}{4} H_2 O )</td>
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<tr>
<td>Nitrate Reduction</td>
<td>( \frac{1}{4} CH_4 O + \frac{1}{5} NO_3^- + \frac{1}{5} H^+ = \frac{1}{4} CO_2 + \frac{1}{10} HS^- + \frac{7}{20} H_2 O )</td>
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<tr>
<td>Sulfate Reduction</td>
<td>( \frac{1}{4} CH_4 O + \frac{1}{8} SO_4^{2-} + \frac{1}{8} H^+ = \frac{1}{4} CO_2 + \frac{1}{8} H_2 S^- + \frac{1}{4} H_2 O )</td>
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Table 1. Microbial respiration pathways in marsh sediment and their accompanying chemical equations.

<table>
<thead>
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<th>Pathway</th>
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<tr>
<td>Oxic Respiration</td>
<td>-114</td>
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<tr>
<td>Nitrate Reduction (denitrification)</td>
<td>-108</td>
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<td>Sulfate Reduction</td>
<td>-20</td>
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</table>

Table 2. A comparison of the energetic efficiencies of three heterotrophic respiration reactions carried out by soil microbes in marsh sediment.

Figure 1. Jorgensen et al. 1980. Distribution of three electron acceptors used for respiration in coastal sediments. Concentration is measured on the x-axis and depth on the y-axis.
**Figure 2.** Jorgensen et al. 1980. Oxygen, nitrate and sulphate respiration rates in marine sediment. Rate of respiration is measured on the x-axis and depth on the y-axis.

**Figure 3.** An artificial comparison of soil decomposition rates over time. The labeled G phases indicate noticeable shifts in decomposition rates as the lignin ratio of the soil increases.
Figure 4. An image of the Plum Island LTER site. The area labeled “West” is a pristine reference site from which I extracted several sediment cores. Cores were also taken from the “TIDE” site, which had been chronically fertilized with mineral nitrogen over a 7-year period. (Google maps).

<table>
<thead>
<tr>
<th>Plot</th>
<th>W/out NH4 + PO4</th>
<th>W/ NH4 + PO4</th>
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<td>Control</td>
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<td>Aerobic Resp. O2</td>
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<tr>
<td>Control</td>
<td>Sulfate Reduction. SO4</td>
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<td>TIDE plot</td>
<td>Nitrate Reduction. NO3</td>
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Table 3. The experimental design.

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<th>NO3 weight (g)</th>
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Table 4. Wet weight in grams of sediment added to the BOD bottles at the beginning of the experiment.
Figure 5. This is a graph of my expected results. I predicted that aerobic respiration would have the highest rate of respiration and sulfate reduction the lowest. I also predicted nutrient addition of ammonium and phosphate would increase respiration rates in all but the sulfate treatment bottles.

Figure 6. Wet weight and dry weight of soil samples collected from the TIDE and West sites in Plum Island sound. The percent water weight of sediment was same at both sites.
**Figure 7.** The C:N ratio of the TIDE sediment was lower than the West sediment. The TIDE sediment appears to be more nitrogen rich.

**Figure 8.** Dissolved oxygen in the oxic treatment bottles declined over time after the bottles were sealed. The bottles were reoxygenated over an eight-day period beginning on Nov. 19th and ending on Nov. 21st. Consumption of dissolved oxygen was higher in the bottles to which nutrients were added.
Figure 9. Nitrate was consumed by nitrate reducing microbes in the nitrate treatment bottles over time. Nitrate uptake was higher in the bottles to which nutrients were added.

Figure 10. A small amount of nitrate was consumed by denitrifying bacteria in the TIDE nitrate treatment bottles.
**Sulfate Treatment**

![Sulfate Treatment Graph]

*Figure 11.* Nitrate was not additionally added to the sulfate treatment bottles and the small amount of nitrate present in the artificial seawater was not consumed by the sulfate reducing bacteria.

**Oxic Treatment**

![Oxic Treatment Graph]

*Figure 12.* Nitrate was not added or consumed by the aerobic respiring bacteria in the oxygen treatment bottles.
Figure 13. Sulfate was consumed in the sulfate treatment bottles between time point 0 and 1. The blank sulfate bottle is used as a reference. Measured sulfate concentrations at time points 2 and 3 were highly variable and therefore not presented in this report.

Figure 14. Ammonium was consumed in the oxic treatment bottles over time. In the nutrient bottles ammonium was added at time points 0, 1 and 2. Excess ammonium increased over time in the nutrient bottles indicating respiration was energetically limited. Ammonium was completely consumed by the aerobic bacteria in the without treatment bottles suggesting that they were nutrient limited.
**Figure 15.** Ammonium was consumed by sulfate reducing bacteria. Excess ammonium increased over time in the sulfate treatment bottles suggesting energetic limitation.

**Figure 16.** Ammonium was consumed by nitrate reducing bacteria. The amount of excess ammonium increased over time in the nutrient and without nutrient bottles, indicating energetic limitation. Ammonium uptake was lower in the nitrate west bottles than in the TIDE nitrate bottles.
Figure 17. Ammonium was consumed by nitrate reducing bacteria. The amount of excess ammonium increased over time in the nutrient bottles and to a lesser degree in the w/out nutrient bottles, indicating energetic limitation. Ammonium uptake was higher in the TIDE bottles than in the Nitrate bottles.

Figure 18. Respiration rates were substantially higher in the sulfate treatment bottles. Populations of denitrifying and aerobic respiring bacteria may not have had enough time grow to comparable levels. There was not a significant difference between sulfate respiration rates in with vs. without nutrient bottles.
Figure 19. Aerobic respiration was higher than nitrate reduction in the TIDE and West sediment. Nitrate reduction was higher in the west sediment than in the TIDE sediment. Chronic fertilization over a seven-year period may have decreased the percent labile carbon in the TIDE plot over the time. Addition of nutrients increased respiration in the oxygen and nitrate bottles.