

**Effects of Anthropogenic Nitrogen Loading on Nitrification
and Denitrification in Atlantic White Cedar Wetlands**

Author:

Kyle Whittinghill
kwhittin@middlebury.edu
Middlebury College
Middlebury, VT 05753

Collaborators:

Megan Hartmann
Lawrence University
Appleton, WI 54912

Meaghan Murphy
Mount Holyoke College
South Hadley, MA 01075

Mentor:

Anne Giblin
Marine Biological Laboratory
Woods Hole, MA 05234

Effects of Anthropogenic Nitrogen Loading on Nitrification and Denitrification in Atlantic White Cedar Wetlands

Author: Kyle Whittinghill¹
Collaborators: Megan Hartmann² and Meaghan Murphy³
Mentor: Anne Giblin

*Semester in Environmental Science
Marine Biological Laboratory, Woods Hole, MA 05234*

¹*Middlebury College, Middlebury; VT 05753*

²*Lawrence University, Appleton; WI 54912*

³*Mount Holyoke College; South Hadley, MA, 01075*

Abstract

I studied the effects of anthropogenic nitrogen loading from septic tanks on the actual and potential denitrification and nitrification rates in two Atlantic white cedar wetlands. I compared these rates from a pristine swamp in the Cape Cod National Seashore near South Wellfleet, Massachusetts and an impacted swamp in Woods Hole, Massachusetts to determine whether higher nitrogen inputs to the system increases denitrification rates or decreases nitrification rates. In a core experiment I measured direct denitrification through the nitrate flux into the sediment and coupled nitrification-denitrification using sediment O₂ and dissolved inorganic nitrogen (DIN) fluxes to calculate the rate of denitrification stoichiometrically. Direct denitrification was positively correlated with nitrate concentration in the cores spiked with nitrate from both sites and was higher at the control site. The coupled nitrification-denitrification rates were lower in the control swamp spiked cores than the impacted swamp spiked cores. I used sediment slurries to measure the potential nitrification and denitrification at the two swamps. Low pH does not appear to inhibit nitrification at either swamp. The rates of potential nitrification and denitrification were higher at the control swamp than the impacted swamp, possibly due to higher surface water nitrate concentrations.

KEY WORDS: Atlantic White Cedar, Forested Wetlands, Nitrification, Denitrification, Impact of Septic Tanks, Anaerobic Respiration

Introduction

In the last 200 years, humans have destroyed thirty to fifty percent of wetlands in the continental United States and Atlantic white cedar wetlands are no exception (Tangley, 1984). Atlantic white cedar wetlands are located in the United States from 44°N latitude southward and most are located from sea level to 50m (Laderman, 1987). Some deciduous species found in Atlantic white cedar swamps include black gum (*Nyssa sylvatica*), red maple (*Acer rubrum*), and sweetbay magnolia (*Magnolia virginiana*) (Ehrenfeld and Scheider). There is a large quantity of relief on the forest floor due to the hummock and hollow topography associated with the tree boles (Ehrenfeld and Schneider). Soils in Atlantic white cedar wetlands are acidic with a pH of 3.1 to 5.5 and are often flooded for at least half of the growing season (Laderman, 1987). The flooded soils create anoxic sediments, with or without an oxic layer at the sediment surface (Tangley, 1984).

The range and number of unaltered Atlantic white cedar wetlands are shrinking due to human impacts (Laderman, 1987). Some of the Atlantic white cedar (*Chamaecyparis thyoides* L.) stands in the United States were removed by sea level rise and beaver dams, however, most of the loss is from harvest practices, altered hydrology, and habitat incursion by humans (Laderman, 1987). Organic chemicals, chloride, heavy metals, and nutrients can contaminate these swamps through septic tank leachate and road runoff (Ehrenfeld and Schneider). Roads can also alter the dynamics and depth of seasonal flooding by acting as dams (Ehrenfeld and Schneider). As Atlantic white cedars are adapted to high acidity and low nutrient levels (Tangley, 1984), increasing the nutrient levels to the swamp, may cause other species to invade. Several rare species including the parula warbler (*Parula Americana*), heartleaf twayblade (*Listera cordata*), the dwarf mistletoe (*Arceuthobium pusillum*) and the Hesselis hairstreak butterfly (*Callophrys hessli*) inhabit cedar bogs and are threatened by this loss and alteration of their habitat (Sarrie and Woolsey, 1987).

In anaerobic environments, such as the sediment in Atlantic white cedar swamps, bacteria must utilize other electron acceptors, such as nitrate or nitrite, for decomposition as oxygen is depleted (Jorgensen, 1980). Denitrification is the process by which nitrate or nitrite is used to decompose organic matter releasing N₂ or N₂O gas (Seitzinger and

Giblin, 1996; Knowles, 1980; Jorgensen, 1980). Henceforth I will use nitrate to refer to both nitrate and nitrite. Denitrification is carried out by facultative anaerobic bacteria, as the process cannot occur in an environment where oxygen is present (Knowles, 1980; Seitzinger, 1988). Denitrification occurs in nearly all lake, river, and coastal marine sediments (Seitzinger 1988) as well as continuously flooded soils (Patrick and Tusnamn, 1972). The nitrate used in denitrification either comes directly from the water column (as in direct denitrification), or from nitrification in the oxic upper sediment layer or water column (as in coupled nitrification-denitrification) (Seitzinger and Giblin, 1996).

Nitrification is a two-step process by which bacteria in the genera *Nitrosomonas* and *Nitrobacter* convert ammonium to nitrate (Seitzinger and Giblin, 1996). Nitrification needs oxygen to occur so cannot continue in anoxic sediments, such as those under an anoxic water column. Therefore, in the flooded Atlantic white cedar wetlands, nitrification cannot occur unless there is an oxic layer in the water above the peat. Nitrate produced by nitrification is taken up by plants, diffuses to the anoxic zone and is denitrified, or is reduced to ammonia (Hemond, 1983). Both nitrification and denitrification are inhibited by low pH (Morris, 1991) but denitrification has been shown to occur in soils with a pH as low as 3.6 (Gilliam and Gambrell, 1978 and Muller et al., 1980). In cypress swamps a pH of 4-5 inhibits nitrification, so the pH typical of Atlantic white cedar wetlands may inhibit or completely prevent nitrification (Morris 1991).

Materials and Methods

I studied the effects of anthropogenic nitrogen loading from septic tanks on the denitrification and nitrification rates in Atlantic white cedar wetlands. I compared actual denitrification, potential denitrification, and potential nitrification rates in two cedar swamps with different disturbance regimes to determine whether increased nitrogen loading to the system increases denitrification rates or decreases nitrification rates. Direct denitrification is measured through the nitrate flux into the sediment and coupled nitrification-denitrification can be measured using sediment O₂ and dissolved inorganic nitrogen (DIN) fluxes to calculate the rate of denitrification stoichiometrically or by measuring the N₂ efflux from the sediment (Seitzinger and Giblin, 1996).

My control study site is an Atlantic white cedar wetland in a twenty-acre glacial kettle located in South Wellfleet, Massachusetts at the Cape Cod National Seashore

(Paterson, 1995). The site has a peat layer 24 ft thick, was colonized by Atlantic white cedar about 3,000 years ago, and is now a mature white cedar forest with some invasion of red maple (Paterson, 1995). The site is the largest Atlantic white cedar stand on the outer arm of Cape Cod and has been undisturbed since 1960, when the National Parks Service acquired the swamp (Paterson, 1995). Before 1960 selective cutting had occurred in the swamp since European settlers came to the area (Paterson, 1995).

My second study site is Devil's Lane swamp located in Woods Hole Massachusetts across the street from the Marine Biological Laboratory Devil's Lane parking lot. It is a disturbed Atlantic white cedar wetland in a kettle depression. Devil's Lane is permanently flooded, as the road has blocked the outflow from the swamp, and several houses with septic tanks are located in the area that drains into the swamp. My third study site is Cumloden swamp, in Woods Hole, Massachusetts. It is an Atlantic white cedar wetland that was flooded in the late 1960's, killing all of the cedar trees (Kelsey, 1997). Several houses with septic tanks are located near the swamp, adding nutrients to the swamp.

We measured the concentration of nitrate, sulfate, and ammonium in the surface water at the center and edge of both the Devil's Lane and the Cumloden swamps. We collected these samples from three transects in each swamp November 12 and 13, 2001. I analyzed all nitrate samples for the study using a Lachat flow injection analyzer (Wood et al., 1967). Throughout the study, I acidified the ammonium samples prior to analysis with 1 μ L/mL of 5N HCl, and analyzed them colorimetrically using an acidified standard curve with 1 μ L/mL of 5N HCl (Solorzano, 1969). Megan Hartmann conducted all of the sulfate analyses.

To measure actual denitrification rates we used peat cores to measure the net flux of dissolved inorganic nitrogen and oxygen into the peat. We collected three peat cores from the edge of the Devil's Lane swamp on November 12, 2001 with 10cm Plexiglas core tubes. Also on November 12, 2001 we filled a 20L carboy of surface water from another site at the edge of the swamp and filtered the water using a nitrogen gas pressure filtration device and a 0.8 μ m glass fiber filter to remove phytoplankton and heterotrophs. We measured the dissolved oxygen at both the core site and the site where we collected

water from Devil's Lane on December 13, 2001. The cores were stored between 6 and 10°C and the filtered water was stored at 6°C until the experiment.

After emptying the overlying water from the core tubes we filled the headspace of the three cores using the filtered water. Within 24 hours of collecting the cores, they were capped and we began our study. The cores were stirred using magnetic stir bars throughout all of the incubations. We took initial water samples for nitrate and ammonium analyses and sampled again after 3, 10, 21, and 26 hours, replacing the sampled water with filtered water each time. After the last sampling time the cores were aerated and the water in the headspace was stirred with the cores uncapped overnight. We then spiked the cores with nitrate to 20µM, 50µM, and 150µM and repeated the previously described incubation, sampling after 0, 4, 7, and 20 hours.

We repeated this experiment with three cores collected from the center of the Marconi swamp on November 26, 2001. There was no surface water at the Marconi swamp when we sampled. However, surface water nitrate and ammonium concentrations were not significantly different between the Devil's lane and Marconi swamps in a previous study (Kelsey, 1997), so to fill the headspace of the Marconi cores we used surface water collected on November 27, 2001 from the same site as the water used for the other set of cores and filtered in the same way. We took an initial sample for nitrate and ammonium once the cores were capped and sampled 4, 10, and 21 hours later, replacing the water each time. After this incubation was complete we bubbled the cores and allowed them to stir open for 12 hours. At the end of the twelve hours we took a water sample from the cores. Then we spiked the headspaces of the cores to concentrations of 20µM, 50µM, and 150µM nitrate, capped the cores, and repeated the incubation sampling the cores 0, 4, 7, and 20 hours after the cores were capped.

At each sampling time for all four incubations we measured the dissolved oxygen in the headspace and Megan Hartmann sampled the cores for carbon dioxide and methane measurements. Throughout all of the incubations the cores were kept between 8 and 11°C and the filtered water was kept at 6°C. After running the core incubations we took a sample of the top layer of each core and analyzed it for carbon and nitrogen content using a Perkin-Elmer CHN analyzer. I calculated the rate of coupled nitrification-denitrification using the Redfield Ratio for a maximum estimate and the carbon to

nitrogen ratio of the cores to give a minimum estimate of the expected release of ammonium through aerobic decomposition. I assumed that the difference between the expected and actual release of ammonium was due to coupled nitrification-denitrification.

We sampled water from the cores sites at the Devil's Lane and Marconi swamps on November 26 and 27, 2001 and analyzed it for nitrate, ammonium and sulfate. Peat samples were collected from the center and edge of the Marconi swamp on November 26, 2001 and the center and edge of the Devil's Lane swamp on November 27, 2001. These samples were kept at 6°C until used for my potential nitrification and potential denitrification sediment slurry experiments and Megan Hartmann's experiment.

I use the sediment slurry method developed by Hanson et al (1981) to measure potential nitrification. I homogenized a subsample of peat from both the edge and center of the two swamps and placed two grams in 14, 50mL centrifuge tubes for each of the four sites. I added 35mL of 300µM ammonium (NH₄Cl) and 100µM phosphate (KPO₄) solution to all 56 tubes and 8 blanks. I placed the tubes on a shaker table at room temperature in the dark for the duration of the experiment to keep the tubes oxygenated. I sampled two tubes from each site for nitrate after 20 minutes of shaking. I then sampled 3 tubes from each site and 2 blanks for nitrate 24 hours later, 71 hours later, 111 hours later, and 143 hours later. I calculated potential nitrification using nitrate appearance.

I also used slurries to calculate potential denitrification from the uptake of nitrate. I placed approximately 15g of homogenized peat from the core sites in 12, 300mL BOD bottles for Marconi and 12, 300mL BOD bottles for Devil's Lane. I then added different amounts of nitrate to three BOD bottles from each site for three replicates of 4 treatments (0µM, 10µM, 50µM, and 150µM nitrate). I bubbled a carboy of nanopure water with He gas for 15 minutes to remove N₂ gas from the water. I then filled the 24 experimental bottles and 3, 60mL BOD bottle blanks with the nanopure water while continuing to bubble the water with He gas. The bottles were incubated for three or four days in the dark at room temperature before being sampled for nitrate. I also sampled Megan Hartmann's experiment for nitrate to determine the denitrification rates for her treatments.

All statistical tests were t-tests unless otherwise noted in the results section. I also analyzed my data using paired t-tests and ANOVAs, with a Tukey's LSD post hoc test.

All of the statistical analyses were conducted using SPSS 9.0 for Windows Student Version.

Results

Although, nitrate concentrations appear to be higher in the center of Cumloden than at the edge (Figure 1); nitrate concentrations were not statistically different between the edge and the center of either Cumloden or Devil's Lane (Cumloden: $df=4$, $t=-1.625$, $p=0.179$; Devil's Lane: $df=4$, $t=-0.673$, $p=0.538$) (Figure 1). Ammonium concentrations appear higher at the center of Devil's Lane than the edge and higher at Devil's Lane than Cumloden (Figure 2). There is no statistical difference in the concentration of NH_4^+ between the center and edge of either swamp (C: $df=4$, $t=-2.352$, $p=0.078$; DL: $df=4$, $t=-0.353$, $p=0.742$) (Figure 2). The ammonium and nitrate concentrations were significantly higher in Devil's Lane than Cumloden (Ammonium: $df=4$, $t=-2.775$, $p=0.020$; Nitrate: $df=4$, $t=-2.240$, $p=0.049$) (Figure 1 and 2).

The ammonium concentration of surface water in Devil's Lane appears to be higher than the ammonium concentration in the pore water from the Marconi core site (Figure 3). The concentration of nitrate appears to be higher at the Marconi core site than at the Devil's Lane core site (Figure 1 and 3). Dissolved oxygen in the surface water at Devil's Lane was less than 1.2mg/L at the core site and less than 2mg/L at the water site. The carbon content of was 47% higher in the Marconi cores than the Devil's Lane cores (Figure 4). The nitrogen content was similar in the two sets of cores, although it was 6% higher in the Marconi cores (Figure 4). The C:N ratio was significantly higher in the Marconi cores than in the Devil's Lane cores ($df=4$, $t=-19.084$, $p<0.001$) (Figure 4).

The oxygen concentration decreased over time in all three Devil's Lane control cores and the Devil's Lane 20 μM , 50 μM , and 150 μM cores (Figure 4 a and b). The oxygen concentration decreased faster in the Devil's Lane spiked cores than the control cores (Figure 4 a and b). Oxygen concentrations in the headspace of both incubations of the Marconi swamp cores also decreased with time (Figure 4 b and c). There does not appear to be a change in oxygen consumption between the control and spiked Marconi cores (Figure 4 b and c). The rate of oxygen consumption was not significantly different between the two sets of control cores ($df=4$, $t=-0.894$, $p=0.422$). There was also no significant correlation between the rate of oxygen consumption and the nitrate

concentration in the cores from either site (Devil's Lane: $df=1$, $F=0.002$, $p=0.963$; Marconi: $df=1$, $F=0.044$, $p=0.843$) (Figure 5 a and b).

In the Devil's Lane control cores both nitrate and ammonium increased over time (Figure 6 a and b). In the Marconi control cores the nitrate concentrations increased or remained fairly constant throughout the incubation (Figure 6 c). The difference in the change in concentration of nitrate between the Devil's Lane and Marconi control cores is marginally significant ($df=4$, $t=2.657$, $p=0.057$). After the third sampling of the Marconi control cores the ammonium concentration was nearly zero, so we did not include the fourth sampling point in our ammonium analysis. The ammonium concentrations decreased throughout the incubation in the Marconi control cores (Figure 6 d). There is a significant difference in the rate of change of ammonium concentration between the two sets of control cores ($df=4$, $t=7.862$, $p=0.001$). Dissolved inorganic nitrogen (DIN) concentrations increased in the Devil's Lane cores and decreased in the Marconi cores over time (Figure 6 e and f). The difference in the rate of change in the concentration of DIN between the two sets of control cores is significant ($df=4$, $t=10.787$, $p<0.001$).

In the spiked Devil's Lane cores the concentration of both nitrate and ammonium decreased over time (Figure 7 a and b). In the spiked Marconi cores the concentration of nitrate also decreased over time (Figure 7 c). In the 20 μ M nitrate Marconi core the ammonium concentration decreased with time (Figure 7 d). However in the 50 and 150 μ M nitrate Marconi cores the ammonium concentration increased over time (Figure 7 d). There is a significant positive correlation between the rate of nitrate consumption and the nitrogen concentration in the Devil's Lane cores ($df=1$, $F=284.530$, $p<0.001$) (Figure 8 a). Although the rate of nitrate consumption appears to increase with nitrogen concentration in the Marconi cores, this correlation is not statistically significant ($df=1$, $F=0.475$, $p=0.528$) (Figure 8 b). The ammonium consumption appears to increase with nitrate concentration in the Devil's Lane cores and decrease with nitrate concentration in the Marconi cores (Figure 9 a and b). There is no significant correlation between the rate of ammonium consumption and the nitrate concentration in the cores from either swamp (Devil's Lane: $df=1$, $F=1.408$, $p=0.301$; Marconi: $df=1$, $F=4.470$, $p=0.120$).

There appear to be higher rates of coupled nitrification-denitrification in the Devil's Lane spiked cores than the Devil's Lane control core (Figure 11). In the Marconi

cores coupled nitrification-denitrification appears to be higher in the control cores than the spiked cores and appears to decrease with the concentration of nitrate (Figure 11). The coupled nitrification-denitrification rates calculated from the Redfield ratio are significantly higher than those calculated from the CHN data for the Marconi and Devil's Lane cores except for the Devil's Lane control cores (Figure 11, Table 2). The rate of coupled nitrification-denitrification rate is significantly lower, when calculated from either the Redfield Ratio or the C:N ratio of the cores, for the Marconi spiked cores than for the Devil's Lane spiked cores (Figure 11) (paired t-test, Redfield: $df=2$, $t=-4.779$, $p=0.041$; C:N: $df=2$, $t=5.151$, $p=0.036$). The rate of coupled nitrification-denitrification also appears to be higher in the Marconi control cores than the Devil's Lane control cores, but the difference is not significant for either calculation (Redfield: $df=2$, $t=0.060$, $p=0.955$; C:N: $df=2$, $t=-2.101$, $p=0.104$) (Figure 11).

The concentration of nitrate decreased in the potential nitrification slurries between 111 hours and 143 hours so the 143-hour data point was not used in analysis. The concentration of nitrate increased in all but one of the potential nitrification slurries during the first 111 hours of the experiment (Figure 12 a). The third replicate from the center of Devil's Lane decreased in nitrate concentration throughout the incubation (Figure 12 b). The potential nitrification rate appears to be higher at the edge of Devil's Lane than in the center (Figure 13). Potential nitrification also appears to be faster in both sites at the Marconi swamp than at the center of Devil's Lane (Figure 13). There are no significant differences overall between sites (ANOVA: $df=3$, $F=2.550$, $p=0.129$), but there is a significant difference between the potential nitrification rates in the center of the two swamps (Table 1).

Potential denitrification rates appear to increase with nitrate concentration at both sites (Figure 14). There is a significant difference in the potential denitrification rates between the two swamps (paired t-test: $t=2.868$, $df=11$, $p=0.015$). The potential denitrification rates at the Devil's Lane swamp, at the Marconi swamp, and overall are also correlated with the increase in nitrate concentrations (Devil's Lane: $df=1$ $t=25.415$, $p<0.001$; Marconi: $df=1$ $t=14.457$, $p<0.001$; Overall: $df=1$ $t=14.550$, $p<0.001$). The rates of denitrification from Megan Hartmann's experiment appear lower than the rates found in my potential denitrification experiment for $150\mu\text{M}$ nitrate (Figure 14 and 15).

Discussion

The low surface and pore water nitrate concentrations at our sites indicate that much of the nitrate in precipitation is removed by plant uptake or denitrification (Figure 1 and 3). Area precipitation has a nitrate concentration of about 30 μ M nitrate (Anne Giblin, personal communication), 5 to 10 times the concentration found in the swamp surface and pore water (Figure 1 and 3). As the concentrations of nitrate and ammonium in the surface water were higher at Devil's Lane than at Cumloden, Devil's Lane appears to be the more impacted swamp in terms of nitrogen (Figure 1 and 2). We chose to use Devil's Lane for our experiments because we were unable to core at Cumloden and Devil's Lane is the more impacted swamp. As there was no significant difference in surface water nitrate and ammonium concentrations between the center and edge of the two swamps we chose to collect cores from only one site in each of the swamps we used in our study (Figure 1 and 2).

Kelsey (1997) found that surface water nitrate concentrations were significantly higher at Marconi than Devil's Lane, which is consistent with our results from the core sites (Figure 3). Unlike our results from the core sites; Kelsey (1997) found no significant differences between the two sites in the concentration of ammonium (Figure 3). It is possible that we sampled our cores at Devil's Lane from near a point source of nitrogen because we took our cores from a site near a house and the road. The core site is also probably affected by a point source of nitrogen because it has a 30 times higher ammonium concentration than the mean of surface water ammonium concentrations at the other sites sampled in Devil's Lane, which is consistent with the concentrations found by Kelsey (1997). As we are interested in the effects of increased nitrogen loading to the swamps and Devil's Lane is our impacted swamp, sampling at a point source of nitrogen in Devil's Lane would not interfere with the study. There may also have been a contamination of our Devil's Lane core site ammonium sample.

The oxygen concentrations decrease in all four core incubations as expected (Figure 4). Oxygen is used in the cores by both aerobic respiration and nitrification. The rate of oxygen consumption does not change with an increase in nitrate in cores from either swamp or between the two swamps (Figure 5 a and b). Organisms gain more energy using oxygen as an electron acceptor for respiration than from other electron

acceptors such as nitrate and sulfate (Jorgensen, 1980; Knowles, 1980). As oxygen is the electron acceptor most readily used in respiration, its consumption would not be affected by changes in the availability of other electron acceptors (Jorgensen, 1980). Thus increasing the concentration of nitrate in a system should not change the rate of aerobic respiration, which is supported by our results.

During the control core incubations the concentration of nitrate increases due to the process of nitrification. Nitrate may be consumed through direct denitrification. Ammonia is released through all processes of decomposition and is consumed during nitrification. In the Devil's Lane and Marconi control cores the amount of nitrification appears to exceed the amount of direct denitrification because the concentration of nitrate increases over time in both sets of cores (Figure 6 a and c). In the Devil's Lane control cores the concentration of ammonium, and total DIN, increase because the amount of ammonium released from the decomposition in the cores exceeds that used for nitrification (Figure 6 b and e). The concentration of ammonium and total DIN in the control cores from the Marconi cores most likely decrease due to the release of N₂ gas through coupled denitrification-nitrification (Figure 6 d and f and Figure 11).

The rates of nitrate appearance were significantly faster in the Devil's Lane control cores than the Marconi control cores, which do not agree with the results of the potential nitrification experiment (Figure 6 a and b and 13). Actual nitrification at the Marconi core site may be lower than that at the Devil's Lane core site because water in the headspace of the Marconi cores started at a higher nitrate concentration than the water in the headspace of the Devil's Lane cores and nitrification is inhibited by nitrite (included in my analyses with nitrate) (Figure 6 a and b) (Jones and Hood, 1980). Both cores ended the incubation at similar nitrate concentrations, $4.22^{\pm}0.25\mu\text{M}$ for Devil's Lane at the final time point and $4.23^{\pm}0.09\mu\text{M}$ for the Marconi cores after 10 hours (The Marconi cores only increased to $4.28^{\pm}0.04\mu\text{M}$ in the last 11 hours of the incubation so this data point was left out of the rate calculation) (Figure 6 a and b). It is possible that actual nitrification rates in the two cores only increase the concentration of nitrate to about $4.25\mu\text{M}$, before the nitrate is used in denitrification.

Nitrate concentrations in the spiked Devil's Lane and Marconi cores decrease due to direct denitrification (Figure 7 a and c). The concentration of ammonium most likely

decreases in the Devil's Lane spiked cores and the Marconi 20 μ M nitrate core due to high amounts of coupled nitrification-denitrification (Figure 7 b and d). Coupled nitrification-denitrification would reduce concentrations of ammonium as ammonium is converted to N₂ gas by this process (Seitzinger and Giblin, 1996). In the 50 and 150 μ M nitrate Marconi cores the concentration of ammonium most likely increases due to decomposition in the sediments. The rate of direct denitrification (indicated by nitrate consumption) increases with the concentration of nitrate in the Devil's Lane cores and the Marconi cores (without the 150 μ M core) (Figure 7). This is as expected because denitrification is correlated with the concentration of nitrate (Seitzinger, 1988). I omitted the 150 μ M Marconi core from this analysis because it did appear to fit the trend in data as it showed a lower rate of direct denitrification than the 50 μ M Marconi core (Figure 7b). The rate of direct denitrification appears to increase faster with the nitrate concentration in the Marconi cores than the Devil's Lane cores, which could be because both the potential denitrification and nitrification rates from the slurry experiments appear to be higher at Marconi than at Devil's Lane (Figure 7, 13, and 14).

The C:N ratio of Devil's Lane (28.5⁺/_{-0.91}) and that of Marconi (39.6⁺/_{-0.43}), are higher than the Redfield Ratio (6.625) (Figure 10). The percent of organic carbon in the Marconi and Devil's Lane cores is enough to maintain a low enough redox potential and supply a substrate sufficient for denitrification (Brinson et al, 1984). It is as expected that the coupled nitrification-denitrification would be less for the CHN estimate than the Redfield ratio estimate because the C:N ratio of the cores is higher than the Redfield ratio of 6.625 (Figure 10).

The Marconi spiked cores may have lower coupled nitrification-denitrification rates (Figure 11) because there is a higher concentration of nitrate in the surface water at the Marconi swamp (Kelsey, 1997) and higher potential nitrification rates (Figure 13) as nitrification is inhibited by nitrite (Jones and Hood, 1980). The actual nitrification rates in the control cores were also significantly lower in the Marconi cores (Figure 6 a and b). Unlike the rate of coupled nitrification-denitrification in the spiked cores the rate of coupled nitrification-denitrification in the control cores appears to be higher in the Marconi cores than the Devil's Lane cores (Figure 11). The rate of coupled nitrification-denitrification in the control cores follows the same trend as the actual denitrification in

the spiked cores, the potential denitrification, and the potential nitrification which all appear greater for Marconi than Devil's Lane (Figure 7 a and b, 11, 13, and 14).

The nitrate concentrations in the potential nitrification slurry experiment decreased between the last two time points most likely because the slurries went anoxic after several days. The oxygen in the headspace must not have been sufficient for the length of the incubation. The center of Devil's Lane remains permanently flooded, while the edge of the Devil's Lane swamp and the entire Marconi Swamp are only seasonally flooded. This could mean that peat in the center of Devil's Lane remains anoxic for most of the year while peat at the other sites are oxygenated for a greater part of the year, which would account for the lower potential nitrification rates at the center of Devil's Lane (Figure 13). Our results for dissolved oxygen support this as the two sites where we measured dissolved oxygen in Devil's Lane are anoxic (dissolved oxygen less than 4 mg/L). Although only the difference between the center of Devil's Lane and Marconi is significant, it is possible other relationships would be significant with more replicates. These results are opposite those of the actual nitrification in the control cores, which is lower in the Marconi cores (Figure 6 a and b and 13).

Potential denitrification rates at both swamps increase with nitrate concentrations because direct denitrification usually increases with nitrate concentration (Seitzinger, 1988) (Figure 14). Potential denitrification rates may be higher at the Marconi swamp because the surface and ground water nitrate concentrations are higher here (Kelsey, 1997) (Figure 14). Potential denitrification may also be higher at the Marconi swamp because potential nitrification rates appear to be higher at the Marconi Swamp (Figure 13 and 14). The potential denitrification rates in the slurry experiment show the same trend as the rates of direct denitrification rates in the spiked cores (represented by nitrate consumption), which are greater in the Marconi cores (Figure 7 a and b and 14). Our potential nitrification rate for the 10 μ M slurries (0.244 μ moles g^{-1} day^{-1} for Devil's Lane and 0.361 μ moles g^{-1} day^{-1}) are below the range found by Muller et al. (1980) of 0.8-1.56 μ moles g^{-1} day^{-1} for a *Rubus chamaemorus* spruce swamp (Figure 14). Our 10 μ M nitrate slurries have higher Denitrification rates than those found by Muller et al (1980) for Sphagnum bogs (-0.005 to 0.026 μ moles g^{-1} day^{-1}) (Figure 14).

The denitrification rate is most likely lower in Megan's experiment than in the potential denitrification slurries because the nitrate was almost completely used at the time of sampling (Figure 14 and 15). It is possible that the nitrate was used at a much higher rate due to the greater dry weight of peat used in her experiment and remained at low levels for the end of the experiment. At a rate of about $4\mu\text{moles g}^{-1} \text{ day}^{-1}$ (approximately that found in the potential denitrification slurries), the $150\mu\text{M}$ nitrate would be used by 7g dry weight of sediment (the amount in Megan's jars) after 6.43hours.

In a study conducted by Dierberg and Brezonik (1983) both the percentage of nitrogen input and the absolute amount used in denitrification were higher in a sewage-enriched cypress dome than a natural dome (Figure 1). In the natural cypress dome 61% of the reduced nitrate was denitrified, whereas in the dome receiving effluent, 75% of the reduced nitrate was denitrified (Dierberg and Brezonik, 1983). As the water column was almost constantly anoxic in the sewage-enriched dome and the pH was low in the natural dome nitrification did not provide nitrate for denitrification in either dome (Dierberg and Brezonik, 1983). In my experiment the potential nitrification and potential denitrification rates were higher in the control swamp than the impacted swamp, which is opposite the results from Dierberg and Brezonik (1983). Also contrary to the results of Dierberg and Brezonik (1983), coupled nitrification-denitrification was an important part of the total denitrification in the control cores from the control swamp and the spiked cores from the impacted swamp.

A study by Brinson et al (1984) in an alluvial floodplain swamp showed that after adding nutrients to the swamp all nitrate was used by denitrification and although ammonia adhering to cation exchange sites in the soils was nitrified when sediments were drier in the summer, nitrate did not accumulate, indicating denitrification is tightly coupled to nitrification in this system. Our study also indicates that most of the nitrate entering the two swamps in precipitation is denitrified and that nitrification plays a role in those parts of the two swamps that are oxic for part of the year. Morris (1991) found that although one swamp had high nitrogen loading for 50 years, the nitrification-denitrification potential did not differ from a control swamp. Unlike Morris (1991), in

our study we found that the nitrification and denitrification potentials to be significantly higher in our control site than in the impacted site.

One source of error for this study is that all samples were collected during the month of November. A possible future study would be to run the experiment during other seasons to compare rates at different times of the year. Another possible future study would be to run more replicates of the spiked cores. The spiked cores were run with water that had already been used for one core incubation, and although it was bubbled it was not as oxygenated as it was at the start of the first incubation. Changing the water between incubations may have reduced the error in our experiment.

It is unknown at what point the potential nitrification slurries went anoxic. If they went anoxic before 111 hours the potential nitrification rates may be higher than my estimates. To prevent this all of the centrifuge tubes should be opened at each sampling time to replace the air in the headspace. It would be interesting to repeat the potential nitrification slurry experiment, opening the tubes at each sampling point, with more replicates to determine whether or not there are statistical differences between the center of Devil's Lane and the edge of Devil's Lane or Marconi.

Although Devil's Lane is the more impacted swamp in terms of septic tank leachate and it has higher surface water ammonium concentrations than Marconi, there are higher surface water nitrate concentrations at Marconi than Devil's Lane. This means that although impacted swamps may see higher ammonium concentrations, nitrate from septic tanks may not affect the swamps, as they are adapted to higher nitrate concentrations. If nitrate concentrations are naturally higher in the pristine sites, possibly due to a shorter period of flooding, there may not be an effect of septic tank leachate on denitrification rates in the swamps.

Direct denitrification was positively correlated with nitrate concentration in the cores from both sites and was higher at the control site than the impacted site. This also suggests that septic tank runoff into the swamps is not affecting the denitrification rate at the swamps. Actual coupled nitrification-denitrification was also higher in the cores from the control swamp, however potential coupled nitrification-denitrification was lower in the control swamp cores. Low pH does not appear to inhibit nitrification at either swamp. The rates of potential nitrification and denitrification were higher at the control

swamp than the impacted swamp, possibly due to higher surface water nitrate concentrations. Permanent flooding, causing anoxic conditions in the sediment throughout most of the year, does appear to decrease the potential nitrification rate in the impacted swamp. However, there do not appear to be effects of septic tank leachate on the surface water nitrate concentrations, the actual or potential denitrification rates, or the potential nitrification rates. Atlantic white cedar wetlands may therefore be useful as wastewater treatment wetlands to remove nitrate from wastewater, if the sediments are flooded for only part of the year.

Acknowledgements

Thanks to my mentor Anne Giblin for I would like to thank all of the help that she gave Megan and I in the lab, in the field and analyzing the data. Thanks to Megan Hartmann, Meagan Murphy, Kris Tholke, Marcus Gay, Tori Ziemann, Aimlee Laderman, Sam Kelsey, the Ecosystems Center at the Marine Biological Laboratory, and the National Park Service for their help with my project idea, choosing study sites, obtaining permits, funding my project, and collecting and analyzing my data.

Literature Cited:

- Atlantic White Cedar Swamp Trail. Pamphlet. Department of the Interior National Park Service
- Brinson, Mark M., H. David Bradshaw, and Emile S. Kane. 1984. Nutrient assimilative capacity of an alluvial floodplain swamp. *Journal of Applied Ecology* **21**:1041-1057.
- Day, Frank P. Jr. 1987. Production and Decay in a *Chamaecyparis thyoides* Swamp in Southeastern Virginia Atlantic White Cedar Wetlands. Pages 123-132 in Aimlee D. Laderman, editor. Atlantic White Cedar Wetlands. Westview Press, Boulder, Colorado, USA.
- Dierberg Forrest E., and Patrick L. Brezonik. 1983. Nitrogen and phosphorous mass balances in natural and sewage-enriched cypress domes. *Journal of Applied Ecology* **20**:323-337.
- Ehrenfeld, Joan G. and John P. Schneider. 1991. *Chamaecyparis thyoides* Wetlands and suburbanization: Effects on Hydrology, water quality and plant community composition. *Journal of Applied Ecology* **28**:467-490.
- Gilliam, J.W. and R.P. Gambrell. Temperature and pH as Limiting Factors in Loss of Nitrate from Saturated Atlantic Coastal Plain Soils. *Journal of Environmental Quality* **7**(4):526-532.
- Gorham, Eville. 1987. The Ecology and Biogeochemistry of Sphagnum Bogs in Central and Eastern North America. Pages 3-15 in Aimlee D. Laderman, editor. Atlantic White Cedar Wetlands. Westview Press, Boulder, Colorado, USA.
- Hansen, Jette I., Kaj. Henriksen, and T. Henry Blackburn. Seasonal Distribution of Nitrifying Bacteria and rates of Nitrification in Coastal Marine Sediments. *Microbial Ecology* **7**:297-304.
- Hemond, Harold, F. 1983. The nitrogen Budget of Thoreau's Bog. *Ecology* **64**(1):99-109.
- Jones, R.D, and Mary A. Hood. 1980. Effects of Temperature, pH, Salinity , and Inorganic Nitrogen on the Rate of Ammonium Oxidation by Nitrifiers Isolated from Wetland Environments. *Microbial Ecology* **6**:339-347.
- Jorgensen, B. B. 1980. Mineralization and the Bacterial Cycling of Carbon, nitrogen and Sulphur in Marine Sediments.
- Kelsey, Samuel W. 1997. Chemical characteristics of surface and groundwater in three Cape Cod Atlantic White Cedar Swamps with varying disturbance histories. (<http://courses.mbl.edu/SES/data/project/1997/1997.htm>)
- Laderman, Aimlee D., editor. 1987. Atlantic White Cedar Wetlands. Westview Press, Boulder, Colorado, USA.
- Morris, James T. 1991. Effects of nitrogen loading on wetland ecosystems with particular reference to atmospheric deposition. *Annual Review of Ecological Systematics* **22**:257-79.
- Muller, Michael M., Veronica Sundman, and J. Skujins. 1980. Denitrification in Low pH spodosols and Peats Determined with the Acetylene Inhibition Method. *Applied and Environmental Microbiology*. **40**:235-239.
- Paterson, William, A. 1987. The composition and successional status of plant communities in the Atlantic White Cedar Swamp, Cape Cod National Seashore,

- So. Wellfleet, Massachusetts. Project Proposal. Department of Forestry and Wildlife Management. University of Massachusetts, Amherst.
- Patrick, W.H. and M. E. Tusneem. 1972. Nitrogen loss from flooded soil. *Ecology* **53**:735-737.
- Sarrie, Bruce A. and Henry L. Woolsey. 1987. The status and distribution of Atlantic White Cedar in Massachusetts. Pages 135-136 *In* Aimlee D. Laderman, editor. Atlantic White Cedar Wetlands. Westview Press, Boulder, Colorado, USA. .
- Seitzinger, Sybil P. 1988. Denitrification in freshwater and coastal marine ecosystems: Ecological and geochemical significance. *Limnology and Oceanography* **33**(4, part 2): 702-724).
- Seitzinger, Sybil P. and Anne E. Giblin. 1996. Estimating denitrification in North Atlantic continental shelf sediments. *Biogeochemistry* **35**:235-260.
- Solorzano, Lucia. 1969. Determination of Ammonia in Natural Waters by the Phenolhypochlorite Method. *Limnology and Oceanography* **14**:799-801.
- Tangley, Laura. 1984. Taking stock of White Cedar Wetlands. *BioScience* **34**(11):682-684.
- Wood, E.D., F.A.G. Armstrong, and F. A. Richards. 1967. Determination of nitrate in seawater by cadmium-copper reduction to nitrite. *Journal of the Marine Biological Association U.K.* **47**:23.

Table 1: Tukey's LSD test for differences between the four sites in the potential nitrification experiment

<u>Comparison</u>	<u>p value</u>
Devil's Lane Edge to Devils Lane Center	0.261
Devil's Lane Edge to Marconi Edge	0.128
Marconi Edge to Marconi Center	0.639
Marconi Center to Devil's Lane Center	0.026
Marconi Center to Devil's Lane Edge	0.169

Table 2: Comparison of coupled nitrification-denitrification rates calculated with the Redfield ratio and the C:N ratios of the cores for all four of the core incubations.

<u>Comparison</u>	<u>Test</u>	<u>df</u>	<u>t value</u>	<u>p value</u>
Marconi spiked cores	paired t-test	2	6.614	0.022
Devil's Lane spiked cores	paired t-test	2	14.506	0.005
Marconi control cores	t-test	4	1.036	0.035
Devil's Lane control cores	t-test	4	3.129	0.359

Figure 1:

The surface water nitrate concentrations at the center and edge of Devil's Lane and Cumloden and at the two core sites

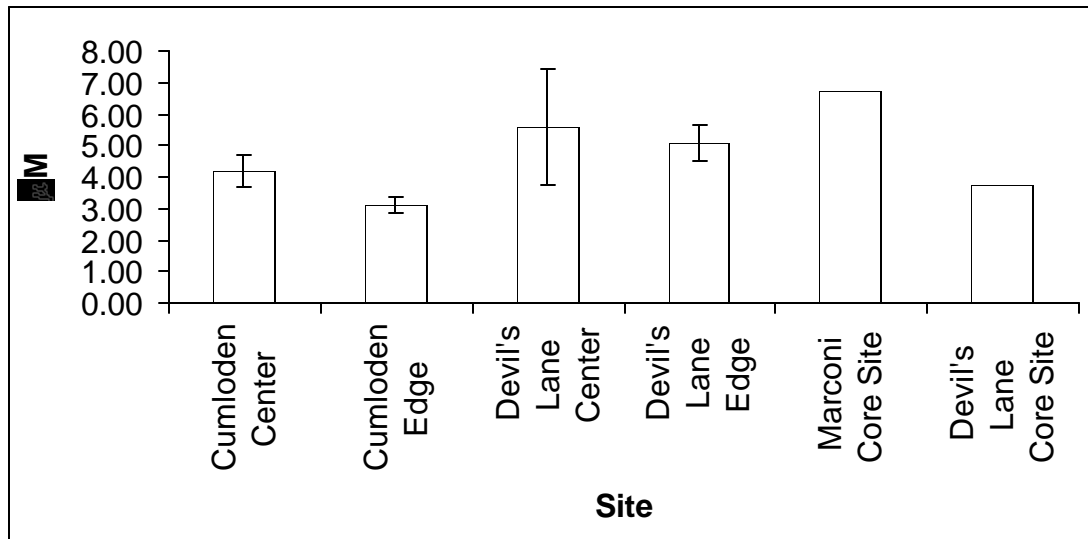


Figure 2:

The surface water ammonium concentrations at the center and edge of Devil's Lane and Cumloden

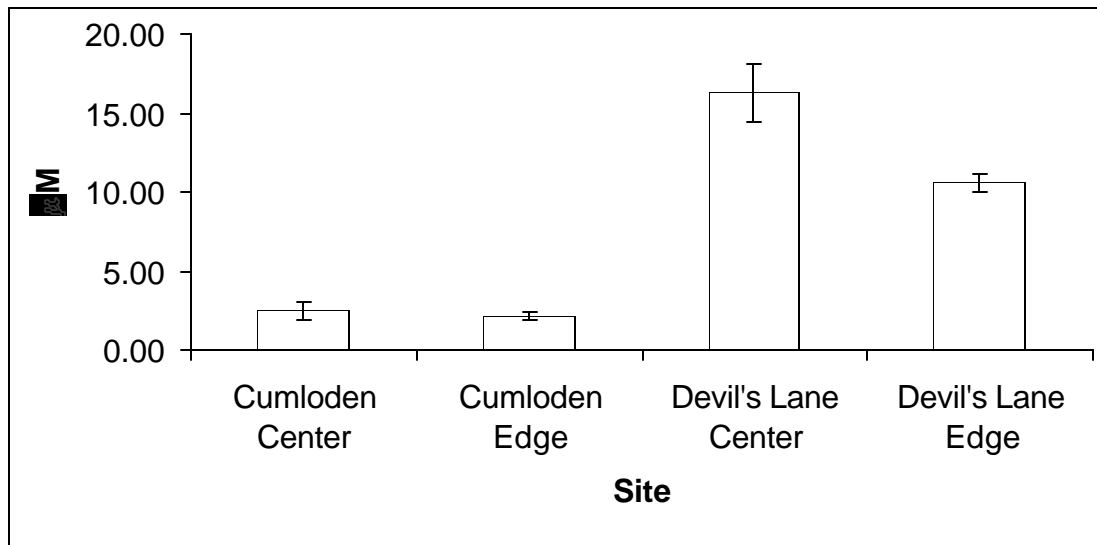


Figure 3:

The nitrate and ammonium concentrations from the core sites. The Devil's Lane data is for surface water and the Marconi data is for pore water.

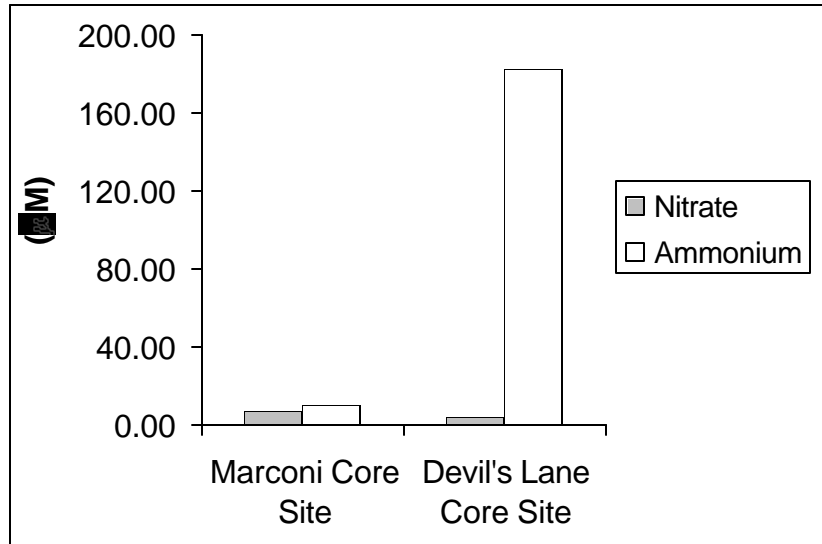


Figure 4:

The percent of carbon in the cores, percent of nitrogen in the cores and the carbon to nitrogen ratio of the cores from Marconi and Devil's Lane

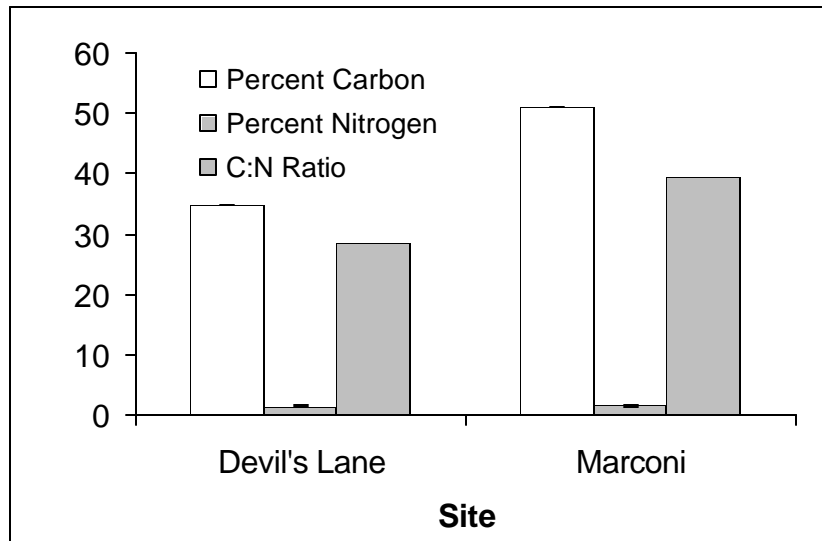
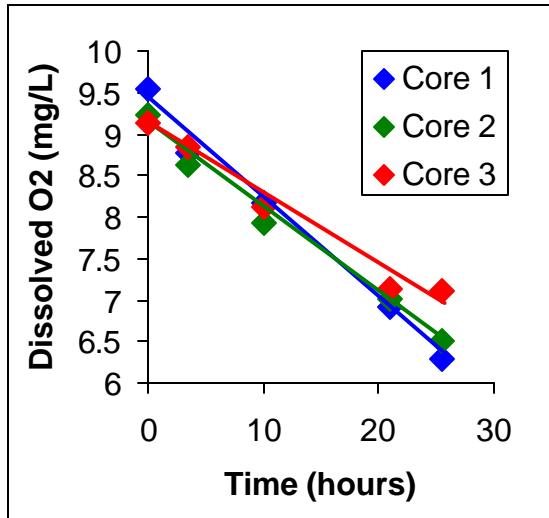
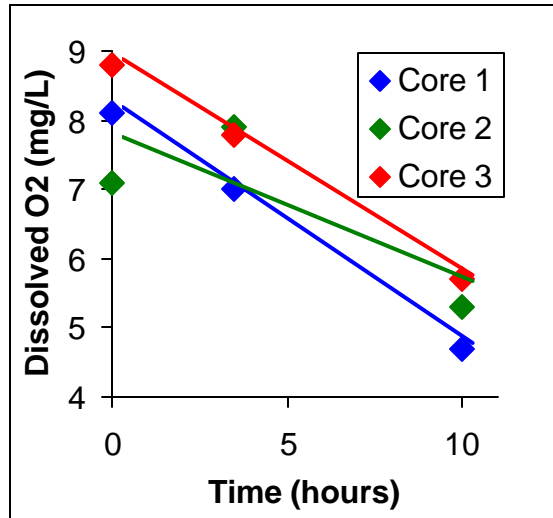


Figure 5:

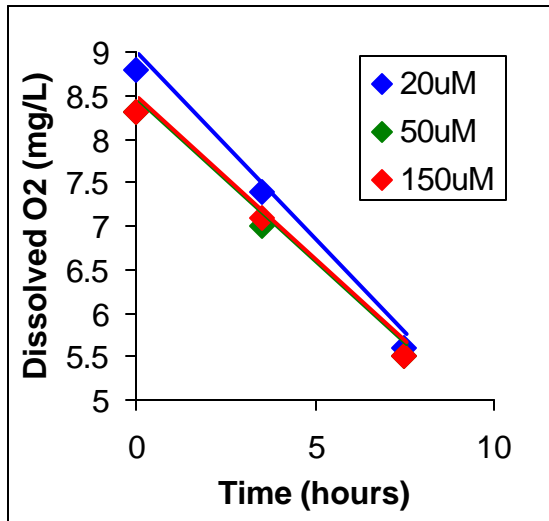
The oxygen concentrations in the headspace of the Devil's Lane and Marconi Cores throughout the incubations



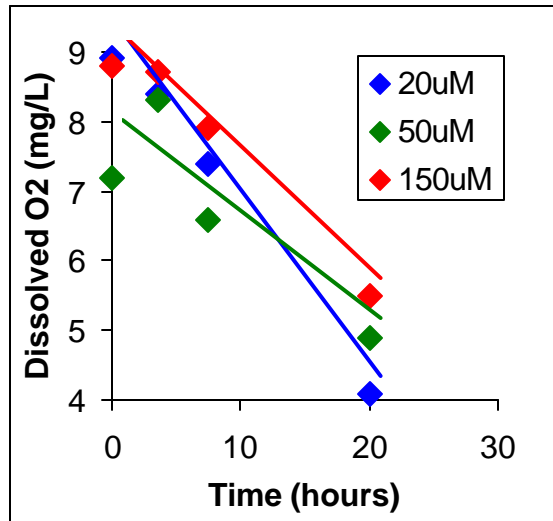
a) Devil's Lane Control Cores



c) Marconi Control Cores



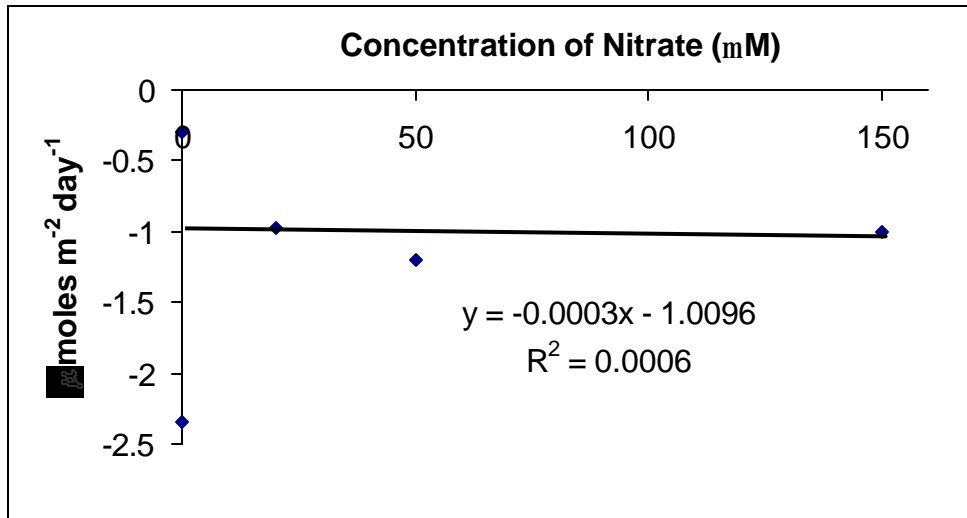
b) Devil's Lane Spiked Cores



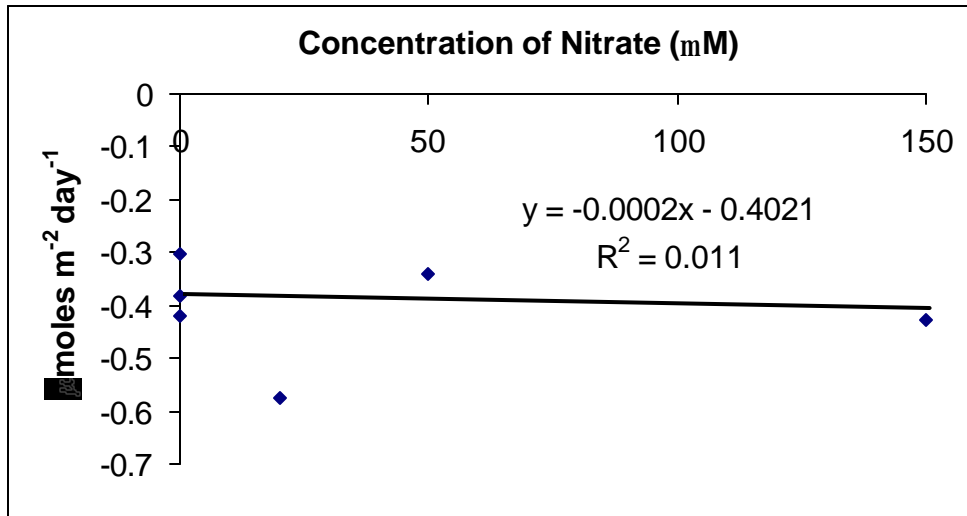
d) Marconi Spiked Cores

Figure 6:

The rate of oxygen consumption in relation to nitrate concentration in the Devil's Lane and Marconi cores throughout the incubations

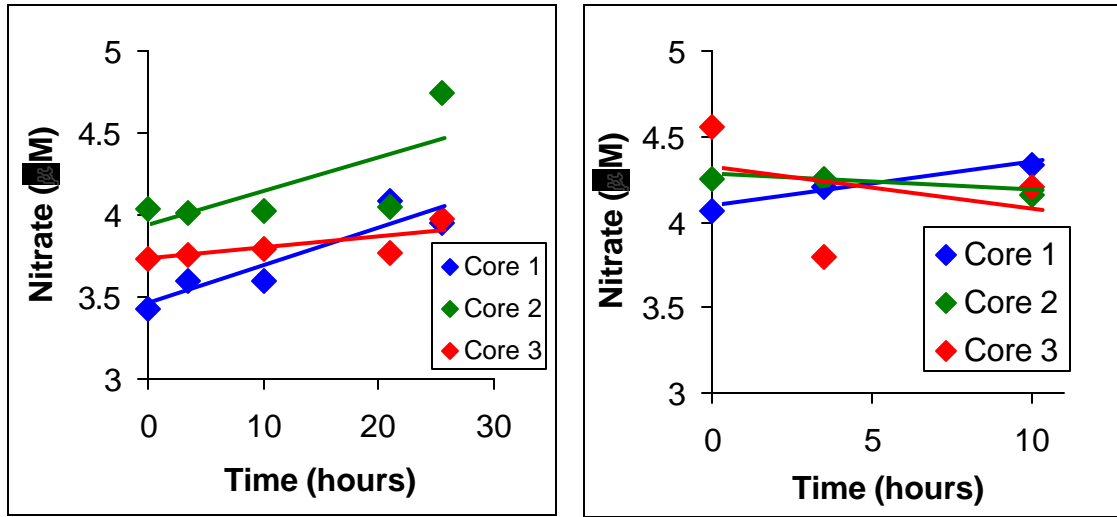


a) Devil's Lane cores



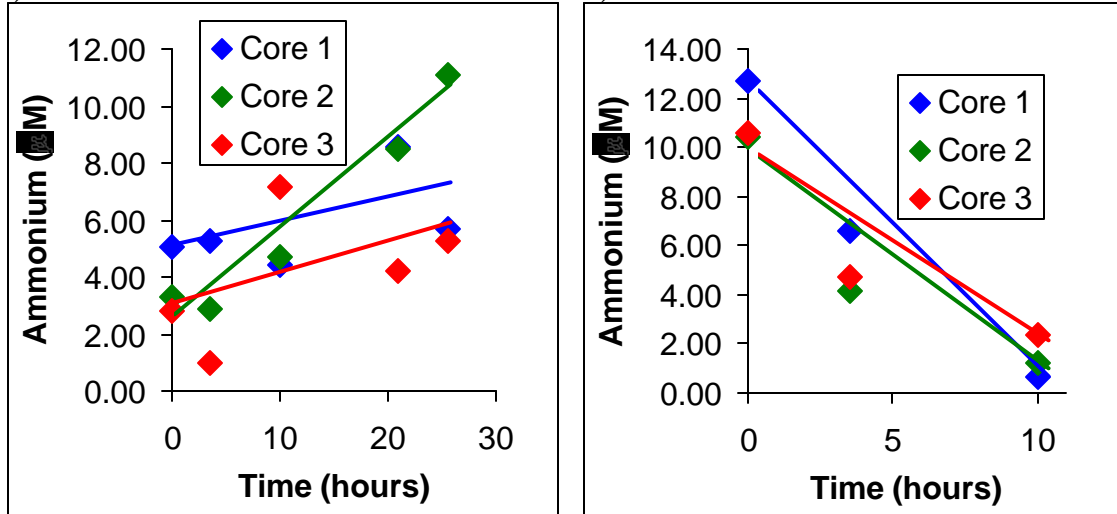
b) Marconi cores

Figure 7: The nitrate, ammonium, and DIN concentrations over time in the Devil's Lane and Marconi control cores



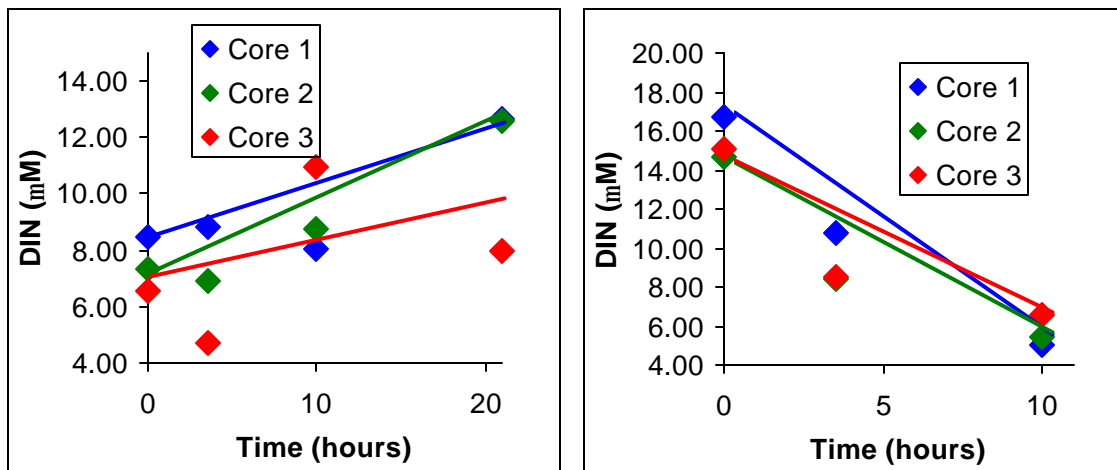
a) Devil's Lane Core Nitrate

b) Marconi Core Nitrate



c) Devil's Lane Core Ammonium

d) Marconi Core Ammonium

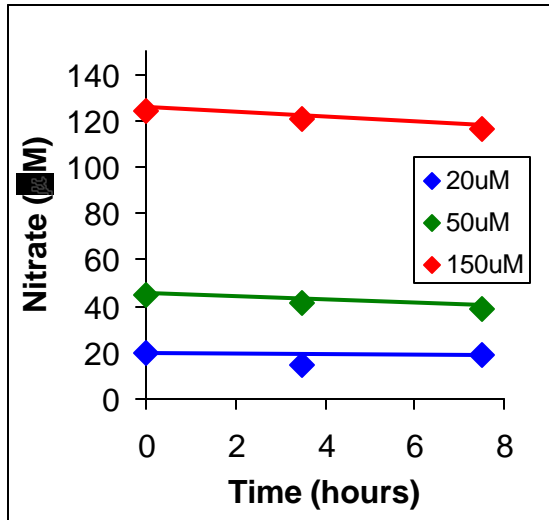


e) Devil's Lane Core DIN

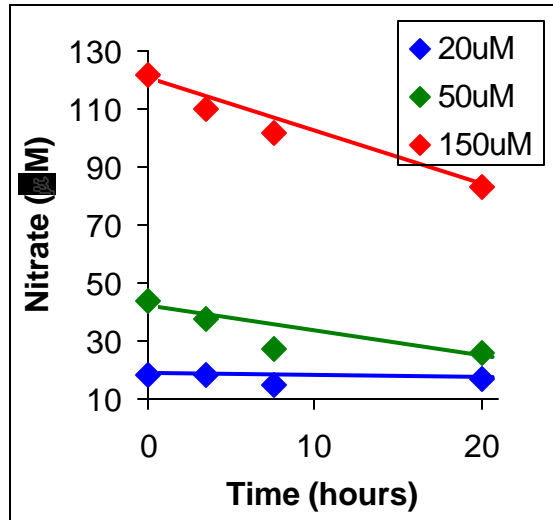
f) Marconi Core DIN

Figure 8:

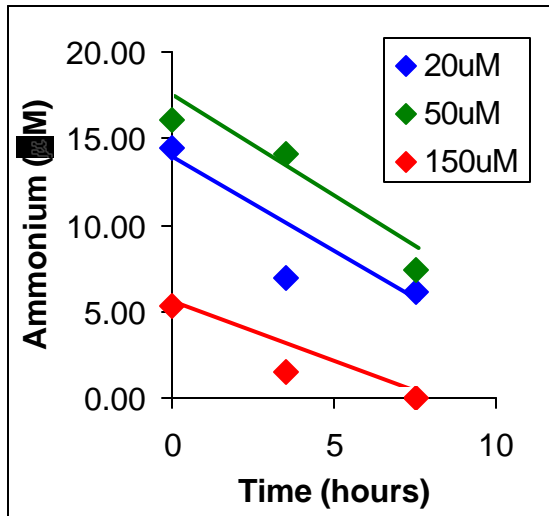
The nitrate and ammonium concentrations over time in the 20 μ M, 50 μ M, and 150 μ M nitrate Devil's Lane and Marconi control cores



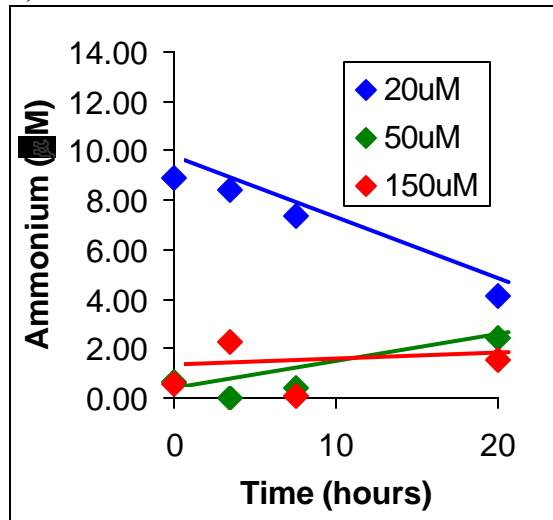
a) Devil's Lane Core Nitrate



b) Marconi Core Nitrate



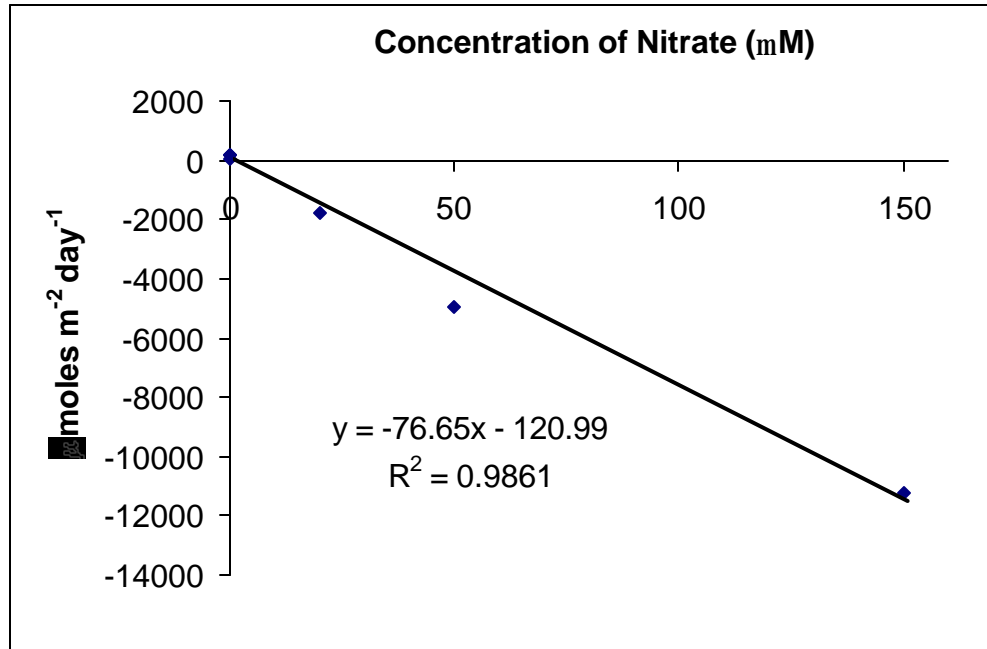
c) Devil's Lane Core Ammonium



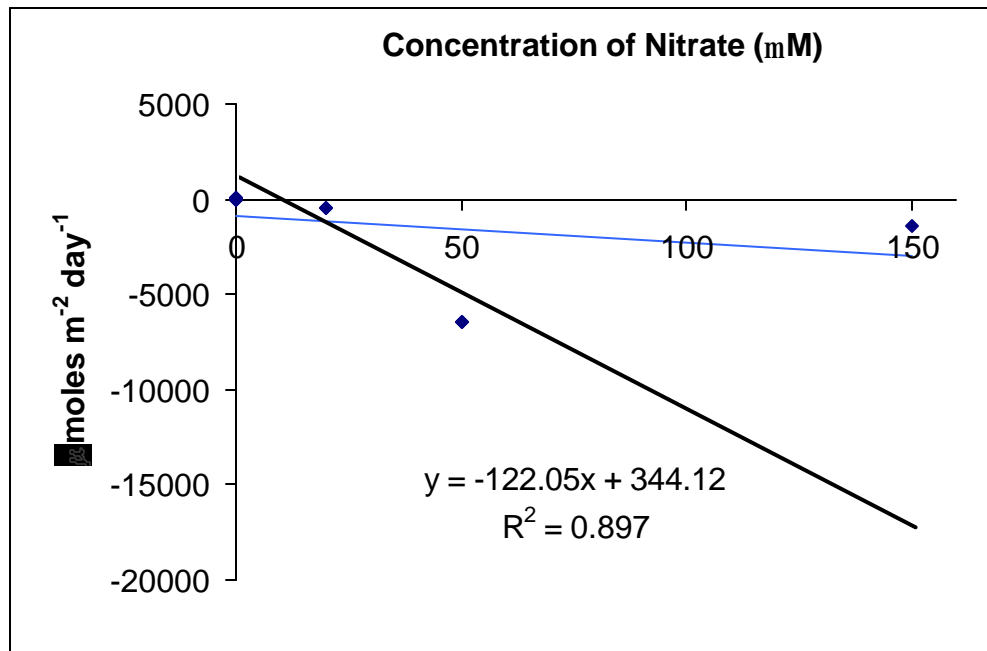
d) Marconi Core Ammonium

Figure 9:

The rate of nitrate appearance in relation to nitrate concentration in the Devil's Lane and Marconi cores throughout the two incubations



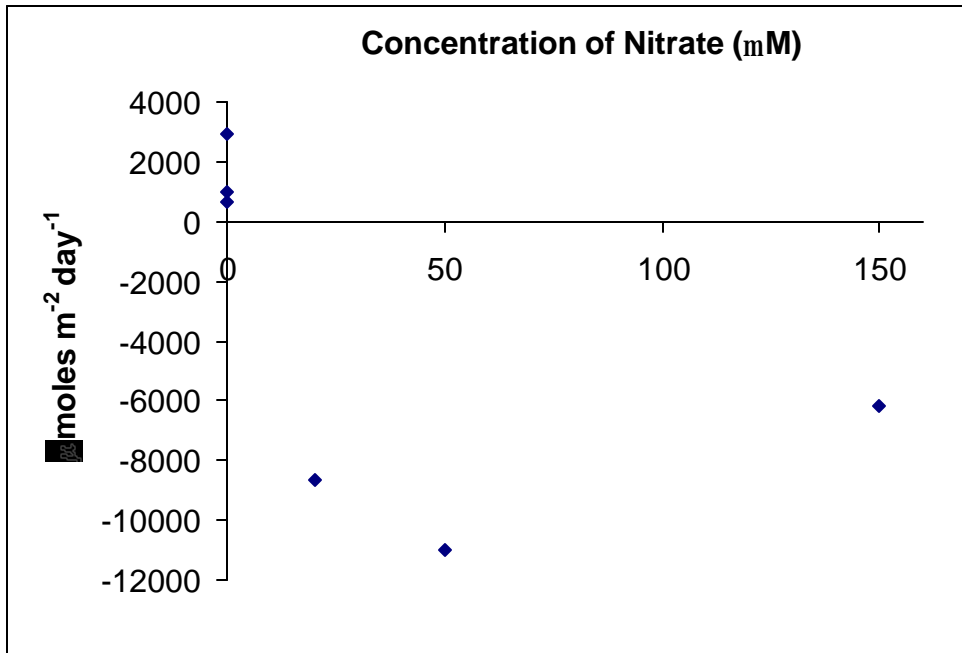
a) Devil's Lane cores



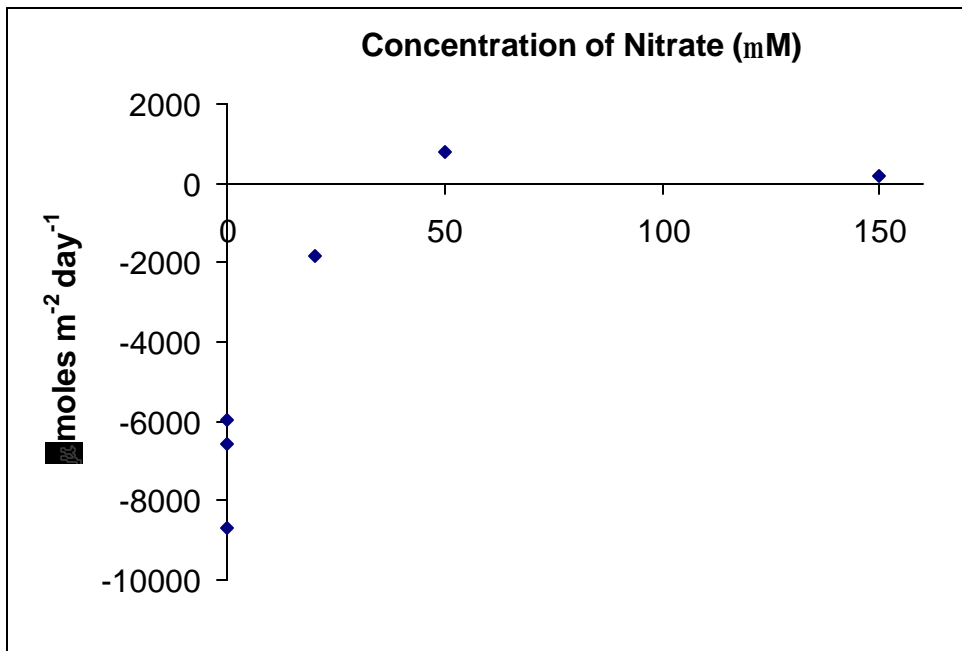
b) Marconi cores (The blue trendline is that will all four points and the black trendline is the trendline using only the first three data points)

Figure 10:

The rate of ammonium appearance in relation to nitrate concentration in the Devil's Lane and Marconi Cores throughout the two incubations



a) Devil's Lane cores



b) Marconi cores

Figure 11:

The coupled nitrification-denitrification rates in the control and spiked cores from both swamps. The rates are calculated using both the Redfield ratio and the C:N ratio of the sediment in the cores.

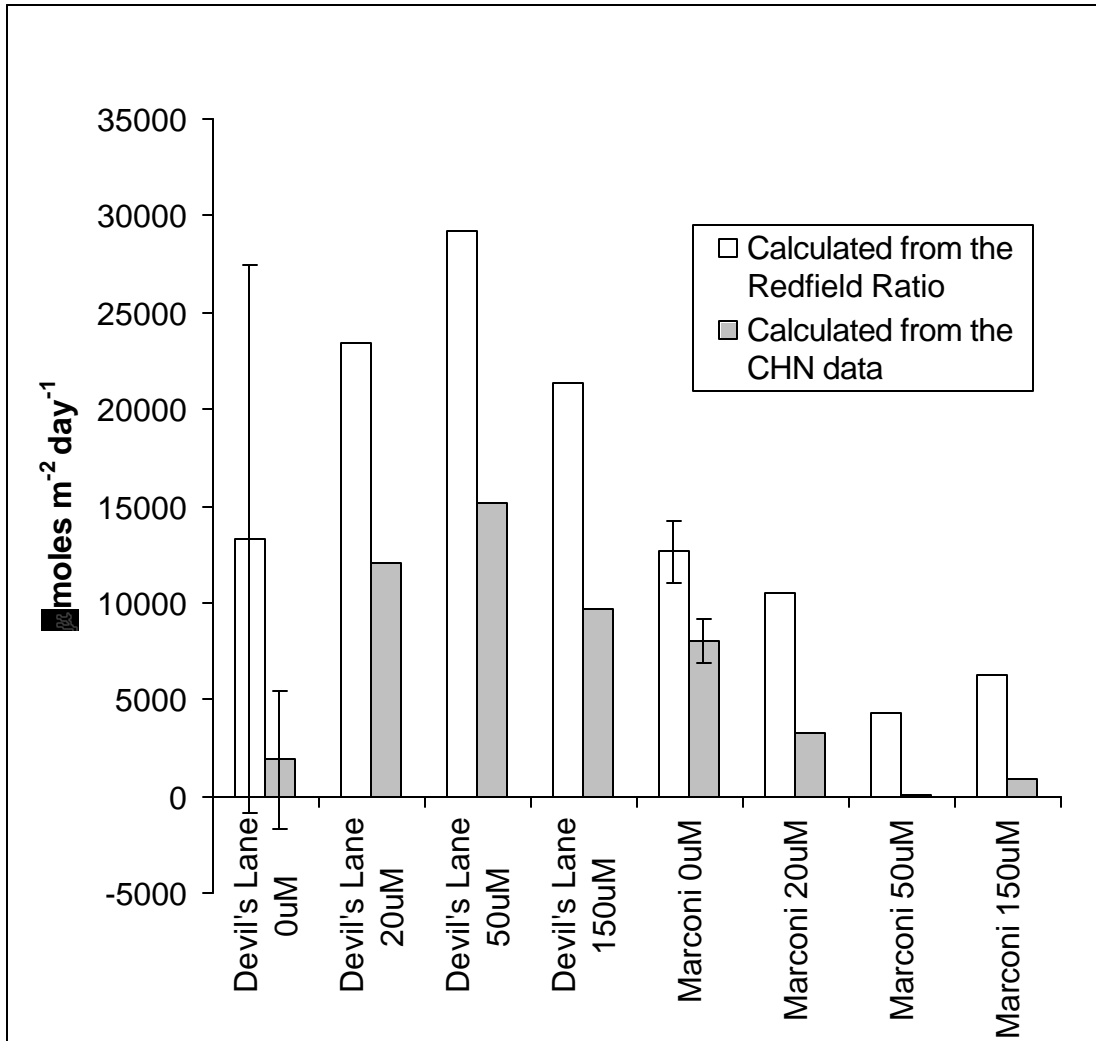
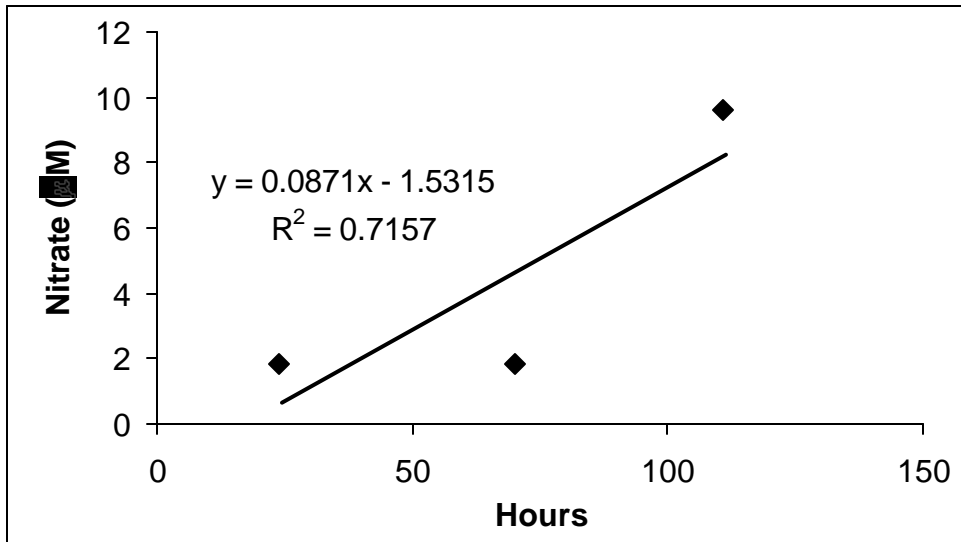
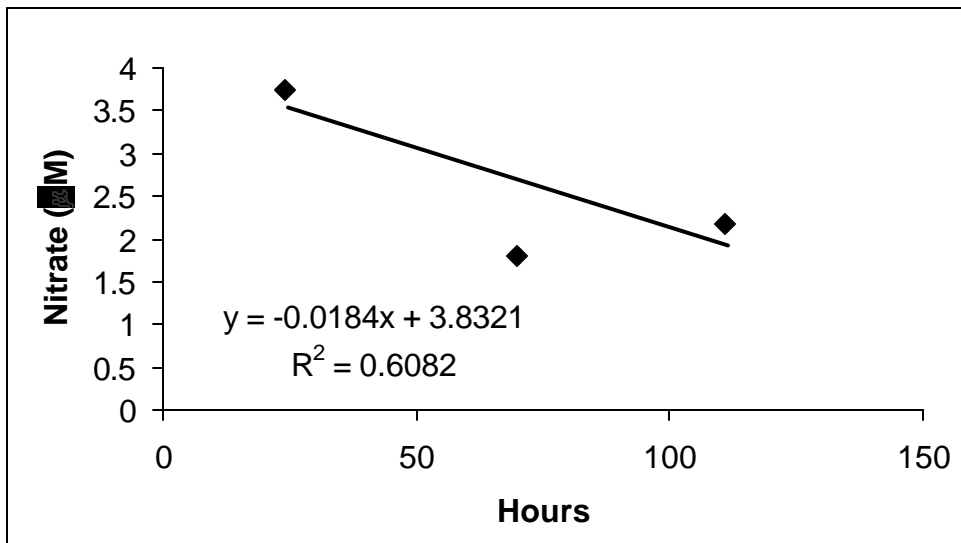


Figure 12:

Nitrate appearance over time in the potential nitrification slurry experiment



a) A representative graph of the change in nitrate concentration. This data is from Devil's Lane Edge 2.



b) Devil's Lane Center 3

Figure 13:

The potential nitrification rates at the two Atlantic white cedar swamps.

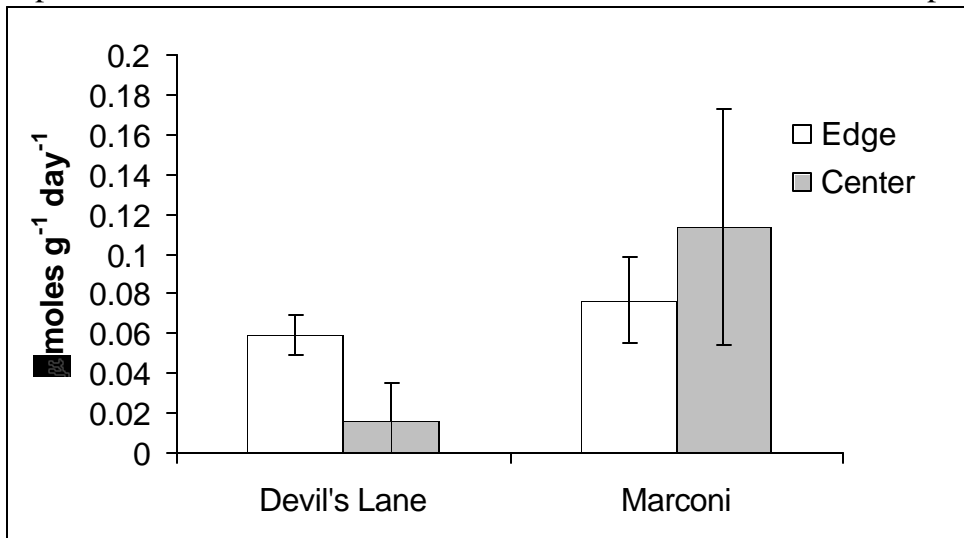


Figure 14:

The potential denitrification rates at the two Atlantic white cedar swamps.

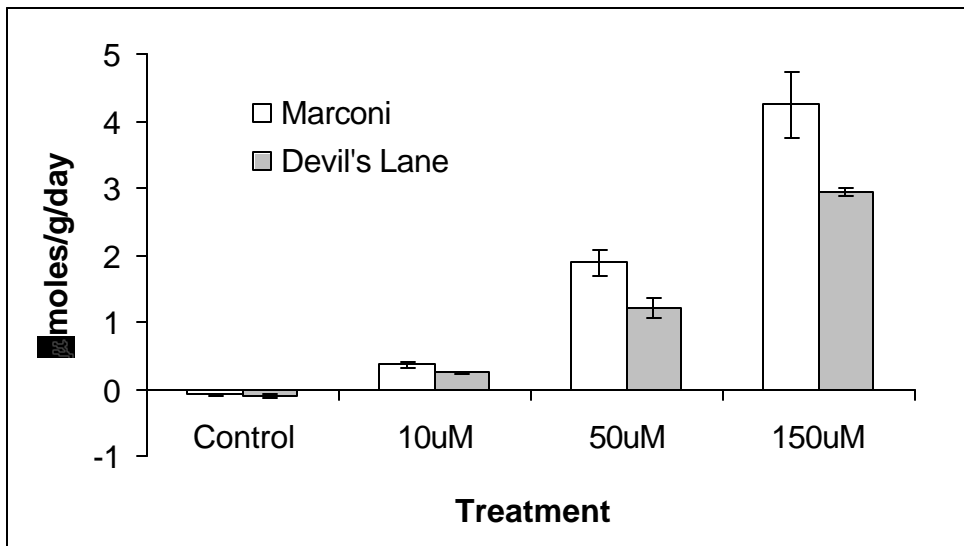


Figure 15:

The potential denitrification rates calculated for Megan Hartmann's experiment

