Impacts of Fertilization on Rates of Autotrophic N2 Fixation in Salt Marshes and Cranberry Bogs of Massachusetts

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Abstract:

Fixation of atmospheric N2 is a significant source of N to ecosystems; however, the energy demanded for the reduction of N2 makes nitrogen uptake more efficient for microbes when soil nitrogen is available. In this study I used the Acetylene Reduction method of measuring fixation to compare the impact of fertilizer nitrogen on rates of autotrophic fixation in salt marshes and cranberry bogs of MA. I measured pools of NH4, NO3, and PO4, in addition to total C and N content of the soils of salt marshes and cranberry bogs, each under two different fertilization treatments and a control treatment. My results indicate that by 1) altering species composition, thereby increasing shading, and 2) making more nitrogen available in the soil for uptake, fertilization inhibits rates of fixation in salt marshes and in cranberry bogs.

Introduction:

The biological fixation of atmospheric N_2 provides a significant annual input of nitrogen to many ecosystems, with an estimated total global input of 175 Tg per year (Paul and Clark, p. 218). Nitrogen fixation varies in different ecosystems, in part because of a diversity in types of nitrogen fixing bacteria, which range from symbiotic heterotrophs to free-living autotrophs. The reduction of N_2, which is catalyzed by the nitrogenase enzyme in N-fixing bacteria, has a high energy-requirement. For fixation to occur, autotrophic fixers demand a high light environment and heterotrophic fixers require carbohydrates typically supplied by a plant host (Chapin, 2001). Many factors in addition to light and carbohydrate supply can influence rates of fixation, including temperature, soil pH, and soil nutrients; and because of constraints on fixation, several ecosystems show nitrogen limitation despite the ample supply of atmospheric nitrogen (Vitousek et al. 1991).

The relative importance of N_2 fixation as an input of nitrogen varies between different environments. In this study I compare rates of autotrophic N_2 fixation and potential factors influencing rates in salt marshes and cranberry bogs in the Falmouth, MA area. High annual rates of fixation, primarily by blue-green algae (Carpenter et al., 1978), have been found in salt marshes, ranging from 0.3-12g N/m2/yr (Valiela, 1983). By contrast, Massachusetts bog studies found much lower rates, on the order of 0.05g N/m2/yr (Schwintzer, 1983) to 1g N/m2/yr (Hemond, 1983), by heterotrophic fixers. Autotrophic fixation rates in the bogs would likely be even lower than those found for heterotrophs that receive carbohydrates from a plant host (Chapin, 2001). In addition to allowing for a comparison of fixation in a natural saltwater ecosystem and a cultivated freshwater system, salt marshes and cranberry bogs in the Falmouth area provide an opportunity to examine the effects of fertilization on rates of N2 fixation. As part of a long-term study by Valiela, Teal, and others, ultra-fertilized plots have received two 25g/m2 doses per week of a 10%N, 6%P, 4% K fertilizer, and moderately fertilized plots have received four 8g/m2 doses per week since 1974. Control plots have received no fertilizer. Cranberry bogs, which have been farmed both inorganically and organically, provide a similar means of examining the effect of fertilization on fixation, with an abandoned bog as the control.

Relative to the energy required for uptake of NH4 and NO3, the energy requirement of N_2 fixation is quite high (Chapin et al., 2001); and according to a model developed by Rastetter et al. (2001), when soil N availability is sufficient, energy is allocated towards uptake rather than fixation. Van Raalte et al. (1974) and Carpenter et al. (1978) found that N input from fertilizer sharply reduced the activity of nitrogen fixers in salt marshes. On the other hand, Hingston et al. (1982) found that phosphorous additions actually enhanced the activity of certain fixers.

Fixed N_2 is not very susceptible to losses (Ed. Paul and Clark, 1996), on the other hand, NO_3^- that is made available by fertilizer may readily leach from the soil. Howes and Teal (1995) found that although plants and sediments in cranberry bogs are effective sinks for nitrogen, fertilizer is a net source of nitrogen to out-flowing stream waters. Nutrients that leach into the streamflow can negatively impact other systems; and in eastern Massachusetts, where many cranberry bogs are located along rivers, the additional inputs of fertilizer nitrogen may be of particular local concern.

In this study I hypothesized that: 1) soil nitrogen availability would inhibit N_2 fixation, 2) phosphorous availability could enhance fixation, 3) sites with high rates of N2 fixation would not be as susceptible to leaching. To examine these hypotheses, I applied the Acetylene Reduction method (Bergerson, 1980) of measuring nitrogen fixation to soil cores incubated in the lab. In addition I measured soil characteristics including pH, respiration, total carbon and nitrogen content of the soils, and NO3, NH4, PO4 concentrations. I sampled from six sites, including three salt marshes: an ultra-fertilized salt marsh plot (F+SM), a moderately fertilized salt marsh plot (FSM), a control salt marsh plot (CSM), and three cranberry bogs: a conventionally fertilized cranberry bog (FCB), an organic cranberry bog (OCB), and an abandoned cranberry bog (ACB) adjacent to FCB that was abandoned about 50 years ago (Mary Kay Fox, personal communication).

Materials and Methods

I sampled at six random points along a 10 meter transect in each of the six study sites. At each random point I collected three soil cores with a tulip-bulb corer. From each site I placed six replicate 80cm^3 cores, 5 cm in diameter and 4 cm in height, into 150ml gas-tight jars. Each jar had a septum from which gas was sampled during the incubation period for Acetylene Reduction measurement. I placed another six replicate 80 cm^3 cores in Falcon Filter cups for respiration measurement. I sub-sampled the top 4cm of the final six replicate cores from each site for pH, CHN analysis, and KCl extraction of NO3, NH4, and PO4.

Nitrogen Fixation

To measure nitrogen fixation, I used the method of acetylene reduction (Bergerson, 1980), in which acetlyene reduced by the nitrogenase enzyme is measured as proportional to the nitrogen fixed. Soil cores were stored at room temperature for 48 hours before introducing 5.5 cc of acetylene gas (10% v/v) to five of the six replicates from each of the sites. I used the sixth replicate core as a soil blank to measure background ethylene production. In addition, I prepared two acetylene blanks to measure ethylene contamination, by introducing 5.5 cc of acetylene to empty jars. I incubated all cores and blanks at constant light and 20C for 2 hours. Following incubation I sampled 5cc from the headspace to measure ethylene concentration (ppm) with a gas chromatograph. I assumed a 3:1 ratio of C_2H_2 reduced to N_2 fixed (Hardy, Burns, and Holsten, 1973).

Respiration

I stored my cores in Falcon Filter cups in the dark for 48 hours. I then calculated the flux of CO2 in the cups by measuring changes in CO2 concentration at 15-second intervals over a 3-minute period with a Licor 6200 Portable Photosynthesis System (SES Lab Manual, 2003).

PH

I prepared a soil slurry with 15g of soil and 50ml of deionized water and then measured the slurry with a Fisher Accumet 20 pH meter.

CHN Analysis

I dried soil samples and recorded fresh and dry weights. I used a Perkin-Elmer Elemental Analyzer to obtain %C and %N of ground soil samples . I removed all large roots, but smaller roots remained in the samples. I applied the fresh to dry ratios I obtained to the 80cm3 cores used for acetylene reduction in order to estimate bulk density of the soils.

KCl extraction of NO3, NH4, and PO4

I added 50 ml of 1M KCl to a 15g soil subsample from the top 4cm of each replicate and to 2 blanks with no soil. After shaking the slurries for 1 hour, I filtered samples using GF/F filters. I measured absorbances of NH4 and PO4 on a Shimadzu 1601 Spectrometer at 640nm and 885nm respectively. I measured NO3 absorbances on the Lachat Quickchem 8000 (SES Lab Manual, 2003).

Results

N2 Fixation Rates

Rates of N_2 fixation given by the acetylene reduction vary greatly between sites and also within sites (Fig. 1 and Fig. 2). Rates of N_2 fixation in salt marshes were generally 2 orders of magnitude greater than fixation rates in the cranberry bogs. FSM and CSM showed the highest rates of fixation and were very similar, with a rate around 273ug/m2/hr. In F+SM, I measured no N_2 fixation, suggesting that the ultra-fertilization treatment inhibited fixation entirely. Although rates of fixation were very low in the cranberry bogs, all sites showed some fixation. The rate of fixation in ACB was highest of the cranberry bogs, at 1ug/m2/hr. Fixation rates did not differ between the conventionally and organically farmed bogs, and showed a rate of fixation around half that of the abandoned bog. This pattern again suggests that fertilizer, both as inorganic and organic nitrogen supplementation, inhibits fixation.

To compare my rates of N_2 fixation to literature values, I scaled hourly rates to annual rates (Table 1). To do so, I assumed that N_2 fixation would occur during 12 hours each day when there is sufficient light, and from May to September, when temperatures are warm enough and light is available to support microbial activity (Gus Shaver, personal communication). My annual N_2 fixation estimates are likely to be low estimates, since I collected cores in late October and early November after microbial activity had probably slowed significantly. I calculated an annual rate of 0.5 g N_2 fixed/m2/y in CSM and FSM. This value falls within the range of 0.3-0.9 calculated by Carpenter et al. 1978 for cyanobacteria under Spartina patens and Spartina alterniflora, respectively, in the Great Sippewissett Marsh. I calculated a rate of 0.002 g N_2 fixed/m2/y in the abandoned cranberry bog, and a rate of 0.001 g N_2 fixed/m2/y in both farmed bogs. I did not find literature values for comparison.

Soil Characteristics and Relationships to Rates of Fixation

pH

The salt marsh soils were more basic than the bog soils, with a pH around 6.3. pH did not differ significantly between treatment types in the salt marshes. FCB and ACB had pHs around 4.5, while OCB had a less acidic pH of 5.3. The basic salt marsh soils supported higher rates of fixation, although many autotrophic fixers prefer more acidic conditions (Paul and Clark, 1996). A comparison of ecosystems with similar rates of fixation but varying in acidity would better reveal any correlation between pH and N_2 fixation.

Respiration Rates

In general, respiration rates were slightly higher in salt marshes than in cranberry bogs (Fig. 3), although FCB also showed a high respiration rate. Salt marsh soils had greater root density and so respiration rates may reflect root respiration rather than microbial respiration. Sites with higher respiration also contained larger pools of carbon and nitrogen (Fig. 4), which may support higher rates of decomposition, and showed higher rates of N_2 fixation (Fig. 1 and Fig. 2). These patterns suggest that the respiration rates do reflect higher levels of activity by decomposers and/or N_2 fixers. Because the nitrogenase enzyme is inhibited by O_2, autotrophic fixers use protection mechanisms including high rates of respiration, N_2 fixing complexes within the cell, slime production, or clump formation to provide anaerobic conditions (Paul and Clark, 1996). A high level of respiration helps protect nitrogenase by maintaining low levels of O_2.

C and N Pools

Carbon pools did not differ greatly between sites, but total nitrogen in salt marshes was about double the nitrogen in cranberry bogs. C:N ratios ranged from 10-20 in the salt marshes, and from 20-30 in the cranberry bogs (Fig. 5). Of the salt marshes, the ultra-fertilized plot had the highest carbon and nitrogen content. Of the cranberry bogs, however, the abandoned bog had the largest pools, followed by the conventionally fertilized bog. Sites in which respiration rates were higher had larger pools of carbon and nitrogen in the soil (Fig. 5); and overall respiration rates showed a positive correlation with total C content (R^2=0.7). This pattern may indicate higher rates of decomposition in F+SM relative to the other salt marshes, and in ACB relative to the other bogs.

Larger pools of carbon and nitrogen very likely reflect greater productivity in F+SM and ACB. The fertilizer inputs significantly increase productivity in the salt marshes (Valiela and Teal, 1974). In farmed cranberry bogs as well, the fertilizer is applied because it increases productivity. In the abandoned bog, where fertilizer is not applied, the presence of tree species could still make productivity even higher. By generating a larger pool of organic matter, higher levels of productivity can stimulate the rates of decomposition, which may have implications for rates of nitrogen fixation. One potential implication of faster rates of decomposition is that nitrogen will more readily become available for uptake. If this were the case I would expect to see that microbes preferentially take up nitrogen made available in the soil, thereby reducing rates of fixation in sites with large pools of nitrogen. In salt marshes I found a pattern that supports high rates of mineralization as a possible explanation for reduced fixation rates, in which there is a negative correlation between nitrogen fixation and total nitrogen content of the soil (R^2=0.97). In the cranberry bogs, fixation was highest in the abandoned bog, despite the large pool of nitrogen.

KCl-extractable NO3, NH4, and PO4

As expected, F+SM contained the most NH4, NO3, and PO4 (Fig. 5). PO4 and NH4 availability (g N or P/ m2) was roughly twice as high in F+SM than in the other salt marshes, and NO3 availability differed even more between the sites. In general, inorganic nutrient availability in salt marshes was an order of magnitude greater than availability in cranberry bogs. Inorganic nutrient availability did not differ as significantly between the three cranberry bogs (Fig. 6). NH4 was slightly higher in ACB, which contained no detectable NO3. The farmed bogs also contained most inorganic nitrogen as NH4 rather than NO3, but the NO3 supply in the conventionally farmed bog was doubled that of the organically farmed bog. The PO4 supply in the farmed bogs was substantially larger than the supply in the abandoned bog. Total DIN was highest in the farmed bogs and the ultra-fertilized salt marsh, and lower in the other marsh sites, which did not differ significantly (Fig. 7). Of the cranberry bogs, the conventionally farmed bog showed the highest total DIN (Fig. 8). The total pool of inorganic nitrogen showed a strong negative correlation to fixation rates in salt marshes (R^2=0.99) and a weaker trend towards negative correlation in cranberry bogs (Fig. 9).

Discussion

The trend in fixation rates, in which the ultra-fertilized salt marsh and the farmed cranberry bogs show lower rates, indicates that fertilization does reduce fixation. In addition to potentially influencing fixation rates by altering the nutrient content of the soils, fertilization also alters plant species composition and thereby changes the availability of light. In a study that looked more closely at light as a factor influencing fixation, Carpenter et al. found that shading by different species of Spartina grasses played an important role in reducing fixation rates (1978). The control salt marsh is primarily dominated by Spartina alterniflora, the moderately fertilized marsh by both S. alterniflora and S. patens, and the fertilized marsh by Distichlis spicata (See Fig. 10) (Valiela, Teal, Sass, 1975).

D. spicata is a dense grass (personal observation), which very likely contributed to the absence of fixation in F+SM by shading the soil surface. This shading alone could possibly stop fixation, although a measurement of the light availability and a closer study of the shade-tolerance of fixers would be necessary to confirm this. The rate of fertilizer application (2x 25g/m2/wk) could also provide nitrogen in a high enough dosage so as to make N_2 fixation and its associated energy costs unnecessary. In FSM, with a lower rate of fertilizer application (4x8g/m2/wk), I would also expect to see reduced N_2 fixation. However, fixation rates in FSM did not differ from rates in the control plot, implying that neither changes in light availability nor nitrogen supplied by the fertilizer affected fixation. It may be the case that the lower rate of fertilization provides enough nitrogen to support increased productivity without saturating the system, such that uptake by vegetation leaves soil nitrogen pools similar in size to nitrogen pools in the control plot. Similar DIN pools and total N pools in FSM and CSM support this hypothesis.

In addition to increased uptake of nutrients by vegetation, leaving soil nitrogen pools similar to unfertilized plots, phosphorous supplied by fertilizer could also potentially allow for fixation under fertilized conditions. Hingston et al. (1982) found that phosphorous enhanced fixation rates. Because nutrients are required in certain ratios, high rates of N uptake or N fixation are not likely to occur when P is not available. However, with the coupling of nitrogen and phosphorous in the fertilized sites, fixation reflected the inhibitory influence of the nitrogen and showed no clear effect from phosphorous. An experiment in which phosphorous alone was supplied to plots would better reveal any relationship between phosphorous availability and fixation rates.

In cranberry bogs, very low rates of fixation may reflect an effect of the disturbance caused by transforming natural systems for agricultural use rather than a strong relationship to nutrient availibility. However, I found no literature values for autotrophic fixation to compare my measured rates with a natural bog system. Heterotrophic rates in two Massachusetts bog studies ranged from 0.05-1gN/m2/yr (Schwintzer, 1983; Hemond, 1988). In addition to extremely low rates of fixation, the doubled rate of fixation in ACB compared to the farmed bogs indicates that the farming practices reduce fixation. Unlike the salt marshes, in which a negative correlation between DIN and fixation ($R2=0.99$) strongly implicates the abundance of DIN in reducing rates, correlation in the cranberry bogs is weak ($R2= 0.24$), leaving doubt as to whether nitrogen availability in the soil is responsible for the reduced rates. Although the dense cranberries provide considerable shading, the trees in the abandoned bog do so as well, and quantification of the light availability would be necessary to determine how light is affecting fixation in these systems.

Conclusions

Although nitrogen fixation appears to be a greater source of nitrogen in the salt marshes, Howarth (1988) noted that nitrogen fixed at a rate of 1g N/m2/yr by heterotrophic fixers constituted 59% of total nitrogen input to Thoreau's Bog, whereas nitrogen fixed at a rate of 11g/m2/yr by heterotrophic fixers in the Sippewisset salt marsh constituted only 1-2% of total nitrogen input to the marsh. Howarth attributed this to the closed nature of freshwater systems as contrasted to the open salt marshes that receive tidal nutrient inputs. Therefore the low rates of fixation in cranberry bogs may still be of significance to the total nitrogen input. A nitrogen budget of the cranberry bogs would provide a context for assessing the importance of fixation as an input.

In addition to providing a means of determining the significance of biological nitrogen fixation in the overall nitrogen budget of an ecosystem, rates of nitrogen fixation can also reveal the level of N-saturation in a system. High rates of fixation will not occur when nitrogen is readily available in the soil for uptake. High rates of fixation may also not necessarily occur in N-limited systems. Nonetheless, in this study, very high rates of fertilization stopped fixation entirely, implying that soil nitrogen was sufficient to support all nitrogen acquisition via uptake. Lower rates of fixation that still supplied substantial nitrogen to the salt marsh did not affect fixation rates, implying that the system had not reached N-saturation, such that expending energy towards fixation was still necessary to meet nitrogen demands. Because systems with an abundance of nitrogen in the soil, especially in the form of NO3, are particularly susceptible to leaching, fixation rates could potentially reveal whether a system is prone towards nitrogen leaching. Nitrogen loss into stream-flow and into groundwater that is associated with fertilizers, but not typically observed in environments with high rates of nitrogen fixation, can have severe consequences for ecosystems receiving the additional nitrogen.

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Figures and Tables

Figures and Tables

Figure 1. Mean hourly rates of N_2 fixation +/- standard error in an ultra-fertilized (F+SM), fertilized (FSM), and control salt marsh (CSM). N_2 fixation rates were measured using the Acetylene Reduction method.

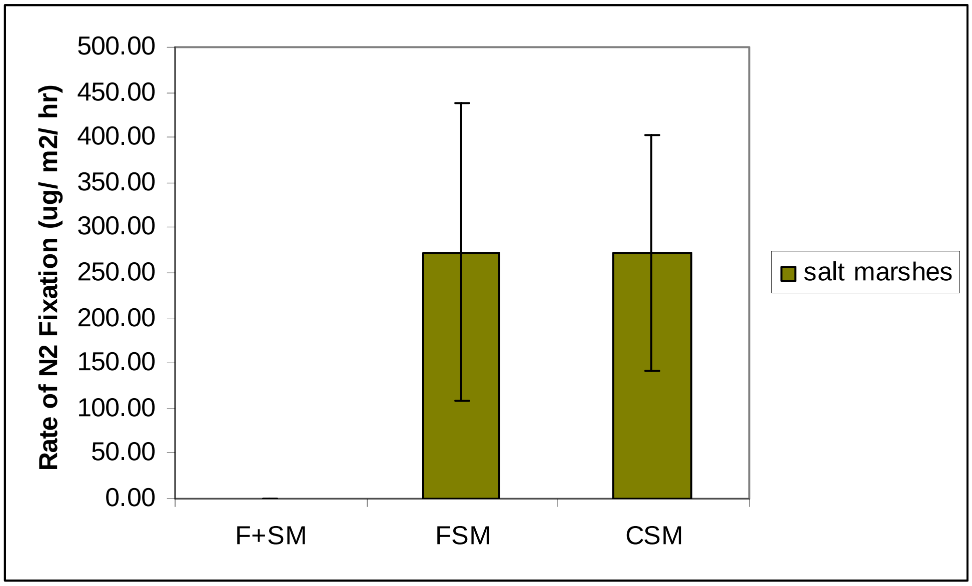

Figure 2. Mean hourly rates of N2 fixation +/- standard error in a conventionally farmed (FCB), organically farmed (OCB), and abandoned cranberry bog (ACB). N_2 fixation rates were measured using the Acetylene Reduction method.

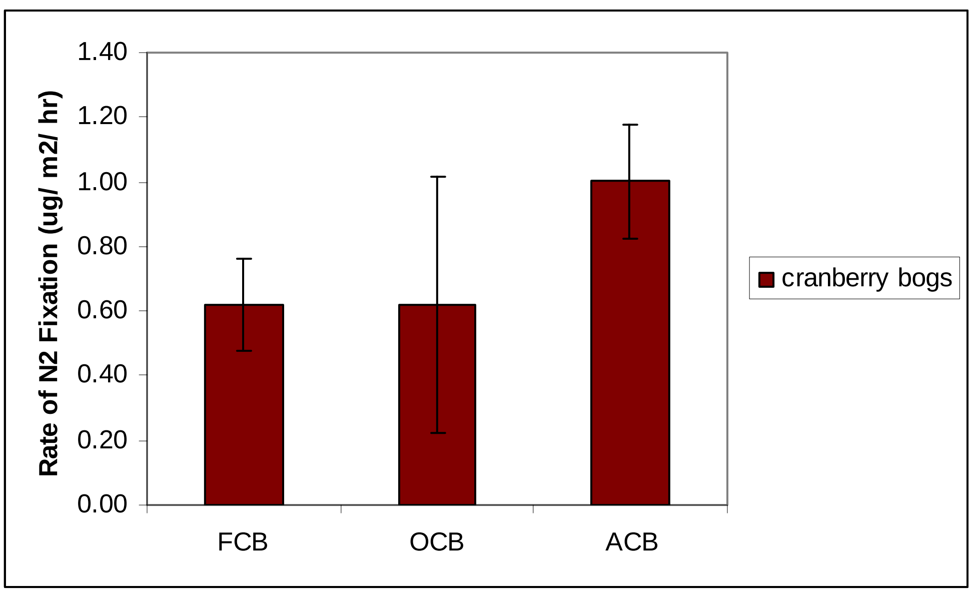

Figure 3. Mean rates of respiration +/- standard error in a conventionally farmed (FCB), organically farmed (OCB), and abandoned cranberry bog (ACB), and in an ultra-fertilized (F+SM), fertilized (FSM), and control salt marsh (CSM).

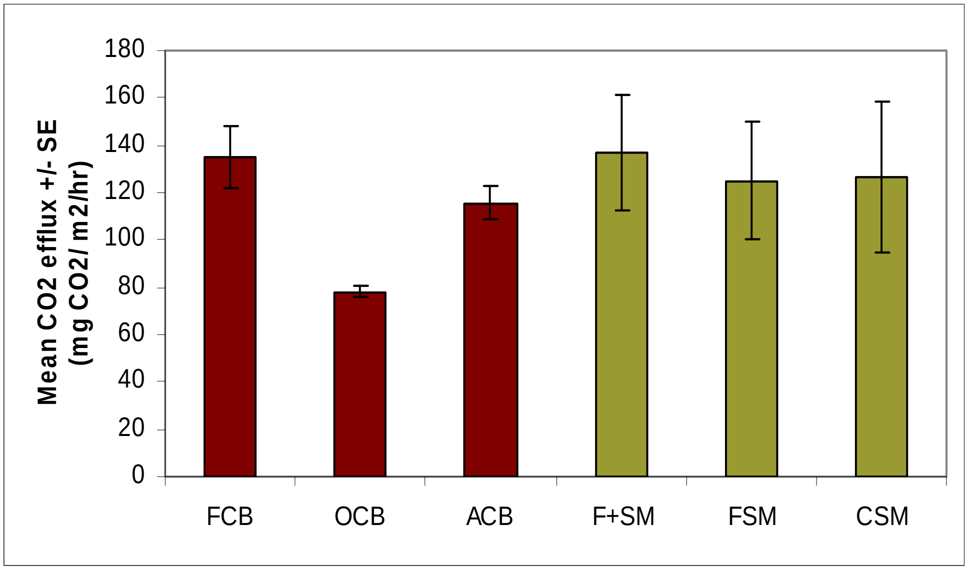

Figure 4. Mean pools of total C and N +/- standard error in the top 4cm of a conventionally farmed (FCB), organically farmed (OCB), and abandoned cranberry bog (ACB), and in an ultra-fertilized (F+SM), fertilized (FSM), and control salt marsh (CSM). Ratio of C:N is included for each site. Carbon and Nitrogen pools follow a similar pattern to respiration rates observed in each site.

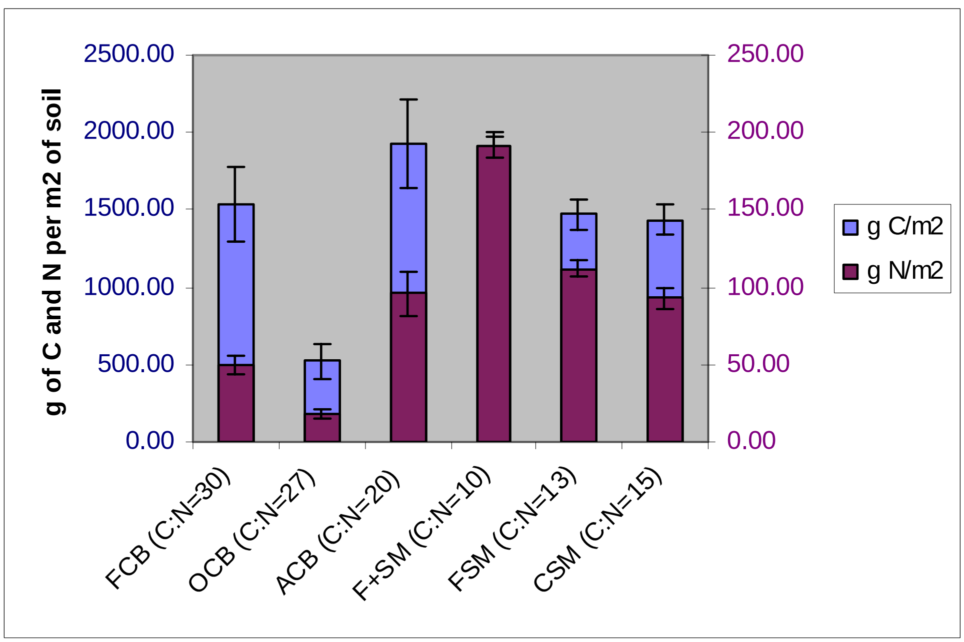

Figure 5. Mean NH4, NO3, and PO4 availability +/- standard error in the top 4cm of an ultra-fertilized (F+SM), fertilized (FSM), and control salt marsh (CSM).

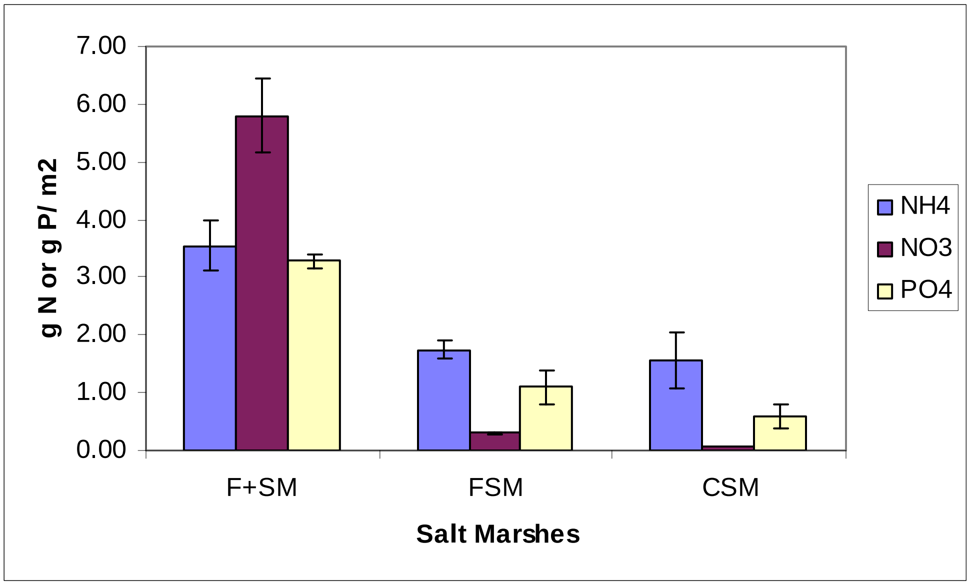

Figure 6. Mean NH4, NO3, and PO4 availability +/- standard error in the top 4cm of a conventionally farmed (FCB), organically farmed (OCB), and abandoned cranberry bog (ACB).

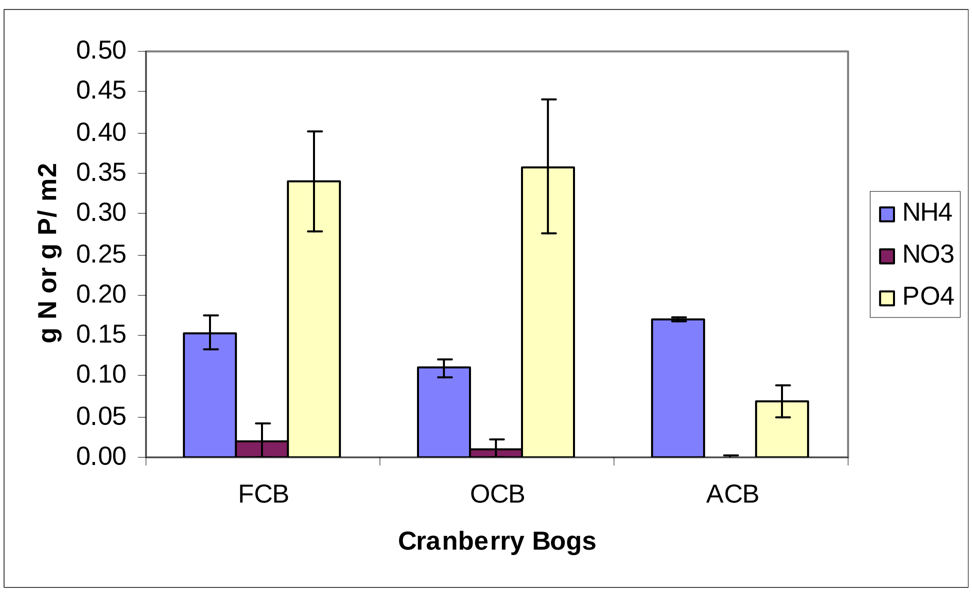

Figure 7. Mean dissolved inorganic nitrogen (DIN) +/- standard error in the top 4cm of an ultra-fertilized (F+SM), fertilized (FSM), and control salt marsh (CSM).

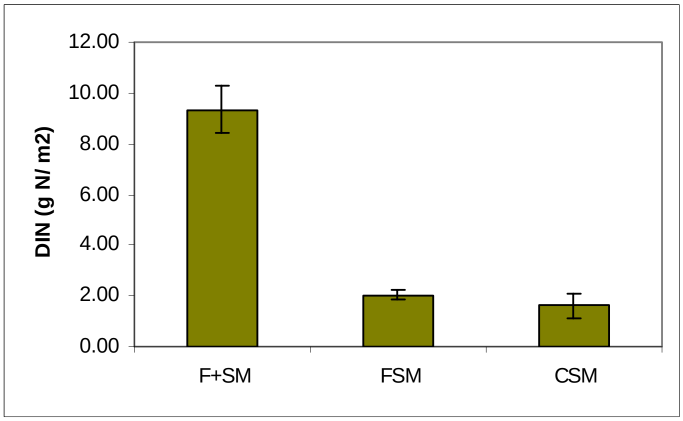

Figure 8. Mean dissolved inorganic nitrogen (DIN) +/- standard error in the top 4cm of a conventionally farmed (FCB), organically farmed (OCB), and abandoned cranberry bog (ACB).

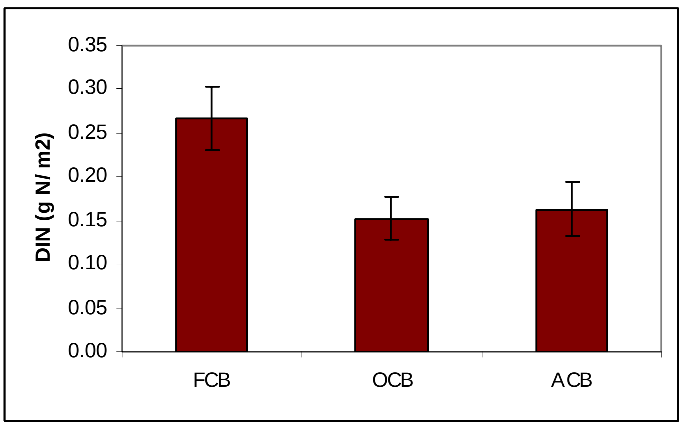

Figure 9. The relationship between the supply of dissolved inorganic nitrogen and the rate of N2 fixation in cranberry bogs and salt marshes.

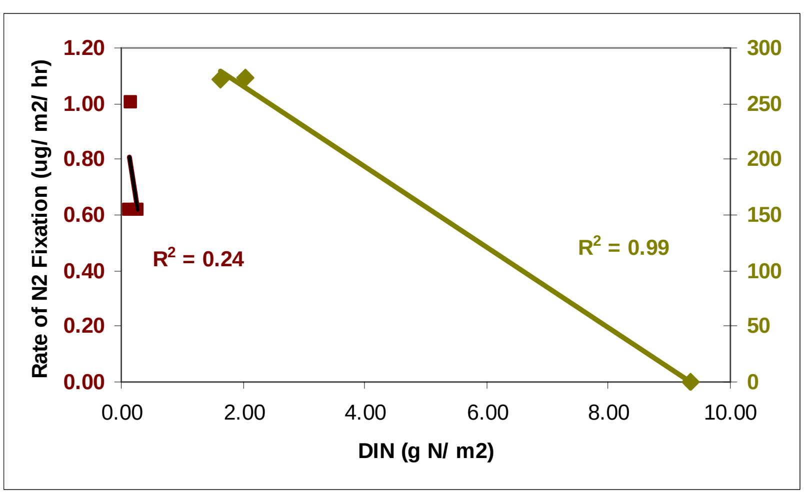

Figure 10. Species composition of different areas of the Great Sippewissett Salt Marsh. a) blue-green algal mats with no grass cover (not measured in this study), b) Spartina alterniflora in control sites, c) Spartina patens in the moderately-fertilized site, d) Distichlis spicata in the ultra-fertilized site. All photos taken by Kendra Myers, 2003.

a) blue-green algal mats

b) Spartina alterniflora

c) Spartina patens

d) Distichlis spicata

Table 1. Mean annual nitrogen fixation rates. Rates were scaled by assuming 12 hours of fixation per day, during May through September.

Study Site	**Mean Annual Fixation** g N2/m2/y
Abandoned Cranberry Bog (ACB)	0.002
Organic Cranberry Bog (OCB)	0.001
Conventional Cranberry Bog (FCB)	0.001
Control Salt Marsh (CSM)	0.490
Fertilized Salt Marsh (FSM)	0.491
Heavily Fertilized Salt Marsh (F+SM)	0.000