

The effects of increases in ambient nitrogen and phosphorous levels on nitrogen cycling in three boreal bogs

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I. INTRODUCTION

Nutrient levels, including nitrogen, have been altered in recent decades, which have sometimes have deleterious effects on plant species (Kooigman 1992), decrease biodiversity, which could make them more susceptible to invasions of exotic species (Stohlgren et al. 1999) and thus ecosystem functioning and predictability (McGrady-Steed et al. 1997, Schlapfer et al. 1999). Anthropogenic sources of fixed nitrogen have increased exponentially over the past fifty years and are now greater than all natural sources combined (Vitousek 1994). These sources include increased planting of legumes to enrich agricultural soils, extended use of fertilizers by the agricultural community, and combustion of fossil fuels. Increased levels of fixed nitrogen can cause eutrophication of previously pristine ecosystems, decreases in species diversity (Chapin 1997), and alteration of nutrient dynamics in various systems (Aerts 1998), which then affect the structure and function of ecosystems. Eutrophication of aquatic environments, caused by an increase in nutrients including nitrogen, can lead to an overabundance of algal activity and production, which can potentially cause declines of other aquatic species. This can be caused by leaching in agriculture areas, greater atmospheric deposition of N, or increasing amount of acid rain (Stepanauskas et al. 1996). Increased nitrogen in ecosystems may also result in decreases of plant or fungal diversity because of alterations in the nature of mutualisms between mycorrhizal fungi and plants (Craft & Richardson 1997). Invasion of exotic species overrunning and forcing native species to

extinction has also been a problem. The concentration of N has been shown to account for up to 83% of exotic species in a given environmental system (Stohlgren et al. 1999). The introduction of exotic species often results in a decrease of species diversity in an ecosystem. Species diversity (McKane et al. 1990), as well as species growth (Gore 1961), can be directly correlated with concentration of N and location and seasonal uptake of N. Levels of N affect alterations in the nutrient dynamics of many ecosystems, and these changes extend to peatlands.

Peatlands are an important system to study in light of these problems because they are often seen as a 'sponge' to soak up excess nutrients, including N (Stepanauskas et al. 1996). This could potentially be helpful in offsetting the aforementioned alterations to the nutrient cycle. The N cycles of peatlands, and the effects of addition of nutrients must be considered to further understand if they can truly be of use in resolving these current problems.

Peatlands are ecosystems dominated by histosols, which are defined as soils in which more than half of the top 80cm is organic material without permafrost (Richardson & Vepraskas 2001). They vary across an ombrotrophic-minerotrophic gradient driven by differences in hydrology. Ombrotrophic bogs occur in wet climates where peatlands are domed above the surrounding landscape and inputs of nutrients and ions are predominately from precipitation. At the other end of the gradient, minerotrophic fens are peatlands that receive water, already rich in bases and nutrients, percolating through the surface as groundwater or overland flow (Gorham 1991). This study focuses on ombrotrophic bogs, which occur in wet climates where peatlands are domed above the surrounding landscape and nutrient, ion, and water inputs are mainly due to precipitation

(Gorham 1991). Bogs are generally acidic, low in alkalinity, and have *Sphagnum*, conifers, and small shrubs as their major vegetation (Verhoeven et al 1990, Bridgham et al. 1996).

Nitrogen is a limiting resource in many terrestrial and aquatic systems, including bogs. The rate of nitrogen cycling affects the availability of this nutrient. The largest pool of nitrogen is atmospheric nitrogen (N_2), which is unavailable to most living organisms. Microorganisms control every aspect of the nitrogen cycle. Microbial N_2 fixation transforms atmospheric nitrogen into a usable form, ammonium. Mineralization occurs when microorganisms break down organic matter into the inorganic constituents, releasing ammonium. Microbes can then transform ammonium to NO_3^- by nitrification, which requires an oxidizing chemical environment. Denitrification is an important process in the nitrogen cycle in which nitrate is anaerobically converted back to N_2 causing a removal of nitrogen from the system.

Ombrotrophic peatlands (commonly known as bogs) have relatively rapid N turnover but the soil N pool is small, so overall net N mineralization and N availability are lower in ombrotrophic than in minerotrophic peatlands (Bridgham et al. 1998). Nitrogen fixation rates are generally low in peatlands and decrease along the ombrotrophic-minerotrophic gradient (Bridgham et al. 1998).

The N cycle in bogs is generally extremely slow and limited to the small aerobic zone of peat, because of the reducing conditions (Urban 1987). In bogs, N is normally cycled as NH_4^+ due to the general absence of nitrification because of reducing conditions (Urban & Eisenrich 1998) and the presence of acidic soils (Bridgham et al. 1998). In addition, NH_4^+ is less mobile than NO_3^- , so bogs are effective at retaining N in the form

of NH_4^+ (Urban 1987). For these reasons, the dominant features of the N cycle in bogs are plant uptake of N, and the mineralization of N; however, microbial uptake of N is also important

Our study focused on determining the mineralization rates in three different ombrotrophic peatlands that have been being fertilized for the last five years with either nitrogen, phosphorous, or nitrogen and phosphorous. We looked at the differences in mineralization rates in the three bogs, as well as across the different treatment plots in each bog. We expected to find an increased N mineralization rate in the areas where N had been added. This is because (1) fertilization increases the N capital of the system, and (2) N generally has a toxic effect on sphagnum, because this plant cannot selectively absorb different nutrients due to their non-vascular nature. As a result, sphagnum takes up the excess N until it reaches toxic levels. In addition, the presence of sphagnum generally has a negative effect on N mineralization because it produces menuric acid, a complex carbon, which is nearly impossible to break down. This increases the C:N ratio, thus lowering N mineralization. Therefore, in the areas where there is a lack of sphagnum, in our case the N treatment plots, N mineralization rates should be higher. These expectations do not necessarily apply to the plots in which nitrogen and phosphorous were added, because it is unclear how altering the nutrient balance by adding phosphorous might mediate the toxicity effect of nitrogen.

It is important to study N mineralization in bogs to add to the previous knowledge about N cycling in peatlands, and the effects of long-term fertilization on the N cycle. Hopefully, this study will act as a base for future studies to look at the effect of hydrology on N cycling and possibly study more minerotrophic peatlands in the same manner. All

of this data could be valuable in further understanding the wetlands importance in recovering from the current problems caused by nitrogen such as eutrophication, lowering species diversity, and increased primary production.

II. MATERIALS AND METHODS

The peatlands in this study are all located on the University of Notre Dame Environmental Research Center (UNDERC) property. Three different sites were studied, each with three different fertilization plots and a control plot of 32m x 32m each. The three different fertilizer regimes consisted of additions of nitrogen, phosphorous, and nitrogen and phosphorous. The fertilizer treatments started in 1998 by adding half of the fertilizer dosage twice during the growing season until 2001 when the enter dose was added at the beginning of the growing season. The doses consisted of 6g N m² as urea (CO(NH₂)₂) for the nitrogen and the nitrogen plus phosphorous regimes and 2g P m² as superphosphate (Ca(H₂PO₄)₂) for the phosphorous and nitrogen plus phosphorous regimes.

A ¹⁵N dilution technique will be used to determine gross nitrification and denitrification rates at the sites (Davidson et al 1991), although that data is not reported in this paper. From each plot, we extracted three core types (Background, Time0, and Time24) with three replications of each for a total of 216 cores. We used a sharp-ended PVP pipe with a 7cm diameter along with a serrated bread knife and clippers to cut and then extract the cores. Each core was 17 cm deep. Background cores were taken to measure the initial ¹⁵N:¹⁴N ratio. They were extracted and immediately placed into a Ziploc gallon freezer bag and stored in a cooler. Time0 cores measured the ¹⁵N:¹⁴N ratio

after initial injections of ^{15}N . These cores were extracted into the PVC pipes in the same manner, and then injected with seven doses of 3mL $^{15}\text{NH}_4\text{Cl}$ using a side-bore 17cm needle and a 3cc syringe. They were immediately put into freezer bags and stored in a cooler. Time24 cores were taken to measure the $^{15}\text{N}:^{14}\text{N}$ ratio after 24 hours of in situ incubation after injection of ^{15}N in the same manner as was done for the Time0 cores. These cores were kept in their PVC pipes with caps on the bottom of the core and returned to the place of removal where they incubated for 24 hours. They were collected, put into Ziploc gallon freezer bags and stored in a cooler. All cores were refrigerated upon arrival at the lab until the N was extracted.

Next, the nitrogen was extracted from the soil cores so the $^{15}\text{N}:^{14}\text{N}$ ratio could be experimentally calculated. The cores were removed from refrigeration, the large roots were removed, and the samples were homogenized. Two different extractions were executed for each sample. Chloroform extractions were carried out to determine the amount of nitrogen in the microbial biomass. 15g of peat was weighted out into a 50mL centrifuge tube, to which 100 μL of chloroform was added. These samples were then capped and left to sit for 18-24 hours. Each sample was then extracted in a glass flask with 50mL of 2M KCl and placed on a shaker table for at least one hour. The samples were then filtered using a Whatman #42 acid washed filter into a Nalgene bottle, and frozen until the time of diffusion.

KCl extractions were used to determine the amounts of inorganic nitrogen. First 40g of peat was weighed into a flask and 100mL of 2M KCl was added. Again, each sample was placed on a shaker table for at least one hour. These samples were also filtered using a Whatman #42 filter into Nalgene bottles, and frozen until diffused.

At this point, some of the extractions were digested. This was done by combining 15mL extract with 3mL digesting solution and then placing these solutions in an autoclave for 20 minutes. They were then removed and cooled until they were diffused.

First, packets, or acid traps, for the diffusions had to be assembled using pre-leached Whatman filters which were leached using four aliquots of 50mL of 2M KCl and then six 50mL aliquots of DI. The filters were then oven-dried at 60°C. Two Whatman filter discs with a diameter of 7mm were placed onto ¾" Teflon tape. 10µL of 2.5M KHSO₄ was added to each filter disc. We folded and sealed the Teflon tape to itself, completing each packet, which consisted of two acid traps each.

The non-digested solutions were diffused first. To test for NH₄⁺ in the non-digested solutions, 15mL of extract was placed in a 120mL specimen cup, which was then diluted to 50mL with DI. A spike of 10µL of ¹⁵NH₄Cl was added to each diffusion. Lastly, 0.2g of MgO was added to make the solutions sufficiently basic. Each diffusion sample was capped, swirled, and left to sit.

The non-digested samples were also tested for NO₃⁻. To accomplish this, 15mL of extract was again placed in a 120mL specimen cup and diluted to 50mL with DI. A spike of 10µL ¹⁵NH₄Cl was again added along with 0.2g of MgO and 0.4g of Devarda's Alloy. An acid trap was added, the sample was capped, swirled, and allowed to sit.

The digested samples were only tested for NO₃⁻. For these tests, 18mL extract was placed in a 120mL specimen cup, which was diluted to 50mL with DI. Then 2.5mL of concentrated NaOH (1:1 by weight) was added to the mixture. Again, 0.2g MgO and 0.4g Devarda's Alloy were added, and the sample was capped and swirled.

Net N mineralization was also determined by subtracting the sum of 2M KCl-extractable NO_3^- plus NH_4^+ at the beginning of the incubation to that at the end of the incubation 24 hours later. Net mineralization includes both microbial mineralization and immobilization of NO_3^- and NH_4^+ . Thus positive values indicate net N mineralization and negative values indicate net N immobilization. Concentrations of NO_3^- plus NH_4^+ were determined on a Lachat autoanalyzer with standard techniques.

III. RESULTS

The effects of the three different bogs and the four different treatments (Table 1) were analyzed using several statistical means. A two-way ANOVA of the sites and treatments showed a significant difference ($p=0.004$) in nitrogen mineralization between the sites and a very significant interaction effect ($p<0.001$) between the sites and the treatments. Thus, sites and treatments were compared separately using first an ANOVA to establish whether a significant difference was measured in the N mineralization rates between either different treatment at the same site or in the same treatment type in the various sites. Then, if a significant difference was detected, a Tukey HSD Multiple Comparison test was run on the data.

For County Road B, the N mineralization rates were significantly different among the different fertilization treatments ($p=0.011$). The N mineralization in the N treatment was significantly greater than that in both the control treatment ($p=0.017$) and the P treatment ($p=0.019$), both of which had net N immobilization (figure 1). In Donut, there was also a significant difference in N mineralization rates among the various treatments ($p=0.009$). In this case, there was only positive net mineralization in the N treatment,

with net immobilization in the control treatment ($p=0.028$), the P treatment ($p=0.034$), and the N+P treatment ($p=0.009$) (figure 2). However, whereas N fertilization increased net N mineralization in South Gate and Donut bogs, an opposite effect was observed in South Gate ($p=0.001$). Here the N treatment caused greater N immobilization than in the control treatment ($p=0.003$), the P treatment ($p=0.003$), and the N+P treatment ($p=0.003$) (Figure 3).

N mineralization in the same treatment types were also compared across the different sites by using a Tukey HSD Multiple Comparison test. There were no significant differences between the N mineralization in the control plots or the P plots in different sites. However, the immobilization of N in the N plot in South Gate was significantly different than the immobilization of N in both County Road B ($p=0.033$) and Donut ($p=0.006$), but the mineralization rates were not significantly different between County Road B and Donut. The N immobilization in the N+P treatment in Donut was significantly different than the net mineralization that occurred in both County Road B ($p=0.008$) and South Gate ($p=0.015$), but in this case County Road B and South Gate were not significantly different.

IV. DISCUSSION

It has been shown that N-mineralization rates vary significantly according to hydrology of a site (Bridgham et al. 1998). As stated in the introduction, this experiment tested sites with similar hydrology with the hypothesis of increased N-inputs leading to an increase in N-mineralization. The data also give some insights into determining how increases in N and P nutrient levels affect N-mineralization rates.

As hypothesized, we found no significant difference in the control plots of the different sites for N-mineralization. This allows us to compare the three sites with the assumption that they have relatively comparable base N-mineralization rates which will not skew the data collected from the treatment plots. This also makes logical sense, given the fact other studies have found that N-mineralization seems to vary primarily across the ombrotrophic-minerotrophic gradient, and all three of these test sites are ombrotrophic.

As expected, there was no significant difference in N-mineralization in the P plots from that in the control plots for each site. Also, when comparing the P plots to the control plots of their respective sites, no noticeable difference can be noted, which is to be expected, considering that inputs of only P should have no significant on the rate of mineralization of N. Significant results in the other two treatments, though, were observed.

There was also no significant difference between the control plots and the N+P treatment plots. This could be due to the complicated interaction that adding both N and P has on the nitrogen's toxic effect on sphagnum. One hypothesis is that the addition on phosphorous in some way mediates the toxic affects of excess nitrogen on *Sphagnum* mosses (explained in further depth in the next paragraph). If this is true, we would expect N-mineralization rates comparable to those observed in the control plots. We indeed see these here. However, more concrete explanations are beyond the scope of this study, and further research is needed.

In both the County Road B and Donut sites, a significant increase in N-mineralization was observed, which was expected. This is may be due to the toxic effect

that N seems to have on *Sphagnum*. *Sphagnum* generally inhibits N-mineralization due to the production of menuric acid, which increases the C:N ratio. It would follow that N-mineralization would be less inhibited in sites where *Sphagnum* was poisoned with added excess N. This impact was observed in both County Road B and Donut bogs. However, it is also possible that adding N simply increased the N concentration in the soil organic matter, and thus directly increased N mineralization rates. In South Gate bog, however, the opposite affect was observed. In the N plot a significant increase in N-immobilization was observed. In light of the hypothesis of nitrogen's toxic effect on *Sphagnum*, this seems counterintuitive. Several hypotheses can be put forth to attempt to explain this phenomenon.

One possible explanation is that the extra N in the N-treatment plot of South Gate was "priming" the microbes, and thus making them more active. The primed microbes are able to take up more nitrogen than was added in the fertilizer, resulting in a net immobilization. A second explanation could involve the added N in the fertilizer resulting in an increase in the overall plant biomass. This would in turn, increase the levels of carbon available to the microbes in the soil, causing them again to become more active and take up larger amounts of nitrogen than they otherwise would. Both of these are possible explanations, which require further study. The results of the present study can neither deny nor substantiate either of them. Further studies would be necessary to make better hypotheses on this phenomenon in the South Gate site.

Unseen differences in the sites seem to be affecting the outcomes of this experiment. To get a greater grasp on trends of N-mineralization in response to increased nutrient inputs (i.e. long term fertilization), more replicates are needed.

One possible problem inherent in this study exists in delineation of treatment plots. Although each treatment plot was treated separately, there could exist the possibility of nutrients from one treatment leaking into another plot (due to groundwater movement for example) especially along the edges of the treatment plots. If some soil cores were taken closer to the edge than others (which is an unavoidable problem when soil cores are taken randomly within a plot) then the data from some cores could have been influenced by the added nutrients in another plot. This would cause discrepancies in the data for any given plot and may mask otherwise significant differences. This could also help to explain some of the large uncertainties in the data. If it were possible to see how great the effects of this leaking were, then one could determine how much of an effect this were having on the data, but this could not be done for this study. It would be helpful to be able to spread the plots out enough were there would be no interaction between sites (if this is possible), but this would have to be in much larger sites than were available to this study.

Further research would be interesting to see how differences in inputs of nutrients would affect N-mineralization across the ombrotrophic-minerotrophic gradient. We recommend more studies to be done which utilize more minerotrophic sites, and compare this data to the data obtained in this experiment.

V. CONCLUSIONS

This study shows that direct or unintentional addition of excess nitrogen to ombrotrophic peatlands can significantly alter N-mineralization rates within those areas.

This has far-reaching implications for peatlands that are affected by N inputs through anthropogenic sources such as acid rain or fertilization runoff from agriculture.

VI. FIGURES

Site	Treatment	Ave M
County Road B	C	-23.6613
	N	39.28718
	N+P	12.58802
	P	-22.5358
Donut	C	-24.4457
	N	265.0667
	N+P	-92.8045
	P	-13.0005
South Gate	C	5.736303
	N	-269.494
	N+P	3.40294
	P	-3.25128

Table 1: Average net N-mineralization rates for each plot. In this table, positive numbers represent net mineralization and negative numbers represent net immobilization.

County Road B: Net N-Mineralization Rates

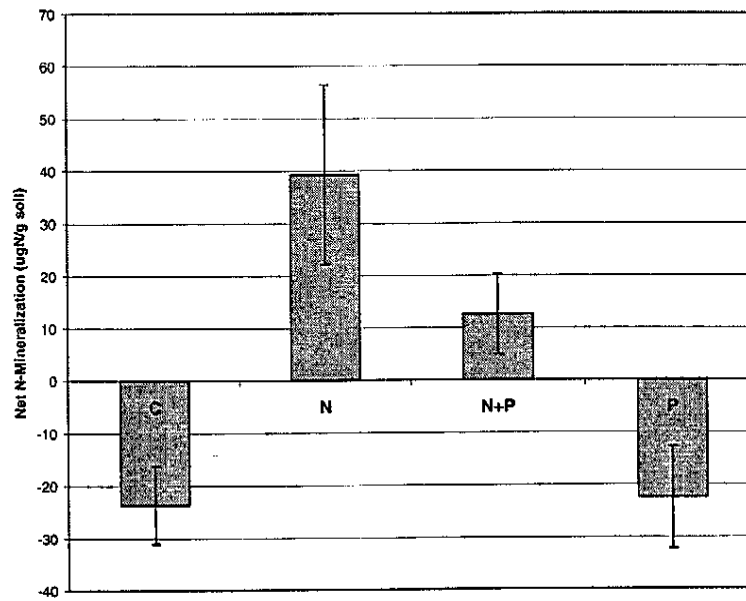


Figure 1: Net mineralization rates within the different treatment plots in the County Road B site show a significant differences in the net N-mineralization rates between the C and N, C and N+P, P and N, and P and N+P treatment plots.

Donut: Net N-Mineralization Rates

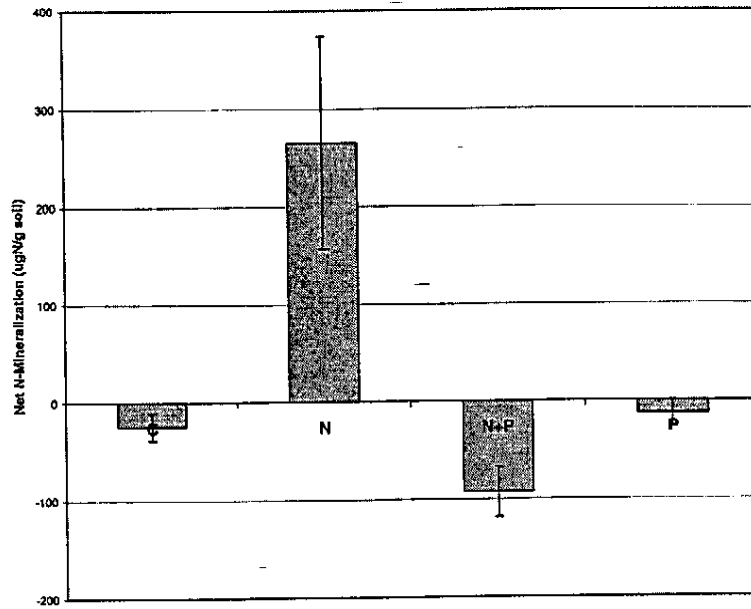


Figure 2: Net mineralization rates within the different treatment plots in the Donut site show significant differences in net N-mineralization rates between the C and N, C and N+P, N and N+P, and N and P treatment plots.

South Gate: Net N-Mineralization Rates

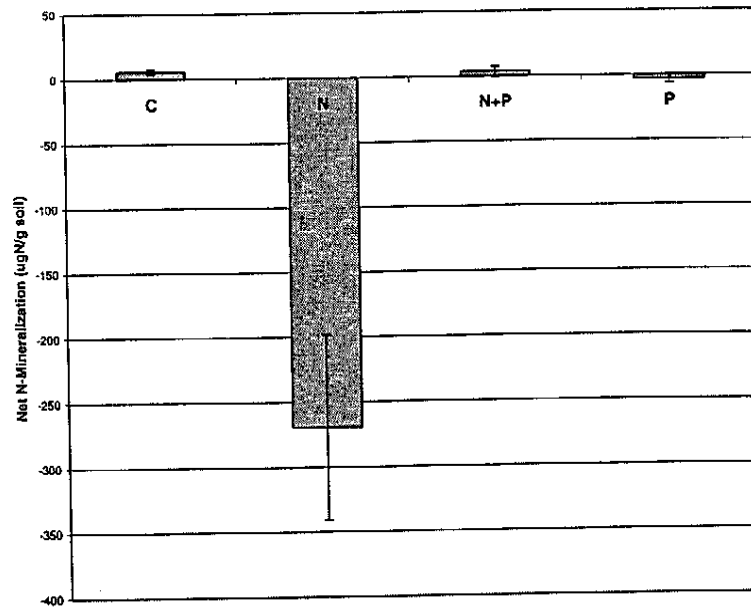


Figure 3: Net mineralization rates within the different treatment plots in the South Gate site show significant differences between the net N-mineralization in the N and C, N and N+P, and N and P treatment plots.

VII. REFERENCES

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