Effects of simulated nitrogen deposition on nitrogen leaching and *Acer saccharum* seedling biomass

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Abstract

Chronic nitrogen (N) deposition can have negative effects on forest plant species and can lead to decreased ecosystem nutrient retention. We examined the impact of simulated N deposition on seedlings of *Acer saccharum* (sugar maple), a common native tree species of eastern United States forests. Seedlings were grown in a greenhouse in soils collected from three forest sites that represent a gradient of N deposition, and received three N treatments: no additional N, N additions corresponding to ambient deposition levels, and five times ambient N deposition. We collected leachate and harvested seedlings after three months to measure biomass and leaf characteristics. If the sites from which soils were collected are nearing N saturation, then we would expect N additions to result in increased N leaching. We also expected to observe differences in seedling biomass among sites and N treatments, in response to N availability. We found that although N leaching was large relative to experimental N additions, N leaching did not increase with N additions. However, there were significant differences in N leaching across sites. Seedling biomass was greater for higher deposition sites, but did not consistently increase in response to N additions. Along with field observations, these results suggest that sites with greater long-term N deposition may be nearing saturation.

Introduction

Nitrogen deposition can have detrimental effects on forests

Nitrogen deposition is the input of reactive forms of N from the atmosphere to biological systems. Globally, atmospheric N deposition has increased in response to increased agricultural and industrial activities (Vitousek et al. 1997). If excess N levels alter plant competitive relationships, this could potentially contribute to shifts in species composition. Chronic N deposition can also lead to N saturation when N inputs are in excess of the potential for biological uptake. N saturated forests may exhibit increased N leaching out of the rooting zone and loss of essential plant nutrients (Aber et al. 1989), precursors of decreased productivity and forest decline.

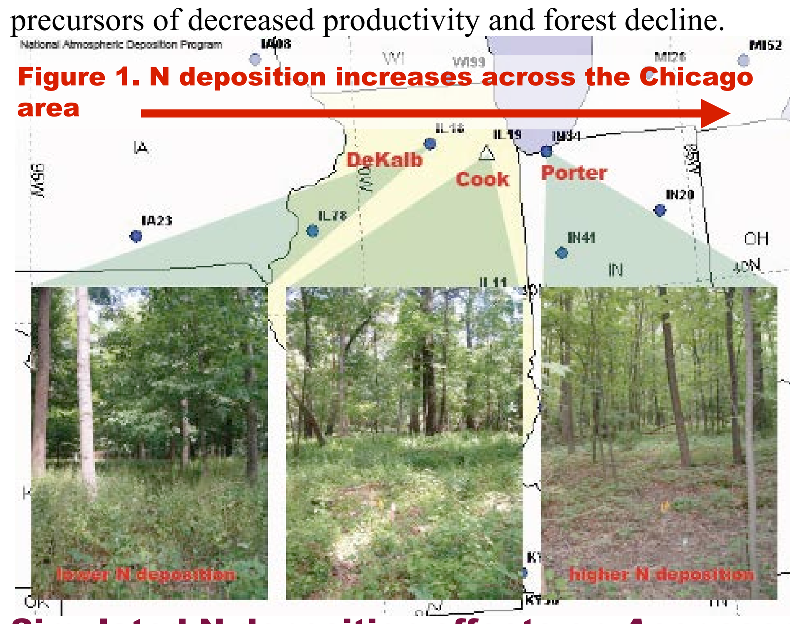


Figure 1. N deposition increases across the Chicago area

Chicago area forests receive some of the highest levels of N deposition in the United States (NADP 2006). Due to the location of agricultural and industrial precursors and prevailing wind patterns, there is a gradient of increasing N deposition across the Chicago area. Studies currently underway at three forest sites are exploring the impacts of N deposition on native forest species.

Simulated N deposition effects on *Acer saccharum* seedlings

In order to learn more about effects of N deposition on a common forest species of the eastern United States, we designed a factorial experiment to simultaneously explore soil and N addition effects on seedling growth. We grew *Acer saccharum* (sugar maple) seedlings in soils collected from three Chicago area forest sites. Seedlings were grown in a greenhouse at one of three N addition levels: control (no N added), ambient (corresponding to average weekly N wet deposition), and five times ambient N deposition levels.

If forests in the Chicago area are nearing N saturation, then further N additions should lead to increased N leaching. **We expected increased nitrate (NO_3^-) leaching in response to N additions, especially from soils at the higher end of the gradient.**

A. saccharum densities increase across the Chicago area, and recent shifts in forest species composition (Bowles et al. 2005) suggest that the species may be responding positively to increased N availability. **We expected greater seedling biomass in soils from the higher end of the gradient, and increased seedling biomass in response to N additions, only if soils have not yet reached N saturation.**

Methods

Soil collection and preparation

Soil was collected from the top 20 cm of forests in DeKalb County and Cook County, Illinois, and Porter County, Indiana on several dates during 2004 and 2005. Prior to this experiment, soils were dried, sieved to remove organic matter and rocks, and homogenized for each site. Sites were selected based on similarities of soil type, canopy species composition, climate and precipitation, and land use histories.

Nitrogen treatments

N treatments were applied weekly in 75 mL increments corresponding to average weekly growing season precipitation. Each treatment included ¼ strength N-free Hoagland's solution. Control plants (Ø N) received no additional N. Seedlings in the ambient N treatment received 60 µM N as NH_4NO_3, approximating the average weekly growing season N wet deposition concentrations recorded at the National Atmospheric Deposition Program sites adjacent to the forests. Seedlings in the elevated N treatment (5x) received five times the average ambient deposition concentration, or 300 µM N as NH_4NO_3.

Leachate collection

Initial leachate samples were collected one week after seedlings were planted, before N applications began, and before leaves unfurled for most seedlings. Deionized water was added to pots in 30 mL increments, and leachate was collected through filters directly into scintillation vials (Figure 2). The volumes of leachate were then measured, and subsamples were analyzed for NH_4^+ and NO_3^- using high performance liquid chromatography (HPLC). Following the same procedure, leachate samples were also collected one week prior to termination of the experiment.

Figure 2. Leachate collection *Leachate samples were collected at the beginning and end of the experiment to measure the volume and NH_4^+ and NO_3^- concentrations of leachate. Differences between initial and final leachate volume and N concentrations were used to determine whether leachate N concentrations changed in response to N treatments or seedling effects.*

Seedling biomass, biomass allocation, and leaf area

There were 10 seedlings per treatment (Figure 3), for a total of 90 seedlings. Seedlings were harvested after three months and separated into leaf, stem, coarse root, and fine root tissues. Immediately after harvest, leaf area was measured on a leaf area meter. Dry mass was recorded for all seedlings after oven drying for three days at 70 °C. For each seedling, we calculated: specific leaf area (SLA = leaf area per unit leaf mass), leaf mass ratio (LMR = proportion of biomass allocated to leaves), and leaf area ratio (LAR = product of SLA and LMR).

Reference soils and seedlings

In addition to pots with seedlings, leachate collection was recorded for pots containing only soil but receiving weekly N treatments.

During the experiment, *A. saccharum* seedlings were also grown in sand, and received 150 mL of low (0.1 mM) N, intermediate (0.5 mM) N, and high (1.0 mM) N as NH_4NO_3 twice weekly, along with ¼ strength N-free Hoagland's solution. Although N application amounts and rates differed from seedlings grown in soil, the seedlings grown in sand served as an additional reference for biomass responses to N availability.

Statistical analysis

Data were analyzed with General Linear Models or ANOVA using MINITAB and SPSS software. Significant differences between treatments are reported as at the α = 0.05 level.

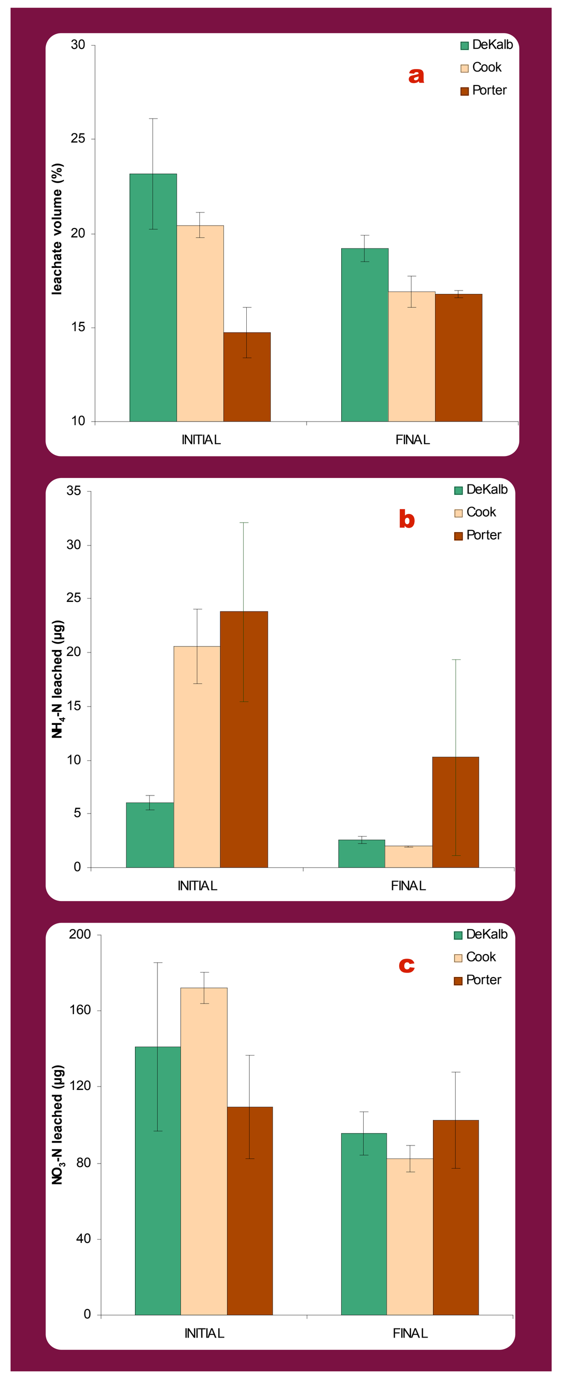
Figure 3. *Acer saccharum* seedlings *were assigned randomly to either control, ambient N, or elevated N treatments, and were grown in soils from either DeKalb, Cook, or Porter County sites for three months prior to harvest.*

Results

Leachate

The proportional volume of leachate was significantly greater for DeKalb soils than for the other two sites on both sampling dates (Figure 4a). There were no significant differences between N treatments. Amounts of the more mobile NO_3^- leached were greater than amounts of NH_4^+ (Figure 4b,c). The amount of NH_4^+ leached was greater during the initial collection than the final collection for all sites. There was significantly less NH_4^+ leached from DeKalb soils than from the other two sites on the initial sampling date. The amount of NO_3^- leached during the initial collection was greater than at the end of the experiment, but differences between sites and N treatments were not significant.

Figure 4. (a) Leachate volume, (b) NH_4-N, and (c) NO_3-N *means ± 1 SE.*



Seedling biomass allocation and leaf characteristics

Seedling biomass differed across sites, but responses to N treatments were not significant. DeKalb seedlings had lower total, leaf, coarse root, and fine root biomass and lower leaf area than Cook or Porter seedlings (Figure 5). Biomass appeared to decrease for Porter seedlings receiving 5x ambient N additions, but differences between treatments were not significant. Leaf characteristics, however, did respond to N treatments. Ambient N seedlings had greater SLA than the control or elevated N treatments. The control seedlings had greater LMR and LAR than seedlings receiving either level of N additions, despite having lower SLA (Table 1).

Table 1. Leaf characteristics *mean ± 1 SE.*

N treatment	Site	SLA ($cm^2\ g^{-1}$)	LMR	LAR
Control	DeKalb	208.6 ± 6.4	0.23 ± 0.03	47.4 ± 1.9
	Cook	204.1 ± 10.3	0.24 ± 0.02	49.2 ± 1.4
	Porter	209.3 ± 11.0	0.22 ± 0.03	45.2 ± 1.6
Ambient	DeKalb	225.2 ± 7.0	0.17 ± 0.01	39.1 ± 0.7
	Cook	211.6 ± 7.4	0.21 ± 0.01	44.0 ± 0.7
	Porter	217.7 ± 6.0	0.20 ± 0.01	42.4 ± 0.8
5x	DeKalb	215.1 ± 7.9	0.18 ± 0.01	37.8 ± 0.9
	Cook	206.5 ± 6.8	0.20 ± 0.01	41.8 ± 0.9
	Porter	189.4 ± 10.6	0.23 ± 0.02	42.6 ± 1.3

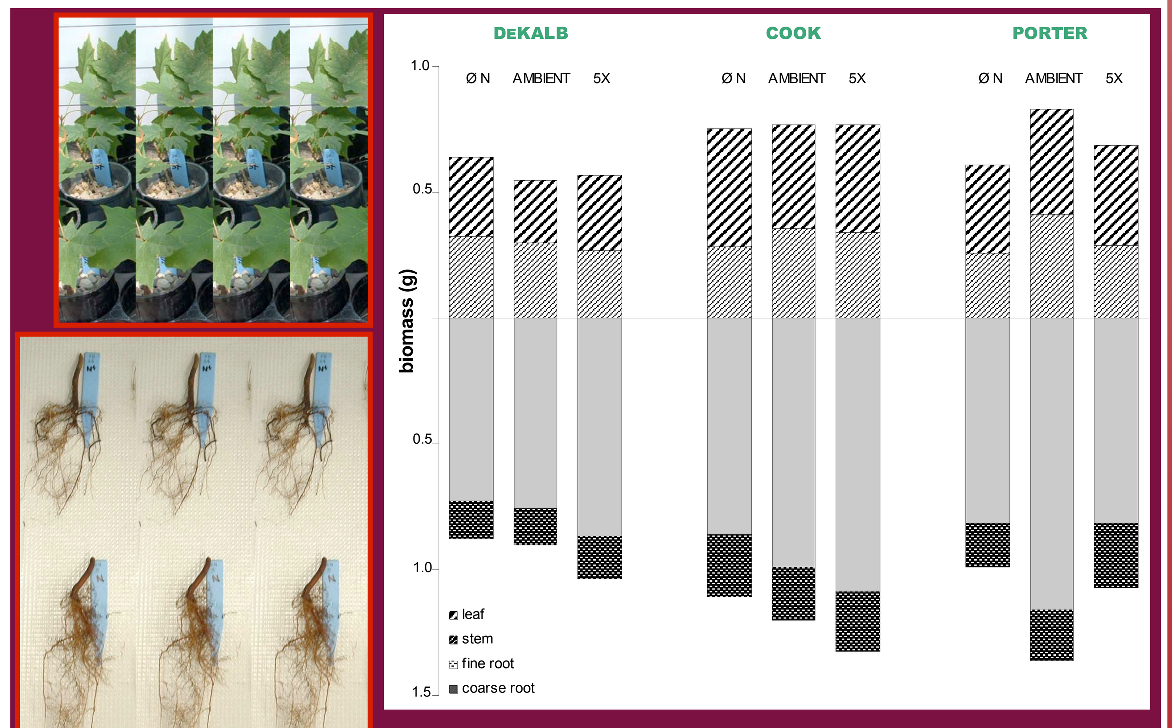


Figure 5. Biomass allocation to leaf, stem, coarse root, and fine root tissues

Conclusions

Soils collected from the three N deposition gradient sites appear to differ in potential for N leaching, but N leaching did not increase in response to N additions. There were initially greater concentrations of NO_3^- in the leachate than in the treatments applied. This suggests that N concentrations in the soils from each site were already relatively high. However, if the soils were at or near saturation, when the 5x N treatment was applied there should have been an increase in the amount of N leached, and this was not observed during our experiment. Field measurements of N leachate and stream concentrations (unpublished data), tend to show much higher NO_3^- concentrations at the DeKalb site than at the Cook or Porter sites. In this experiment, when controlling for N and water inputs to homogenized soil, these site differences in NO_3^- were not observed, although the volume of leachate from DeKalb soils was consistently greater than from the other sites.

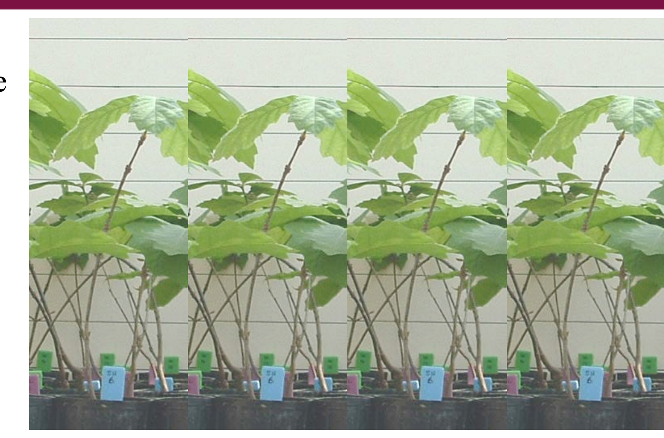
Seedling biomass and leaf characteristic responses to N treatments were not consistent across sites. However, these results indicate that biomass of seedlings at the Porter site may have responded negatively to the 5x N addition treatment. This would correspond to observations of growth for *A. saccharum* seedlings in the field; growth is greater at the Cook and Porter sites than at the DeKalb site, and seedling growth has decreased at the Porter site after four years of N additions (HL Sehtiya, unpublished data). Similarly, the greater SLA, LMR, and LAR of control and ambient N plants indicate the potential for these plants to have greater growth than plants receiving 5x N additions. Seedling biomass was weakly but significantly ($r^2 = 0.1$, $P < 0.01$) positively correlated with NH_4-N leached, indicating that growth differences between sites may have been partly in response to NH_4^+ availability, the preferred N form of *A. saccharum* seedlings (Templer and Dawson 2004).

Surprisingly, reference seedlings grown in sand across a range of N treatments did not show any biomass response to increased N availability. It is then possible that responses to N treatments would have been observed had differences between N treatments been greater. However, seedlings grown in soils are not likely to be exposed to N additions greater than those applied in this experiment, and these N additions should represent the extreme ranges of N deposition encountered by field-grown seedlings.

Results from this experiment indicate that differences in soil characteristics of the three N deposition gradient sites may have a greater influence on N leaching than do N additions. Seedling biomass and leaf characteristics, however, did show some significant responses to N treatments. We will use a mass balance approach along with additional measurements of soil and plant tissue N content to gain further information about the observed differences between sites in N retention.

Future Directions

Red oak (*Quercus rubra*) seedlings subjected to the same treatments are currently being grown in the greenhouse, and will be harvested after 5 months. This will allow us to determine whether seedlings of a different species respond similarly to the soils and N treatments, and whether these responses may have implications for soil N retention.



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