THE EFFECTS OF EXPERIMENTAL NITROGEN AND PHOSPHORUS ADDITION ON A TEMPERATE DECIDUOUS FOREST ECOSYSTEM

By

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ABSTRACT

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The effects of experimental nitrogen and phosphorus addition on a
temperate deciduous forest
(Under the direction of Robert K. Peet)

As human activities continue to increase nitrogen inputs into temperate ecosystems via atmospheric nitrogen deposition, understanding the impact of increased nitrogen levels on plant community composition and ecosystem functioning is of vital importance. I added nitrogen and phosphorus to a forest near Mountain Lake Biological Station (VA) from 1994 through 1996. I then tested whether nitrogen addition reduced the species diversity of the community or altered the rates of nutrient cycling and nitrogen uptake in the ecosystem. I also tested whether phosphorus availability influenced the community's or the ecosystem's response to elevated nitrogen inputs.

Nitrogen addition increased the abundance and biomass of the dominant understory species, *Aster acuminatus*, and decreased the species diversity of the plant community. However, contrary to expectations based on observations following N addition in other ecosystems, the number of species in the community was not affected by nitrogen inputs. Phosphorus addition did not affect the response of species diversity or the species richness of the community to nitrogen addition.

Nitrogen inputs did not saturate the capacity of the Mountain Lake ecosystem to retain nitrogen. Soil N and, to a lesser extent, canopy vegetation were the ecosystem components that appeared most responsible for N retention. Nitrogen content of microbial populations did not increase. Phosphorus inputs did not affect the ecosystem's ability to retain nitrogen, as neither nitrogen cycling nor nitrogen contained in ecosystem pools were significantly different in plots receiving phosphorus addition.

Dramatic changes in composition and diversity of European plant communities indicate that atmospheric nitrogen deposition may impact North American communities in the future. Regions in North America receiving high rates of atmospheric nitrogen deposition, such as high-elevation forests in the northeastern United States, are likely to face substantial changes in community composition and ecosystem function. Grasslands, prairies, and savannas existing in areas of moderate-high levels of nitrogen deposition are also especially vulnerable to future community changes because grass species have been especially able to increase dominance following increased N inputs in other regions. Greater monitoring of community composition and diversity in areas of high-nitrogen inputs will be necessary to track future community changes.

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Chapter 1

General Introduction

Human release of the precursors of nitrate and ammonium from the burning of fossil fuels and from agricultural activities, respectively, has caused a dramatic increase in atmospheric nitrogen (N) deposition onto terrestrial ecosystems in industrialized areas of the world. Large areas of eastern North America receive as much as 25 kg N ha^{-1} yr^{-1} whereas areas of Europe may receive as much as 50 kg N ha^{-1} yr^{-1} in wet and dry deposition (Aber et al. 1989; Gunderson 1991; Gunderson 1995). N deposition directly downwind of industrial sources may be even greater. By contrast, atmospheric N inputs in areas largely unaffected by human activities are typically 1-2 kg N ha^{-1} yr^{-1} (Galloway, Likens, and Hawley 1984; Aber et al. 1989; National Atmospheric Deposition Program 1997).

N levels play an important role in the species composition and richness of plant communities. For example, increased N inputs have been shown to alter the species composition and diversity of plant communities (Tilman 1987; Bobbink 1991; Thimonier, Dupouey, and Timbal 1992; Kellner 1993; Tilman et al. 1994). The consequences have been particularly striking in European grasslands and heathlands where a few species successful in the high-N conditions created by atmospheric N deposition have experienced a dramatic increase in dominance (Heil and Diemont 1983; Aerts et al. 1990; Bobbink 1991; Willems, Peet, and Bik 1993). European forest communities have experienced increased abundance of species characteristic of N-rich habitats, especially the grass *Deschampsia flexuosa* (Van Breemen and van Dijk 1988; Hogbom and Hogberg 1991; Rosen et al. 1992; DeVries et al. 1995); (but see Liu and Brakenhielm 1996).

Increased N inputs also have important effects on such ecosystem processes as primary productivity, decomposition, and groundwater transport of nutrients. The amount of N contained in such ecosystem components as microbial populations, vegetation, and soil N pools frequently increases following increased N deposition (Nadelhoffer et al. 1995; Koopmans, Tietema, and Boxman 1996; Magill et al. 1997). Elevated N deposition can also lead to increased primary productivity (Emmett et al. 1995; Magill et al. 1997) and more rapid cycling of N (McNulty et al. 1990). Ecosystems impacted most severely by elevated N inputs, however, may lose the ability to retain N inputs and, consequently, export large amounts of N via leaching losses. Such ecosystems have been termed N-saturated (Aber et al. 1989; Aber 1992) and are likely to experience greater concentrations of nitrate in streamwater (Van Miegroet, Lovett, and Cole 1992; Stoddard 1994; Dise and Wright 1995; Gunderson 1995).

It is not clear what impact atmospheric N deposition will have on North American plant communities. Changes in community composition or diversity as a result of increased atmospheric N deposition have not been reported in the eastern United States. Experimental N addition in grassland and old-field communities in North America have seen increased dominance by one or a few species and a decrease in species diversity similar to the response in European communities (Bakelaar and Odum 1978; Tilman 1987; Wedin and Tilman 1996). However, few studies have addressed the question of how forest communities respond to increased N inputs. It is not clear whether understory species diversity and species richness will decline in eastern forests as has been seen in grassland communities and if so which species are likely to become dominant. N inputs could also allow the invasion of new species well-adapted to high-N conditions, as has been seen in European forests (e.g., Rosen et al. 1992; DeVries et al. 1995).

Understanding the impact of elevated N inputs on ecosystems in the eastern United States is of vital importance. This region receives high levels of atmospheric N input (National Atmospheric Deposition Program 1997), and many ecosystems in the northeastern United States and in the southern Appalachians already exhibited symptoms of N-saturation (Van Miegroet, Lovett, and Cole 1992; Stoddard 1994; Gilliam, Adams, and Yurish 1996; Adams, Angradi, and Kochenderfer 1997). Progressively more ecosystems face N-saturation in the future as elevated N deposition continues.

As N availability increases in North American ecosystems, the importance of other essential nutrients, such as phosphorus (P), for community composition and nutrient cycling can be expected to increase. As N-stimulated growth increases nutrient uptake, P should be expected to become more scarce. Differences in the ability of species to take up P should influence community composition and diversity in high-N conditions. P availability also may control the ability of ecosystem components to retain N in the ecosystem and prevent leaching losses of N. By experimentally adding P to plots receiving elevated N inputs, it is possible to assess the influence that P availability has on the plant community and ecosystem under high-N conditions.

In this volume I describe the results of a three-year experiment to test the effects of experimental N and P additions on the plant community and N cycling in a deciduous forest in southwestern Virginia. I address the following general questions.

1. Do increased N inputs change species composition or diversity of a forest understory community?
2. Does P availability influence species composition or diversity under high-N conditions?
3. Are three years of high levels of N addition sufficient to lead to the N saturation of a forest ecosystem?
4. What ecosystem components increase N retention following fertilization?
5. Does P addition increase N retention under high-N conditions?

In Chapter 2 I investigate the effect of experimental N and P additions on the forest understory community. In particular, I test the effects of increased N availability on species richness and diversity, primary productivity, and tissue N concentrations. I also test the impact of P addition on the plant community. In chapter 3 I focus on the effects of N and P additions on the forest ecosystem, including N leaching losses, the rates of net N mineralization and net nitrification, and N uptake by various ecosystem components. Finally, in chapter 4 I review reports from the scientific community of the consequences of atmospheric N deposition for ecosystems and plant communities in eastern North America and Europe and place the results of chapters 2 and 3 in global perspective. I also discuss the likelihood of vegetation changes in North American communities as N inputs continue.

Literature Cited

Aber J. D. 1992. Nitrogen cycling and nitrogen saturation in temperate forest ecosystems. Trends in Ecology and Evolution 7:220-4.

Aber J. D., Nadelhoffer K. J., Steudler P., Melillo J. M. 1989. Nitrogen saturation in northern forest ecosystems. BioScience 39:378-86.

Adams M. B., Angradi T. R., Kochenderfer J. N. 1997. Stream water and soil solution responses to 5 years of nitrogen and sulfur additions at the Fernow Experimental Forest, West Virginia. Forest Ecology and Management 95:79-91.

Aerts R., Berendse F., deCaluwe H., Schmitz M. 1990. Competition in heathland along an experimental gradient of nutrient availability. Oikos 57:310-8.

Bakelaar R. G., Odum E. P. 1978. Community and population level responses to fertilization in an old-field ecosystem. Ecology 59:660-5.

Bobbink R. 1991. Effects of nutrient enrichment in Dutch chalk grasslands. Journal of Applied Ecology 28:28-41.

DeVries W., Leeters E.E.J.M., Hendriks C.M.A., Van Dobben H., Van den Burg J., Boumans L.J.M. 1995. Large scale impact of acid deposition on forests and forest soils in The Netherlands. In: Heij G.J., Erisman J.W., editors. Acid rain research: Do we have enough answers? Amsterdam:Elsevier. p 261-77.

Dise N. B., Wright R. F. 1995. Nitrogen leaching from European forests in relation to nitrogen deposition. Forest Ecology and Management 71:153-61.

Emmett B. A., Brittain S. A., Hughes S., Kennedy V. 1995. Nitrogen additions ($NaNO_3$ and NH_4NO_3) at Aber forest, Wales: II. Response of trees and soil nitrogen transformations. Forest Ecology and Management 71:61-73.

Galloway J. N., Likens G. E., Hawley M. E. 1984. Acid precipitation: Natural versus anthropogenic components. Science 226:829-31.

Gilliam F. S., Adams M. B., Yurish B. M. 1996. Ecosystem nutrient responses to chronic nitrogen inputs at Fernow Experimental Forest, West Virginia. Canadian Journal of Forest Research 26:196-205.

Gunderson P. 1991. Nitrogen deposition and the forest nitrogen cycle: role of denitrification. Forest Ecology and Management 44:15-28.

Gunderson P. 1995. Nitrogen deposition and leaching in European forests - preliminary results from a data compilation. Water, Air, and Soil Pollution 85:1179-84.

Heil G. W., Diemont W. H. 1983. Raised nutrient levels change heathland into grassland. Vegetatio 53:113-20.

Hogbom L., Hogberg P. 1991. Nitrate nutrition of *Deschampsia flexuosa* (L.) Trin. in relation to nitrogen deposition in Sweden. Ecologia 87:488-94.

Kellner O. 1993. Effects on associated flora of sylvicultural nitrogen fertilization repeated at long intervals. Journal of Applied Ecology 30:563-74.

Koopmans C. J., Tietema A., Boxman A. W. 1996. The fate of ^{15}N enriched throughfall in two coniferous forest stands at different nitrogen levels. Biogeochemistry 34:19-44.

Liu Q., Brakenhielm S. 1996. Variability of plant species diversity in Swedish natural forest and its relation to atmospheric deposition. Vegetatio 125:163-72.

Magill A. H., Aber J. D., Hendricks J. J., Bowden R. D., Melillo J. M., Steudler P. A. 1997. Biogeochemical response of forest ecosystems to simulated chronic nitrogen deposition. Ecological Applications 7:402-15.

McNulty S., Aber J. D., McLellan T. M., Katt S. M. 1990. Nitrogen cycling in high elevation forests in the northeastern U.S. in relation to nitrogen deposition. Ambio 19:38-40.

Nadelhoffer K. J., Downs M. R., Fry B., Aber J. D., Magill A. H., Mellilo J. M. 1995. The fate of ^{15}N-labelled nitrate additions to a northern hardwood forest in eastern Maine, USA. Oecologia 103:292-301.

National Atmospheric Deposition Program (NRSP-3) / National Trends Network. 1997. NADP/NTN Coordination Office, Natural Resource Ecology Laboratory, Colorodo State University, Fort Collins, CO 80523.

Rosen K., Gunderson P., Tegnhammer L., Johnansson M., Frogner T. 1992. Nitrogen enrichment of Nordic forests: the concept of critical loads. Ambio 21:364-8.

Stoddard J.L. 1994. Long-term changes in watershed retention of nitrogen: its causes and aquatic consequences. In: Baker L.A., editor. Environmental chemistry of lakes and reservoirs. Washington D.C.:Advances in Chemistry Series. p 223-83.

Thimonier A., Dupouey J. L., Timbal J. 1992. Floristic changes in the herb-layer vegetation of a deciduous forest in the Lorraine Plain under the influence of atmospheric deposition. Forest Ecology and Management 55:149-67.

Tilman D., Dodd M.E., Silvertown J., Poulton P.R., Johnston A.E., Crawley M.J., Leigh R.A. 1994. The Park Grass Experiment: Insights from the most long-term ecological study. In: Leigh R.A., Johnston A.E., editors. Long-term experiments in agricultural and ecological sciences. Oxon, UK:CAB International. p 287-303.

Tilman D. 1987. Secondary succession and the pattern of plant dominance along experimental nitrogen gradients. Ecological Monographs 57:189-214.

Van Breemen N., van Dijk H. F. G. 1988. Ecosystem effects of atmospheric deposition of nitrogen in The Netherlands. Environmental pollution 54:249-74.

Van Miegroet H., Lovett G.M., Cole D.W. 1992. Summary and Conclusions - Atmospheric deposition and forest nutrient cycling. In: Johnson D.W., Lindberg S.E., editors. Atmospheric deposition and forest nutrient cycling. New York:Springer-Verlag. p 202-7.

Wedin D. A., Tilman D. 1996. Influence of nitrogen loading and species composition on the carbon balance of grasslands. Science 274:1720-3.

Willems J. H., Peet R. K., Bik L. 1993. Changes in chalk-grassland structure and species richness resulting from selective nutrient additions. Journal of Vegetation Science 4:203-12.

Chapter 2

Responses of a deciduous forest understory community to experimental nitrogen and phosphorus addition

Introduction

As human activities continue to increase N inputs into temperate ecosystems via atmospheric N deposition (Galloway, Likens, and Hawley 1984; Vitousek and Matson 1993; DeVries et al. 1995a; Vitousek et al. 1997), understanding the impact of increased N levels on plant community composition is of vital importance (van Breemen and van Dijk 1988; Ellenberg 1988; Rosen et al. 1992; Woodin and Farmer 1993; Schlesinger 1994; Chapter 4). Large areas of eastern North America receive as much as 25 kg N ha^{-1} yr^{-1} whereas areas of Europe may receive as much as 50 kg N ha^{-1} yr^{-1} in wet and dry deposition (Aber et al. 1989; Gunderson 1991; Gunderson 1995). By contrast, areas largely unaffected by anthropogenic sources receive < 2 kg N ha^{-1} yr^{-1} (Galloway, Likens, and Hawley 1984; Aber et al. 1989; National Atmospheric Deposition Program 1997). This deposition significantly increases the input of a nutrient that is frequently in short supply in the temperate region (Vitousek and Howarth 1991).

Many studies have shown that changes in N availability can alter the species composition and diversity of plant communities (e.g., Tilman 1987; Bobbink 1991;

Thimonier. Dupouey, and Timbal 1992: Kellner 1993; Tilman et al. 1994), and a number of European communities have changed significantly as a result of increased atmospheric N deposition (Chapter 4. this volume). For example. the diversity of vascular plants. bryophytes. and lichens has declined in Dutch chalk grasslands as *Brachypodium pinnatum*. a perennial grass. has increased in abundance (During and Willems 1986: Bobbink and Willems 1987: Bobbink and Willems 1991: Bobbink 1991). Increased N inputs combined with outbreaks of heather beetles (*Lochmaea sturalis*) in the Netherlands and Britain have shifted dominance in wet and dry heathlands from dwarf shrub species such as *Calluna vulgaris* and *Erica tetralix* to the grass species *Molinia caerulea. Festuca ovina. and* especially *Deschampsia flexuosa* (Heil and Diemont 1983: Aerts and Berendse 1988: Aerts et al. 1990: Woodin and Farmer 1993). The abundances of species characteristic of N-rich habitats. such as *Deschampsia*. have increased due to increased N deposition in forests in France (Thimonier. Dupouey. and Timbal 1992), the Netherlands (van Breemen and van Dijk 1988; DeVries et al. 1995a; DeVries. Leeters. and Hendricks 1995b), and Sweden. Norway. and Finland (Falkengren-Grerup 1986; Hogbom and Hogberg 1991: Rosen et al. 1992); (though see Liu and Brakenhielm 1996), while diversity of herbs has generally decreased.

The consequences of increased atmospheric N input for North American plant communities are less well known. Though the levels of atmospheric N deposition are higher and have proceeded for a longer period of time in Europe. large areas of eastern North America receive substantially higher inputs of N from atmospheric deposition than they received prior to human influence (Galloway. Likens, and Hawley 1984). Although studies have frequently examined the impact of elevated N deposition on ecosystem nutrient cycling (e.g., Aber et al. 1989; Van Miegroet. Cole. and Foster 1992; McNulty. Aber, and Newman

1996; Magill et al. 1997; Adams, Angradi, and Kochenderfer 1997; Chapter 3), the ecosystem-level responses of North American forest communities to increased N availability have been little studied.

The impacts that increased N inputs have on plant community composition may be influenced by the availability of other essential soil nutrients, such as phosphorus (P) (Tilman 1982; Tilman 1985; Bobbink 1991; Willems, Peet, and Bik 1993; Wilson, Wells, and Sparks 1995). For example, increasing dominance and loss of species in chalk grasslands due to increased N deposition has been related to the ability of *Brachypodium pinnatum* to compete effectively for low levels of P in the soil (Bobbink 1991; Willems, Peet, and Bik 1993; Wilson, Wells, and Sparks 1995). P is an essential nutrient frequently in short supply in forest soils, and ecosystem responses to N addition (e.g., increased biomass production) may make P even more scarce. The response of forest understory vegetation to P input has not been tested, though P availability may influence a forest communities' responses to increased N inputs.

I added supplemental N and P to a deciduous forest in southwestern Virginia for 3 years to test the responses of the forest ecosystem and community to increased N and P availability. Atmospheric N deposition in this region is low compared to other parts of the eastern United States (National Atmospheric Deposition Program 1997), so N cycling and species composition are less likely to have been affected by previous N inputs. Chapter 3 describes the effect of N and P fertilization on soil N cycling, ecosystem N storage in understory and microbial biomass, and leaching losses of N.

In this chapter I discuss the response of the understory plant community. Specifically, I tested the following hypotheses:

1. *N addition will increase above-ground production and tissue N concentration of understory species.* Primary productivity in the temperate region is frequently limited by N availability (Vitousek and Howarth 1991), and species frequently respond to increased N availability by increasing growth and/or increasing storage of N in leaf tissue (Chapin 1980; DiTommaso and Aarssen 1989). An increase in growth and/or tissue N content by understory species would indicate that increased N inputs impact species' N uptake and N allocation.

2. *N addition will decrease the species richness or species diversity of the forest understory community.* Though experimental addition of N on grasslands frequently results in a decrease in the species richness of the community (e.g., Thurston 1969; Tilman 1987; Bobbink 1991; Willems, Peet, and Bik 1993; Tilman et al. 1994; Wedin and Tilman 1996), declines in species richness is typically much less intense in European coniferous forests that have been fertilized with N (e.g., Tyler et al. 1992; Falkengren-Grerup 1993; Kellner 1993; Rodenkirchen 1995; Makipaa 1995). It is not known whether the response of North American forests will be more similar to that of grassland communities or the European coniferous forests. Measurements of community diversity, which take into account both the number of species in the community and their relative abundances, are sensitive to shifts in community dominance in favor of one or a few species that may take place even if the number of species is not affected by N inputs. Understanding the likelihood of changes in species diversity and species richness in North American forests is essential for predicting the effects that increasing atmospheric N inputs are likely to have.

3. *P addition will increase species richness and species diversity under high-N conditions.* Increased growth rates and N uptake by vegetation receiving elevated N inputs (Hypothesis #1) may decrease availability of other essential nutrients, such as P. Reduced allocation of resources to roots relative to shoots may also reduce P uptake by some species (Bobbink 1991). Greater competition for P or low P uptake under high-N conditions could lead to the exclusion of members of the plant community. If reduced P availability or uptake negatively affect species' competitive abilities under high-N conditions, then species richness and/or diversity would be expected to be higher in plots receiving both N and P than in plots receiving only N.

Methods

Study area

This study was conducted at the University of Virginia's Mountain Lake Biological Station (MLBS) in the Ridge and Valley Province of Virginia, located at an elevation of 1158m. The mean annual temperature is 8° C, ranging from -3° C in January to +19° C in July. The mean annual precipitation is 130.1 cm. Monthly precipitation is highest from May-July, reaching ca. 12 cm. Precipitation is lowest from December-February, during which monthly precipitation is ca. 8.5 cm. The soils are acidic (pH 3.5 - 4.1) and highly weathered. Soil texture is approximately 15% clay, 60% silt, and 25% sand.

The canopy vegetation resembles the *Quercus rubra- Acer rubrum* community described by Stephenson and Adams (1991). *Q. rubra and A. rubrum* are approximately codominant, with *Q. alba*, *Amelanchier arborea*, and *Q. velutina* of secondary importance. *Aster acuminatus* is the most abundant understory species throughout the study area. *Amianthium muscaetoxicum*, *Vaccinium pallidum*, *V. angustifolium*, and *A. rubrum* seedlings are the next-most important understory species.

Logging in the 1920's gave rise to an even-aged stand with the exception of a few >150 year-old *Q. alba*. The site's exposed aspect and periodic winter ice-storms have combined to keep the canopy relatively open. Gaps in the canopy remain from a severe ice-storm in March 1994. There is no record of the area having burned, and there are no fire scars on trees. However, soil cores do contain small (< 0.5 cm) charcoal fragments in the top 5 cm, indicating burning in the past.

The Mountain Lake region supports a large deer population, and browsing of understory vegetation is extensive. Seedlings are rarely able to reach a height sufficient to escape consumption by the deer. As a result, regeneration of canopy species is, at best, sporadic (J. Kastendeik and S. Swank, pers. comm.). Herbaceous species are consumed as well, and the composition of the understory community is certainly affected by differences in deer preferences and the differential ability of species to recover from herbivory.

A recording station for the National Atmospheric Deposition Program (NADP) located <10 km from the study site reported N inputs as wet deposition of nitrate and ammonium of 4.50 kg N ha^{-1} in 1994, 3.95 kg N ha^{-1} in 1995, and 5.24 kg N ha^{-1} in 1996 (National Atmospheric Deposition Program 1997). N inputs have been increasing since data recording began in 1978 (Figure 2.1). The current level of N deposition is higher than this region would receive in the absence of human influences. Thus, N availability in this ecosystem is increasing, irrespective of experimental applications. The ecosystem is not N saturated, however, as low nitrate concentrations in lysimeters installed below the rooting zone indicate that the ecosystem retains nearly 100% of N inputs (Chapter 3).

Experimental design

In June 1994 I established a series of eight replicate blocks in a 0.25 km^2 area of relatively homogenous understory vegetation and level topography. I divided each of the eight blocks into four 10m x 10m plots in a randomized complete block design (Figure 2.2). Thus, there were a total of 32 plots (4 treatments X 8 blocks). I randomly assigned the plots in each block to one of four treatments: +N, +P, +N+P, and Control (which received no experimental N or P addition). I established a 1m wide buffer around each plot to minimize edge effects, and confined all measurements to the central 64m^2 of each plot. Plots were separated from adjacent plots by at least 1m. No trenching was done to exclude root extension into adjacent plots.

N (NPK 34-0-0 using commercial NH_4NO_3) and P (NPK 0-22-0 using reagent grade Na_2HPO_4) fertilizer pellets were cast by hand throughout the entire 100m^2 of a plot. 408 g NH_4NO_3 were added to +N and +N+P plots and 197 g Na_2HPO_4 were added to +P and +N+P plots. Additions were made each month from April through October to simulate the periodic nutrient addition characteristic of atmospheric deposition. The experimental addition amounted to a yearly input of 97 kg N ha^{-1} yr^{-1} and 30 kg P ha^{-1} yr^{-1}. The level of experimental N inputs is 2-4 times the level of atmospheric N deposition in heavily impacted areas of North America. The level of yearly P addition was chosen to equal approximately half of the P contained in above-ground vegetation and the forest floor in a survey of temperate deciduous forests (Cole and Rapp 1981). Treatments began in July 1994 and continued through October 1996. To minimize foliar burning from fertilizer contact, I brushed any fertilizer settling on the vegetation to the ground using a cotton kitchen mop. All treatment and control plots were mopped.

Vegetation sampling

In 1995 and 1996 I measured species' abundances and species diversity by counting the number of stems of each species in four randomly located $4m^2$ subplots within each of the 32 $100m^2$ plots. I relocated the same subplots for sampling each year. Data was collected from June 9 - July 23 in 1995 and from June 11 - July 1 in 1996. The period of sampling was chosen so that data collection began after all individuals had emerged and ended before any species had senesced.

I sampled above-ground biomass production of the understory in 1996. I clipped all individuals < 50cm tall at ground-level in three $4m^2$ subplots within each of the 32 $100m^2$ plots. Subplots chosen for harvesting were selected pseudo-randomly: subplots used for species sampling were not chosen for harvesting. Harvesting took place from July 5 - July 19, 1996. Minimal regrowth was seen following harvesting. During harvest, species were separated and placed in paper bags. I dried the clippings in paper bags at 60^0 C for 24 hours the day of harvest, then again for 48 hours immediately prior to weighing.

Dried leaves from three species, *Acer rubrum*, *Amianthium muscaetoxicum*, and *Aster acuminatus*, were reserved after weighing and used for measuring tissue N and P concentrations. The dried samples were ground in a Wiley Mill to pass through a 20-mesh screen. Each sample (0.04g) was digested in sulfuric and salicylic acid, using the standard Kjeldahl method (Haynes 1980; Lowther 1980; Wolf 1982), in a block digester (Bran-

Luebbe, Elmsford, New York). Within each batch of 40 samples, two sucrose blanks and a range of N and P standards were also digested. Digested samples, blanks, and N and P standards were analyzed for N and P concentration on a continuous flow autoanalyzer (Bran-Luebbe Technicon). Accuracy in each batch was assured using pine needle samples from the National Bureau of Standards.

Data analysis

I described the community composition using measures of species richness and species diversity. Species richness in each $100m^2$ plot was calculated as the mean of the number of species present in the four $4m^2$ subplots in which species' abundances were counted. Two indices of diversity, Simpson's Index of Diversity ($D=1/\sum p_i$), and the Shannon-Weaver Index ($H'=-\sum p_i \ln p_i$) were calculated in order to take into account both the number of species in the community and the species' relative proportion. The indicies were calculated for each $4m^2$ subplot., where p_i = proportion of stems of the i^{th} species in the $4m^2$ area. The diversity of each $100m^2$ plot was estimated by calculating the mean diversity of the four subplots sampled.

I constructed dominance-diversity plots for each $100m^2$ plot by plotting species abundance on a log scale against species rank in 1995 and 1996. Abundances for each species were calculated by summing the abundances in the four $4m^2$ subplots in each $100m^2$ plot. If nutrient inputs result in increasing dominance by species in the community and suppression of other species, the treated plots should have shorter, steeper dominance-diversity plots. The resulting plots approximated linear functions, allowing comparison of regression coefficients for each treatment (Kleinbaum, Kupper, and Muller 1988).

I performed all statistical analyses using PROC GLM (SAS version 6.10). The experimental design permitted a full factorial analysis, with two levels of N addition (0, 97 kg N ha^{-1} yr^{-1} added), and two levels of P addition (0, 30 kg P ha^{-1} yr^{-1} added). For each measured variable, the effects of N and P addition were tested using analysis of variance (ANOVA). Each ANOVA model included the main effects of block, N, and P, and the N*P interaction: X = Block + N + P + (N*P). In this form, the F-test for N in the ANOVA tests the null hypothesis that there is no difference in the level of the test variable between plots that receive N addition (i.e. +N and +N+P plots) and plots that do not receive N addition (i.e. control and +P plots).

Because species richness, Simpson's D, and Shannon-Weaver H', and species' abundances were calculated for both 1995 and 1996, repeated measures analysis of variance (ANOVAR) was used for analysis of these variables. ANOVAR differs from standard ANOVA analysis in that the variables are tested against different levels of error (Milliken and Johnson 1992). In this analysis, the effects of Block, N, P, and the N*P interaction are tested using the within-plot-error, whereas the effects of Year, Year*N, Year*P, and Year*N*P are tested using the between-plot-error. Because only two measurements were made on each plot, in 1995 and 1996, circularity analysis is unnecessary and uncorrected F-statistics were used in the ANOVAR.

Total understory, herbaceous, woody, and *Aster acuminatus* biomass were log transformed and *Aster acuminatus* abundances were square-root transformed to correct non-normality.

Results

Community changes

Species richness did not differ between plots that received supplemental N (+N and +N+P plots) and those that did not (control and +P plots), as tested by the main effect of N in the ANOVAR model (Table 2.1). Nor was there a significant Year*N interaction. Plots receiving experimental P addition (+P and +N+P plots) had, on average, 2 fewer species per $4m^2$ in 1995 and 1996 than plots that did not receive P (control and +N plots) (Table 2.1). The main effect of P addition was significant in the ANOVAR model, but there was no significant Year*P interaction (Table 2.1). There was no significant N*P interaction on species richness.

Species diversity, as measured by Simpson's D and the Shannon-Weaver index (H'), was significantly lower in plots receiving N, and the difference became more pronounced over the two-year period (Year*N; Table 2.2). Simpson's D and H' were significantly lower in plots receiving supplemental P as compared to those that did not (Table 2.2). There was no significant Year*P interaction for either Simpson's D or H'. There was no significant N*P interaction on either measure of species diversity (Table 2.2). The negative impact that N and P addition had on the two diversity indices was more pronounced for Simpson's D than H' (Table 2.2B).

The abundance of the dominant herbaceous herb, *Aster acuminatus*, was significantly greater in plots receiving experimental N (Table 2.3). ANOVAR revealed a significant Year*N interaction, as *Aster* abundance increased from 1995 to 1996 in +N and +N+P plots as compared with Control and +P plots. The number of individuals of the other 12 most abundant species were not significantly different in plots receiving N addition versus plots that did not receive N.

Aster was more abundant in plots receiving experimental P, as well, though the effect did not change between 1995 and 1996 (Table 2.3). There were fewer *Acer rubrum* and *Amelanchier arborea* individuals in +P and +N+P plots than in Control and +N plots. The difference in *Acer rubrum* abundance between plots receiving P addition and those that did not increased from 1995 to 1996 (Year*P – Table 2.3) due to a sharp increase in the number of *Acer rubrum* individuals in Control and +N plots in 1996 relative to +P and +N+P plots. There was no significant N*P interaction for any of the 12 most abundant species in the MLBS community.

There was little difference between dominance-diversity curves for plots receiving experimental N addition and plots that did not receive experimental N addition (Figure 2.3). Comparison of the slopes of +N and +N+P plots with Control and +P plots revealed no significant difference in either 1995 (F=0.06; p>0.2) or 1996 (F=0.67; p>0.2). The slopes of +P and +N+P plots, however, were significantly steeper than Control and +N plots in 1995 (F=45.09; p<0.0001) and 1996 (F=25.74; p<0.0001).

The total above-ground biomass of the understory community (all stems <50 cm in height) was not significantly greater in plots receiving experimental addition of N (Figure 2.4a; Table 2.4). Herbaceous species made up >70% of the biomass in all plots, and their biomass was significantly greater in plots fertilized with N (Figure 2.4a, b; Table 2.4). The increased biomass production by understory species was due almost exclusively to the positive response of the dominant understory species, *Aster acuminatus*, which had significantly higher biomass in +N and +N+P plots relative to the Control and +P plots

(Figure 2.4a; Table 2.4). As a result of its increased production, *Aster's* proportion of the understory community's above-ground biomass increased in plots receiving experimental N addition (Figure 2.4b). In contrast, other herbaceous species besides *Aster* contributed a lower proportion to the total biomass in plots receiving N addition (Figure 2.4b). Above-ground biomass by sampled woody species (<50 cm) in the understory community was not significantly affected by N addition (Table 2.4).

Plots receiving experimental P addition had lower above-ground biomass of the entire understory community, herbaceous species, *Aster acuminatus*, and understory woody species than plots that did not receive experimental P addition (Figure 2.4a; Table 2.4). P addition had no effect on the relative contribution of *Aster*, other herbaceous species, and woody species to the understory biomass (Figure 2.4b).

No interaction between N and P was detected for variables related to above-ground biomass (Table 2.4).

Effects of herbivory by deer on understory biomass

At the time of harvesting, 21 of the 96 2m X 2m subplots harvested were classified as experiencing "heavy" browsing, most likely from deer. Of the 21 subplots, 9 were P-treated, 11 were N- and P-treated, and 1 was N-treated. When estimates of above-ground production from these heavily browsed areas are excluded from the analysis of the effect of N and P on biomass, the negative impact of P addition on *Aster* biomass is not significant ($F=2.52$; $p<0.12$). No other relationship between biomass and N or P input differed from the analyses using all subplots.

Tissue N and P concentrations

Tissue N concentration of *Acer rubrum, Amianthium muscaetoxicum,* and *Aster acuminatus* leaves were significantly higher in +N and +N+P plots as compared to control and +P plots (Table 2.5; Figure 2.5). P fertilization resulted in a significant decrease in the tissue N concentration of *Aster* (Table 2.5), particularly in +N+P plots as compared to +N plots (Figure 2.5). Tissue N concentrations of *Acer* and *Amianthium* were not significantly different between plots that received experimental P addition and those that did not, and there was no significant N*P interaction on tissue N concentration for any of the three species tested (Table 2.5; Figure 2.5).

Tissue P concentration increased in all three species following P addition (Table 2.6; Figure 2.6). In *Aster* and *Acer*, N addition significantly decreased the tissue P concentration (Table 2.6; Figure 2.6). The negative effect of N on tissue P concentration was more pronounced in plots receiving experimental P addition than in plots that did not (N*P interaction, Table 2.6).

The ratio of N:P in leaf tissue of all three species was significantly higher in plots that received experimental N addition (+N and +N+P plots) as compared to plots that did not receive N addition (Control and +P plots) (Table 2.7; Figure 2.7). P addition significantly decreased the N:P ratio in *Acer, Amianthium,* and *Aster* (Table 2.7; Figure 2.7).

Discussion

Effects of N addition on species diversity and species richness

Species diversity declined following N addition, primarily because of the increased abundance of the dominant understory species, *Aster acuminatus*. None of the other important species' abundances was different in plots receiving experimental N addition (Table 2.3), and species richness in the community was not significantly different in N-treated plots from plots that did not receive N. There is no evidence that N addition resulted in decreased abundance of rarer species in the community. Differences in the responses to N addition of the two diversity indices indicate that the negative effect of N on diversity was due to the positive response of *Aster*. Simpson's D is more sensitive to increasing dominance by abundant species than H' (Peet 1974), and the greater response of Simpson's D than H' to N addition (Table 2.2C) suggests that the decline in diversity was due to increasing dominance by *Aster*, rather than decreased importance of rare species in the community. If the abundance of rare species was lower in plots that received experimental N addition, the slopes of dominance-diversity curves from plots that received experimental N would also be expected to be steeper than for plots that did not receive experimental N. Instead, there was no significant difference in slopes. Processes promoting *Aster* abundance may continue with further N addition; however, it is not clear whether increased dominance of *Aster* would lead to a decline in species richness if N addition were to continue for a longer period of time. All of the species in the MLBS community are either woody or herbaceous perennials capable of maintaining below-ground reserves from year-to-year. Once established, such species may be able to persist for some time in spite of strong competitive pressure (Grime 1979).

Species richness typically declines following N addition to grasslands (Thurston 1969; Bobbink 1991; Willems, Peet, and Bik 1993; Tilman et al. 1994) and successional old-field communities (Bakelaar and Odum 1978; Tilman 1984; Tilman 1987; Wedin and Tilman 1996). The lack of a negative response of species number to N addition at MLBS, however, is consistent with other nutrient addition experiments in forest communities, where species richness is frequently unaffected by N inputs (e.g. Tyler et al. 1992; Falkengren-Grerup 1993; Kellner 1993; Rodenkirchen 1995; Makipaa 1995). I propose a number of mechanisms responsible for the maintenance of species richness in the MLBS community following N inputs:

1. Variation in light availability through the understory canopy due to gaps in vegetation cover. Decline in species richness in grasslands and old-field communities fertilized with N has been related to sharp reductions in light penetration through the herbaceous canopy as a result of increased production by competitive dominants (Al Mufti et al. 1977; Grime 1979; Tilman 1987; Grime 1990; Bobbink 1991; Willems, Peet, and Bik 1993). Plants low in the canopy receive insufficient light and are lost from the community. Openings in the canopy may allow species closer to the soil surface to receive sufficient light and persist in the community. In the MLBS community, total cover of understory species was <100% in all treatments (J. Corbin, pers. observation). Spatial variation in plant cover resulted in patches where light was able to penetrate through the canopy to plants closer to the soil surface. Willems, Peet, and Bik (1993) found that variations in light availability and canopy heterogeneity played an important role in species coexistence in chalk grasslands, as predicted by Tilman (1982, 1985). The presence of mechanisms creating and maintaining canopy openings at MLBS may explain why there was no decline in species richness despite an increase in the abundance and biomass of the dominant species.
2. Variation in light availability through the growing season. Virtually all species in the community are deciduous, and litter accumulation from year to year does not limit plant emergence in the spring. Early in the growing season, before *Aster* individuals have

emerged or reached their maximum heights, opportunities exist for other species to receive more light than is available later in the season. This may enable species to survive by growth early in the season, even if canopy closure reduces light availability later in the season.

3. Browsing by deer may further reduce canopy closure by *Aster* and promote species coexistence at MLBS. Grazing or clipping of competitive dominants can have significant positive effects on other members of the community (Bobbink and Willems 1991; Wilson, Wells, and Sparks 1995). Consumption of *Aster* was more extensive than was the case for any other species (unpublished data), which impacted the community by reducing the abundance of *Aster* stems and limiting *Aster* canopy closure. Deer may act as "keystone predators" in the MLBS forest understory, targeting the dominant understory species disproportionately and permitting coexistence of other species.

Future research should examine the importance of each mechanism in maintaining species richness, and whether the mechanisms contribute to the apparent differences in responses to N addition between grassland/old field communities and forest communities.

Effect of N addition on above-ground production and leaf tissue N concentration

Above-ground productivity of the herbaceous species in the community increased following N addition, though the increase was nearly completely due to the response of a single species, *Aster* (Table 2.3; Figure 2.3). Experimental addition of N frequently results in increased production by one or several constituents of the community (e.g., Bakelaar and Odum 1978; Tilman 1987; Bobbink 1991; Willems, Peet, and Bik 1993; Tilman et al. 1994; Wedin and Tilman 1996). However, the absence of a response by other herbaceous species in the community does not necessarily indicate a lack of N limitation or an inability to respond to changes in nutrient availability. In the absence of competitive pressure from *Aster*, other herbaceous species may have increased production following N addition. Chalk grassland and heathland species that respond negatively to N addition in the presence of competitive dominants are capable of increasing production in the absence of competitors (Aerts et al. 1990; Wilson, Wells, and Sparks 1995). No increase in understory (<50 cm height) woody biomass was detected, though production of vegetation >50 cm in height was not sampled. Tree biomass has frequently responded positively to N addition (e.g. Magill et al. 1997), but the present study did not estimate total woody production in the ecosystem.

The concentration of N in leaf tissue increased in all three species sampled, including one species that showed positive responses to N addition (*Aster*) and two species that did not respond significantly (*Acer rubrum*, *Amianthium muscaetoxicum*). The higher N content in the plants' leaf tissue indicates that the availability of N and N uptake by vegetation was greater in +N and +N+P plots than in Control and +P plots. The higher concentration of N in the leaves could have long-term impacts on the population of each species, even in species that experienced no increase in productivity in N-amended plots. For example, leaf N concentration has a strong positive relationship with photosynthetic rates. Plants' ability to photosynthesize at a greater rate per unit leaf area could result in higher fecundity and/or improved chemical defenses by increasing the availability of carbohydrate. Eventually, increased fecundity or decreased susceptibility to herbivory could lead to increased population sizes by species experiencing higher N availability.

Effect of P addition on species diversity and above-ground production

Species diversity declined in plots receiving P addition due to both the increased abundance of dominant species relative to other community members and the loss of rare species from the community. The abundances of *Aster*, *Acer rubrum*, and *Amelanchier arborea*, together making up 55% of the individuals in the MLBS community, declined in P-amended plots. Species richness and the dominance-diversity curves of P-amended plots illustrate the negative impact of P inputs on rare species. Species richness was lower in the +P and +N+P plots, as rare species were not present in the P-amended communities. The steeper slopes of dominance-diversity curves from plots receiving P addition indicates that rarer species were less abundant, as well. In addition to the negative impact of P addition on species richness and diversity, the growth of even the dominant species, *Aster*, was reduced in P-amended plots.

The most likely explanation for the lower species richness, diversity, and biomass in the +P and +N+P plots is the impact of deer herbivory on P-amended plots. +P and +N+P plots experienced the heaviest levels of herbivory, resulting in an obvious negative impact on above-ground production in these plots. Intense herbivory is also likely to have reduced the number of individuals and the number of species in a sample area.

The loss of species richness and reduced biomass production following P addition may have also been the result of nutrient imbalances within plant tissues as a result of suboptimal ratios of N:P. Ratios of N:P in plant tissues have been found to be significantly correlated with the ratio of N:P in nutrient inputs in desert shrubs (Lajtha and Klein 1988) and freshwater marsh species (Shaver and Melillo 1984). The N:P ratio in all three of the species measured in the present study was significantly lower in plots receiving P input (Figure 2.6). Plants with low N:P ratios can exhibit low photosynthetic efficiency (van den Driessche 1974; Ingestad 1979; Rundel 1982; Verhoeven, Koerselman, and Meuleman 1996) and growth rates (Lajtha and Klein 1988). Increased stress from imbalanced nutrient status could have increased mortality in some species and reduced species richness following P addition to the MLBS community.

The sodium in the P fertilizer may have influenced the understory community. Sodium levels in the soil were higher in P-amended plots ($F=65.92$; $p<0.0001$), which is not surprising considering that 45 kg Na ha^{-1} yr^{-1} was added to +P and +N+P plots in the sodium phosphate fertilizer. Sodium can affect the availability of other soil nutrients by displacing other cations from cation exchange sites, thereby making those nutrients vulnerable to loss from the ecosystem (Schlesinger 1997). However, levels of ammonium, calcium, magnesium, and potassium were not significantly different in plots receiving P addition ($p>0.10$; Chapter 3). Plants also may have experienced direct toxic effects from elevated sodium levels, though the sensitivity of forest herbs to sodium toxicity is not well described.

Role of P in the response of the community to N addition

Increased competition for secondary nutrients such as P has been suggested as a mechanism for loss of species richness following N addition (Tilman 1982; Bobbink 1991; Willems, Peet, and Bik 1993). With increased production due to N addition, there may be increased uptake of secondary nutrients by dominant species and increased competition for secondary nutrients. Addition of secondary nutrients may allow for species persistence under high-N conditions. However, levels of extractable P were not significantly different in plots receiving N addition and plots receiving no N addition (Chapter 3), and there is no evidence that the number of species able to coexist in the community is higher in +N+P plots than in +N plots at MLBS. The effect of N addition on species diversity was no different with experimental P addition than with no P (N*P interaction, Table 2.2).

The possibility exists that the present experiment was not able to test for the importance of P availability under the conditions most likely to evoke a response from the community. Competition for P would be expected to be strongest when N availability is so high that N is no longer limiting. Three years of N addition, even at the rate of 100 kg N ha^{-1} yr^{-1} may not have been enough to relax N limitation and bring about P limitation. Analysis of ecosystem nutrient cycling indicates that N-amended plots were not saturated with N (Chapter 3). Fertilization for a longer time period would make it more likely that the vegetation is not N-limited and that competition for P would be important in the community. The possibility also exists that consequences of the selective browsing by deer on P-fertilized plots swamped out any effect of P in the +N+P plots. An experimental design controlling for herbivory would permit the examination of the role of P in high-N environments without the influence of grazing patterns.

Conclusions

This study demonstrated the ability of increased N inputs to affect the plant community of a forest, as the species diversity decreased while the dominance of *Aster acuminatus* increased. Above-ground biomass production of the understory was higher in plots receiving N addition, due primarily to the positive response of *Aster*. Leaf N concentrations in all three species analyzed showed positive responses to increased N inputs. However, contrary to expectations based on observations following N addition in other ecosystems, there was no decline in species richness following N addition. Nor was there any significant interaction between N and P addition on either species richness or species diversity. Mechanisms creating herbaceous canopy openings may have allowed high enough light levels beneath the *Aster* canopy to permit coexistence of species following N addition in spite of increased production by *Aster*.

Results from this experiment suggest that the impact of atmospheric N deposition on forest communities is likely to be different from the impact on grassland and old field communities. Increased N inputs appear unlikely to cause a precipitous decline in species richness in forests, in contrast to observations in grassland communities. A substantial unknown factor in predicting the impact of atmospheric N deposition on eastern North American forests, however, is the effect of immigration of species well-adapted to high-N conditions. N addition experiments that proceed long enough to follow species' immigration and extinction within a community would provide better predictions as to how increasing N inputs will affect forest communities.

Literature Cited

Aber J. D., Nadelhoffer K. J., Steudler P., Melillo J. M. 1989. Nitrogen saturation in northern forest ecosystems. BioScience 39:378-86.

Adams M. B., Angradi T. R., Kochenderfer J. N. 1997. Stream water and soil solution responses to 5 years of nitrogen and sulfur additions at the Fernow Experimental Forest, West Virginia. Forest Ecology and Management 95:79-91.

Aerts R., Berendse F., deCaluwe H., Schmitz M. 1990. Competition in heathland along an experimental gradient of nutrient availability. Oikos 57:310-8.

Aerts R., Berendse F. 1988. The effect of increased nutrient availability on vegetation dynamics in wet heathlands. Vegetatio 76:63-9.

Al Mufti M. M., Sydes C. L., Furness S. B., Grime J. P., Band S. R. 1977. A quantitative analysis of shoot phenology and dominance in herbaceous vegetation. Journal of Ecology 65:759-91.

Bakelaar R. G., Odum E. P. 1978. Community and population level responses to fertilization in an old-field ecosystem. Ecology 59:660-5.

Bobbink R. 1991. Effects of nutrient enrichment in Dutch chalk grasslands. Journal of Applied Ecology 28:28-41.

Bobbink R., Willems J. H. 1987. Increasing dominance of *Brachypodium pinnatum* (L.) Beauv. in Chalk Grasslands: A threat to a species-rich ecosystem. Biological Conservation 40:301-14.

Bobbink R., Willems J. H. 1991. Impact of different cutting regimes on the performance of Brachypodium pinnatum in Dutch Chalk Grassland. Biological Conservation 56:1-21.

Chapin F. S. III. 1980. The mineral nutrition of wild plants. Annual Review of Ecology and Systematics 11:233-60.

Cole D.W., Rapp M. 1981. Elemental cycling in forest ecosystems. In: Reichle D.E.. editor. Dynamic Principles of Forest Ecosystem. London:Cambridge University Press. p 341-409.

DeVries W., Leeters E.E.J.M., Hendriks C.M.A., Van Dobben H., Van den Burg J., Boumans L.J.M. 1995a. Large scale impact of acid deposition on forests and forest soils in The Netherlands. In: Heij G.J., Erisman J.W., editors. Acid rain research: Do we have enough answers? Amsterdam:Elsevier. p 261-77.

DeVries W., Leeters E. M., Hendricks C. A. 1995b. Effects of acid deposition on Dutch forest ecosystems. Water, Air, and Soil Pollution 85:1063-8.

DiTommaso A., Aarssen L. W. 1989. Resource manipulations in natural vegetation: a review. Vegetatio 84:9-29.

During H. J., Willems J. H. 1986. The impoverishment of the bryophyte and lichen flora of the Dutch chalk grasslands in the thirty years 1953-1983. Biological Conservation 36:143-58.

Ellenberg H. 1988. Floristic changes due to nitrogen deposition in Central Europe. In: Nilsson J, Grennfelt P, editors. Critical loads for sulphur and nitrogen. Skokloster, Sweden:UN-ECE. p 375-83.

Falkengren-Grerup U. 1986. Soil acidification and vegetation changes in deciduous forests in southern Sweden. Oecologia 70:339-47.

Falkengren-Grerup U. 1993. Effects on beech forest species of experimentally enhanced nitrogen deposition. Flora 188:85-91.

Galloway J. N., Likens G. E., Hawley M. E. 1984. Acid precipitation: Natural versus anthropogenic components. Science 226:29-831.

Grime J.P. 1979. Plant strategies and vegetation processes. Chinchester, UK. John Wiley and Sons. 222p.

Grime J.P. 1990. Mechanisms promoting floristic diversity in calcareous grasslands. In: Hillier S.H., Walton D.W.H., Wells D.A., editors. Calcareous grasslands: ecology and management. Bluntisham:Bluntisham Books. p 51-6.

Gunderson P. 1991. Nitrogen deposition and the forest nitrogen cycle: role of denitrification. Forest Ecology and Management 44:15-28.

Gunderson P. 1995. Nitrogen deposition and leaching in European forests - preliminary results from a data compilation. Water, Air, and Soil Pollution 85:1179-84.

Haynes R. J. 1980. A comparison of two modified Kjeldahl digestion techniques for multi-element plant analysis with conventional wet and dry ashing methods. Communications in Soil Science and Plant Analysis 11:459-67.

Heil G. W., Diemont W. H. 1983. Raised nutrient levels change heathland into grassland. Vegetatio 53:113-20.

Hogbom L., Hogberg P. 1991. Nitrate nutrition of *Deschampsia flexuosa* (L.) Trin. in relation to nitrogen deposition in Sweden. Ecologia 87:488-94.

Ingestad T. 1979. Nitrogen stress in birch seedlings II. N, K, P, Ca, and Mg nutrition. Physiologia Plantarum 45:149-57.

Kellner O. 1993. Effects on associated flora of sylvicultural nitrogen fertilization repeated at long intervals. Journal of Applied Ecology 30:563-74.

Kleinbaum D.G., Kupper L. L., Muller K. E. 1988. Applied regression analysis and other multivariate methods. 2nd edition. Boston. PWS-Kent Publishing Company. -718p.

Lajtha K., Klein M. 1988. The effect of varying nitrogen and phosphorus availability on nutrient use by *Larrea tridentata*, a desert evergreen shrub. Oecologia 75:348-53.

Liu Q., Brakenhielm S. 1996. Variability of plant species diversity in Swedish natural forest and its relation to atmospheric deposition. Vegetatio 125:63-72.

Lowther J. R. 1980. Use of a single sulfuric acid-hydrogen peroxide digest for the analysis of *Pinus radiata* needles. Communications in Soil Science and Plant Analysis 11:175-88.

Magill A. H., Aber J. D., Hendricks J. J., Bowden R. D., Melillo J. M., Steudler P. A. 1997. Biogeochemical response of forest ecosystems to simulated chronic nitrogen deposition. Ecological Applications 7:402-15.

Mäkipää R. 1995. Effect of nitrogen input on carbon accumulation of boreal forest soils and ground vegetation. Forest Ecology and Management 79:217-26.

McNulty S. J., Aber J. D., Newman S. D. 1996. Nitrogen saturation in a high elevation New England spruce-fir stand. Forest Ecology and Management 84:109-21.

Milliken G.A., Johnson D. E. 1992. Analysis of messy data: Volume 1 - Designed experiments. London. Chapman and Hall. 473p.

National Atmospheric Deposition Program (NRSP-3) / National Trends Network. 1997. NADP/NTN Coordination Office, Natural Resource Ecology Laboratory, Colorodo State University, Fort Collins, CO 80523.

Peet R. K. 1974. The measurement of species diversity. Annual Review of Ecology and Systematics 5:285-307.

Rodenkirchen H. 1995. Nutrient pools and fluxes of the ground vegetation in coniferous forests due to fertilizing, liming, and amelioration. Plant and Soil 168-169:383-90.

Rosen K., Gunderson P., Tegnhammer L., Johnansson M., Frogner T. 1992. Nitrogen enrichment of Nordic forests: the concept of critical loads. Ambio 21:364-8.

Rundel R. W. 1982. Nitrogen utilization efficiencies in Mediterranean-climate shrubs of California and Chile. Oecologia 55:409-13.

Schlesinger W.H. 1997. Biogeochemistry: An analysis of global change. 2nd edition. San Diego. Academic Press. -588p.

Schlesinger W.H. 1994. The vulnerability of biotic diversity. In: Socolow R., Andrews C., Berkhout F., Thomas V., editors. Industrial Ecology and Global Change. Cambridge:Cambridge University Press. p 245-60.

Shaver G. R., Melillo J. M. 1984. Nutrient budgets of marsh plants: efficiency concepts and relation to availability. Ecology 65:1491-510.

Stephenson S. L., Adams H. S. 1991. Upland oak forests of the Ridge and Valley Province in Southwestern Virginia. Virginia Journal of Science 42:371-80.

Thimonier A., Dupouey J. L., Timbal J. 1992. Floristic changes in the herb-layer vegetation of a deciduous forest in the Lorraine Plain under the influence of atmospheric deposition. Forest Ecology and Management 55:149-67.

Thurston J.M. 1969. The effect of liming and fertilizers on the botanical composition of a permanent grassland, and on the yield of hay. In: Rorison I., editor. Ecological aspects of the mineral nutrition of plants. Oxford:Blackwell Scientific Publications. p 3-10.

Tilman D., Dodd M.E., Silvertown J, Poulton PR, Johnston AE, Crawley MJ, Leigh RA, eds. 1994. The Park Grass Experiment: Insights from the most long-term ecological study. In: Leigh R.A., Johnston A.E., editors. Long-term experiments in agricultural and ecological sciences. Oxon, UK:CAB International. p 287-303.

Tilman D. 1982. Resource competition and community structure. Princeton, NJ. Princeton University Press. 296p.

Tilman D. 1984. Plant dominance along an experimental nutrient gradient. Ecology 65:1445-53.

Tilman D. 1985. The resource ratio hypothesis of plant succession. American Naturalist 125:827-52.

Tilman D. 1987. Secondary succession and the pattern of plant dominance along experimental nitrogen gradients. Ecological Monographs 57:189-214.

Tyler G., Pahlsson A.B., Bergkvist B., Falkengren-Grerup U., Folkeson L., Nihlgard B., Ruhling A., Stjernquist I. 1992. Chemical and biological effects of artificially increased nitrogen deposition to the ground in a Swedish beech forest. Scandinavian Journal of Forest Research 7:515-32.

van Breemen N., van Dijk H. F. G. 1988. Ecosystem effects of atmospheric deposition of nitrogen in The Netherlands. Environmental Pollution 54:249-74.

van den Driessche R. 1974. Prediction of mineral nutrient status of trees by foliar analysis. Botanical Review 40:347-94.

Van Miegroet H., Cole D.W., Foster N.W. 1992. Nitrogen distribution and cycling. In: Johnson D.W., Lindberg S.E., editors. Atmospheric deposition and forest nutrient cycling. New York, New York:Springer-Verlag. p 178-96.

Verhoeven J. T. A., Koerselman W., Meuleman A. F. M. 1996. Nitrogen- or phosphorus-limited growth in herbaceous, wet vegetation: relations with atmospheric inputs and management regimes. Trends in Ecology and Evolution 11:494-7.

Vitousek P. M., Aber J. D., Howarth R. H., Likens G. E., Matson P. A., Schindler D. W., Schlesinger W. H., Tilman D.G. 1997. Human alterations of the global nitrogen cycle: sources and consequences. Ecological Applications 7:737-50.

Vitousek P. M., Howarth R. W. 1991. Nitrogen limitation on land and sea: How can it occur? Biogeochemistry 13:87-115.

Vitousek P.M., Matson P.A. 1993. Agriculture, the global N cycle, and trace gas flux. In: Oremland R.S., editor. The biogeochemistry of global change: radiative gases. New York, New York:Chapman and Hall. p 193-208.

Wedin D. A., Tilman D. 1996. Influence of nitrogen loading and species composition on the carbon balance of grasslands. Science 274:1720-3.

Willems J. H., Peet R. K., Bik L. 1993. Changes in chalk-grassland structure and species richness resulting from selective nutrient additions. Journal of Vegetation Science 4:203-12.

Wilson E. J., Wells T. C. E., Sparks T. H. 1995. Are calcareous grasslands in the UK under threat from nitrogen deposition? an experimental determination of a critical load. Journal of Ecology 83:823-32.

Wolf B. 1982. A comprehensive system of leaf analyses and its use for dignosing crop nutrient status. Communications in Soil Science and Plant Analysis 13:1035-59.

Woodin S. J., Farmer A. M. 1993. Impacts of sulfur and nitrogen deposition on sites and species of nature conservation importance in Great Britain. Biological Conservation 63:23-30.

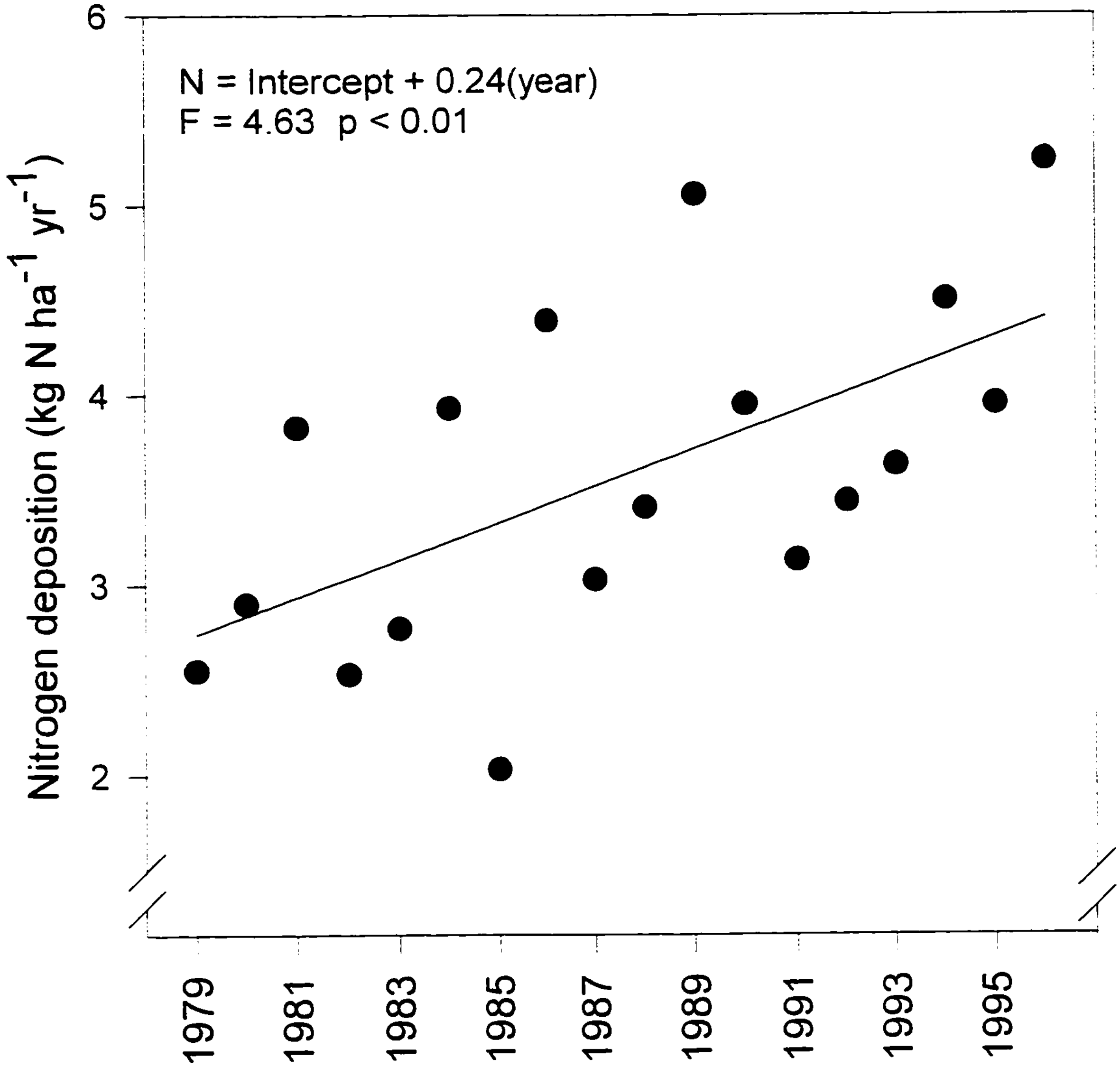

Figure 2.1. Regression of wet deposition of nitrogen at Horton's Station, adjacent to Mountain Lake, VA. The F-test and p-value test the null hypothesis that the slope of the regression=0. Data from National Atmospheric Deposition Program (1997).

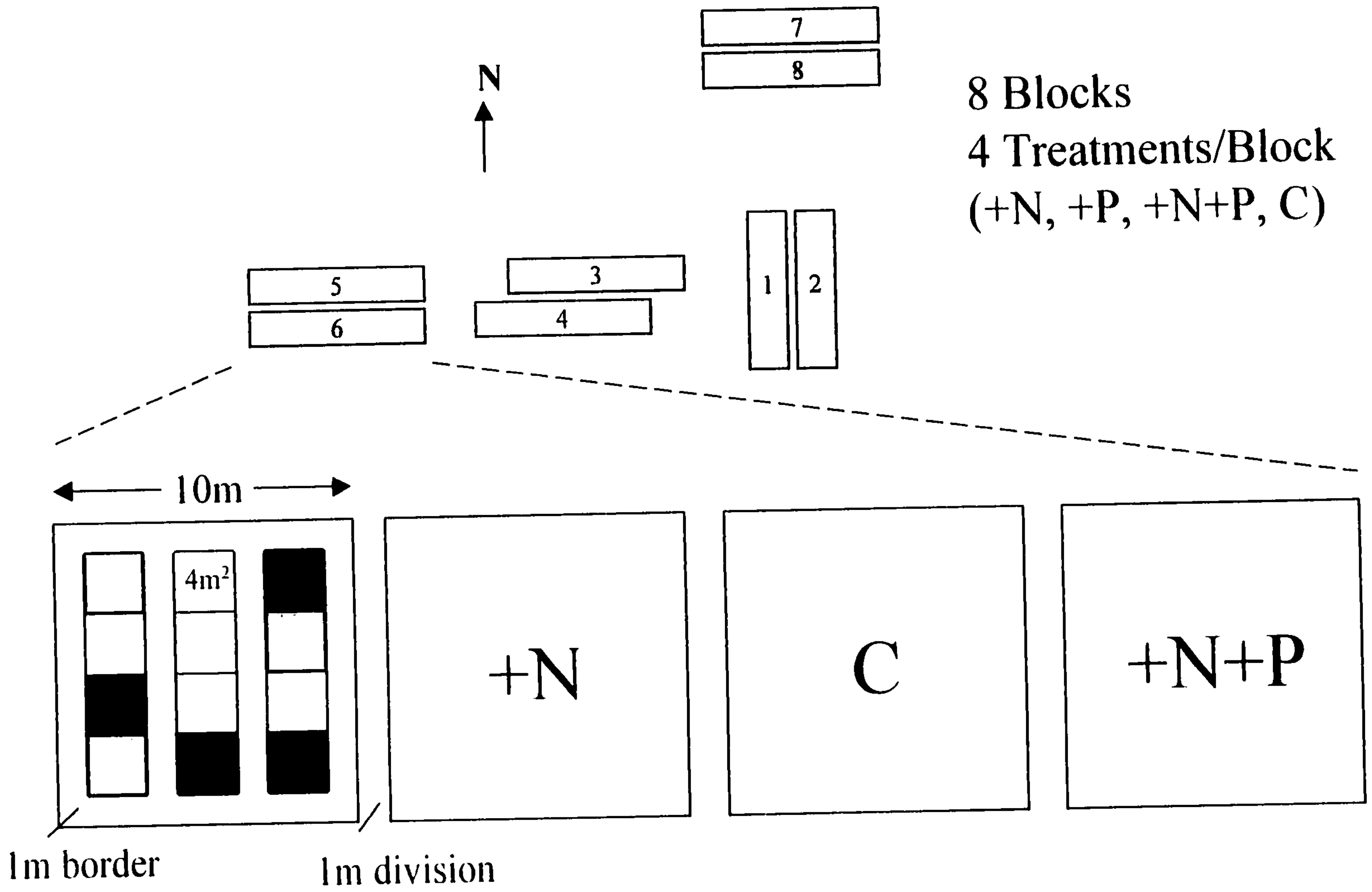

Figure 2.2. Layout of experimental plots at Mountain Lake Biological Station. Below is a detailed illustration of one block, with four experimental treatments, +P, +N, Control, and +N+P. Each plot is divided into 12 $4m^2$ subplots for biomass sampling.

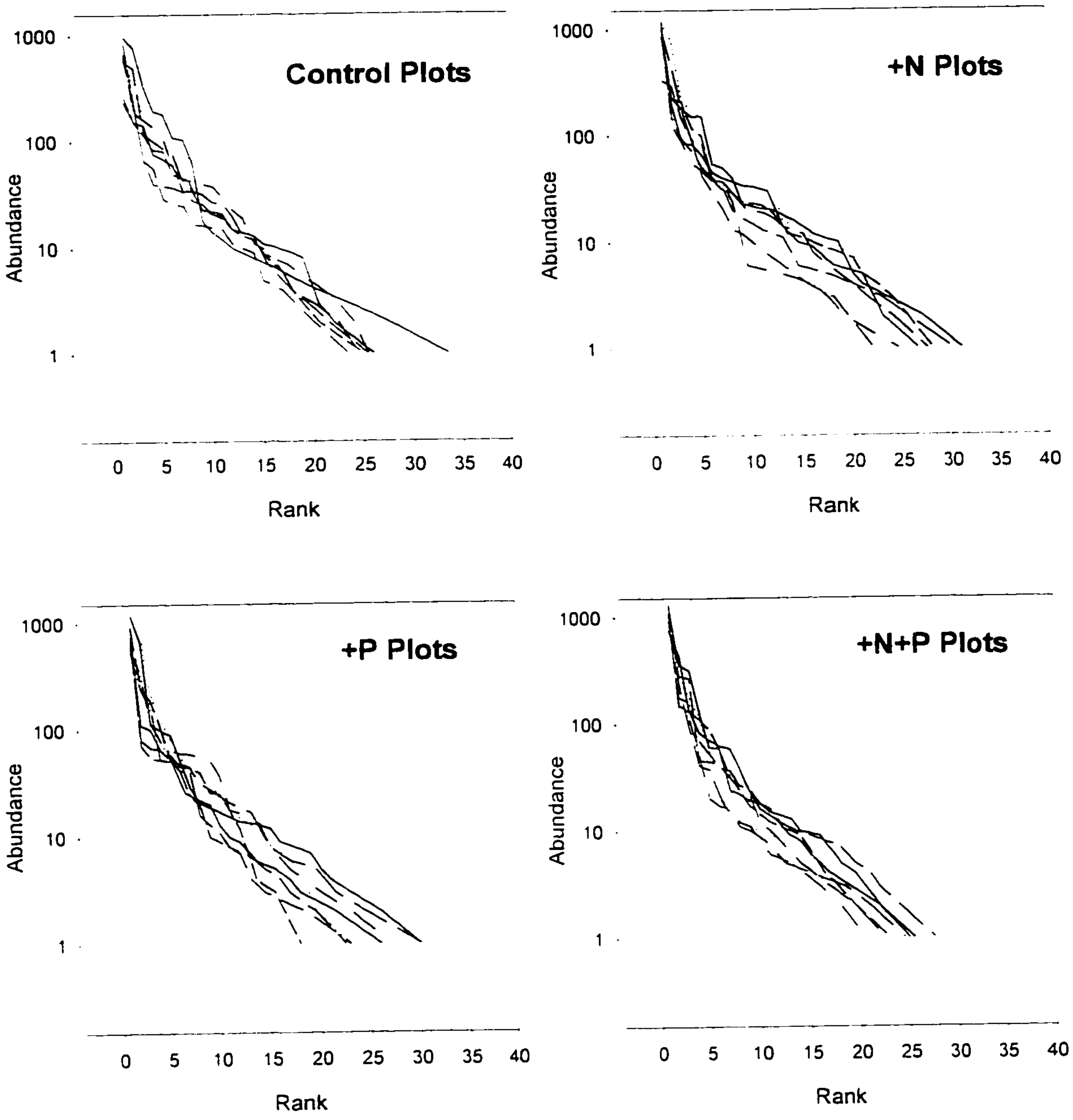

Figure 2.3. Dominance-diversity plots for Control, +N, +P and +N+P plots in 1996. Vertical axis is abundance of a given species plotted on a log scale; horizontal axis is a species rank, from most abundant to least abundant.

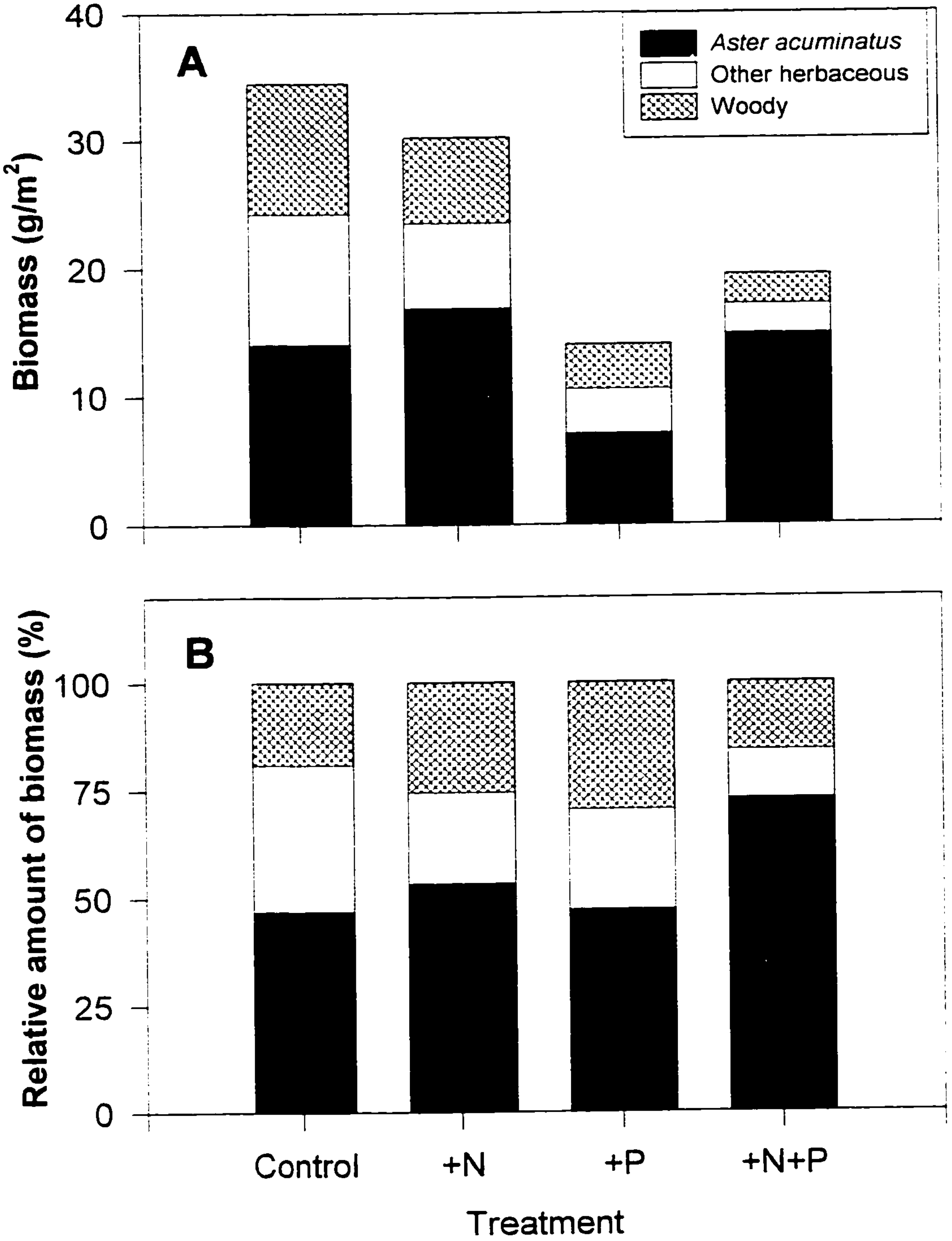

Figure 2.4. Absolute (A) and relative (B) amounts of biomass in each of three fractions: *Aster acuminatus*; other herbaceous species; and woody species <50 cm in height.

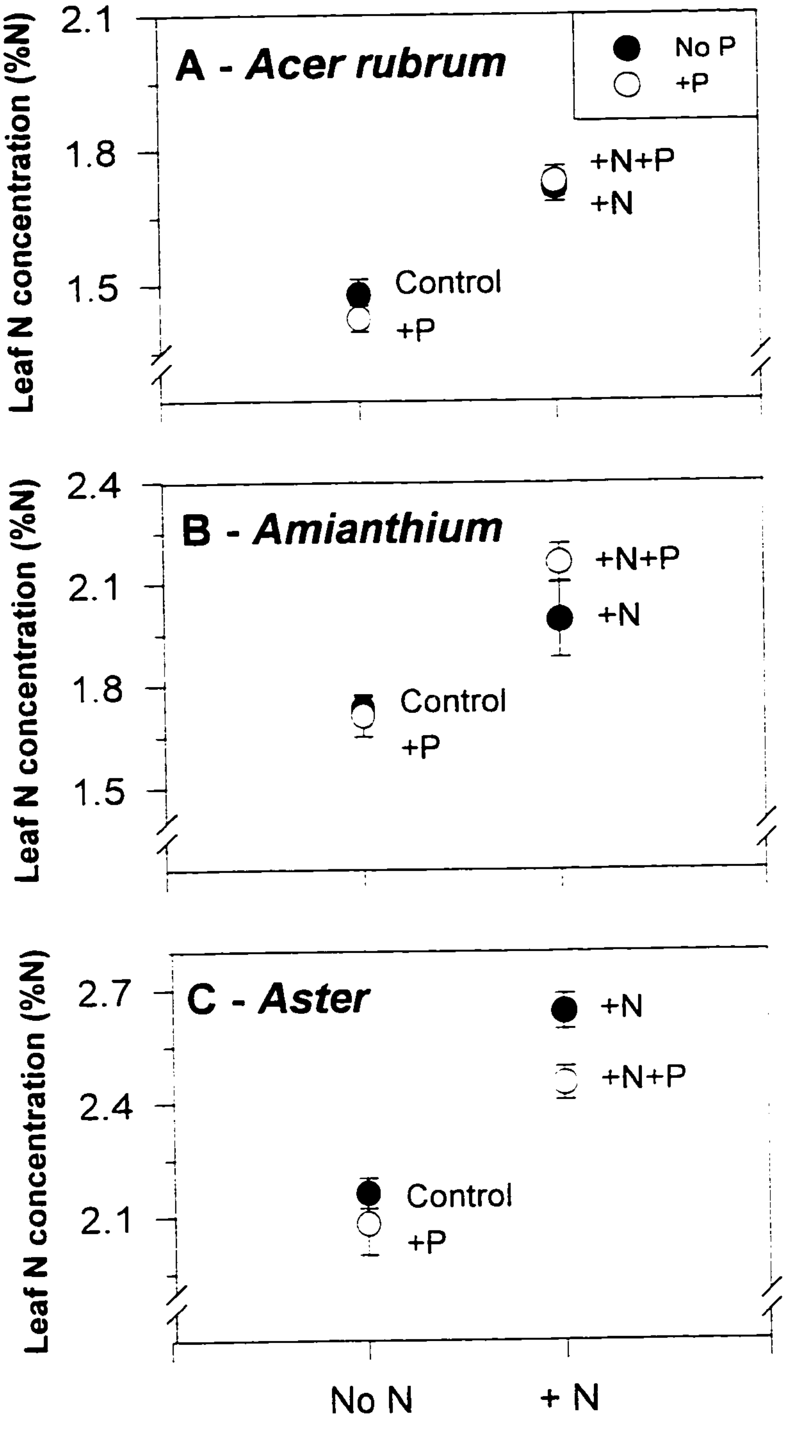

Figure 2.5. Tissue N concentration in (A) *Acer rubrum*, (B) *Amianthium muscaetoxicum*, and (C) *Aster acuminatus*. Labels identify treatment. Error bars represent 1 S.E.

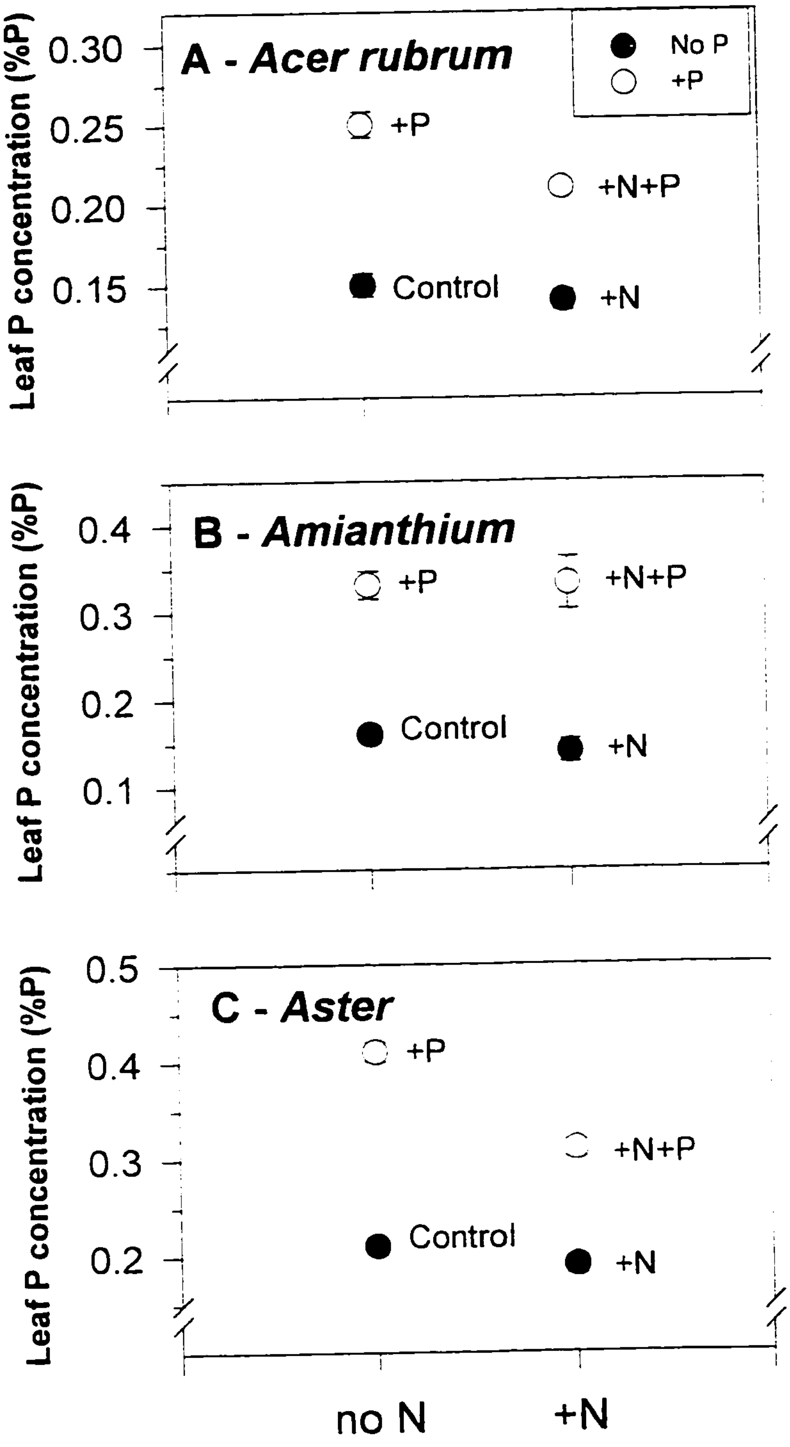

Figure 2.6. Tissue P concentration in (A) *Acer rubrum*, (B) *Amianthium muscaetoxicum*, and (C) *Aster acuminatus*. Labels identify treatment. Error bars represent 1 S.E.

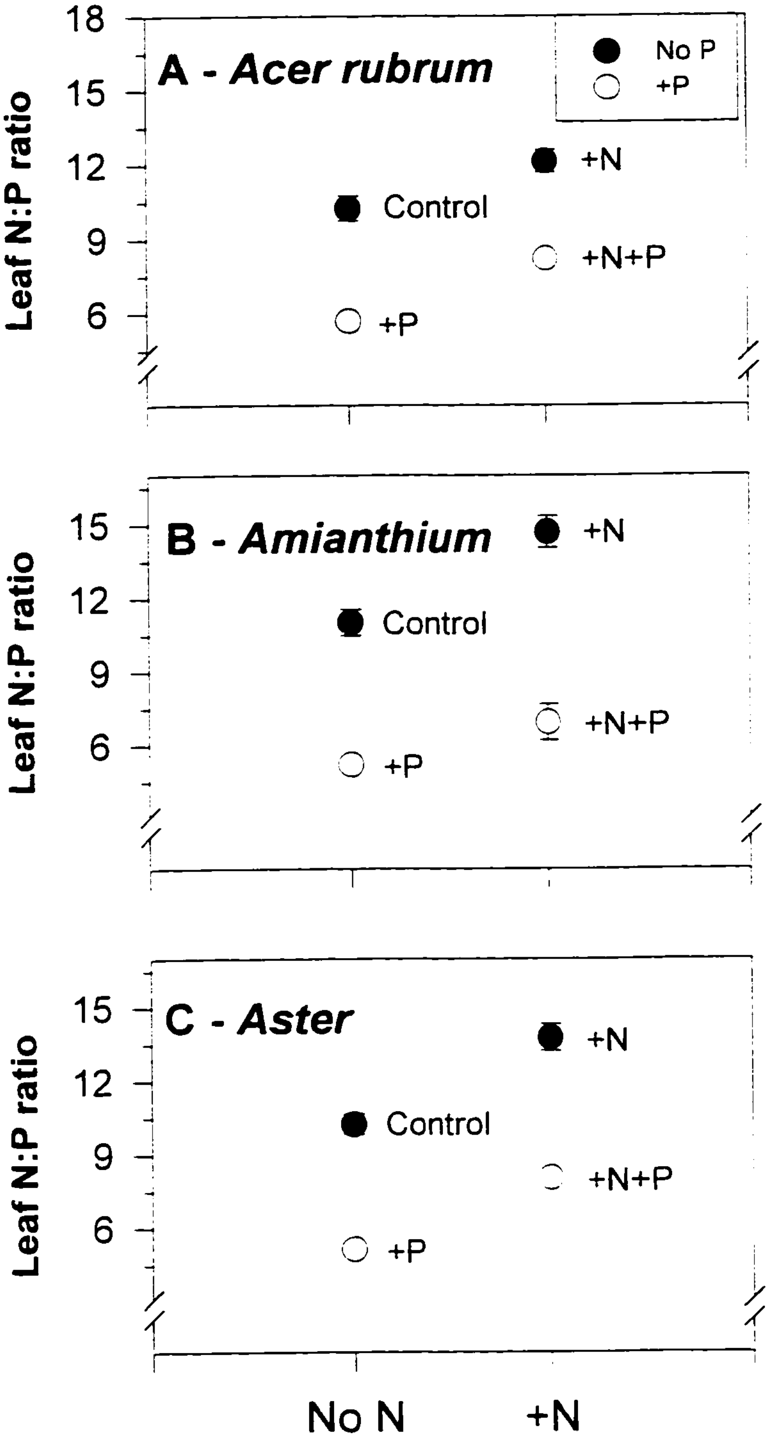

Figure 2.7. Ratio of N:P concentrations in (A) *Acer rubrum*, (B) *Amianthium muscaetoxicum*, and (C) *Aster acuminatus*. Labels identify treatment. Error bars represent 1 S.E.

Table 2.1. A) Species richness per $4m^2$ in each treatment in 1995 and 1996. Values are means with standard errors. B) ANOVAR of species richness. For brevity, not all components of the model are given.

A

Treatment	**Species Richness**	
	1995	**1996**
Control	17.8 (0.7)	18.0 (0.8)
+N	17.9 (1.2)	18.2 (1.0)
+P	16.1 (0.8)	16.0 (0.7)
+N+P	15.6 (0.7)	15.7 (0.8)

B

ANOVAR of Species Richness			
Source	**d.f.**	**F-ratio**	**p-value**
Block	7	2.57	**0.044**
N	1	0.04	0.85
P	1	8.83	**0.007**
N*P	1	0.15	0.70
Within plot error	21		
Year	1	0.21	0.65
Year*N	1	0.06	0.81
Year*P	1	0.21	0.65
Year*Block	7	0.82	0.58
Between plot error	21		
Total	62		

Table 2.2. A) Simpson' D and Shannon-Weaver Index (H') in each treatment in 1995 and 1996. Values are means with standard errors. B) The average value for each diversity index in each year in plots that do not receive N (No N), plots that do receive N (+N), plots that do not receive P (No P) and plots that receive P (+P). % decrease is calculated by dividing the difference between two averages by the larger average. C) ANOVAR of Simpson's D and H'. For brevity, not all components of the model are given.

A

Plot	**Simpson's D**		**H'**	
	1995	**1996**	**1995**	**1996**
Control	4.27 (0.56)	4.59 (0.41)	1.78 (0.10)	1.86 (0.08)
+N	3.32 (0.27)	3.29 (0.29)	1.62 (0.07)	1.62 (0.08)
+P	2.94 (0.27)	3.16 (0.24)	1.51 (0.08)	1.59 (0.06)
+N+P	2.66 (0.12)	2.47 (0.09)	1.42 (0.05)	1.35 (0.05)

B

Nutrient	**Simpson's D**		**H'**	
	1995	**1996**	**1995**	**1996**
No N	3.61	3.88	1.65	1.73
+N	2.99	2.88	1.52	1.49
% Decrease	**17%**	**26%**	**9%**	**14%**
No P	3.80	3.94	1.70	1.74
+P	2.82	2.82	1.47	1.47
% Decrease	**26%**	**29%**	**14%**	**16%**

C

ANOVAR of Simpson's D and H'					
		Simpson's D		**H'**	
Source	**d.f.**	**F-ratio**	**p-value**	**F- ratio**	**p-value**
Block	7	0.41	0.89	0.25	0.97
N	1	6.11	**0.02**	5.40	**0.03**
P	1	10.53	**0.004**	10.26	**0.004**
N*P	1	0.95	0.34	0.08	0.78
Within plot error	21	--	--	--	--
Year	1	0.83	0.37	1.20	0.29
Year*N	1	4.45	**0.047**	12.46	**0.002**
Year*P	1	0.55	0.47	1.25	0.28
Year*Block	7	1.41	0.26	3.27	**0.02**
Between plot error	21	--	--	--	--
Total	62	--	--	--	--

Table 2.3. F-values from ANOVAR of abundance of all species with at least 1% of total vegetation abundance. See text for direction of significant results.

Species	Proportion of abundance	N	Year*N	P	Year*P
Acer rubrum	0.09	1.82	0.65	**5.38***	**5.34***
Amelanchier arborea	0.02	2.52	1.51	**4.38***	3.08
Amianthium muscaetoxicum	0.07	0.93	2.66	0.02	0.16
Aster acuminatus	0.44	**4.08**$^+$	**4.33***	**6.14***	0.06
Dichanthelium boscii	0.01	0.26	0.02	1.66	0.02
Galax aphylla	0.01	4.45	**10.80***	2.00	0.93
Maianthemum canadensis	0.02	0.07	3.46	0.12	0.15
Medeola virginica	0.03	0.04	0.07	0.61	0.00
Rubus hispidus	0.04	1.18	0.51	0.75	**12.39****
Thelypteris novaborecensis	0.09	2.34	1.90	1.34	1.36
Vaccinium angustifolium	0.01	0.27	0.55	0.13	0.01
Viola spp.	0.04	0.09	0.29	0.61	1.25

\+ - $p < 0.056$
* - $p < 0.05$
** - $p < 0.01$

Table 2.4. ANOVA tables of tests for effect of N and P addition on the above-ground biomass of all species, all herbaceous species, *Aster acuminatus*, and all woody species in 1996.

Variable	**Source**	**d.f.**	**F-ratio**	**p-value**
Total biomass	Block	7	4.81	**0.002**
	N	1	3.34	0.08
	P	1	13.85	**0.01**
	N*P	1	1.17	0.3
	Error	21	--	--
Herbaceous biomass	Block	7	4.92	**0.002**
	N	1	5.19	**0.03**
	P	1	8.37	**0.009**
	N*P	1	2.23	0.15
	Error	21	--	--
Aster acuminatus biomass	Block	7	2.54	**0.05**
	N	1	6.79	**0.02**
	P	1	1.32	0.3
	N*P	1	1.84	0.2
	Error	21	--	--
Woody biomass	Block	7	1.19	0.4
	N	1	0.69	0.4
	P	1	8.14	**0.01**
	N*P	1	1.00	0.3
	Error	21	--	--

Table 2.5. ANOVA tables of tests for effect of N and P addition on the concentration of N in leaf tissue for *Acer rubrum*, *Amianthium muscaetoxicum*, and *Aster acuminatus*.

Species	**Source**	**d.f.**	**F-ratio**	**p-value**
Acer rubrum	Block	7	0.77	0.62
	N	1	66.44	**0.0001**
	P	1	0.39	0.54
	N*P	1	1.00	0.33
	Error	21	--	--
Amianthium muscaetoxicum	Block	7	0.82	0.58
	N	1	24.77	**0.0001**
	P	1	1.12	0.30
	N*P	1	1.90	0.18
	Error	21	--	--
Aster acuminatus	Block	7	0.56	0.78
	N	1	49.53	**0.0001**
	P	1	5.34	**0.03**
	N*P	1	0.83	0.37
	Error	21	--	--

Table 2.6. ANOVA tables of tests for effect of N and P addition on the concentration of P in leaf tissue for *Acer rubrum*, *Amianthium muscaetoxicum*, and *Aster acuminatus*.

Species	**Source**	**d.f.**	**F-ratio**	**p-value**
Acer rubrum	Block	7	0.29	0.95
	N	1	9.98	**0.005**
	P	1	155.05	**0.0001**
	N*P	1	7.13	**0.01**
	Error	21	--	--
Amianthium muscaetoxicum	Block	7	2.60	**0.04**
	N	1	0.43	0.52
	P	1	132.56	**0.0001**
	N*P	1	0.48	0.50
	Error	21	--	--
Aster acuminatus	Block	7	1.14	0.38
	N	1	46.87	**0.0001**
	P	1	294.71	**0.0001**
	N*P	1	21.75	**0.0001**
	Error	21	--	--

Table 2.7. ANOVA tables of tests for effect of N and P addition on the ratio of N:P in leaf tissue for *Acer rubrum*, *Amianthium muscaetoxicum*, and *Aster acuminatus*.

Species	**Source**	**d.f.**	**F-ratio**	**p-value**
Acer rubrum	Block	7	0.52	0.81
	N	1	34.41	**0.0001**
	P	1	127.17	**0.0001**
	N*P	1	0.71	0.41
	Error	21	--	--
Amianthium muscaetoxicum	Block	7	1.94	0.11
	N	1	28.44	**0.0001**
	P	1	180.25	**0.0001**
	N*P	1	3.73	0.07
	Error	21	--	--
Aster acuminatus	Block	7	1.10	0.40
	N	1	82.14	**0.0001**
	P	1	233.61	**0.0001**
	N*P	1	0.74	0.40
	Error	21	--	--

Chapter 3

Ecosystem responses to three years of nitrogen and phosphorus addition to a deciduous forest understory

Introduction

Nitrogen (N) inputs via atmospheric deposition have increased dramatically in eastern North America and northern Europe in the last 20-40 years (Galloway, Likens, and Hawley 1984; Mayewski et al. 1988), and have begun to alter the nutrient status of ecosystems over much of this region (Aber et al. 1989; Aber 1992; Vitousek et al. 1997). Changes in N cycling (McNulty et al. 1990) and higher streamwater nitrate concentrations (Van Miegroet, Lovett, and Cole 1992; Stoddard 1994; Dise and Wright 1995; Gunderson 1995) have been reported in a number of forest systems.

As elevated N deposition continues, it is critical that we understand the impacts of increased N inputs on ecosystem processes such as N mineralization rates, nitrification, and N retention. Greater N deposition has the potential to increase rates of net nitrification relative to net N mineralization, leading to increased abundance of nitrate in the soil solution (McNulty et al. 1990; Aber 1992). Once nitrate levels exceed the capacity of the ecosystem to retain N, leaching losses of nitrate can be expected to increase (Aber et al. 1989; Stoddard 1994). Such a shift to a N-saturated ecosystem exhibiting leaching losses of N would represent a profound change from the highly conservative N cycling in N-limited ecosystems where immobilization of ammonium and nitrate in the soil is rapid and leaching losses of inorganic N is low (Hedin, Armesto, and Johnson 1995; Schlesinger 1997).

Although impacts of increased N inputs have been demonstrated in the northeastern United States and northern Europe (Aber et al. 1993; Wright and van Breeman 1995; Gunderson and Rasmussen 1995; Christ et al. 1995; Wright and Tietema 1995; Koopmans, Tietema, and Boxman 1996; Magill et al. 1996; Magill et al. 1997), the responses of ecosystems in the southeastern United States are not as well-known. Gilliam and colleagues (Gilliam, Adams, and Yurish 1996; Peterjohn, Adams, and Gilliam 1996; Adams, Angradi, and Kochenderfer 1997) have studied the impacts of experimental N inputs to a N-saturated hardwood forest ecosystem at Fernow Experimental Forest, WV. However, the impact of elevated N inputs to more ecosystems must be documented before a general model of ecosystem response to atmospheric N deposition in North America can be developed.

I established a nutrient addition experiment in a deciduous forest in southwestern Virginia to test whether three years of experimental N inputs are capable of driving the ecosystem to N saturation, and what factors control N retention in the ecosystem. Atmospheric N deposition in this region is low compared to other parts of the eastern United States (National Atmospheric Deposition Program 1997), so N cycling and species composition are less likely to have been affected by previous N inputs.

In this chapter I discuss the effects of N and P addition on N cycling and N retention in the ecosystem. Specifically, I address the following questions:

1. *Does three years of high levels of experimental N enrichment lead to N saturation of the ecosystem?* N addition experiments can be used to assess the degree of N saturation of an

ecosystem (Aber et al. 1993; Wright and van Breeman 1995; Gilliam, Adams, and Yurish 1996; Magill et al. 1997). A number of changes in the cycling of N are would be expected where N inputs have exceeded the capacity of the ecosystem to retain N. Specifically, I tested the impacts of N addition on the rate of net nitrification and on N leaching losses. If experimental N inputs lead to N saturation of the MLBS ecosystem, then the rate of net nitrification relative to net N mineralization would be expected to increase, as has been observed in a number of ecosystems experiencing high N inputs (McNulty et al. 1990; Aber et al. 1993; Gilliam, Adams, and Yurish 1996; McNulty, Aber, and Newman 1996; Magill et al. 1997). In addition, leaching losses of N, particularly as nitrate, can be expected to increase if N inputs saturate the capacity of the MLBS ecosystem to retain N (Aber et al. 1989; Aber 1992; Moldan et al. 1995).

2. *Do N pools in the ecosystem, such as soil inorganic N, total soil N, microbial biomass, and understory vegetation increase N retention following experimental N inputs?* Identification of the ecosystem pools that contribute to N retention is essential to understanding the development of N saturation in an ecosystem. The amount of N that can be taken up by ecosystem components such as soil N, vegetation, and microbial populations may determine the likelihood that an ecosystem will become N saturated. In this study I have focussed on soil inorganic N, total soil N, microbial biomass, and understory vegetation as the pools most likely to increase N uptake following N addition. If the increased N inputs do not lead to N saturation (Question #1), then increased uptake of N by one or more of the ecosystem N pools likely explains the maintenance of N-limitation in the ecosystem. Canopy vegetation has been shown to be an important factor

in N retention in other ecosystems (e.g., Nadelhoffer et al. 1995; Magill et al. 1997; Tietema et al. 1998) but N uptake by trees was not measured in this study.

3. *Does P addition increase N retention and slow the development of N saturation in the ecosystem?* An ecosystem's capacity to retain N and limit N leaching into groundwater may be influenced by the availability of other soil nutrients. The role of P in ecosystem responses to elevated N inputs has not been studied. Phosphorus (P) is often in short supply in temperate ecosystems (DiTommaso and Aarssen 1989), and as N availability increases, plant and microbial capacity to take up N may be influenced by P availability. If increased N inputs induce P limitation of vegetation or microbial biomass production, N uptake by plants and N immobilization by microbes may be limited by P availability. In that case, addition of P would result in increased N retention by vegetation and microbial populations, and a reduction in the degree of N saturation of the ecosystem.

Methods

The study was conducted in a *Quercus rubra - Acer rubrum* forest adjacent to the Mountain Lake Biological Station (MLBS) in southwestern Virginia. The plant community is described in greater detail in Chapter 2. Logging in the 1920's gave rise to a relatively even-aged stand with the exception of a few <150 year old *Quercus alba*. The forest is likely still aggrading, which can impact ecosystem responses to nutrient addition. Aggrading ecosystems tend to be highly conservative in their retention of essential nutrients such as N (Bormann and Likens 1994), and can be expected to be capable of receiving elevated N inputs without experiencing leaching losses (Aber et al. 1989; Hedin, Armesto, and Johnson 1995; Emmett et al. 1995).

The mean annual temperature at MLBS is 8° C, ranging from -3° C in January to +19° C in July. The mean annual precipitation is 130.1 cm. Monthly precipitation is highest from May-July, reaching ca. 12 cm. Precipitation is lowest from December-February, during which monthly precipitation is ca. 8.5 cm. The soils are acidic (pH 3.5 - 4.1) and highly weathered. Soil texture is approximately 15% clay, 60% silt, and 25% sand.

A recording station for the National Atmospheric Deposition Program (NADP) located <10 km from the study site reported N inputs as wet deposition of nitrate and ammonium of 4.50 kg N ha^{-1} in 1994, 3.95 kg N ha^{-1} in 1995, and 5.24 kg N ha^{-1} in 1996 (National Atmospheric Deposition Program 1997). N inputs from precipitation have been increasing since data recording began in 1978 (Figure 3.1). Dry deposition of N at MLBS was not measured, though its contribution to N inputs may be as high as 50% of wet deposition levels (Ollinger et al. 1993).

Experimental design

In June 1994 I established a series of eight replicate blocks in a 0.25 km^2 area of relatively homogenous understory vegetation and level topography. I divided each of the eight blocks into four 10m x 10m plots in a randomized complete block design (Figure 3.2) for a total of 32 plots (4 treatments X 8 blocks). I randomly assigned the plots in each block to one of four treatments: +N, +P, +N+P, and control (which received no experimental N or P addition). I established a 1m wide buffer within each plot to minimize edge effects, and confined all measurements to the central $64m^2$. Each plot was separated from adjacent plots by at least 1m. No trenching was done to exclude root extension into adjacent plots.

N (NPK 34-0-0 using commercial NH_4NO_3) and P (NPK 0-22-0 using reagent grade Na_2HPO_4) fertilizer pellets were cast by hand throughout the entire $100m^2$ of a plot. 408 g NH_4NO_3 were added to +N and +N+P plots and 197 g Na_2HPO_4 were added to +P and +N+P plots. Additions were made each month from April through October to simulate the periodic nutrient addition characteristic of atmospheric deposition. The experimental addition amounted to a yearly input of 97 kg N ha^{-1} yr^{-1} and 30 kg P ha^{-1} yr^{-1}. The level of yearly P addition was chosen to equal approximately half of the P contained in above-ground vegetation and the forest floor found in a survey of temperate deciduous forests (Cole and Rapp 1981). Treatments began in July 1994 and continued through October 1996. To minimize foliar burning from fertilizer contact, I brushed any fertilizer settling on the vegetation to the ground using a cotton kitchen mop. All treatment and control plots were mopped.

Field sampling

Soil samples were collected 14 days after the monthly nutrient addition in July 1995 and each month in May-August 1996. The sampling date was chosen half-way between any two nutrient additions so that transient responses to the nutrient pulse were not measured. Three sample points were randomly located in each plot in 4 of the eight blocks in 1995 and in all eight blocks in 1996. In each plot, ~250g of soil was collected from each sample point with a hand trowel to a depth of 10cm. The forest floor and O horizon, where present, were separated from the mineral soil. The three soil samples taken at each sample period from a plot were pooled to yield one composite sample for each plot.

Above-ground biomass production of the understory was sampled between July 10 and July 24, 1996 by harvesting three $4m^2$ subplots within each of the 32 plots. The three subplots were chosen randomly from a pool in which subplots used for species frequency sampling (Chapter 2) were excluded. I did not harvest subplots used for frequency sampling so disturbance associated with biomass harvest would not influence future sampling of the plant community. All individuals < 50cm tall were clipped at ground-level in three randomly located $4m^2$ subplots within each $100m^2$ plots. Regrowth following harvest was minimal. During the harvesting, species were separated and placed in paper bags. Clippings were dried in paper bags at 60° C for 24 hours the day of harvest, then again for 48 hours immediately prior to weighing. For the purpose of analysis, measurements of biomass for the three subplots in each plot were pooled, resulting in one estimate of above-ground biomass for each of the 32 plots.

Biomass of fine roots was sampled in control and +N plots once in 1996. 15 cm deep cores were removed from three randomly located points in each plot using a 9.5 cm diameter hand auger. Roots larger than 5 mm were discarded as they were not expected to respond to 3 years of nutrient addition. Easily separated roots (~5mm-2mm in diameter) were removed, washed, and dried in paper bags at 55°C for 48 hours. The remaining root-soil mixture taken from each core was homogenized, and one quarter of the mixture (by weight) was reserved for collecting small roots. The root-soil mixture was washed through a double layer of sieves, the first sieve 2mm in diameter and the second 0.5mm in diameter. Roots were hand-picked from the material collected on the sieves, oven-dried, and weighed as above. The weight of roots in the homogenized sample for each soil core was estimated by extrapolating from the weights of the roots in the subsample. This amount was added to the weight of easily separated roots to estimate the total root biomass <5 mm in each soil core. The root biomass in the three soil cores in each plot were pooled to provide one estimate of root biomass <5mm in each of the 32 plots.

In May 1996 porous-cup lysimeters (Soil Moisture Corp, Santa Barbara, CA) were installed in +N and control plots in six of the eight blocks. Rocks in the soil prevented installation of lysimeters in two of the blocks. Some tree roots may be encountered between 50-75 cm depths, but the density of roots is very low below 15 cm depths (J. Corbin, pers. observation). Lysimeter cup depth ranged from 45 cm to 98 cm, depending on the depth that bedrock was encountered. Lysimeter cups were surrounded by 200-mesh silica to ensure lysimeter-soil contact sufficient to permit water collection. Water leaching below the rooting zone was collected into the lysimeters by establishing 40 centibars of suction in the lysimeters for 24 hours. 100ml of the water collected in the lysimeter cups after the 24 hour period was removed from the lysimeters via suction and frozen until further analysis. Collections were made once per month from June-September.

Laboratory methods

Soil extractions and incubations - Following sifting (<2mm), six samples of soil (5g each) were collected from each plot's pooled soil sample. Three subsamples were immediately extracted with 50 ml 2.0M KCl for determination of extractable ammonium and nitrate. The other three subsamples were incubated in the laboratory for 14 days at 20°C. Each subsample was incubated in capped polyethylene sample bottles at field moisture. The incubated subsamples were extracted with KCl as above. The filtered extracts were frozen until further analysis.

NO_3-N and NH_4-N concentrations in the KCl extracts were measured using a Bran & Lube (Technicon) Traacs 800 Autoanalyzer. NO_3-N (Method 782-86T) and NH_4-N (Method 780-86T) were analyzed using standard methods for determining ammonium and nitrate in wastewater. ERA standards were used to assure accuracy of the measurements. The ammonium and nitrate contents for the three subplots in each plot were averaged to yield one value for soil ammonium content and one value for soil nitrate content in each of the 32 plots for each sample period.

The water content of each plot's soil sample was determined so that analyses of soil ammonium and nitrate levels could be done based on dry weights. 5g of soil were dried at 60°C for 24 hours. The difference in weight before and after drying was assumed to be due to water loss. Bulk density measurements were made on sieved soil.

Net mineralization of nitrogen was calculated as extractable nitrate + ammonium in the incubated sample minus extractable nitrate + ammonium in the initial extracts. Net nitrification was calculated as incubated minus initial extractable nitrate.

Microbial biomass - N - N content of microbial biomass was determined using the chloroform fumigation-extraction method (Brookes et al. 1985). Four subsamples of soil (10g) were collected from each plot's pooled soil sample. Two subsamples were immediately extracted with 30ml 0.5M K_2SO_4 for determination of initial extractable organic N. The other two soil subsamples were fumigated with 2ml chloroform for 5 days. The fumigated subsamples were extracted with K_2SO_4 as above. K_2SO_4 extracts were frozen until further analysis.

Organic N in the K_2SO_4 extracts was converted to NO_3-N using a persulfate digestion (D'Elia, Steudler, and Corwin 1977) and analyzed for NO_3-N as above.

Soil total N and soil elemental analysis - One subsample of soil (100-120g) from each plot's pooled soil sample was dried and sent to Brookside Laboratories (New Knoxville. Ohio) for analysis of soil total N, extractable P. Ca, K. Mg, and Na , % soil organic matter. and cation exchange capacity (CEC). Total N was analyzed by combustion followed by gas chromotography using a Carlo-Erba 1500 N-C-S Autoanalyzer. Extractable P. Ca, K. Mg, and Na were analyzed using standard Mehlich III methods (Mehlich 1984). The soil organic matter content was analyzed by loss on ignition.

Tissue chemistry - Tissue chemistry was analyzed for three species collected during above-ground biomass harvesting. Following weighing, leaves from *Acer rubrum*. *Amianthium muscaetoxicum*, and *Aster acuminatus* were ground in a Wiley Mill to pass through a 20-mesh screen. Each sample (0.04g) was digested in sulfuric and salicylic acid. using the standard Kjeldahl method (Haynes 1980; Lowther 1980: Wolf 1982), in a block digester (Bran-Luebbe, Elmsford. New York). Within each batch of 40 samples. two sucrose blanks and a range of N and P standards were also digested. Digested samples. blanks. and N and P standards were analyzed for N and P concentration on a continuous flow autoanalyzer (Bran-Luebbe Technicon). Accuracy in each batch was assured using pine needle samples from the National Bureau of Standards.

Lysimeters - Lysimeter samples were analyzed for NO_3-N and NH_4-N on the Bran-Luebbe Technicon autoanalyzer using the same techniques as described above for determination of extractable soil ammonium and nitrate.

Statistical analysis

I performed all statistical analyses using PROC GLM (SAS version 6.10). The experimental design permitted a full factorial analysis, with two levels of N addition (0, 97 kg N ha^{-1} yr^{-1} added), and two levels of P addition (0, 30 kg P ha^{-1} yr^{-1} added). For each measured variable, the effects of N and P addition were tested using analysis of variance (ANOVA). Each ANOVA model included the main effects of block, N, and P, and the N*P interaction: X = Block + N + P + (N*P). In this form, the F-test for N in the ANOVA tests the null hypothesis that there is no difference in the level of the test variable between plots that receive N addition (i.e. +N and +N+P plots) and plots that do not receive N addition (i.e. control and +P plots).

Because extractable ammonium and nitrate, net mineralization and nitrification, microbial N content, and lysimeter ammonium and nitrate concentrations were measured multiple times in 1996, repeated measures analysis of variance (ANOVAR) was used for analysis of these variables in 1996. ANOVAR differs from standard ANOVA analysis in that the variables are tested against different levels of error (Milliken and Johnson 1992). In this analysis, the effects of Block, N, P, and the N*P interaction are tested using the within-plot-error, whereas the effects of Month, Month*N, Month*P, and Month*N*P are tested using the between-plot-error. The tested variables did not violate circularity assumptions, and uncorrected F-statistics were used in the ANOVAR (Milliken and Johnson 1992).

Power analysis was performed on the effect of N addition on soil total N using the SAS macro %POWER (Latour 1992).

Results

Extractable ammonium and nitrate

Extractable ammonium and nitrate levels were significantly higher in plots receiving supplemental N (+N and +N+P plots) than in plots that did not receive N (control and +P plots) (Table 3.1a). The influence of N input changed over the course of the season, however. Ammonium in N-treated plots decreased from May-August 1996 relative to ammonium in plots that did not receive N (Time*N, Table 3.1a; Figure 3.3a). Extracted nitrate in N-treated plots was highly variable through the season. Nitrate in +N plots was at least twice as abundant as in +P and control plots in every sample period except June 1995 and June 1996 (Figure 3.3b). Nitrate in +N+P plots showed a large amount of variation from sample period to sample period (Figure 3.3b). Nitrate in control and +P plots was at or near detection limits (~0.25 μg/g soil) for each sample period and did not change between May and August 1996 (Figure 3.3b).

P addition had no significant effect on either extracted ammonium or nitrate (Table 3.1a). There was no interaction between N and P for either ammonium or nitrate (Table 3.1a), indicating that the response of extractable inorganic N to addition of N or P did not depend on the addition the other nutrient.

Net mineralization and nitrification

Net mineralization of N in 1996 was greater in plots receiving N addition than in plots that did not receive additional N (Table 3.1b; Figure 3.4a).

N addition only affected nitrification in July and August of the third year of N additions, when nitrification in N-fertilized plots was significantly lower than nitrification on plots that did not receive N (July: F=10.57, $p < 0.004$); August: F=6.29, $p < 0.02$). Net nitrification showed a strong seasonal trend through the course of 1996, as the nitrification decreased through the growing season until August, when it increased in all treatments (Month, Table 3.1b; Figure 3.4b). In June and July 1996, net nitrification was negative in all treatments except +P plots in July 1996.

Net nitrification was 2-5% of the total net N mineralization in all treatments, and neither the main effects of N or P nor the interaction between N and P were significant for the proportion of mineralized N that was nitrified.

Lysimeter concentrations of ammonium and nitrate

There was no significant difference in either ammonium (F=1.00, $p < 0.39$) or nitrate (F=1.00, $p < 0.37$) concentrations in groundwater collected from lysimeters between +N and Control plots (the only two treatments into which lysimeters were installed; Figure 3.5). The concentration of nitrate in groundwater decreased significantly (F=4.04, $p<0.04$) over the course of the summer. Loss of N into groundwater occurred primarily as ammonium during the early portion of the sample period.

Soil elemental analysis

Extractable P concentrations were twice as high in plots receiving P addition as compared to plots that did not receive P (Table 3.2). There was no effect of N addition or an interaction between N and P on extractable P. Plots receiving P addition also had greater levels of sodium (Na) and magnesium (Mg) (Table 3.2).

The N content of the mineral soil was not significantly greater in plots receiving additional N (Table 3.2). Variation between samples was high, and power analysis indicated that the probability of detecting a true difference between treatments was only 14%. Neither the main effect of P nor the interaction between N and P were significant in the ANOVA of soil N. N in the soil was strongly related to the amount of soil organic matter ($r^2 = 0.61$, $t = 6.802$, $p < 0.0001$).

Microbial biomass N content

Neither N ($F=0.98$, $p < 0.33$) nor P ($F=0.09$, $p < 0.77$) addition had any effect on microbial-N (Table 3.3). Nor was there a significant N*P interaction ($F=3.04$, $p < 0.096$). The N content of microbial biomass in all treatments increased between July and August (Table 3.3); this increase was significant ($F=35.34$, $p < 0.0001$).

Vegetation N content

Understory vegetation biomass (herbaceous + woody species) was not significantly higher in +N and +N+P plots than in +P and control plots ($F = 3.34$, $p < 0.082$; Figure 3.6). Understory above-ground biomass was significantly lower in plots receiving P addition ($F=13.85$, $p < 0.01$; Figure 3.6). There was no significant interaction between N and P ($F=1.17$; $p< 0.29$).

The concentration of N in the leaf tissue of the three most abundant understory species, *Acer rubrum*, *Amianthium muscaetoxicum*, and *Aster acuminatus*, increased significantly when N was added (Chapter 2, this volume). By taking both the above-ground biomass of these three species (Chapter 2, this volume) and the concentration of N in their leaf tissue into account, I estimated the N stored in the above-ground biomass of the dominant species in the understory (Tables 3.3, 3.4). N storage was greater in N-fertilized plots ($F=5.29$, $p < 0.032$), primarily due to the response of *Aster acuminatus*, which contributed 70 - 90% of the N stored by the 3 species (see Table 3.5 in Chapter 2). The increase in N storage by vegetation was primarily via increased incorporation of N into tissue rather than increased above-ground biomass, though a modest increase in above-ground biomass by *Aster* was observed. Extrapolated to a per hectare basis, N content of these three species in +N and +N+P plots was 4.6 kg N ha^{-1}, as compared to 3.0 kg N ha^{-1} in Control and +P plots.

N storage in the three understory species was significantly lower in plots receiving P addition ($F=7.38$, $p < 0.013$; Tables 3.3, 3.4). There was no interaction between N and P on N storage ($F=0.65$, $p < 0.43$).

Below-ground biomass

Estimated fine root biomass was not significantly higher in +N treatments than in Control treatments ($F=2.85$, $p < 0.19$).

Nitrogen distribution and ecosystem N budget

Soil N was the largest of the pools of N I assessed in the ecosystem, accounting for over ten times the N found in any other measured pool (Table 3.3). Microbial biomass was the next-largest pool, followed by extractable inorganic N and understory vegetation. Though not measured in the present study, N contained in canopy vegetation and below-ground biomass likely represent sizeable amounts of N in the ecosystem.

When N inputs and outputs are compared between plots that received experimental N inputs and plots that did not receive experimental N, a substantial portion of added N remains unaccounted for (Table 3.5). Inputs from wet deposition and experimental N addition totaled 304.7 kg N ha^{-1} during the three years of experimental manipulation. N losses via leaching were not significantly different between the +N and +N+P treatments and the control and +P treatments, so N retention in the ecosystem is assumed to have increased. Based on N retention in a similarly-aged mixed hardwood stand receiving three years of N addition at Harvard Forest (Aber et al. 1993; Magill et al. 1997), I assume that the trees at Mountain Lake are capable of retaining 15-30 kg N ha^{-1} during three years of elevated N inputs. Increased extractable NH_4 and NO_3 pools and N contained in understory vegetation in plots receiving experimental N addition are responsible for the uptake of an additional 5.9 kg N ha^{-1}. N contained in fine root biomass, microbial biomass, and in unextractable portions of the soil did not increase retention. The net result of the response of ecosystem N pools is that 88-93% of the experimental N added was not found in a measured pool.

Discussion

Status of Mountain Lake with respect to nitrogen saturation

Leaching losses of N and increasing rates of net nitrification relative to net N mineralization have been suggested as indications of N saturation (Aber et al. 1989; Aber 1992). Based on measurements of N leaching and net nitrification, I conclude that atmospheric N deposition and experimental N addition have not led to the N saturation of the MLBS ecosystem. There was no significant difference in N losses to groundwater between plots that received N addition (+N and +N+P plots) and plots that did not receive N addition (control and +P plots), assuming similar volume of water passing below the rooting zone. Losses of ammonium early in the summer were likely related to the disturbance of the soil matrix surrounding the lysimeters following installation in May 1996. Delays of 6 months are common between installation and the first soil water collections (Aber et al. 1993); I allowed only 2 months for the soil processes to recover. As the time since installation progressed, the N collected in the lysimeters decreased dramatically (Figure 3.3.3). The possibility exists that significant leaching losses took place in N-amended plots during the winter, as N leaching was only measured during the growing season. Such a pattern of high N losses during the winter would indicate that the ecosystem N retention is in the early stages of N saturation (Aber et al. 1989), and significant leaching losses in the growing season would be expected with continued N inputs.

Net nitrification rates provide further evidence that the MLBS ecosystem is not N-saturated. Net immobilization of nitrate was highly efficient, particularly in plots receiving additional N. Net nitrification was negative for all treatments in two out of four sample periods, and addition of N resulted in even greater sequestration of nitrate. The low proportion of mineralized N that was nitrified (2-5% in all treatments) is characteristic of ecosystems that are strongly N-limited (McNulty et al. 1990; Aber et al. 1993).

The response of the MLBS ecosystem to three years of experimental N addition resembles results from strongly N-limited forests in the northeastern United States. N leaching in a spruce-fir forest at Mount Ascutney, VT (McNulty and Aber 1993) and a mixed hardwoods forest at Harvard Forest, MA (Aber et al. 1993) was essentially nonexistent for the first 3 years of N addition. Although net N mineralization increased following N addition, net nitrification was very low at both sites (Aber et al. 1993; McNulty and Aber 1993). In contrast, N addition to Fernow Experimental Forest, WV (Gilliam, Adams, and Yurish 1996), a pine forest at Harvard Forest (Aber et al. 1993; Magill et al. 1997), and Bear Brook Watersheds, ME (Magill et al. 1996) resulted in increases in lysimeter leaching, net nitrification, and the ratio of net nitrification : net mineralization (Aber et al. 1993 - Pine plots; Gilliam et al. 1996; Magill et al. 1996).

Aber and colleagues (Aber et al. 1989; Aber 1992; Nadelhoffer et al. 1995; Magill et al. 1996) have suggested that differences in the degree of ecosystem N saturation are responsible for the different responses to N addition. Indeed, patterns of nitrification and N leaching at Harvard Forest and Mount Ascutney came to resemble those in other ecosystems when N addition was continued at the N-limited sites for >3 years (McNulty, Aber, and Newman 1996; Magill et al. 1997). For example, after 7 years of N addition at Mount Ascutney, the quantity of N leaching onto resin exchange bags was higher, and the percentage of mineralized N that was nitrified increased (McNulty, Aber, and Newman 1996). Net nitrification was higher in N-amended plots than in control plots at the Harvard Forest mixed hardwood site by the fourth year of experimental N addition, although N leaching losses were nearly zero, even after six years of N addition (Magill et al. 1997).

The positive response of net N mineralization rates and ammonium concentrations to N addition suggests that N cycling in the MLBS ecosystem has begun to change. I expect that as longer-term inputs of N and N mineralization continue to increase ammonium availability, the MLBS ecosystem will begin to experience net nitrification. At that point, processes controlling nitrate immobilization will likely be N saturated and nitrate leaching into groundwater will be detectable throughout the year.

Distribution of N inputs in Mountain Lake ecosystem

The retention of added N in ecosystems is controlled by such processes as N uptake by microbial populations or by vegetation, incorporation of N into soil organic matter, or increasing quantities of N in pools of extractable inorganic N. This study examined the ability of understory vegetation, microbial biomass in the mineral soil, and soil N pools to retain more N under increased N inputs. Of these ecosystem components, extractable inorganic N pools and understory vegetation contained increased N in plots receiving elevated N inputs. However, only a small portion of the N at MLBS could be accounted for in a measured pool (Table 3.5). N uptake by canopy individuals, which was not measured in this study, has been shown to be important in retaining added N (e.g., Magill et al. 1997; Christ et al. 1995). However, it is unlikely that canopy vegetation could be responsible for retention of more than 30 kg N ha^{-1} yr^{-1}.

Though no statistically significant difference was found between soil N pools in N-amended plots and plots that received no experimental N addition, the mean N content of soil in +N and +N+P plots was >400 kg N ha^{-1} greater than in control and +P plots (Table 3.3). The experimentally-added N at MLBS was most likely retained in this large and biogeochemically active pool. Power analysis indicated that the heterogeneity of the soil N pools made detection of a significant difference difficult. Other studies, using application of ^{15}N tracers, have demonstrated the importance of soil N pools for N retention. For example, Nadelhoffer et al. (1995) found that soil organic and mineral horizons were responsible for nearly half of the N retained by the ecosystem. Similarly, Koopmans, Tietema and Boxman (1996) found that the soil to 70cm depth retained 37% of the added ^{15}N in Scots pine and

Douglas fir sites. It is likely that retention of N in soil accounts for a substantial portion of the N retained in the MLBS ecosystem, as well.

Microbial populations play an important role in N retention in disturbed environments (Vitousek and Matson 1984; Vitousek and Matson 1985) and in regulating N availability to vegetation in low-nutrient conditions (Chapin, Vitousek, and Van Cleve 1986). However, the N content of microbial populations at MLBS was not higher in plots receiving experimental N addition. The conclusion that N does not limit microbial N immobilization indicates that microbial populations are not capable of increasing N retention in ecosystems experiencing increased N inputs.

The observation that microbial populations at MLBS do not respond to experimental N additions is consistent with other studies examining microbial response to increased N availability (Gallardo and Schlesinger 1994; Micks 1994; Magill et al. 1997). Gallardo and Schlesinger (1994) concluded that N availability limited microbial biomass in the forest floor of a mixed hardwood forest in North Carolina, though P availability limited microbial biomass in the mineral soil. However, P addition had no impact on microbial N content in the mineral soil at MLBS. Microbial N immobilization at MLBS is most likely limited by the supply of labile carbon (C). As the availability of inorganic N increases and the pool of C substrates for microbial heterotrophic activity decreases, C availability may limit microbial immobilization of N (Aber 1992; Hart et al. 1994). Zak et al. (1994) found evidence of C limitation of microbial activity and N immobilization across a wide range of ecosystem types, including forests. If C limitation of microbial activity is the rule in areas receiving elevated N deposition, then microbial populations will be unable to increase N immobilization as N inputs increase. Increased microbial uptake of N, therefore, will not be an important mechanism for retention of N as N deposition continues.

This study demonstrates the ability of understory vegetation to contribute to N retention in forested ecosystems. The three species analyzed for N content accounted for retention of 1.6 kg N ha^{-1} of the N added to +N and +N+P plots. The estimated biomass of fine roots was unaffected by N treatments, so below-ground storage of N did not increase following N addition, assuming that the concentration of N in fine roots did not change. Understory species, particularly spring ephemerals, have been shown to contribute to N retention during the period in the spring before forest canopy development (Muller and Bormann 1976). Results from MLBS suggest that understory species' ability to take up increased N inputs is an important factor in ecosystem N retention throughout the growing season.

The size of extractable pools of N frequently respond to N addition (Aber et al. 1993; Stuanes, Kjonaas, and van Miegroet 1995; Christ et al. 1995; Gilliam, Adams, and Yurish 1996; Magill et al. 1997), though extractable nitrate is more likely to increase in ecosystems in which N saturation is more developed (Aber 1992; Aber et al. 1993; Magill et al. 1997). Quantities of extractable ammonium and nitrate in soil at MLBS increased following N addition as well, but these pools are unlikely to be responsible for long-term retention. Soil inorganic N is highly reactive, and nitrate, in particular, possesses a strong potential for leaching out of the ecosystem (Schlesinger 1997). Increased availability of inorganic N, however, may result in greater uptake of N by biota, which can result in long-term N retention.

The role of phosphorus in N cycling at MLBS

P addition increased P availability, but had little effect on the cycling of N within the ecosystem. Neither the quantity of extractable ammonium and nitrate nor the rates of net N mineralization and net nitrification were significantly affected by P addition. The effect of P addition on N leaching was not measured since lysimeters were only installed in control and +N plots. However, the lack of a response of either net N mineralization or net nitrification indicates that P addition does not influence N cycling in the MLBS ecosystem.

P addition also had little effect on the ecosystem's response to N addition, as measured by the N*P interaction in the ANOVA models. Even after three years of N addition, P was not a significant factor in ecosystem N dynamics. Continuing N inputs could saturate the ecosystem's demand for N and lead to limitation by another nutrient, such as P, in the future. If primary production becomes P limited, then P availability could control N uptake by plants and microbes and, therefore, how much N is stored by biota.

However, the responses of the understory vegetation and the microbial populations to P addition indicate that P availability will have a negligible impact on the N cycle, even if N is no longer limiting to biota. Microbial biomass-N did not respond to either N or P addition. As I suggest above, immobilization of N at MLBS by the soil microbial pool is most likely governed by the availability of labile C, and changes in P availability would be expected to have few long-term effects on the quantity of labile C. The above-ground storage of N by understory vegetation was lower in P-amended plots, indicating that, far from increasing N storage, P has a negative effect on vegetation N levels. If anything, increasing concentrations of PO_4^{2-} may reduce nitrate's access to anion exchange sites and reduce the soil's capacity to maintain nitrate in the soil and contribute to nitrate leaching losses.

P addition resulted in greater concentrations of Na and Mg in the soil. It is unknown whether these elements played a role in ecosystem responses to P. Na, added to P-amended plots as part of the Na_2PO_4 fertilizer, could have had direct effects on biotic activity in the soil, though the sensitivity of forest soils to elevated Na levels is not well known.

Conclusions

This study indicates that experimental addition of 97 kg N ha^{-1} yr^{-1} for three years was not sufficient to saturate the capacity of the MLBS ecosystem to retain N inputs. Neither N leaching losses nor net nitrification rates were significantly higher in plots that received three years of experimental N inputs than in plots that received no experimental N inputs.

A substantial portion of the added N was not found in any of the measured pools at MLBS. The ecosystem components that were most likely responsible for N retention were soil N, and to a lesser extent canopy vegetation. Inorganic N pools and the understory vegetation increased N content, though the N retained by these pools were relatively minor compared with the level of N inputs. Despite the importance of microbial populations in N cycling and evidence of their role in N retention in other systems, N content of microbial populations at MLBS did not increase following N addition.

The availability of P was hypothesized to influence N cycling and N retention when N inputs are high. However, neither net N mineralization nor net nitrification under high-N conditions varied with P inputs. N content of various ecosystem pools were similarly unaffected by P addition. Though P availability may be important once the ecosystem is N-saturated, the response of the MLBS ecosystem to N addition appears to be unrelated to P inputs.

The fact that N inputs of over 300 kg N ha^{-1} did not lead to N saturation indicates that the current atmospheric N deposition rates, below 10 kg N ha^{-1} yr^{-1}, will not saturate the MLBS ecosystem in the short-term. N-saturated ecosystems have been documented in the southern Appalachians, in such ecosystems as Great Smoky Mountains National Park, TN (Van Miegroet, Lovett, and Cole 1992) and Fernow Experimental Forest, WV (Stoddard 1994; Gilliam, Adams, and Yurish 1996; Adams, Angradi, and Kochenderfer 1997), though N deposition rates at these ecosystems were substantially higher than at MLBS. Wet N deposition to Fernow Experimental Forest is 13 kg N ha^{-1} yr^{-1}, twice the rate of N inputs at MLBS. This study indicates that the phenomenon of N saturation is not taking place across the entire region. Further assessment of the condition of other southern Appalachian ecosystems with respect to N saturation is necessary to better understand the impacts of elevated N deposition on the region.

Literature Cited

Aber J. D. 1992. Nitrogen cycling and nitrogen saturation in temperate forest ecosystems. Trends in Ecology and Evolution 7:220-4.

Aber J. D., Magill A., Boone R., Melillo J. M., Steudler P., Bowden R. 1993. Plant and soil responses to chronic nitrogen additions at the Harvard Forest, MA. Ecological Applications 3:156-66.

Aber J. D., Nadelhoffer K. J., Steudler P., Melillo J. M. 1989. Nitrogen saturation in northern forest ecosystems. BioScience 39:378-86.

Adams M. B., Angradi T. R., Kochenderfer J. N. 1997. Stream water and soil solution responses to 5 years of nitrogen and sulfur additions at the Fernow Experimental Forest, West Virginia. Forest Ecology and Management 95:79-91.

Bormann F.H., Likens G. E. 1994. Pattern and process in a forested ecosystem. New York. Springer-Verlag. 253p.

Brookes P. C., Landman A., Pruden G., Jenkinson D. S. 1985. Chloroform fumigation and the release of soil nitrogen: a rapid and direct extraction method to measure microbial biomass in soil. Soil Biology and Biochemistry 17:837-42.

Chapin F. S., III, Vitousek P. M., Van Cleve K. 1986. The nature of nutrient limitation in plant communities. American Naturalist 127:48-58.

Christ M., Zhang Y., Likens G. E., Driscoll C. T. 1995. Nitrogen retention capacity of a northern hardwood forest soil under ammonium sulfate additions. Ecological Applications 5:802-12.

Cole D.W., Rapp M. 1981. Elemental cycling in forest ecosystems. In: Reichle D.E., editor. Dynamic Principles of Forest Ecosystem. London:Cambridge University Press. p 341-409.

D'Elia C. F., Steudler P., Corwin N. 1977. Determination of total nitrogen in aqueous samples using persulfate digestion. Limnology and Oceanography 22:760-4.

Dise N. B., Wright R. F. 1995. Nitrogen leaching from European forests in relation to nitrogen deposition. Forest Ecology and Management 71:153-61.

DiTommaso A., Aarssen L. W. 1989. Resource manipulations in natural vegetation: a review. Vegetatio 84:9-29.

Emmett B. A., Brittain S. A., Hughes S., Kennedy V. 1995. Nitrogen additions ($NaNO_3$ and NH_4NO_3) at Aber forest, Wales: II. Response of trees and soil nitrogen transformations. Forest Ecology and Management 71:61-73.

Gallardo A., Schlesinger W. H. 1994. Factors limiting microbial biomass in the mineral soil and forest floor of a warm-temperate forest. Soil Biology and Biochemistry 26:1409-15.

Galloway J. N., Likens G. E., Hawley M. E. 1984. Acid precipitation: Natural versus anthropogenic components. Science 226:829-31.

Gilliam F. S., Adams M. B., Yurish B. M. 1996. Ecosystem nutrient responses to chronic nitrogen inputs at Fernow Experimental Forest, West Virginia. Canadian Journal of Forest Research 26:196-205.

Gunderson P. 1995. Nitrogen deposition and leaching in European forests - preliminary results from a data compilation. Water, Air, and Soil Pollution 85:1179-84.

Gunderson P., Rasmussen L. 1995. Nitrogen mobility in a nitrogen limited forest at Klosterhede, Denmark, examined by NH_4NO_3 addition. Forest Ecology and Management 71:75-88.

Hart S. C., Nason G. E., Myrold D. D., Perry D. A. 1994. Dynamics of gross nitrogen transformations in an old-growth forest - the carbon connection. Ecology 75:880-91.

Haynes R. J. 1980. A comparison of two modified Kjeldahl digestion techniques for multi-element plant analysis with conventional wet and dry ashing methods. Communications in Soil Science and Plant Analysis 11:459-67.

Hedin L. O., Armesto J. J., Johnson A. H. 1995. Patterns of nutrient loss from unpolluted. old-grouwth temperate forests: evaluation of biogeochemical theory. Ecology 76:493-509.

Koopmans C. J., Tietema A., Boxman A. W. 1996. The fate of ^{15}N enriched throughfall in two coniferous forest stands at different nitrogen levels. Biogeochemistry 34:19-44.

Latour K.R. 1992. %Power: A simple macro for power and sample size calculations. In: SAS Institute, editor. Cary, NC:SAS Institute, INC. p 1173-7.

Lowther J. R. 1980. Use of a single sulfuric acid-hydrogen peroxide digest for the analysis of *Pinus radiata* needles. Communications in Soil Science and Plant Analysis 11:175-88.

Magill A.H., Downs M. R., Nadelhoffer K. J., Hallett R. A., Aber J. D. 1996. Forest ecosystem response to four years of chronic nitrate and sulfate additions at Bear Brook Watershed, Maine, USA. Forest Ecology and Management 84:29-37.

Magill A. H., Aber J. D., Hendricks J. J., Bowden R. D., Melillo J. M., Steudler P. A. 1997. Biogeochemical response of forest ecosystems to simulated chronic nitrogen deposition. Ecological Applications 7:402-15.

Mayewski P. A., Lyons W. B., Spencer M. J., Twickler M., Dansgaard W., Koci B., Davidson C. I., Honrath R. E. 1988. Sulfate and nitrate concentrations from a south Greenland ice core. Science 232:975-7.

McNulty S., Aber J. D., McLellan T. M., Katt S. M. 1990. Nitrogen cycling in high elevation forests in the northeastern U.S. in relation to nitrogen deposition. Ambio 19:38-40.

McNulty S. G., Aber J. D. 1993. Effects of chronic nitrogen additions on nitrogen cycling in a high-elevation spruce-fir stand. Canadian Journal of Forest Research 23:1252-63.

McNulty S. J., Aber J. D., Newman S. D. 1996. Nitrogen saturation in a high elevation New England spruce-fir stand. Forest Ecology and Management 84:109-21.

Mehlich A. 1984. Mehlich-3 soil test extractant: a modification of Mehlich-2 extractant. Communications in Soil Science and Plant Analysis 15:1409-1416.

Micks P. 1994. Soil respiration and nitrogen applications in two forest stands: short- and long-term responses. Thesis, University of New Hampshire. Durham, New Hampshire.

Milliken G.A., Johnson D. E. 1992. Analysis of messy data: Volume 1 - Designed experiments. London. Chapman and Hall. 473p.

Moldan F., Hultberg H., Nystrom U., Wright R. F. 1995. Nitrogen saturation at Gardsjon, southwestern Sweden, induced by experimental addition of ammonium nitrate. Forest Ecology and Management 71:89-97.

Muller R. N., Bormann F. H. 1976. Role of *Erythronium americanum* Ker. in energy flow and nutrient dynamics of a Northern Hardwood forest. Science 193:1126-8.

Nadelhoffer K. J., Downs M. R., Fry B., Aber J. D., Magill A. H., Mellilo J. M. 1995. The fate of ^{15}N-labelled nitrate additions to a northern hardwood forest in eastern Maine, USA. Oecologia 103:292-301.

National Atmospheric Deposition Program (NRSP-3) / National Trends Network. 1997. NADP/NTN Coordination Office, Natural Resource Ecology Laboratory, Colorodo State University, Fort Collins, CO 80523.

Ollinger S. V., Aber J. D., Lovett G. M., Millham S. E., Lathrop R. G., Ellis J. M. 1993. A spatial model of atmospheric deposition for the northeastern U.S. Ecological Applications 3:459-72.

Peterjohn W. T., Adams M. B., Gilliam F.S. 1996. Symptoms of nitrogen saturation in two central Appalachian hardwood forest ecosystems. Biogeochemistry 35:507-22.

Schlesinger W.H. 1997. Biogeochemistry: An analysis of global change. 2 edition. San Diego. Academic Press. -588p.

Stoddard J.L. 1994. Long-term changes in watershed retention of nitrogen: its causes and aquatic consequences. In: Baker L.A., editor. Environmental chemistry of lakes and reservoirs. Washington D.C.:Advances in Chemistry Series. p 223-83.

Stuanes A. O., Kjonaas O. J., van Miegroet H. 1995. Soil solution response to experimental addition of nitrogen to a forested catchment at Gardsjon, Sweden. Forest Ecology and Management 71:99-110.

Tietema, A., Emmett B.A., Gunderson P., Kjonaas O.J., Koopmans C.J. 1998. The fate of ^{15}N-labelled nitrogen deposition in coniferous forest ecosystems. Forest Ecology and Management 101:19-27.

Van Miegroet H., Lovett G.M., Cole D.W. 1992. Summary and Conclusions - Atmospheric deposition and forest nutrient cycling. In: Johnson D.W., Lindberg S.E., editors. Atmospheric deposition and forest nutrient cycling. New York:Springer-Verlag. p 202-7.

Vitousek P. M., Aber J. D., Howarth R. H., Likens G. E., Matson P. A., Schindler D. W., Schlesinger W. H., Tilman D.G. 1997. Human alterations of the global nitrogen cycle: sources and consequences. Ecological Applications 7:737-50.

Vitousek P. M., Matson P. A. 1984. Mechanisms of nitrogen retention in forested ecosystems: a field experiment. Science 225:51-2.

Vitousek P. M., Matson P. A. 1985. Disturbance, nitrogen availability, and nitrogen losses in an intensively managed loblolly pine plantation. Ecology 66:1360-76.

Wolf B. 1982. A comprehensive system of leaf analyses and its use for dignosing crop nutrient status. Communications in Soil Science and Plant Analysis 13:1035-59.

Wright R. F., van Breeman N. 1995. The NITREX project: an introduction. Forest Ecology and Management 71:1-5.

Wright R. F., Tietema A. 1995. Ecosystem responses to 9 years of nitrogen addition at Sogndal, Norway. Forest Ecology and Management 71:133-42.

Zak D. R., Tilman D., Parmenter R. R., Rice C. W., Fisher F. M., Vose J., Milchunas D., Martin C. W. 1994. Plant production and soil microorganisms in late-successional ecosystems: a continental-scale study. Ecology 75:2333-47.

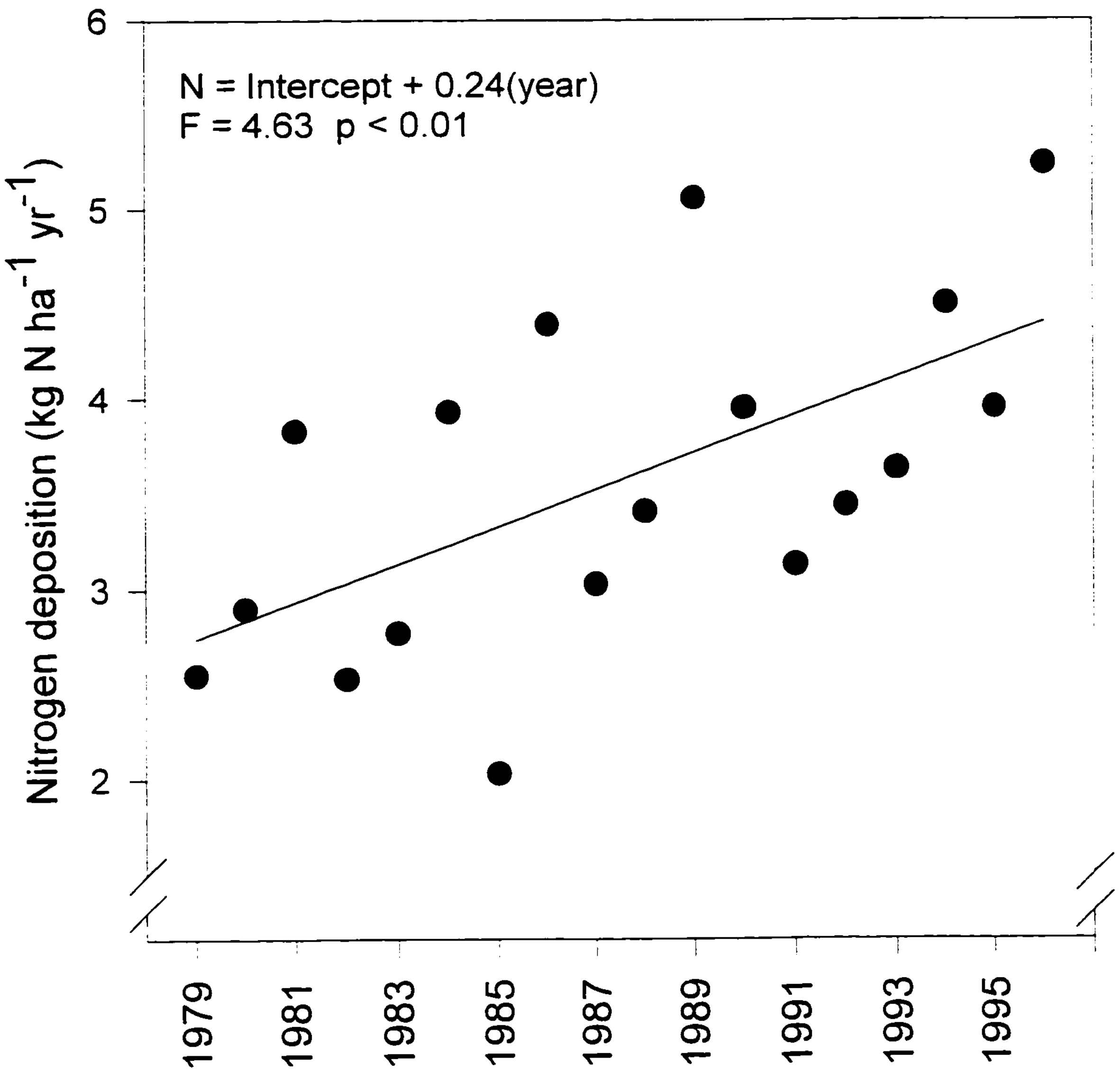

Figure 3.1. Regression of wet deposition of nitrogen at Horton's Station, adjacent to Mountain Lake, VA. The F-test and p-value test the null hypothesis that the slope of the regression=0. Data from National Atmospheric Deposition Program (1997).

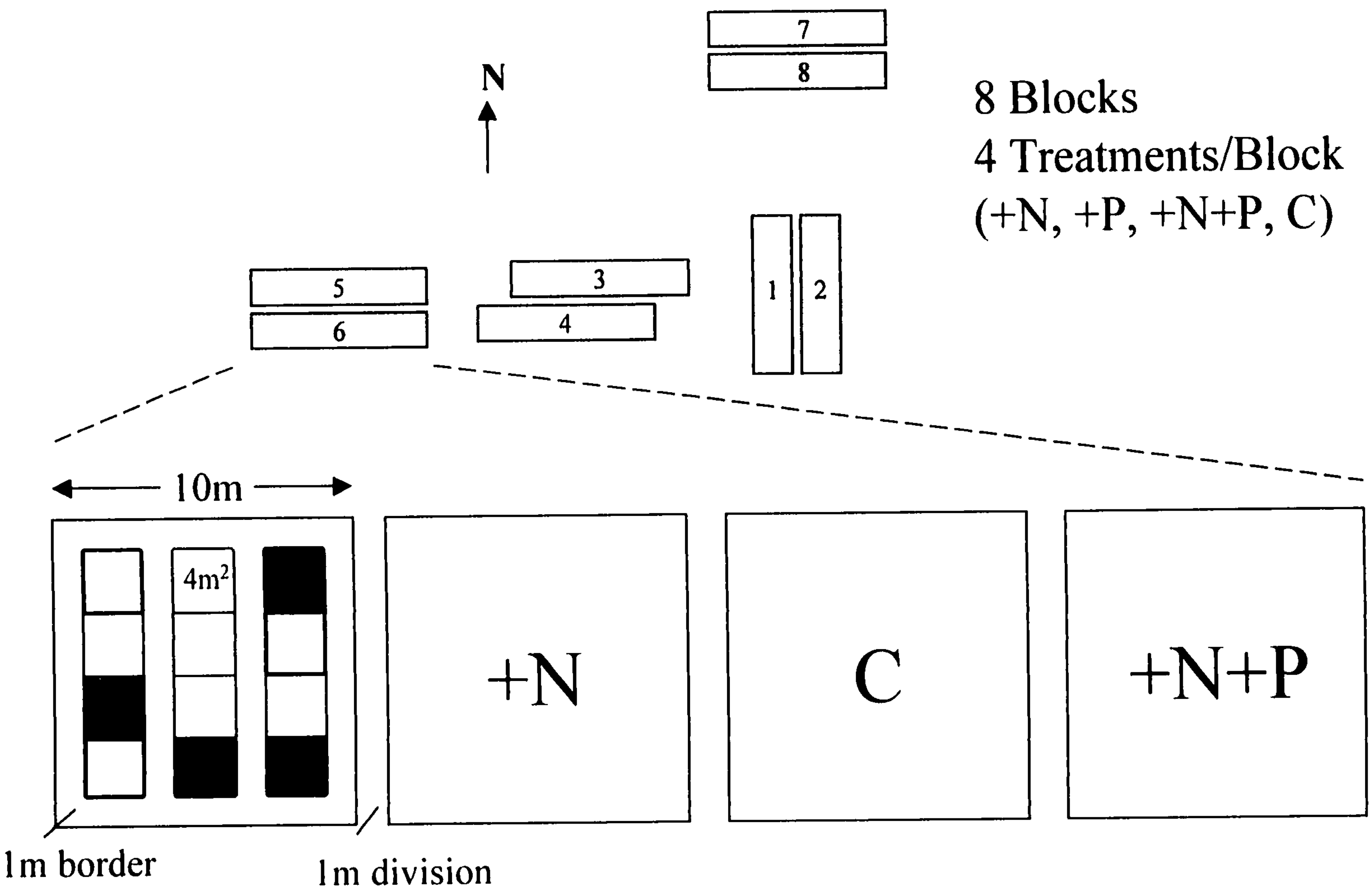

Figure 3.2. Layout of experimental plots at Mountain Lake Biological Station. Below is a detailed illustration of one block, with four experimental treatments, +P, +N, Control, and +N+P. Each plot is divided into 12 4m^2 subplots for biomass sampling.

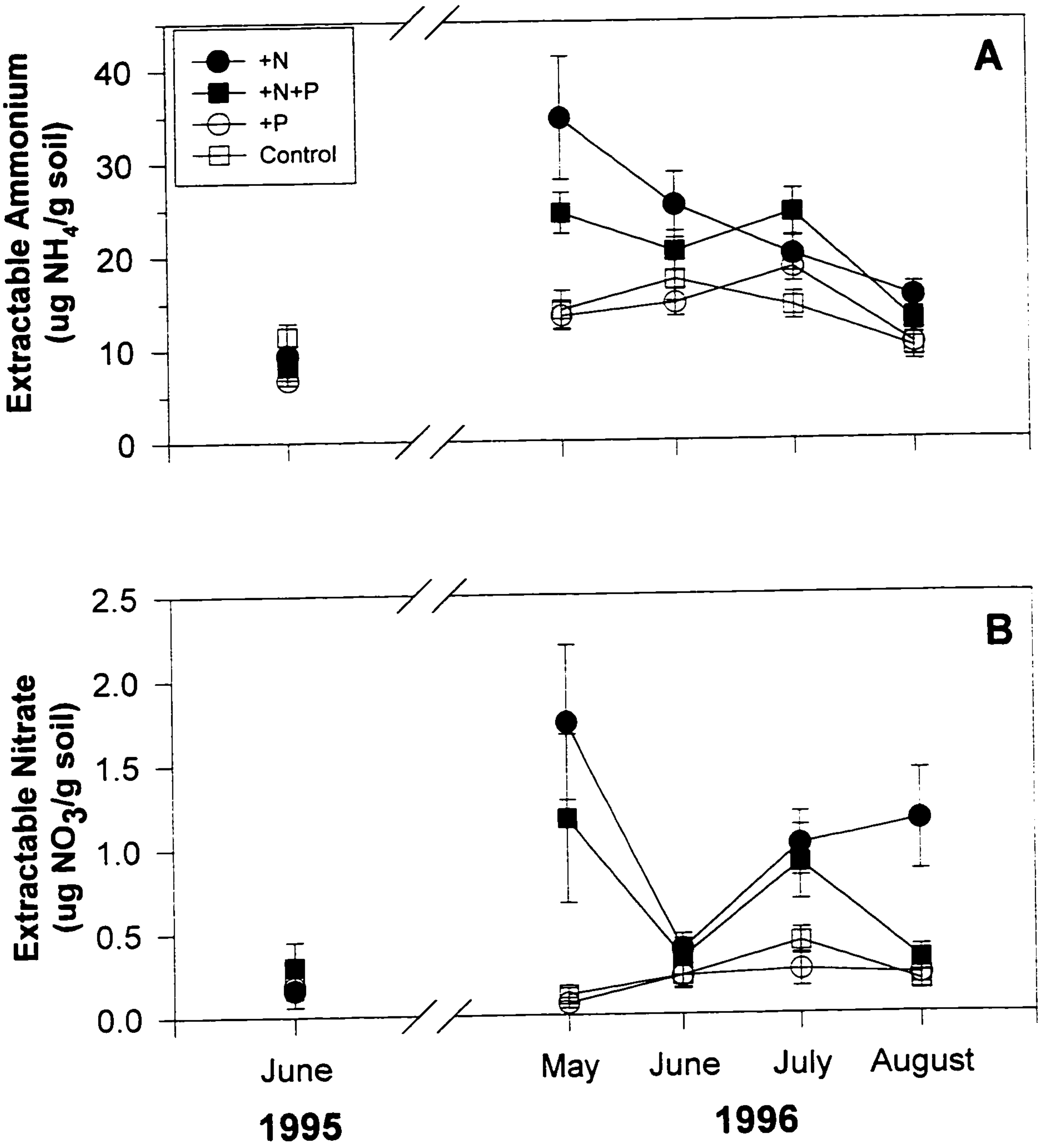

Figure 3.3. Extractable (A) ammonium and (B) nitrate in soil samples at MLBS. Each data point represents the mean of four (1995) or eight (1996) plots. Error bars represent one S.E.

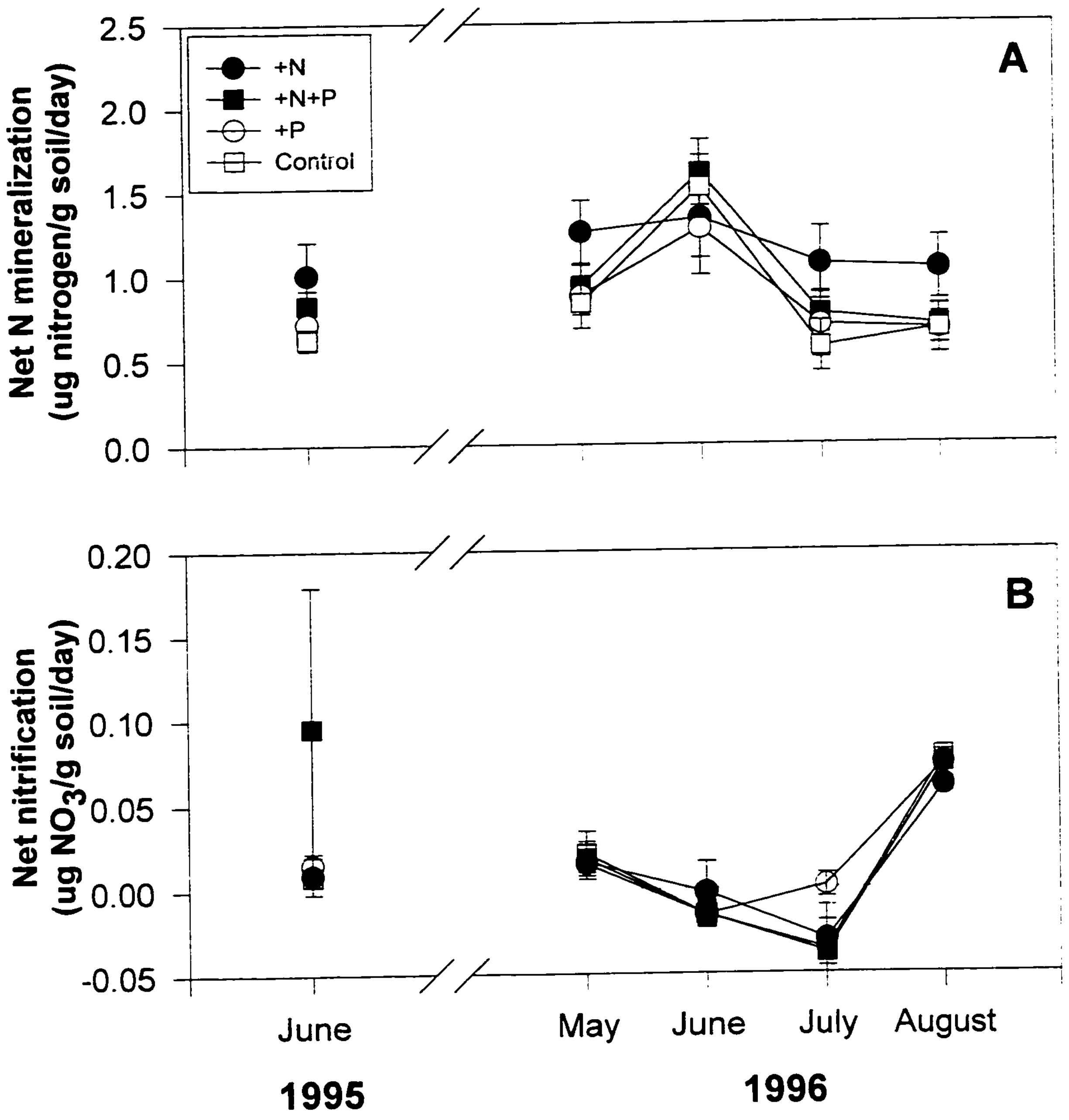

Figure 3.4. (A) Net N mineralization and (B) net nitrification in soil samples at MLBS. Data points represent means of four (1995) or eight (1996) plots. Error bars represent one S.E.

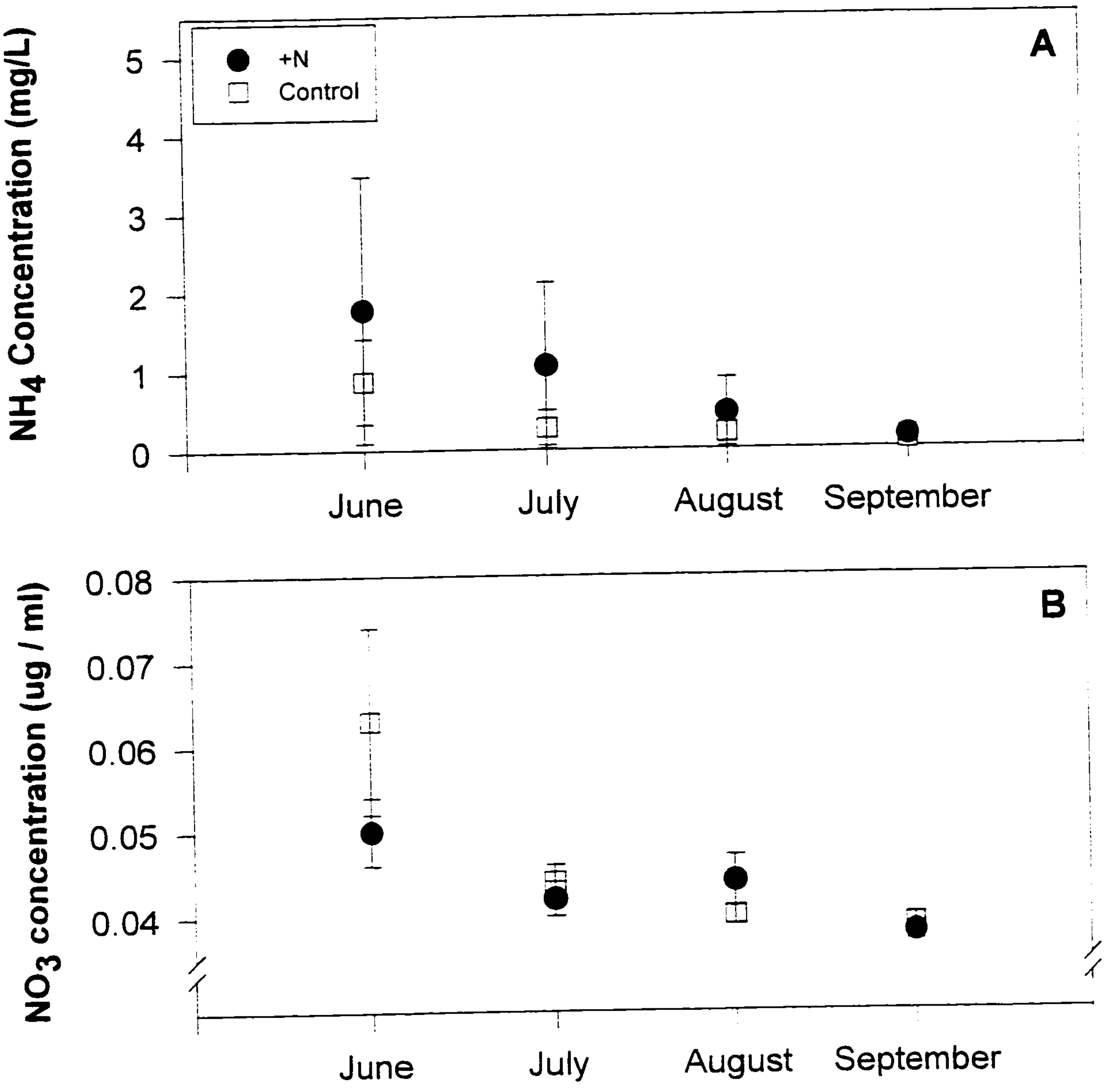

Figure 3.5. (A) Ammonium and (B) nitrate concentrations in 1996 from lysimeters installed at MLBS. Note the different units in each graph. Each data point represents the mean of six plots and one S.E. Lysimeters were installed May 1996.

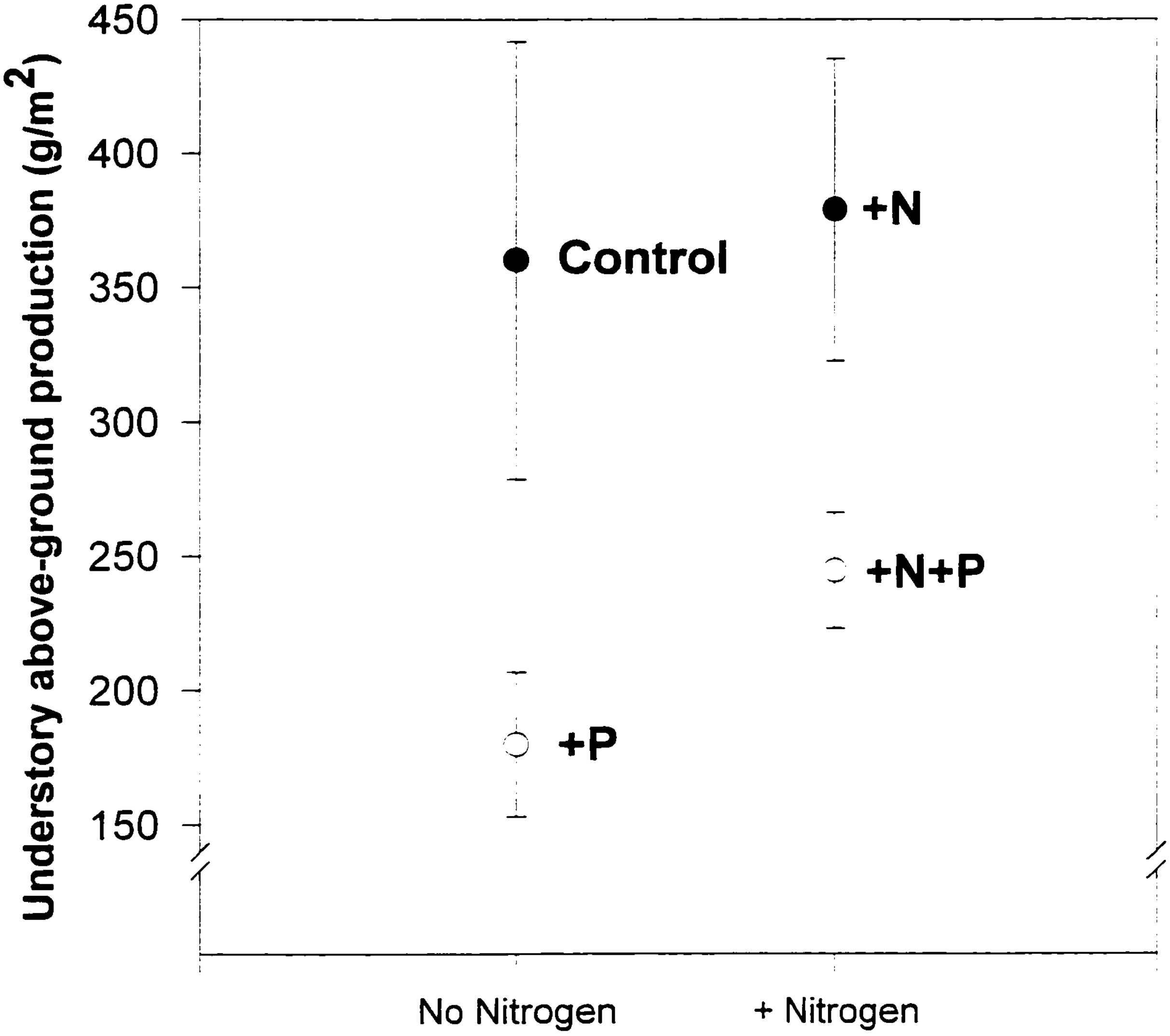

Figure 3.6. Above-ground biomass of all understory species (<50 cm) in 1996. Error bars represent one S.E.

Table 3.1. Repeated measures analysis of variance for (A) Extracted ammonium and nitrate and (B) Net N mineralization and net nitrification in 1996.

(A)		**Extracted Ammonium**		**Extracted Nitrate**	
Source	**d.f.**	**F**	**p < F**	**F**	**p < F**
Block	7	1.44	0.24	0.54	0.79
N	1	58.52	**0.0001**	21.49	**0.0001**
P	1	2.00	0.17	3.66	0.07
N*P	1	3.05	0.10	0.98	0.33
Error (Plot)	21	--	--	--	--
Month	3	10.22	**0.0001**	5.38	**0.009**
Month*Block	21	0.96	0.53	1.52	0.15
Month*N	3	4.06	**0.01**	7.34	**0.002**
Month*P	3	2.66	0.06	0.67	0.51
Month*N*P	3	0.64	0.59	1.97	0.15
Error (Month)	63	--	--	--	--
Total	127	--	--	--	--

(B)		**Net N mineralization**		**Net nitrification**	
Source	**d.f.**	**F**	**p < F**	**F**	**p < F**
Block	7	1.69	0.17	1.15	0.37
N	1	4.65	**0.04**	3.72	0.07
P	1	1.20	0.29	0.02	0.89
N*P	1	0.67	0.42	0.63	0.44
Error (Plot)	21	--	--	--	--
Month	3	14.76	**0.0001**	106.6	**0.0001**
Month*Block	21	1.30	0.21	2.49	**0.003**
Month*N	3	0.27	0.84	3.10	**0.03**
Month*P	3	0.24	0.87	0.37	0.77
Month*N*P	3	1.86	0.14	1.07	0.37
Error (Month)	63	--	--	--	--
Total	127	--	--	--	--

Table 3.2. (A) Soil chemistry in each treatment at Mountain Lake in 1996. (B) F-values for main effects of N and P and N*P interaction for each soil chemistry variable. See Materials and Methods for ANOVA model.

A

	CEC M.E./100g	**Total N kg/ha**	**P kg/ha**	**Na kg/ha**	**Ca kg/ha**	**K kg/ha**	**Mg kg/ha**
Control	7.53	5402	149.5	29.1	483.0	125.2	108.1
+P	8.17	4915	315.8	75.0	603.1	119.0	114.5
+N	8.64	5763	164.6	38.6	630.8	123.2	103.0
+N+P	8.59	5468	368.2	78.4	564.8	129.6	123.2

B

	CEC	**Total N**	**P**	**Na**	**Ca**	**K**	**Mg**
N	0.76	0.83	1.52	1.49	0.55	0.14	0.10
P	0.12	0.61	45.69**	65.92**	0.13	0.00	5.23*
N*P	0.16	0.04	0.46	0.34	1.59	0.29	1.39

* $p<0.05$

** $p<0.0001$

CEC = Cation Exchange Capacity

Table 3.3. N in selected pools in each treatment at Mountain Lake in 1996. Values are means of 8 replicates with SE in parentheses. Units are kg N ha^{-1}.

	Control		+N		+P		+N+P	
Vegetation								
Overstory	..	..	..	..	..	..	..	..
Understory	4.2	(1.0)	5.2	(0.9)	1.8	(0.4)	3.9	(0.4)
Microbial biomass								
July	199.8	(14.8)	223.5	(25.0)	231.2	(21.8)	192.4	(32.6)
August	255.5	(21.1)	256.7	(29.3)	268.9	(17.6)	235.9	(12.3)
Mean	227.6		240.1		250.1		214.2	
Soil N pools[1]								
Extractable NH_4-N	6.60	(0.53)	11.49	(1.11)	7.14	(0.76)	10.09	(0.83)
Extractable NO_3-N	0.12	(0.02)	0.51	(0.07)	0.10	(0.02)	0.35	(0.11)
Total soil N[2]	5402	(297)	5763	(626)	4915	(694)	5468	(200)

[1] Value for each of eight replicate calculated from mean amount of extractable NH_4-N or NO_3-N sampled in all four time periods during 1996.

[2] Total soil N is assumed to include all organic and inorganic N contained in the soil.

Table 3.4. N content in each treatment of three common understory species, and the sum of all three species' N content. N content was calculated by multiplying above-ground biomass by tissue N content. Values are means (in kg N ha^{-1}) of eight replicates with one S.E. in parentheses.

Treatment	***Acer rubrum***	***Amianthium muscaetoxicum***	***Aster acuminatus***	**Sum**
Control	0.33 (0.25)	0.84 (0.22)	3.05 (0.86)	4.22 (1.03)
+N	0.23 (0.12)	0.53 (0.13)	4.47 (0.95)	5.24 (0.94)
+P	0.03 (0.00)	0.24 (0.07)	1.55 (0.41)	1.82 (0.41)
+N+P	0.10 (0.03)	0.19 (0.06)	3.64 (3.80)	3.93 (0.40)

Table 3.5. Inputs, outputs, retention, and distribution of nitrogen in 1996 at Mountain Lake. Distribution of N estimated by the difference in N content of each pool in plots receiving experimental N addition (+N, +N+P plots) and plots that received no experimental N addition (control, +P plots).

	Effect of N addition
N inputs (kg/ha)	
Atmospheric deposition (wet only)	13.7
Fertilization	291.0
Total	**304.7**
Distribution of N (kg/ha)	
Gaseous losses	..
Leaching losses	≈0
Understory vegetation	1.7
Trees	(15-30)[1]
Fine roots	0[2]
Microbial biomass	0
Extractable N	4.2
Soil organic matter	0
Total N measured (kg/ha)	**20.9-35.9**
Percent N unaccounted for	**88-93%**

[1] - Estimated from Aber et al. (1993), Magill et al. (1997)

[2] - Assumes constant N concentration of fine root biomass

Chapter 4

Current and future impacts of atmospheric nitrogen deposition on North American plant communities and ecosystems

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4.1.Introduction

Human release of the precursors of nitrate and ammonium from burning of fossil fuels and from agriculture, respectively, has caused a dramatic increase in atmospheric nitrogen (N) deposition onto terrestrial ecosystems in industrialized areas of the world. Large areas of eastern North America receive as much as 25 kg N ha^{-1} yr^{-1} whereas areas of Europe may receive as much as 50 kg N ha^{-1} yr^{-1} in wet and dry deposition (Aber et al. 1989; Gunderson 1991; Gunderson 1995). N deposition directly downwind of industrial sources may be even greater. In contrast, atmospheric N inputs in areas largely unaffected by human activities are typically 1-2 kg N ha^{-1} yr^{-1} (Galloway, Likens, and Hawley 1984; Aber et al. 1989; National Atmospheric Deposition Program 1997).

Ice cores from Greenland provide historical records of emissions of N from North America and Europe due to the influence of both North American and Eurasian air masses on

Greenland precipitation (Mayewski et al. 1990). The record of nitrate in Greenland ice cores indicates that atmospheric deposition of significant amounts of N is a relatively recent phenomenon (Herron 1982; Lyons and Mayewski 1983; Neftel et al. 1985; Mayewski et al. 1988). Nitrate concentrations in snow deposited in Greenland after 1960 were twice as high as nitrate concentrations in snow deposited in 1767-1903 (Mayewski et al. 1990). Nitrate concentrations during the period 1976-1984 were three times as high as in the period 1767-1903. Although natural sources such as wildfires, volcanic eruptions, or biotic production of nitric and nitrous oxide may influence deposition of nitrate, the pattern of increasing nitrate deposition in Greenland is thought to be human-induced (Risbo, Clausen, and Rasmussen 1981; Herron 1982; Neftel et al. 1985; Mayewski et al. 1990). Ice cores in areas relatively isolated from industrial activity such as Antarctica and the Himalayas do not show a similar trend of increasing nitrate concentrations in recent decades (Lyons and Mayewski 1983).

Changes in nutrient cycling (McNulty et al. 1990; Johnson and Lindberg 1992; DeVries et al. 1995a; DeVries, Leeters, and Hendricks 1995b) and in the composition of plant communities (Aerts and Berendse 1988; Van Breemen and van Dijk 1988; Schultze 1989; Aerts et al. 1990; Bobbink 1991; Johnson et al. 1992; DeVries et al. 1995a; DeVries, Leeters, and Hendricks 1995b) in response to increases in N deposition have been recognized in many ecosystems, particularly in the eastern United States and in northern and central Europe. Though the effects in other regions of the world are not as widespread, N deposition has impacted ecosystems in a variety of locations, including California (Fenn, Poth, and Johnson 1996), the Rocky Mountains (Williams et al. 1996), Japan (Ohrui and Mitchell 1997), and China (Galloway et al. 1996). As population growth and industrialization increase the amounts of fossil fuel burned and the area of land under cultivation, even more regions of the world will experience increased atmospheric N deposition levels (Vitousek and Matson 1993; DeVries et al. 1994; Galloway, Levy, and Kasibhatla 1994; Galloway et al. 1996).

This chapter reviews the impacts of atmospheric N deposition on terrestrial systems in eastern North America and Europe. I describe the patterns of N saturation in the temperate region and factors that contribute to the development of N saturation. I also summarize the effects that atmospheric N deposition has had on terrestrial communities, and the mechanisms by which increased N inputs may lead to community change. I conclude by considering the likelihood of various future changes in community composition in North America.

4.2.Definitions of nitrogen saturation

Atmospheric N inputs to ecosystems can reach levels high enough to fundamentally change the internal cycling of N. Primary productivity in most temperate ecosystems is limited by N availability. Rapid biotic and abiotic uptake of N inputs in N-limited ecosystems limit N outputs and conserve N within the ecosystem (Schlesinger 1997). However, N inputs may exceed the capacity of the biotic and abiotic components of the ecosystem to take up N, and N outputs via leaching loss into groundwater and denitrification become significant. Ecosystems experiencing large losses of N have been described as "nitrogen saturated" (Agren and Bosatta 1988; Aber et al. 1989; Gunderson 1991; Aber 1992).

As Gunderson (1991) and Binkley and Hogberg (1997) point out, the criteria for the identification of N-saturated ecosystems have not been consistent in the literature. Gunderson (Gunderson 1991) defines N saturation as the condition where the availability of mineral N exceeds the capacity of the organisms to absorb N. This definition, unfortunately, ignores the role of abiotic mechanisms of retention that may operate in many ecosystems (e.g., Nadelhoffer et al. 1995; Koopmans, Tietema, and Boxman 1996; Tietema et al. 1998). Aber et al. (1989; Aber 1992) describe N saturation in terms of fundamental changes in ecosystem functioning, such as significant leaching losses of N or elevated rates of net nitrification. Agren and Bosatta (1988) and Binkley and Hogberg (1997) restricted their definition to the hypothesized extreme impact of elevated N deposition, namely 0% retention of N inputs. In such ecosystems, losses of N via denitrification or leaching into groundwater are equal to N inputs from N fixation and atmospheric deposition.

I employ the strictest definition of N saturation, namely that inputs of N into an ecosystem equal the outputs because of the consistency with standard English usage of the word "saturation" (Binkley and Hogberg 1997). Furthermore, a "process"-based definition such as that favored by Aber (1992) relies on comparisons of ecosystem functioning before and after anthropogenic disturbance. Data on ecosystem functioning prior to anthropogenic disturbance, as required for application of Aber's (1992) model, may not be available (i.e.. the rate of nitrate leaching losses in the past). Recognizing that N inputs may change ecosystem processes in important ways prior to the onset of complete N saturation (Aber et al. 1989; Gunderson 1991; Aber 1992), I will characterize ecosystems in which N retention is less than 100%, but in which inputs do not equal outputs, as "nitrogen leaky" (*sensu* Christ et al. 1995).

4.3.To what extent are ecosystems in North America and Europe N-saturated?

In spite of the elevated atmospheric N inputs that much of eastern North America and Europe have received for the last 20-40 years, only a handful of sites in either region appear to experience leaching losses of N similar in magnitude to N inputs. In North America, nitrate leaching losses at or close to measured throughfall inputs were found in two of 16 sites in which input-output budgets were measured: a northern hardwoods forest in Ontario (Foster et al. 1989; Van Miegroet, Lovett, and Cole 1992) and a hardwoods forest in the Cascade Mountains of Washington State dominated by the N-fixing tree, red alder. In addition, a mixed hardwoods forest in West Virginia has been reported to be N saturated (Stoddard 1994; Gilliam, Adams, and Yurish 1996; Peterjohn, Adams, and Gilliam 1996; Adams, Angradi, and Kochenderfer 1997). N saturation of European ecosystems is more frequent; nine of 64 ecosystems measured as part of the "Elemental Cycling and Output-fluxes in Forest Ecosystems in Europe" (ECOFEE) survey were apparently N-saturated (Gunderson 1995).

Although few ecosystems experience leaching losses high enough to qualify as N-saturated, N-leakiness is relatively common in North America and Europe. Van Miegroet, Lovett and Cole (1992) reported that six of the 14 sites in North America that were not N-saturated experienced significant leaching losses of N: two old-growth spruce-fir forests and one beech forest in Great Smoky Mountains National Park in Tennessee, a spruce-fir forest at Whiteface Mountain in New York, a mixed hardwood forest in Huntington Forest, New York, and a loblolly pine forest at Oak Ridge, Tennessee. High-elevation forests in the eastern United States appear to be most vulnerable to N-leakiness, perhaps because of greater levels of N inputs at higher elevations in the east relative to low-elevation sites and other regions in North America. 60% of the sites sampled in Europe by ECOFEE leached more than 5 kg N ha^{-1} yr^{-1} (Gunderson 1995). N-leakiness in Europe is most prevalent in the central and northern parts of the continent (Dise and Wright 1995).

Denitrification is another mechanism by which N may be lost from an ecosystem, but the amount of N lost from most ecosystems via denitrification is small compared to leaching losses of ammonium and nitrate. Denitrification results in the gaseous loss of nitrogen gases such as NO, N_2O, and N_2 from ecosystems. The rate of denitrification is greatest in wet or heavily fertilized soils with high concentrations of nitrate, though denitrification has been shown to occur even in well-drained, upland soils (Schlesinger 1997). Although increases in trace gas flux have been observed following N-fertilization (Emmett, Stevens, and Reynolds 1995), the total loss of N via denitrification in even N-saturated ecosystems has been less than 5 kg N ha^{-1} yr^{-1} (Magill et al. 1997). Such a level of N loss is likely to be of minor importance relative to leaching losses of inorganic N, and the loss of N via denitrification is frequently assumed to be negligible in N-fertilization studies (e.g., Tietema et al. 1998; Koopmans et al. 1995; Christ et al. 1995; Chapter 3, this volume).

4.4. What factors influence the development of N saturation?

Aber et al. (1989; Aber 1992) proposed a conceptual model to summarize ecosystem responses to increased N inputs and the development of N saturation (Table 1). Ecosystem responses to continuing N inputs are described in a series of stages defined by soil N cycling and N retention by the ecosystem. Briefly, as N inputs increase N availability in an ecosystem, the ability of vegetation and microbial populations to take up that new N decreases and N limitation of biotic productivity is replaced by limitation by other environmental factors (such as the availability of water or labile C). As a result, inorganic N pools increase in size and the rate of net nitrification, very close to zero in strongly N-limited ecosystems, increases. The nitrate produced via net nitrification accumulates in the soil and is exposed to leaching loss and transport into groundwater.

A number of landscape-scale studies in North America and Europe have tried to relate N leaching losses to ecosystem characteristics in various watersheds (Van Miegroet, Lovett, and Cole 1992; Dise and Wright 1995; Gunderson 1995). The amount of N entering an ecosystem, the form of N that dominates the N input, and the amount of mineralized N that becomes nitrified are the most important factors in the development of N saturation in an ecosystem:

1) The greater the level of N input, the greater the level of N leaching losses in European ecosystems. The level of N input was a strong predictor of N leaching losses from European sites sampled as part of the Evaluation of Nitrogen and Sulfur Fluxes (ENSF), explaining 69% of the variation in N leaching losses (Dise and Wright 1995). All sites receiving N inputs above 25 kg N ha^{-1} yr^{-1} leached large amounts of nitrate. N deposition rates between 10 and 25 kg N ha^{-1} yr^{-1} displayed wide range of nitrate leaching rates, with some sites retaining N and some sites losing up to 80% of the N inputs via leaching loss. At N-input levels below 10 kg N ha^{-1} yr^{-1}, nitrate leaching losses were minimal. N leaching from ECOFEE sites in Europe showed a similar sensitivity to the level of N input (Gunderson 1995).

   The correlation between N input levels and N leaching losses was much weaker in North American ecosystems (Van Miegroet, Lovett, and Cole 1992). N inputs explained only 11% of the variation in N leaching losses. However, relatively few ecosystems in North America as compared to European ecosystems experience N deposition rates above 25 kg N ha^{-1} yr^{-1} (Figure 1). As a result, few North American ecosystems experience N input rates high enough that variation in ecosystem N retention does not influence N leaching.

Duration of elevated N deposition is another factor that likely affects N leaching losses. The total amount of N input into an ecosystem may play a larger role in ecosystem N cycling than present-day deposition rates. Obviously, historical rates of N deposition are impossible to estimate where no monitoring took place, but extensive regional deposition networks were established in North America and Europe in the mid-1970's (Dise and Wright 1995; National Atmospheric Deposition Program 1997). Estimates of N deposition over time should be factored into future attempts to relate N leaching losses to environmental variables.

2) The capacity of ecosystems to retain ammonium appears to be greater than their ability to retain nitrate. Ecosystems in Europe receiving high levels of ammonium-dominated N inputs were able to retain a higher proportion of the added N than ecosystems receiving nitrate-dominated N inputs. (Dise and Wright 1995; Gunderson 1995). Nitrate is a highly mobile ion that is more vulnerable to leaching into groundwater than ammonium, and its mobility likely explains its heightened impact on ecosystems. The proportion of N deposition as nitrate is greater in North America than in Europe due to differences in the origin of N emissions. A given level of N input may affect North American ecosystems more than European ecosystems receiving similar rates of N deposition due to the greater importance of nitrate deposition in North America.

3) The greater the amount of N and the lower the C:N ratio in the soil, the greater the probability that the ecosystem is N-leaky or N-saturated. Soil C:N ratios were negatively correlated with nitrate losses from ecosystems in North America (Van Miegroet, Lovett, and Cole 1992), southwestern Sweden (Nohrstedt et al. 1996), and Finland (Kortelainen, Saukkonen, and Mattsson 1997). Soil C:N ratios were not sampled in the ENSF study of European ecosystems, but a positive relationship was found between %N in the mineral soil and N leaching losses in Europe (Dise and Wright 1995). High C:N ratios and low %N in the soil favor microbial immobilization of N. Immobilization of ammonium and nitrate by microbial populations can reduce the rate of net nitrification and the amount of nitrate available for leaching (Vitousek and Matson 1985; Davidson, Hart, and Firestone 1992; Hart et al. 1994) and forms a key part of Aber's model of the development of N saturation (Aber et al. 1989; Aber 1992).

4) The land-use and disturbance history in an ecosystem have been suggested as factors in the development of N saturation (Magill et al. 1997; Aber and Driscoll 1997). Land-use practices such as fertilization for agricultural or silvicultural use should increase N pools in the ecosystem, thereby speeding the development of N saturation (see above). Meanwhile, ecosystems recovering from a disturbance such as harvest or fire may experience greater uptake of N by vegetation during the aggrading stage of ecosystem development (*sensu* Bormann and Likens 1994) and lower N losses. Thus far, no consistent relationship between either land-use practices or disturbance history and N leaching has been found. Magill et al. (1997) attributed differences in N retention between pine and hardwood forests in Harvard Forest, MA to the fact that

the pine site was in the process of succeeding from an old field, whereas the hardwood site had never been cleared or treated. However, attempts to relate land-use or disturbance histories to ecosystem N cycling across a range of sites have not been successful. Aber and Driscoll (1997) found no significant relationship between land-use history or disturbance and nitrate leaching in six watersheds in the White Mountains, NH. Kortelainen, Saukkonen, and Mattsson (1997) and Dise and Wright (1995) obtained similar results for Finland and the rest of Europe, respectively. Thus, the importance of land-use history for the development of N saturation in areas experiencing elevated N deposition has not been established.

4.5. What ecosystem pools are responsible for N retention?

The amount of N that leaches out of an ecosystem is strongly influenced by the capacity of biotic and abiotic pools to take up and store inorganic N as it accumulates in the soil solution. An ecosystem's capacity for N retention can be thought of as the sum of the ecosystem components' individual net N storage. The strength of a particular component of the ecosystem to act as a N sink can be demonstrated by comparing the N content of the compartment before and after N addition or in treatment and control areas (e.g., Tamm et al. 1995; Magill et al. 1997; Chapter 3, this volume). The use of ^{15}N tracers have proven to be especially effective in measuring the importance of large and heterogeneous N pools such as soil organic matter (e.g., Nadelhoffer et al. 1995; Koopmans, Tietema, and Boxman 1996; Tietema et al. 1998).

Vegetation

Vegetation has been shown to be an important factor in N retention due to both the amount of N retained and the slow turnover of N in vegetative structures. Forest vegetation is the second largest pool of N in many ecosystems, and is capable of taking up as much as one-third of experimental N additions (Table 2). Once incorporated into biomass, N in vegetation may be isolated from N cycling in the ecosystem for a long period due to the longevity of wood and other plant parts, translocation of nutrients prior to leaf loss, and the resistance of plant litter to decomposition.

Most studies have found that trees allocate a greater proportion of N taken up to foliage than to woody structures (Table 2). The increase in foliar N takes place as both an increase in leaf biomass and an increase in concentration of N in leaf tissue. A significant gap in our understanding of the importance of vegetation in N retention is the role that growth of and N allocation to fine roots plays in N dynamics. Magill et al. (1997) found that increased fine root production was responsible for retention of 6.5-7.8 kg N ha^{-1} yr^{-1} in N-fertilized pine plots and 8.5-12.5 kg N ha^{-1} yr^{-1} in N-fertilized hardwood plots. Conversely, Corbin and Levy (Chapter 3, this volume) found no significant change in fine root production in N-fertilized mixed hardwood plots at Mountain Lake, VA. The inconsistent results between the studies by Magill et al. (1997) and Corbin and Levy (Chapter 3, this volume) may be due to the fact that fine root biomass is a large and heterogeneous N pool. ^{15}N studies, which are better-able to resolve changes in N content of large N pools, may be more appropriate to assess the importance of fine roots in N retention than non-isotope techniques. Future budgets of ecosystem N retention using ^{15}N addition should quantify N retained in fine roots.

The response of fine roots, potentially a large ecosystem N pool, to N addition should be quantified in future budgets.

The capacity of vegetation to take up added N may become saturated by large N inputs or N inputs over an extended period of time. N uptake by vegetation frequently reaches a maximum level beyond which increasing N inputs have little effect. Magill et al (1997) found that vegetation in a red pine forest receiving 324 kg N ha^{-1} over a six-year period took up 103 kg N ha^{-1}, while plots receiving 874 kg N ha^{-1} took up 109 kg N ha^{-1}. The difference between low-N (324 kg N ha^{-1}) and high-N (874 kg N ha^{-1}) treatments in a nearby northern hardwoods forest was 54 kg N ha^{-1}. Christ et al. (1995) found that the amount of N taken up by vegetation was only slightly greater in plots receiving 1040 kg N ha^{-1} than the amount of N taken up by vegetation in plots receiving 320 kg N ha^{-1} at Hubbard Brook, NH. The capacity of vegetation to take up N may decrease over time, as well. Nadelhoffer et al. (1995) found that the amount of ^{15}N assimilated into beech foliage at Bear Brook Watershed, ME during the four-year experiment decreased each year. The attenuation of the amount of N taken up by trees over time or with increasing N inputs is likely the result of vegetation growth by another factor, such as water or a soil nutrient besides N, that places an upper limit on N uptake.

Understory vegetation can contribute to ecosystem N retention, as well. Corbin (Chapter 3, this volume) found that addition of 97 kg N ha^{-1} yr^{-1} resulted in an increase in N storage by the understory plant community by 1.6 kg N ha^{-1} yr^{-1}. N addition increased the N storage by both stimulating biomass production of the dominant understory species, *Aster acuminatus*, and by increasing the concentration of N in leaf tissue of understory species.

Vegetation uptake of N is not an important mechanism of N retention in all ecosystems. For example, Wright and Tietema (1995) added 7 kg N ha^{-1} yr^{-1} for 9 years to a treeless alpine ecosystem receiving <1 kg N ha^{-1} yr^{-1} as atmospheric deposition in Sogndal, Norway. N treatments did not increase the tissue N concentration of the five most common shrub species.

Microbial populations

Microbial populations also represent a large pool of N in the ecosystem, and microbial immobilization of N is an important component of N retention in some ecosystems. In ecosystems in which N availability is low and a large pool of labile carbon (C) is available, microbial populations may immobilize large amounts of added N and prevent N leaching losses. Vitousek and Matson (1985; Vitousek and Andariese 1986) found that microbial biomass was responsible for retention of 69% of ^{15}N added to a clear-cut pine plantation. By comparison, only 16% was retained in soil organic matter and 6% as inorganic N in the plantation (Vitousek and Matson 1985; Vitousek and Andariese 1986). Koopmans, Tietema, and Boxman (1996) estimated that microbial populations took up only 10% of the added ^{15}N in the Netherlands during the first 9 months of N addition. Gallardo and Schlesinger (1994) found that microbial immobilization of N increased with N addition growth in a loblolly pine forest in North Carolina was limited by N in the forest litter layer, though P limited microbial populations in the mineral soil.

Microbial N retention has frequently shown no positive response to N addition. Microbial capacity to take up N is often limited by C availability (Zak et al. 1994), particularly when the availability of inorganic N is already high (Hart et al. 1994). As a result, the capacity of microbial populations to contribute to retention of added N will be low in some systems. Magill et al. (1997) concluded that microbial activity at Harvard Forest did not increase following N addition, based on the observation that soil respiration was not higher in high-N plots than in controls. Corbin (Chapter 3) estimated microbial N-content using the chloroform fumigation-extraction technique in a mixed hardwood ecosystem after three years of fertilization with 97 kg N ha^{-1} yr^{-1} and found no difference in microbial N between the control and N-amended plots.

Thus far, indications are that although microbial populations may play an important role in the initial immobilization of inorganic N in N-limited ecosystems (Vitousek and Matson 1985; Koopmans, Tietema, and Boxman 1996), microbes are not responsible for long-term N-storage. Inorganic N converted into microbial biomass can be quickly remineralized if microbial growth slows or becomes limited by another factor (Koopmans, Tietema, and Boxman 1996). Unfortunately, many studies of ecosystem responses to N addition have failed to expressly test the response of microbial populations (Table 2), and instead lump microbial biomass into the pool of total soil N (e.g., Nadelhoffer et al. 1995; Koopmans, Tietema, and Boxman 1996; Tietema et al. 1998). Future research should focus on the ultimate fate of the N taken up by microbes. Specifically, under what circumstances is N immobilized by microbes remineralized and returned to the inorganic-N pool, and when is the N transferred to a pool capable of long-term retention, such as the soil organic matter?

Soil N pools

^{15}N studies have demonstrated that soil N pools may be responsible for over half of the N retention in some systems (Table 2). Soil N is the largest pool of N in most ecosystems, exceeding the amount of N contained in living biomass (Aber 1992). The turnover of microbial biomass, roots and mycorryizae that have assimilated N results in the accumulation of organic N in the soil (Nadelhoffer et al. 1995; Schlesinger 1997). N may also be incorporated into soil through the abiotic isolation of N in clay minerals or soil organic molecules (Nommik and Vahtras 1982; Vitousek and Matson 1985; Foster et al. 1989). The input of N into soil N pools is balanced by net N mineralization by microbial heterotrophs, resulting in the conversion of organic N back into NH_4-N. The relative importance of these processes in most ecosystems, however, is poorly understood and is deserving of further study.

In general, the capacity of soil layers to retain N decreases as the depth increases. The greatest amount of N retention takes place in the organic layers (Nadelhoffer et al. 1995; Koopmans, Tietema, and Boxman 1996) where microbial activity is likely to be greatest and organic molecules capable of incorporating N into the soil abiotically are likely to be plentiful. Deeper soil depths have less microbial activity and fewer organic molecules to assimilate N into the soil, and hence less N is retained in deeper soil horizons.

Increases in the size of soil inorganic N pools, particularly of ammonium pools, are frequently observed following N addition. For example, extractable ammonium in the pine plot at Harvard Forest receiving 150 kg N ha^{-1} yr^{-1} were 60% higher than extracted ammonium in the control plot after one year of N addition, and 120% higher after six years (Magill et al. 1997). During the six-year time period, extractable nitrate increased five-fold in the pine high-N plot, whereas extractable nitrate decreased in the pine control plot. Similarly increases in inorganic N concentrations occurred in a hardwood forest at Harvard Forest receiving N addition. However, the amount of N retained in inorganic N pools is generally low compared to the N retained in other soil N pools (Vitousek and Matson 1985; Zak et al. 1990). Furthermore, the high reactivity of inorganic N and the likelihood of leaching losses of nitrate make inorganic N pools unlikely mechanisms of long-term N retention. Rather, increases in inorganic N pool sizes following N addition are important because inorganic N is the dominant source of N for biotic uptake. Greater quantities of N in soil solution permits greater biotic uptake of N and N retention until another environmental factor limits biotic activity.

4.6.How has increased atmospheric N deposition impacted plant communities?

European plant communities

Grasslands

Chalk grasslands in the Netherlands are the best examples of communities that have been affected by atmospheric N deposition. Dutch chalk grasslands are among the most species-rich communities in the world, containing a large number of rare and endangered species and supporting, not uncommonly, up to 40 species m^{-2} (Bobbink and Willems 1987). In the last decade, these communities have experienced increasing dominance of *Brachypodium pinnatum* and decreasing species richness (Bobbink and Willems 1987; Bobbink and Willems 1991; Berendse, Aerts, and Bobbink 1994; Bobbink and Roelofs 1995). The increase in N in the chalk grasslands and the concomitant increase in abundance of *Brachypodium* have resulted in the loss of short-lived and low-stature forbs (Bobbink and Willems 1987), bryophytes, and lichens (During and Willems 1986) from these communities. As a result, chalk grasslands are being converted into species-poor communities where forbs species have been out competed by *Brachypodium*.

Experimental addition of N has convincingly demonstrated that the increased N inputs from atmospheric deposition caused the community changes in the Dutch chalk grasslands. Within 3-5 years, addition of 100 kg N ha^{-1} yr^{-1} increases the abundance of *Brachypodium* and decreases species richness (Bobbink, Bik, and Willems 1988; Bobbink 1991; Willems, Peet, and Bik 1993). For example, Bobbink (1991) found that 3 years of N or NPK addition roughly doubled the above-ground biomass of *Brachypodium* relative to Control, +P, and +K plots; meanwhile, the total number of vascular plant species decreased from twenty-seven per 0.25 m^{-2} in control plots to seventeen per 0.25 m^{-2} in N plots.

British chalk grasslands have experienced some increase in *Brachypodium* dominance and a decrease in species richness, but decreasing diversity and increasing dominance by grasses are not as widespread in Britain as in the Netherlands (Pitcairn et al. 1991). Furthermore, neither Morecroft et al. (1994) nor Wilson, Wells, and Sparks (1995) reported significant changes in species dominance or species richness in British chalk grasslands following experimental input of N at rates as high as 80-140 kg N ha^{-1} yr^{-1}.

A number of factors have been suggested to explain the greater decrease in diversity in Dutch chalk grasslands than British chalk grasslands:

1) Chalk grasslands in the Netherlands may have reacted more dramatically to N addition than British grasslands because a greater amount of N has accumulated in the Dutch ecosystems from background N deposition. N deposition levels are higher in the Netherlands than in Britain. Deposition in communities in the Netherlands affected by anthropogenic N addition may reach 50-80 kg N ha^{-1} yr^{-1} (Bobbink and Willems 1987), whereas N deposition in Great Britain is typically below 20 kg N ha^{-1} yr^{-1} (Wilson, Wells, and Sparks 1995);

2) Livestock grazing, used in Britain for grassland management, may be more effective in maintaining species diversity than hay-cutting, used in the Netherlands. Today, most Dutch chalk grasslands are managed with hay-cutting rather than livestock grazing, while the traditional grazing still takes place in the UK (Wilson, Wells, and Sparks 1995). Simulated grazing has been shown to be more successful than hay cutting at controlling *Brachypodium* dominance and maintaining species richness following increased N inputs (Bobbink and Willems 1991; Wilson, Wells, and Sparks 1995);
3) Differences in the pool of species in British and Dutch chalk grasslands may be responsible for the different responses to N addition. *Brachypodium* is absent from many chalk grassland sites in the UK. The grass species that more commonly exist in British chalk grasslands, such as *Bromus erectus*, *Festuca ovina*, *F. rubra* and *Avenula pratense*, may be less able than *Brachypodium* to competitively exclude other species following increased N inputs;
4) Greater P availability in Dutch soils than in British soils may contribute to *Brachypodium* dominance and decreased species richness. Dutch chalk grasslands were fertilized with P in the past (Bobbink, Bik, and Willems 1988), whereas British chalk grasslands were generally not fertilized with P (Wilson, Wells, and Sparks 1995). P availability in British chalk grasslands may be too low for *Brachypodium* to efficiently utilize added N, whereas *Brachypodium* is not limited by P availability in the Netherlands and is able to increase its growth rate following N addition (Morecroft, Sellers, and Lee 1994; Wilson, Wells, and Sparks 1995);

5) Dutch chalk grasslands are more fragmented than the British chalk grasslands, and this fragmentation may make the communities in the Netherlands more susceptible to species invasion.

These five factors, background N inputs, land use/disturbance history, species pool, availability of other essential nutrients, and susceptibility to species invasion, likely mediate community responses to increased N inputs. Differences in grassland management strategies (grazing vs. hay-cutting) and differences in availability of P are the best-supported suggestions for the different responses to elevated N inputs of grasslands in the Netherlands and the UK (Bobbink, Bik, and Willems 1988; Bobbink and Willems 1991; Morecroft, Sellers, and Lee 1994; Wilson, Wells, and Sparks 1995).

Differences in N inputs alone are likely not responsible for the greater decrease in diversity in Dutch communities than in British communities, as experimental N addition of 80-140 kg N ha^{-1} yr^{-1} to British chalk grasslands is not sufficient to transform chalk grasslands (Morecroft, Sellers, and Lee 1994; Wilson, Wells, and Sparks 1995). The absence of *Brachypodium pinnatum* from many British chalk grasslands is not the only explanation, either, as N-fertilization of British grasslands planted with eight common species including *Brachypodium* did not result in either increased growth by *Brachypodium* or decreased species diversity (Wilson, Wells, and Sparks 1995). Competition experiments using chalk grassland species grown in soils from both the Netherlands and Britain under controlled conditions would help explain whether soil conditions or species differences are more important in explaining the greater dominance of *Brachypodium* in Dutch grasslands. If the results in the two soil types are similar, then species differences between the two communities are the most likely explanation for the greater effect that *Brachypodium* has had on Dutch chalk grasslands than British chalk grasslands. In contrast, if *Brachypodium* dominance is greater in Dutch soils, then soil differences such as P availability would be the best explanation.

Experimental N addition to other types of grassland communities in a variety of regions has impacted community dominance and species richness in a similar fashion as in Dutch chalk grasslands. Experimental N addition to calcareous and acidic grasslands in Europe and the United States have resulted in shifts in dominance and decreases in species diversity. Experiments in Great Britain (Thurston 1969; Silvertown 1980; Tilman 1982; Tilman 1985; Tilman et al. 1994; Mountford , Lakhani, and Kirkham 1993) Greece (Elisseou, Veresoglou, and Mamolos 1995), Belgium (Van Hecke, Impens, and Behaeghe 1981), and the United States (Bakelaar and Odum 1978; Tilman 1987; Tilman 1988; Wedin and Tilman 1996; Tilman 1996) have resulted in increases in dominance by grass species and a decline in the number of species, especially forb species, in the community. Some characteristics of grasslands that contribute to the decline in species richness following N addition will be discussed in section 4.7.

Heathlands

Wet and dry heathlands in the Netherlands and the UK have experienced a shift in dominance from heath species, such as the dwarf shrubs *Erica tetralix* and *Calluna vulgaris*, to the grass species *Molinia caerulea*, *Deschampsia flexuosa*, and *Festuca ovina* (Heil and Diemont 1983; Aerts and Berendse 1988; Aerts et al. 1990; Woodin and Farmer 1993). Analysis of aerial photographs suggests that greater than 35% of heathlands in the Netherlands have been replaced by grassland in recent decades (Van Kootwijk and Van der Voet 1989). Although grass species have historically been a component of the understory in heathland vegetation, they have not dominated heathland communities until recently (Heil and Diemont 1983). Increased soil N availability from atmospheric N deposition interacting with periodic disturbances such as drought, frost, and outbreaks of heather beetles (*Lochmaea saturalis*) has been shown to explain the increase in dominance of grasses in the formerly shrub-dominant heathlands of Europe.

Increasing N inputs favor grass dominance in heathlands because grass species' growth rates are greater than shrub species' growth rates in the higher-N conditions created by atmospheric N deposition. Heathland communities are typically characterized by low nutrient availability, and in these conditions shrub species are able to grow faster than grass species (Aerts and Berendse 1988; Aerts et al. 1990). However, a number of studies have demonstrated that, when N and P levels are high, the grass species are capable of higher growth rates and maximum heights than *Erica* or *Calluna* (Heil and Bruggink 1987; Aerts and Berendse 1988; Aerts et al. 1990; Prins, Berdowski, and Latuhihin 1991). Grass species' faster growth rates under high-N conditions created by atmospheric N deposition are an important competitive advantage over shrub species.

Disturbances such as beetle outbreaks facilitate the shift to grass dominance by creating openings in the shrub canopy that can be colonized by grasses. Berdowski and Zeilinga (1987) examined patterns of damage and recovery of a *Calluna* heathland that experienced a beetle outbreak in 1980-1981. The outbreak resulted in widespread mortality of *Calluna* and a mosaic of patches of dead shrubs and surviving shrubs. *Deschampsia* and other grasses expanded in 30% of the plots studied by Berdowski and Zeilinga (1987), especially where *Calluna* damage was severe. Once canopy openings were created by the death or damage of *Calluna* individuals, grass species were capable of faster growth under the new high-N conditions created by the atmospheric N inputs and were more likely to colonize the newly-created gaps.

There is also evidence that atmospheric N deposition has indirectly increased the severity of *Lochmaea* outbreaks. Experimental addition of N has been shown to increase N content of *Calluna* leaves (Heil and Bruggink 1987), and it is likely that insect population dynamics are affected by the nutrient content of available food. Heil and Diemont (1983) found that damage to shrub vegetation from a natural *Lochmaea* outbreak was more severe in N fertilized plots. This suggests that atmospheric N deposition not only affects competitive relationships between the grass and shrub species but also the disturbance regime in the heathland ecosystems.

The decline of shrub vegetation in formerly heathland communities appears to be the result of an interaction and synergy between the increased N inputs and the periodic beetle outbreaks. In spite of grass species' higher growth rates in high-N conditions, replacement of shrubs by grasses is slow in intact shrub canopies where shrub species are able to intercept incoming light and limit the growth of grass seedlings (Aerts et al. 1990; Prins, Berdowski, and Latuhihin 1991). However, disturbance events that open up the shrub canopy and increase light availability to grass seedlings contribute to grass dominance under high-N conditions (Heil and Diemont 1983; Heil and Bruggink 1987; Aerts et al. 1990; Prins, Berdowski, and Latuhihin 1991). Atmospheric N inputs have increased both the availability of N in the ecosystem and the severity of beetle outbreaks, leading to the rapid transformation of the heath shrublands to grass-dominated communities.

European forests

Coniferous forests - Atmospheric N deposition to European coniferous forests leads to increased abundance of species characteristic of N-rich habitats, though species richness in these communities appears to be less sensitive to N addition than in grassland communities. Invasions and increased abundance of the grass *Deschampsia flexuosa*, the most widespread observations, have been reported in coniferous forests in the Netherlands (Van Breemen and van Dijk 1988; DeVries, Leeters, and Hendricks 1995b) and in Sweden, Norway, and Finland (Hogbom and Hogberg 1991; Rosen et al. 1992); (but see Liu and Brakenhielm 1996). The effect of increased *Deschampsia* dominance on species richness has not been addressed.

Long-term (i.e. 10-20 years) experimental addition of N to European coniferous forests has resulted in increased dominance of species characteristic of N-rich habitats. Kellner (1993) examined four boreal forests in central-northern Sweden that had been fertilized with 120 to 600 kg N ha^{-1} yr^{-1} every 5-7 years for 15-20 years. N addition caused a shift in community composition in favor of species characteristic of N-rich habitats. The response was most pronounced at the sites receiving the most N. There was no effect of N addition on species richness, though species turnover resulted in different species present in the most-fertilized treatment than in the control. Rodenkirchen (1995) found, in a 10-year N addition to an acid pine and spruce forest, increased dominance of *Deschampsia* and nitrophilous herbs such as *Epilobium*, and decreased biomass of ericaceous shrubs and cryptogams. Becker et al. (1992) reported that the abundance of nitrophilous species increased in a fir forest from 1969-1989 in plots receiving experimental N addition, as well as plots receiving only background N inputs. The results from the N additions by Kellner

(1993), Rodenkirchen (1995) and Becker (1992) are consistent with the increasing importance of *Deschampsia* and other nitrophilous species seen in unmanipulated coniferous forest communities in Europe.

Broadleaf forests – European broadleaf forests have also experienced increased abundance of species characteristic of N-rich habitats in the last 20 years. Falkengren-Grerup (1986) saw an increase in cover of nitrophilous species such as *Rubus idaeus*, *Chamaenerium angustifolium*, *Aegopodium podagraria*, and *Stellaria nemorum* in Swedish deciduous forests. The number of species in the forests increased. Thimonier, Dupouey, and Timbal (1992) reported an increase in cover of nitrophilous species from 1970-1990 in a *Quercus-Carpinus* forest in northeastern France, but no significant effect on species richness.

A number of short-term (i.e. 2-5 year) studies in broadleaf forests in Europe have found modest changes in species' cover or biomass in response to experimental N addition, but the response is less dramatic than the responses of European grassland, heathland and coniferous forests. N addition to a Swedish forest increased the abundance of *Rubus idaeus* and *Oxalis acetosella* (Gerhart and Kellner 1986). Tyler et al. (1992) and Falkengren-Grerup (1993) studied the effects of N addition to two beech forests in southern Sweden. In one forest, only *Impatiens parviflora* and *Anemone nemorosa* increased cover following N addition; otherwise, the understory vegetation showed little response. At the second site, no species' cover increased, and the cover of a number of species, including *Anemone nemorosa*, decreased. Understory biomass at both sites increased slightly following N addition. The dramatic decrease in species diversity seen in grassland communities or the shift in species composition toward nitrophilous species seen in European heathland and coniferous forest communities has not taken place in Swedish broadleaf forests receiving experimental N inputs.

It is possible that European broadleaf forests are no less vulnerable to increased N inputs than other communities and that experimental studies have not continued long enough for shifts in community composition to take place. Given the observations by Falkengren-Grerup (1986) and Thimonier et al. (1992), that beech and oak forest community composition is shifting in favor of nitrophilous species, it is unlikely that European broadleaf forests are as immune to the impacts of increased N deposition as suggested by the short-term studies of experimental N addition. On the other hand, European broadleaf forests may be less susceptible to community changes following increased N inputs than other European communities. The availability of N in these communities may be less important in structuring vegetation composition than in other European communities, perhaps because of strong light limitation. Another reason that broadleaf forest communities may not respond to N inputs is that species able to invade communities and increase in abundance following N addition are not present in the region.

North American plant communities

Observational studies

The impact of increased atmospheric N deposition has been far less visible in North American communities than in European communities. Attempts to demonstrate the impact of increasing N inputs on North American plant communities are hindered by a paucity of information on past community composition. Detection of community changes taking place on a regional basis may be detected, as the recognition in the early 1980's that populations of red spruce (*Picea rubens*) were declining as a result of acidic precipitation (Johnson and Fernandez 1992) demonstrates. However, future shifts in dominance or declines in species diversity in areas of high N deposition would be more readily identified if permanent plots to which future composition could be compared are established (see Section 4.9, below).

Experimental N addition

Although observations of plant community changes in response to background atmospheric N deposition have not been as dramatic in North America as in Europe, North American plant communities respond to experimental N addition in a similar manner as European communities. Experimental N addition to grasslands in the midwestern United States (Tilman 1984; Tilman 1987; Wedin and Tilman 1996) and the southeastern United States (Bakelaar and Odum 1978; R.K. Peet, unpublished data) result in dramatic decreases in species diversity and species richness, just as reported from N-fertilized European grasslands. Responses of North American forests to experimental N addition have been little studied. The few experiments that have altered N inputs to North American forests and followed plant community responses have seen little response of the community during the experimental period. Corbin (Chapter 2, this volume) added N to a mixed-*Quercus* forest understory in Virginia for 3 years, and found that the abundance and dominance and biomass of the dominant understory herbaceous species, *Aster acuminatus*, increased. Otherwise, the understory biomass and species richness remained unchanged. Wilson and Schure (1993) fertilized a Southern Appalachian forest during the first two years of recovery from a clear-cut harvest and found that the growth of some species was enhanced with NPK addition. Again, the species richness did not vary as a result of the nutrient addition. The results of N addition experiments on southern Appalachian deciduous forests are consistent with the changes in species cover and biomass following N addition to European broadleaf forests.

4.7.What are the mechanisms of community change following increased N inputs?

A variety of mechanisms may be responsible for changes in plant community composition following increased N inputs. No single mechanism is likely to explain the N-induced changes in all North American and European plant communities. However, as I discuss below, tests of the importance of each mechanism should be a goal of future investigations of the effects of increased N availability on plant communities.

Competition

The influence of competitive interactions on species composition has received the greatest attention in attempts to explain community changes following N addition (e.g., Al Mufti et al. 1977; Grime 1979, 1990; Tilman 1982, 1988; Goldberg and Miller 1990; Willems et al. 1993).

Above-ground competition

Decline in species richness in grassland and old-field communities has been related to increased production by competitive dominants (Al Mufti et al. 1977; Grime 1979, 1990; Tilman 1987; Bobbink 1991; Willems et al. 1993). Sharp reductions in light penetration through the herbaceous canopy may lead to the exclusion of some species as light competition intensifies. For example, increased above-ground growth by *Brachypodium pinnatum* decreased light penetration through the vegetation (Bobbink 1991; Willems, Peet, and Bik 1993). Forb species close to the soil surface were lost from the community, probably due to the reduced light levels. Tilman (1987) saw decreasing light availability, decreasing species richness, and increasing height of surviving species with increasing N input levels in an old field community at Cedar Creek, MN. As species capable of increasing growth rates or increasing allocation to photosynthetic machinery increase above-ground production, plants low in the canopy may receive less light and be lost from the community.

Increased above-ground biomass does not necessarily intensify above-ground competition to the extent that species richness declines. Corbin (Chapter 2, this volume) saw an increase in the above-ground biomass of an eastern deciduous forest understory following three years of N addition, but there was no decrease in the number of species. Falkengren-Grerup (1986) also found no decrease in species richness of a Swedish beech forest, despite an increase in the above-ground biomass of the understory. Competition for light in forest understory communities apparently had less of an impact on the species richness than in grassland communities. Forest understory species, well-adapted to low-light environments, may be less sensitive to shading by neighbors than grassland species are.

Above-ground competition would not be expected to increase following N addition to communities that experience no increase in above-ground production following N addition. Ecosystems in which N does not limit primary production, such as N-saturated ecosystems or ecosystems in which the availability of other essential nutrients or water are low, would not be expected to increase above-ground growth in response to increased N inputs. Increased above-ground competition in such ecosystems would not be expected to play a role in changes in species richness of the community

Below-ground competition

Plant species differ in their allocation of resources to roots versus shoots following N addition, and these differences can have a significant impact on community responses to N addition. *Brachypodium pinnatum*, the dominant grass species in Dutch chalk grassland communities, increased its root biomass following N addition (Bobbink 1991). Maintenance of root structures likely provides *Brachypodium* an advantage in competition for other essential nutrients such as P. As N inputs increase above-ground growth by vegetation, the availability of other essential nutrients such as P decreases. Bobbink (1991) and Willems et al. (1993) found that *Brachypodium's* success under high-N conditions can be explained, in part, by the ability of *Brachypodium* to compete effectively for low levels of P in the soil under the high-N conditions. *Brachypodium* is able to take up scarce amounts of soil P and translocate large amounts to rhizomes for storage after the growing season (Bobbink 1991). In contrast, other species in the Dutch chalk grassland communities reduce allocation to roots following N addition and are unable to compete effectively for scarce P (Bobbink 1991). The low availability of P and other essential nutrients in the Dutch chalk grassland community, and the difference in allocation to below-ground structures between *Brachypodium* and other community members likely contributes to the dramatic increase in dominance by *Brachypodium* in the Dutch chalk grasslands.

The only test of the importance of below-ground competition for P in forest communities indicated that competition for P does not become important in the forest community following N addition. Corbin (Chapter 2, this volume) added N and P to a deciduous forest understory for three years. Though N addition increased above-ground production of the understory community (and likely of the canopy vegetation, as well), levels of extractable P in the soil did not decrease in plots receiving N addition. Nor were there any significant differences in species' abundances, species' biomass, or species richness between plots that received both N and P and those that received only N or P. Despite the increased production following N addition, below-ground competition for P did not increase in the deciduous forest community.

pH sensitivity

Increased acidity from N inputs may have direct negative effects on vegetation and contribute to species loss from the community. N addition lowers soil pH by increasing the ionic strength of the soil solution as ammonium is converted into nitrate, and by accelerating the removal of base cations as nitrate is taken up by vegetation or lost from the ecosystem via leaching (Schlesinger 1997). Species' tolerance for low pH can vary, and species' loss from the communities receiving elevated N may be related to sensitivity to low pH. Falkengren-Grerup (1986) reported that changes in species composition in Swedish forests were correlated with decreasing pH. Five species, including *Mercurialis perennis*, *Galium odoratum*, and *Oxalis acetosella*, decreased in cover in the more acidic soils in the period since original sampling in 1949-1970. Falkengren-Grerup (1986) concluded that the decreasing acidity, influenced by atmospheric N deposition, likely contributed to the species' decrease.

The impact of increasing N inputs on soil acidity are likely to be minimal on well-buffered soils. For example, chalk grassland soils have a pH of 7-8 (Bobbink 1991) and are likely well-buffered by abundant Ca. Direct effects of increasing acidity are likely not important factors for the community changes in chalk grassland communities.

Species invasion

Species invasion may have dramatic effects on competitive interactions within a community (Vitousek 1990), and the ability of species to colonize a community has been shown to be influenced by N availability (Heddle and Specht 1975; Gerrish and Mueller-Dombois 1980; Vitousek and Walker 1989). Increased availability of N would permit the invasion of species characteristic of high-N conditions into formally low- or moderate-N habitats. Increased N inputs into European forests may have permitted the colonization of new areas by N-demanding species such as *Deschampsia flexuosa* that would be unable to become established in low-N conditions (Rosen et al. 1992). The consequences of species invasions on communities can be far-reaching, affecting nutrient supply, trophic interactions, and disturbance regime (Vitousek 1990; D'Antonio and Vitousek 1992; Asner and Beatty 1996). To the extent that successful invaders are frequently characterized by fast growth rates under high-N conditions, increased atmospheric N deposition should enhance the success of invaders and contribute to community change.

Species invasion has been less important in Dutch chalk grasslands and heathlands than in European forests. *Brachypodium pinnatum* is a natural component of the Dutch chalk grassland flora, and the grass species dominating heathland communities are frequently found in the shrub understory. There are reports that *Brachypodium* is invading British chalk grasslands, and the introduction of this species may lead to dramatic changes in communities in the UK (Woodin and Farmer 1993).

Nutrient availability and disturbance

The historical availability of nutrients, including N, in a habitat influences how species are able to respond to increases in N inputs. Increased N inputs to high-N habitats would be expected to have a minimal impact on the growth rate of the species in the community or on the community composition. In this case, another environmental factor, such as water or light availability, would be expected to limit vegetation growth and increased N inputs would have minimal impact on growth rates. On the other hand, species in low-N habitats may be unable to respond to increased N availability due to growth limitations (Chapin 1980; Chapin, Vitousek, and Van Cleve 1986). The greatest community response to N addition would be expected at a level of N availability above the point at which species' growth responses to N addition are minimal but below the point at which N no longer limits growth.

The availability of other essential nutrients besides N can influence community responses to N addition, as well. If the availability of nutrients such as P or Ca are low enough that they limit primary productivity, then N inputs would be expected to have minimal impacts on growth of species in the plant community. Communities in habitats with high levels of essential nutrients besides N would be expected to respond more dramatically than communities in habitats with low nutrient levels.

Disturbance frequency and intensity can have strong effects on the direction of community change following N addition. Periodic disturbances that create openings for colonizers or other members of the community may facilitate shifts in community dominance, as in the case of periodic beetle outbreaks in Dutch heathlands (Heil and Diemont 1983; Aerts et al. 1990; Prins, Berdowski, and Latuhihin 1991). Such disturbances that could contribute to community change include grazing, disease, and wind damage. In the absence of the disturbance, species capable of succeeding in the high-N conditions may have difficulty establishing in the community or in attaining resources necessary for growth.

Nutrient availability and disturbance may interact to influence community composition. The combination of periodic beetle outbreaks and high-N conditions that favor grass species has led to rapid replacement of shrub species by grass species in Dutch heathlands (Heil and Diemont 1983; Aerts et al. 1990; Prins, Berdowski, and Latuhihin 1991). The combination of disturbances that permit establishment by species successful in high-N conditions and increasing N inputs can influence community dynamics sufficiently to lead to shifts in dominance.

Disturbances may also limit community change by reducing the success of the dominant species. Disturbances that act as "keystone predators" by impacting the dominant species disproportionately may slow the shift in dominance in the community. For example, grazing has proven more successful in controlling *Brachypodium* dominance in chalk grasslands than hay cutting (Bobbink and Willems 1991; Wilson, Wells, and Sparks 1995), perhaps because *Brachypodium* is the most heavily grazed species in the community. Corbin (Chapter 2, this volume) suggested that deer herbivory on *Aster acuminatus* prevented exclusion of other community members in a deciduous forest understory.

4.8. What communities in North America are most likely to be affected by increasing N deposition?

The forested regions in eastern North America, particularly at high elevation sites, are likely to face changes in community composition because of high levels of N deposition. Changes in community composition are most likely in regions receiving high levels of atmospheric N inputs. The higher the rate of N deposition, the more likely that N availability to vegetation will increase and affect community composition. Deposition levels are highest in the northeastern United States and in areas of the southern Appalachians (Figure 1; National Atmospheric Deposition Program 1997). Furthermore, deposition rates frequently increase with elevation due to increased precipitation and the importance of cloud-borne N at high elevations.

Though community change has not been detected in North American forests in spite of increased levels of N deposition, species capable of increasing dominance exist in forest communities in eastern North America. Species such as *Deschampsia flexuosa* and other grass species capable of invading high-N habitats are present in North American forests. Particular emphasis should be placed in following the community composition of forests where grass species such as *Deschampsia* are important components of the understory and may be increasing in abundance.

Grasslands, prairies, and savannas existing in areas of moderate-high levels of N deposition are especially vulnerable to future community change because many grass species are well-adapted to high-N conditions. The presence of species capable of increasing growth rates following N inputs in a community is another important factor influencing whether a community will be affected by increased N inputs. Prairie communities in the midwestern United States are of considerable conservation importance due to their isolation and high frequency of rare or endangered plants. N deposition rates of over 5 kg N ha^{-1} yr^{-1} (National Atmospheric Deposition Program 1997) in large areas of the Midwest may lead to increasing dominance by a few grass species in the future.

The number of species in *Pinus palustris* savannas in the southeastern United States may reach as high as 50 m^{-2} (Walker and Peet 1983), but continued N inputs may have similar consequences as N deposition has in Dutch chalk grasslands. Diversity in *P. palustris* savannas in North Carolina and Mississippi is sensitive to experimental N addition (R.K. Peet, unpublished data), and these unique communities appear to be especially vulnerable to changes in species composition with continued N deposition. Deposition in the southeastern Coastal Plain is currently 5-10 kg N ha^{-1} yr^{-1}, well below the deposition levels experienced in Dutch grasslands. However, the sensitivity *P. palustris* savannas have shown to N addition warrants careful monitoring for decreasing species richness or loss of forb species.

Grasslands in California are also at risk of community changes due to atmospheric deposition. Many ecosystems downwind of large population centers in California are experiencing high levels of atmospheric N deposition. The increasing N availability may impact communities by favoring grass species and decreasing forb diversity. N addition has been shown to shift California serpentine grassland communities from annual forb-domination to non-native grass domination (Hobbs et al. 1988; Huenneke et al. 1990). Non-serpentine grasslands may be vulnerable, as well, as N inputs continue.

Fragmented communities are especially vulnerable to changes following N inputs. Landscape fragmentation caused by roads, logging, or land conversion may facilitate the invasion and spread of weedy species (Saunders et al. 1991; Hobbs and Huenneke 1992). Such weedy species often possess characteristics that are successful under high-N conditions, such as rapid growth rates, rapid N uptake, and high fecundity. The high-N conditions found in areas of elevated atmospheric N deposition may favor the establishment of weedy species in fragmented habitats and provide the newcomers an advantage in competitive interactions within the community.

Communities where the availabilities of essential nutrients such as P and Ca are too low to permit a growth response to N inputs are unlikely to be affected by increased N deposition. Communities with highly weathered soils as in the piedmont region of the southeastern United States may not show dramatic growth responses to N addition because primary productivity rapidly becomes limited by another nutrient.

4.9.A call for more extensive community monitoring and experimentation

In spite of the possibility that atmospheric N deposition is leading to shifts in the composition of plant communities, the ability to track future community changes is severely limited. Basic community information, such as composition, diversity and , or species' cover is unknown for most areas receiving elevated N deposition. The monitoring of communities for increasing abundance of weedy species, increasing total plant cover, or decreasing diversity is impossible without such baseline data. Attempts should be made to increase the establishment of permanent plots from which regular surveys of community characteristics can be made.

Besides greater monitoring of communities in areas receiving elevated N inputs, future research should focus on the understanding of the susceptibility of North American forest communities to increases in N availability. Thus far, attempts to predict the impact increased N deposition will have on plant communities, especially forest communities, have been limited by the number of studies documenting vegetation responses to N addition. Generalization from a small number of disconnected studies is difficult, and even dangerous. Whereas ecosystem scientists investigating the impact of elevated N deposition on nutrient cycling and ecosystem functioning have set up networks in which sites spanning a range of regions, vegetation types, and levels of background deposition linked into a common experimental design (e.g., Aber et al. 1995 ; Gunderson et al. 1998), studies of community responses to N addition have usually focused on a single site (e.g., Tilman 1987; Chapter 2, this volume). Instead, plant community composition and diversity responses to experimental N addition should be monitored in a variety of sites chosen to maximize variation in vegetation type, level of background N deposition, and soil fertility. This will allow better predictions as to what types of plant communities are most susceptible to changes as N deposition continues. A coordinated nutrient addition network would also suggest likely candidates for species capable of responding aggressively to increases in N availability.

4.10. Conclusions

Atmospheric N deposition has contributed to the development of N-saturation and N-leakiness in a variety of ecosystems in eastern North America and Europe. The rate of N deposition and the amount of N in the soil are positively correlated with the extent of N-leaching losses from ecosystems. Soil N pools and, to a lesser extent, vegetation, are the major ecosystem components responsible for retention of N inputs.

The effects of atmospheric N deposition on plant communities has been most striking in European chalk grasslands and heathland communities. The dominance of grass species has increased dramatically in these communities at the expense of other species. Thus far, responses of North American communities to elevated N inputs have not been reported. However, forests in the eastern United States, where N deposition levels are highest, and grasslands, savannas, and prairies in areas of moderate N deposition levels should be closely monitored for future shifts in community composition or species diversity.

4.11. Literature Cited

Aber J. D. 1992. Nitrogen cycling and nitrogen saturation in temperate forest ecosystems. Trends in Ecology and Evolution 7:220-4.

Aber J. D., Driscoll C. T. 1997. Effects of land use, climate variation, and N deposition on N cycling and C storage in northern hardwood forests. Global Biogeochemical Cycles 11:639-48.

Aber J. D., Nadelhoffer K. J., Steudler P., Melillo J. M. 1989. Nitrogen saturation in northern forest ecosystems. BioScience 39:378-86.

Adams M. B., Angradi T. R., Kochenderfer J. N. 1997. Stream water and soil solution responses to 5 years of nitrogen and sulfur additions at the Fernow Experimental Forest, West Virginia. Forest Ecology and Management 95:79-91.

Aerts R., Berendse F., deCaluwe H., Schmitz M. 1990. Competition in heathland along an experimental gradient of nutrient availability. Oikos 57:310-8.

Aerts R., Berendse F. 1988. The effect of increased nutrient availability on vegetation dynamics in wet heathlands. Vegetatio 76:63-9.

Agren G. I., Bosatta E. 1988. Nitrogen saturation of terrestrial ecosystems. Environmental pollution 54:185-97.

Al Mufti M. M., Sydes C. L., Furness S. B., Grime J. P., Band S. R. 1977. A quantitative analysis of shoot phenology and dominance in herbaceous vegetation. Journal of Ecology 65:759-91.

Asner G. P., Beatty S. W. 1996. Effects of an African grass invasion on Hawaiian shrubland nitrogen biogeochemistry. Plant and Soil 186:205-11.

Bakelaar R. G., Odum E. P. 1978. Community and population level responses to fertilization in an old-field ecosystem. Ecology 59:660-5.

Becker M., Bonneau M., LeTacon F. 1992. Long-term changes in an *Abies alba* forest: natural development compared with response to fertilization. Journal of Vegetation Science 3:467-74.

Berdowski J. J. M., Zeilinga R. 1987. Transition from heathland to grassland: damaging effects of the heather beetle. Journal of Ecology 75:159-75.

Berendse F., Aerts R., Bobbink R. 1994. Atmospheric deposition and its impact on terrestrial ecosystems. In: Vos C.C., Opdam P., editors. Landscape ecology of a stressed environment. London:Chapman and Hall. p 104-21.

Binkley D., Hogberg P. 1997. Does atmospheric deposition of nitrogen threaten Swedish forests? Forest Ecology and Management 92:119-52.

Bobbink R. 1991. Effects of nutrient enrichment in Dutch chalk grasslands. Journal of Applied Ecology 28:28-41.

Bobbink R., Bik L., Willems J. H. 1988. Effects of nitrogen fertilization on vegetation structure and dominance of *Brachypodium pinnatum* (L.) Beauv. in chalk grassland. Acta Botanica Neerlandica 37:231-42.

Bobbink R., Roelofs J.G.M. 1995. Ecological effects of atmospheric deposition on non-forested ecosystems in Western Europe. In: Heij G.J., Erisman J.W., editors. Acid Rain Research: Do we have enough answers? Amsterdam:Elsevier. p 279-92.

Bobbink R., Willems J. H. 1987. Increasing dominance of *Brachypodium pinnatum* (L.) Beauv. in Chalk Grasslands: A threat to a species-rich ecosystem. Biological Conservation 40:301-14.

Bobbink R., Willems J. H. 1991. Impact of different cutting regimes on the performance of *Brachypodium pinnatum* in Dutch Chalk Grassland. Biological Conservation 56:1-21.

Bormann F.H., Likens G. E. 1994. Pattern and process in a forested ecosystem. New York. Springer-Verlag. 253p.

Chapin F. S., III. 1980. The mineral nutrition of wild plants. Annual Review of Ecology and Systematics 11:233-60.

Chapin F. S., III, Vitousek P. M., Van Cleve K. 1986. The nature of nutrient limitation in plant communities. American Naturalist 127:48-58.

Christ M., Zhang Y., Likens G. E., Driscoll C. T. 1995. Nitrogen retention capacity of a northern hardwood forest soil under ammonium sulfate additions. Ecological Applications 5:802-12.

D'Antonio C. M., Vitousek P. M. 1992. Biological invasions by exotic grasses, the grass/fire cycle, and global change. Annual Review of Ecology and Systematics 23:63-87.

Davidson E. A., Hart S. C., Firestone M. K. 1992. Internal cycling of nitrate in soils of a mature coniferous forest. Ecology 73:1148-56.

DeVries W., Leeters E.E.J.M., Hendriks C.M.A., Van Dobben H., Van den Burg J., Boumans L.J.M. 1995a. Large scale impact of acid deposition on forests and forest soils in The Netherlands. In: Heij G.J., Erisman J.W., editors. Acid rain research: Do we have enough answers? Amsterdam:Elsevier. p 261-77.

DeVries W., Leeters E. M., Hendricks C. A. 1995b. Effects of acid deposition on Dutch forest ecosystems. Water, Air, and Soil Pollution 85:1063-8.

DeVries W., Reinds G.J., Posch M., Kamara J. 1994. Simulation of soil response to acidic deposition scenarios in Europe. Water, Air, and Soil Pollution 78:215-46.

Dise N. B., Wright R. F. 1995. Nitrogen leaching from European forests in relation to nitrogen deposition. Forest Ecology and Management 71:153-61.

During H. J., Willems J. H. 1986. The impoverishment of the bryophyte and lichen flora of the Dutch chalk grasslands in the thirty years 1953-1983. Biological Conservation 36:143-58.

Elisseou G. C., Veresoglou D. S., Mamolos A. P. 1995. Vegetation productivity and diversity of acid grasslands in Northern Greece as influenced by winter rainfall and limiting nutrients. Acta Oecologia 16:687-702.

Emmett B.A., Quarmby C. 1991. The effect of harvesting intensity on the fate of applied ^{15}N-ammonium to the organic horizons of a coniferous forest in North Wales. Biogeochemistry 15:47-63.

Emmett B. A., Stevens P. A., Reynolds B. 1995. Factors influencing nitrogen saturation in Sitka spruce stands in Wales, UK. Water, Air, and Soil Pollution 85:1629-34.

Falkengren-Grerup U. 1986. Soil acidification and vegetation changes in deciduous forests in southern Sweden. Oecologia 70:339-47.

Falkengren-Grerup U. 1993. Effects on beech forest species of experimentally enhanced nitrogen deposition. Flora 188:85-91.

Fenn M. E., Poth M. A., Johnson D. W. 1996. Evidence for nitrogen saturation in the San Bernardino Mountains in southern California. Forest Ecology and Management 82:211-30.

Foster N. W., Hazlett P. W., Nicolson J. A., Morrison I. K. 1989. Ion leaching from a sugar maple forest in response to acidic deposition and nitrification. Water, Air, and Soil Pollution 48:251-62.

Gallardo A., Schlesinger W. H. 1994. Factors limiting microbial biomass in the mineral soil and forest floor of a warm-temperate forest. Soil Biology and Biochemistry 26:1409-15.

Galloway J. N., Dianwu Z., Thomson V. E., Chang L. H. 1996. Nitrogen mobility in the United States of America and the People's Republic of China. Atmospheric Environment 30:1551-61.

Galloway J. N., Levy I. I., Kasibhatla P. S. 1994. Year 2020: Consequences of population growth and development on deposition of oxidized nitrogen. Ambio 23:120-3.

Galloway J. N., Likens G. E., Hawley M. E. 1984. Acid precipitation: Natural versus anthropogenic components. Science 226:829-31.

Gerhart K., Kellner O. 1986. Effects of nitrogen fertilizers on the field- and bottom-layer species in some Swedish coniferous forests. *Meddelanden fran Vaxtbiologiska Institutionen*, 1986:1, pp. 1-47. Institute of Ecological Botany, Uppsala, Sweden.

Gerrish G., Mueller-Dombois D. 1980. Behavior of native and non-native plants in two tropical rain forests on Oahu, Hawaiian Islands. Phytocoenologia 8:237-295.

Gilliam F. S., Adams M. B., Yurish B. M. 1996. Ecosystem nutrient responses to chronic nitrogen inputs at Fernow Experimental Forest, West Virginia. Canadian Journal of Forest Research 26:196-205.

Goldberg D.E., Miller T.E. 1990. Effects of different resource additions on species diversity in an annual plant community. Ecology 71:213-225.

Grime J.P. 1979. Plant strategies and vegetation processes. Chinchester, UK. John Wiley and Sons. 222p.

Grime J.P. 1990. Mechanisms promoting floristic diversity in calcareous grasslands. In: Hillier S.H., Walton D.W.H., Wells D.A., editors. Calcareous grasslands: ecology and management. Bluntisham:Bluntisham Books. p 51-6.

Gunderson P. 1991. Nitrogen deposition and the forest nitrogen cycle: role of denitrification. Forest Ecology and Management 44:15-28.

Gunderson P. 1995. Nitrogen deposition and leaching in European forests - preliminary results from a data compilation. Water, Air, and Soil Pollution 85:1179-84.

Gunderson P., Emmett B. A., Kjonaas O. J., Koopmans C. J., Tietema A. 1998. Impact of nitrogen deposition on nitrogen cycling in forests: a synthesis of NITREX data. Forest Ecology and Management 101:37-55.

Hart S. C., Nason G. E., Myrold D. D., Perry D. A. 1994. Dynamics of gross nitrogen transformations in an old-growth forest - the carbon connection. Ecology 75:880-91.

Heddle E.M., Specht R.L. 1975. Dark Island Heath (Ninety-Mile Plain, South Australia), VIII. The effects of fertilizers on composition and growth. Australian Journal of Botany 23:151-164.

Heil G. W., Bruggink M. 1987. Competition for nutrients between *Calluna vulgaris* (L.) Hull and *Molinia caerulea* (L.) Moench. Oecologia 73:105-8.

Heil G. W., Diemont W. H. 1983. Raised nutrient levels change heathland into grassland. Vegetatio 53:113-20.

Herron M. M. 1982. Impurity sources of F^- , Cl^-, NO^{3-}, and SO^{4-} in Greenland and Antarctic precipitation. Journal of Geophysical Research 87:3052-60.

Hobbs R.J. Huenneke L.F. 1992. Disturbance, diversity, and invasion: implications for conservation. Conservation Biology 6:324-337.

Hobbs R.J., Gulmon S.L., Hobbs V.J., Mooney H.A. 1988. Effects of fertilizer addition and subsequent gopher disturbance on serpentine annual grassland dynamics in Northern California. Oecologia 75:291-295.

Hogbom L., Hogberg P. 1991. Nitrate nutrition of *Deschampsia flexuosa* (L.) Trin. in relation to nitrogen deposition in Sweden. Ecologia 87:488-94.

Huenneke L.F., Hamburg S.P., Koide R., Mooney H.A., Vitousek P.M. 1990. Effects of soil resources on plant invasion and community structure in Californian serpentine grassland. Ecology 71:478-491.

Johnson A.H., McLaughlin S.B., Adams M.B., Cook E.R., Hayes D.H., Eagar C., Fernandez I.J., Johnson D.W., Kohut R.J., Mohnen V.A., and others. 1992. Synthesis and conclusions from epidemiological and mechanistic studies of Red Spruce decline. In: Eagar C., Adams M.B., editors. Ecology and decline of the Red Spruce in the Eastern United States. New York, New York:Springer-Verlag. p 385-411.

Johnson D. W., Lindberg S. E. 1992. Atmospheric deposition and forest nutrient cycling. New York:Springer-Verlag. 707pp.

Johnson D.W., Fernandez I.J. 1992. Soil mediated effects of atmospheric deposition of eastern U.S. Spruce-Fir forests. In: Eagar C., Adams M.B., editors. Ecology and decline of the Red Spruce in the Eastern United States. New York. New York:Springer-Verlag. p 235-70.

Kellner O. 1993. Effects on associated flora of sylvicultural nitrogen fertilization repeated at long intervals. Journal of Applied Ecology 30:563-74.

Koopmans C.J., Lubrecht W.C., Tietema A. 1995. Nitrogen transformations in two nitrogen saturated forest ecosystems subjected to an experimental decrease in nitrogen deposition. Plant and Soil 175:205-218.

Koopmans C. J., Tietema A., Boxman A. W. 1996. The fate of ^{15}N enriched throughfall in two coniferous forest stands at different nitrogen levels. Biogeochemistry 34:19-44.

Kortelainen P., Saukkonen S., Mattsson T. 1997. Leaching of nitrogen from forested catchments in Finland. Global Biogeochemical Cycles 11:627-38.

Liu Q., Brakenhielm S. 1996. Variability of plant species diversity in Swedish natural forest and its relation to atmospheric deposition. Vegetatio 125:163-72.

Lyons W., Mayewski P. A. 1983. Nitrate plus nitrite concentrations in a Himalayan ice core. Geophys.Res.Letters 10:1160-3.

Magill A. H., Aber J. D., Hendricks J. J., Bowden R. D., Melillo J. M., Steudler P. A. 1997. Biogeochemical response of forest ecosystems to simulated chronic nitrogen deposition. Ecological Applications 7:402-15.

Magusha A.G., Pluth D.J. 1994. Distribution and recovery of ^{15}N-urea in a tamarack - black spruce mixed stand on a drained minerotrophic peatland. Forest Ecology and Management 68:353-363.

Mayewski P. A., Lyons W. B., Spencer M. J., Twickler M., Dansgaard W., Koci B., Davidson C. I., Honrath R. E. 1988. Sulfate and nitrate concentrations from a south Greenland ice core. Science 232:975-7.

Mayewski P. A., Lyons W. B., Spencer M. J., Twickler M. S., Beck C. F., Whitlow S. 1990. An ice-core record of atmospheric response to anthropogenic sulfate and nitrate. Nature 346:554-6.

McNulty S., Aber J. D., McLellan T. M., Katt S. M. 1990. Nitrogen cycling in high elevation forests in the northeastern U.S. in relation to nitrogen deposition. Ambio 19:38-40.

Morecroft M. D., Sellers E. K., Lee J. A. 1994. An experimental investigation into the effects of atmospheric nitrogen deposition on two semi-natural grasslands. Journal of Ecology 82:475-83.

Mountford J.O., Lakhani K.H., Kirkham F.W. 1993. Experimental assessment of the effects of nitrogen addition under hay-cutting and aftermath grazing on the vegetation of meadows on a Somerset peat moor. Journal of Applied Ecology 30:321-332.

Nadelhoffer K. J., Downs M. R., Fry B., Aber J. D., Magill A. H., Mellilo J. M. 1995. The fate of ^{15}N-labelled nitrate additions to a northern hardwood forest in eastern Maine, USA. Oecologia 103:292-301.

National National Atmospheric Deposition Program (NRSP-3) / National Trends Network. 1997. NADP/NTN Coordination Office, Natural Resource Ecology Laboratory, Colorodo State University, Fort Collins, CO 80523.

Neftel A., Beer J., Oeschger H., Zurcher F., Finkel R. C. 1985. Sulfate and nitrate concentrations in snow from south Greenland 1895-1978. Nature 314:611-3.

Nohrstedt H. O., Sikstrom U., Ring E., Nasholm T., Hogberg P., Persson T. 1996. Nitrate in soil water in three Norway spruce stands in southwestern Sweden as related to nitrogen deposition and soil, stand, and foliage properties. Canadian Journal of Forest Research 26:836-48.

Nommik H., Vahtras K. 1982. Retention and fixation of ammonium and ammonia in soils. In: Stevenson F., editor. Nitrogen in agricultural soils. Madison, Wisconsin:American Society of Agronomy. p 123-71.

Ohrui K., Mitchell M. J. 1997. Nitrogen saturation in Japanese forested watershed. Ecological Applications 7:391-401.

Peterjohn W. T., Adams M. B., Gilliam F.S. 1996. Symptoms of nitrogen saturation in two central Appalachian hardwood forest ecosystems. Biogeochemistry 35:507-22.

Pitcairn C.E.R., Fowler D. Grace J. 1991. Changes in species composition of semi-natural vegetation associated with the increase in atmospheric inputs of nitrogen. CSD Report No. 1246 to Nature Conservancy Council, Petersborough.

Preston C.M., Marshall V.G., McCullough K., Mead D.J. 1990. Fate of ^{15}N-labelled fertilizer applied on snow at forest sites in British Columbia (Canada). Canadian Journal of Forest Research 20:1583-1592.

Prins A. H., Berdowski J. J. M., Latuhihin M. J. 1991. Effects of NH_4 fertilization on the maintenance of a *Calluna vulgaris* vegetation. Acta Botanica Neerlandica 40:269-79.

Risbo T., Clausen H. B., Rasmussen K. L. 1981. Supernovae and nitrate in the Greenland Ice Sheet. Nature 294:637-9.

Rodenkirchen H. 1995. Nutrient pools and fluxes of the ground vegetation in coniferous forests due to fertilizing, liming, and amelioration. Plant and Soil 168-169:383-90.

Rosen K., Gunderson P., Tegnhammer L., Johnansson M., Frogner T. 1992. Nitrogen enrichment of Nordic forests: the concept of critical loads. Ambio 21:364-8.

Saunders D.A., Hobbs R.J., Margules C.R. 1991. Biological consequences of ecosystem fragmentation. Conservation Biology 5:18-32.

Schlesinger W.H. 1997. Biogeochemistry: An analysis of global change. 2 edition. San Diego. Academic Press. -588p.

Schultze E. D. 1989. Air pollution and forest decline in a Spruce (*Picea abies*) forest. Science 244:776-83.

Silvertown J. W. 1980. The dynamics of a grassland ecosystem: botanical equilibrium in the Park Grass Experiment. Journal of Applied Ecology 17:491-504.

Stoddard J.L. 1994. Long-term changes in watershed retention of nitrogen: its causes and aquatic consequences. In: Baker L.A., editor. Environmental chemistry of lakes and reservoirs. Washington D.C.:Advances in Chemistry Series. p 223-83.

Tamm C.O.. Aronsson A.. Popovic B. 1995. Nitrogen saturation in a long-term forest experiment with annual additions of nitrogen. Water. Air. and Soil Pollution 85:1683-8.

Thimonier A.. Dupouey J. L.. Timbal J. 1992. Floristic changes in the herb-layer vegetation of a deciduous forest in the Lorraine Plain under the influence of atmospheric deposition. Forest Ecology and Management 55:149-67.

Thurston J.M. 1969. The effect of liming and fertilizers on the botanical composition of a permanent grassland. and on the yield of hay. In: Rorison I.. editor. Ecological aspects of the mineral nutrition of plants. Oxford:Blackwell Scientific Publications. p 3-10.

Tietema. A.. Emmett B.A.. Gunderson P.. Kjonaas O.J.. Koopmans C.J. 1998. The fate of ^{15}N-labelled nitrogen deposition in coniferous forest ecosystems. Forest Ecology and Management 101:19-27.

Tilman D.. Dodd M.E.. Silvertown J.. Poulton P.R.. Johnston A.E.. Crawley M.J.. Leigh R.A. 1994. The Park Grass Experiment: Insights from the most long-term ecological study. In: Leigh R.A.. Johnston A.E.. editors. Long-term experiments in agricultural and ecological sciences. Oxon. UK:CAB International. p 287-303.

Tilman D. 1982. Resource competition and community structure. Princeton. NJ. Princeton University Press. 296p.

Tilman D. 1985. The resource ratio hypothesis of plant succession. American Naturalist 125:827-52.

Tilman D. 1987. Secondary succession and the pattern of plant dominance along experimental nitrogen gradients. Ecological Monographs 57:189-214.

Tilman D. 1988. Plant strategies and the dynamics and structure of plant communities. Princeton. NJ. Princeton University Press. 360p.

Tilman D. 1996. Biodiversity: Population versus ecosystem stability. Ecology 77:350-63.

Tyler G.. Pahlsson A.B.. Bergkvist B.. Falkengren-Grerup U.. Folkeson L.. Nihlgard B.. Ruhling A.. Stjernquist I. 1992. Chemical and biological effects of artificially increased nitrogen deposition to the ground in a Swedish beech forest. Scandinavian Journal of Forest Research 7:515-32.

Van Breemen N.. van Dijk H. F. G. 1988. Ecosystem effects of atmospheric deposition of nitrogen in The Netherlands. Environmental pollution 54:249-74.

Van Hecke J.. Impens I.. Behaeghe T. J. 1981. Temporal variation of species composition and species diversity in permanent grassland plots with different fertilizer treatments. Vegetatio 47:221-32.

Van Kootwijk E.J.. Van der Voet H. 1989. De kartering van heidevergrassing in Nederland met de Landsat Thematic mapper sattelietbeelden. Report RIN 89/2. Arnhem (In Dutch).

Van Miegroet H.. Lovett G.M.. Cole D.W. 1992. Summary and Conclusions - Atmospheric deposition and forest nutrient cycling. In: Johnson D.W.. Lindberg S.E.. editors. Atmospheric deposition and forest nutrient cycling. New York:Springer-Verlag. p 202-7.

Vitousek P. M. 1990. Biological invasions and ecosystem processes: towards an integration of population biology and ecosystem studies. Oikos 57:7-13.

Vitousek P. M.. Matson P. A. 1985. Disturbance. nitrogen availability. and nitrogen losses in an intensively managed loblolly pine plantation. Ecology 66:1360-76.

Vitousek P.M.. Andariese. 1986. Microbial transformations of labelled nitrogen in a clear-cut pine plantation. Oecologia 68:601-605.

Vitousek P.M.. Walker L.R. 1989. Biological invasion by *Myrica faya* in Hawai'I: Plant demography. nitrogen fixation. ecosystem effects. Ecological Monographs 59:247-265.

Vitousek P.M.. Matson P.A. 1993. Agriculture. the global N cycle. and trace gas flux. In: Oremland R.S.. editor. The biogeochemistry of global change: radiative gases. New York. New York:Chapman and Hall. p 193-208.

Walker J.. Peet R.K. 1983. Composition and species diversity of pine-wiregrass savannas of the Green Swamp. North Carolina. Vegetatio 55:163-179.

Wedin D. A.. Tilman D. 1996. Influence of nitrogen loading and species composition on the carbon balance of grasslands. Science 274:1720-3.

Willems J. H.. Peet R. K.. Bik L. 1993. Changes in chalk-grassland structure and species richness resulting from selective nutrient additions. Journal of Vegetation Science 4:203-12.

Williams M. W.. Baron J. S.. Caine N.. Sommerfeld R.. Sanford J. 1996. Nitrogen saturation in the Rocky Mountains. Environmental Science and Technology 30:640-6.

Wilson A.D.. Schure D.H. 1993. Plant competition and nutrient limitation during early succession in the Southern Appalachian Mountains. American Midland Naturalist 129:1-9.

Wilson E. J.. Wells T. C. E.. Sparks T. H. 1995. Are calcareous grasslands in the UK under threat from nitrogen deposition? an experimental determination of a critical load. Journal of Ecology 83:823-32.

Woodin S. J., Farmer A. M. 1993. Impacts of sulfur and nitrogen deposition on sites and species of nature conservation importance in Great Britain. Biological Conservation 63:23-30.

Wright R., Tietema A. 1995. Ecosystem responses to 9 years of nitrogen addition at Sogndal, Norway. Forest Ecology and Management 71:133-42.

Zak D.R., Groffman P.M., Pregitzer K.S., Christensen S., Tiedje J.M. 1990. The Vernal Dam: Plant-microbe competition for nitrogen in Northern Hardwood forests. Ecology 71:651-656.

Zak D. R., Tilman D., Parmenter R. R., Rice C. W., Fisher F. M., Vose J., Milchunas D., Martin C. W. 1994. Plant production and soil microorganisms in late-successional ecosystems: a continental-scale study. Ecology 75:2333-47.

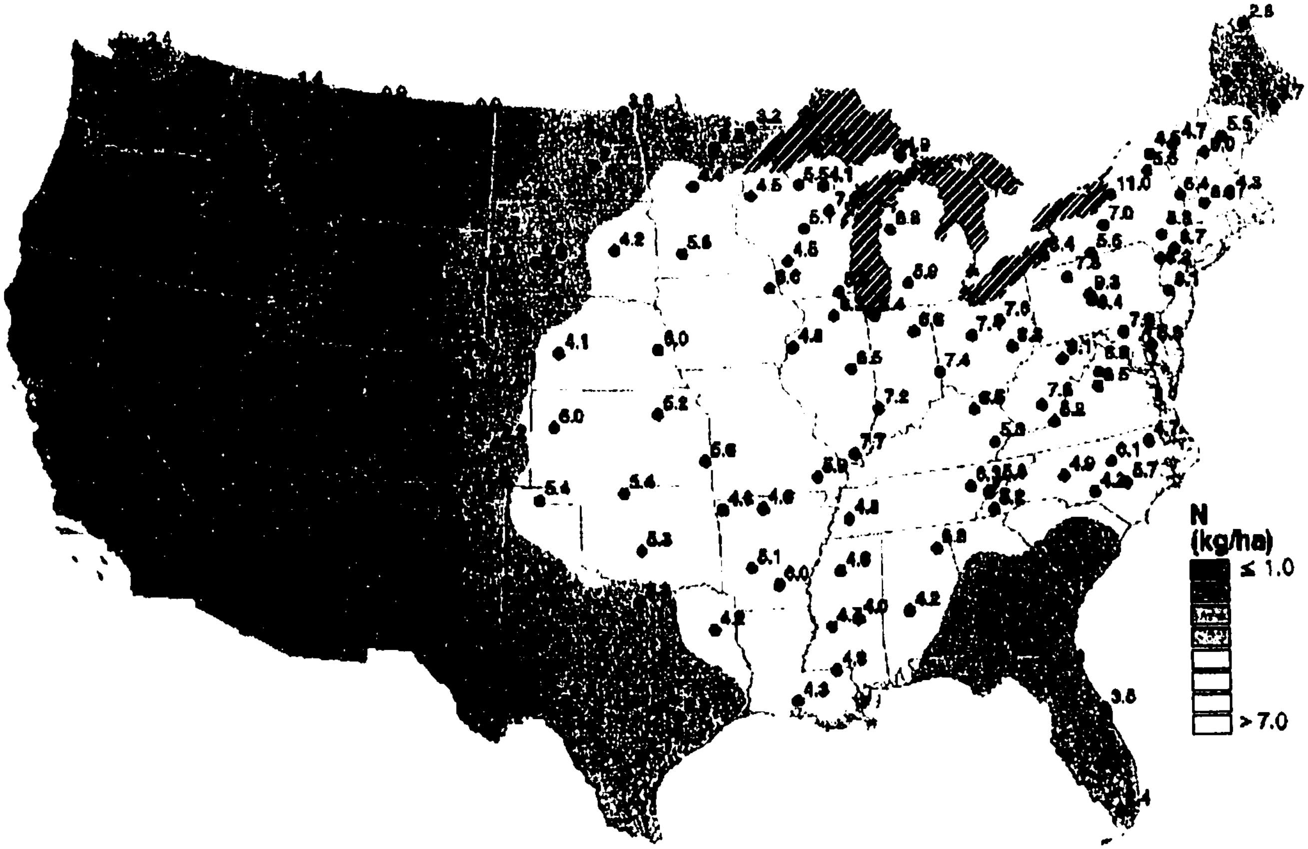

Figure 4.1: 1996 atmospheric N deposition in the United States (kg/ha). From National Atmospheric Deposition Network / National Trends Network.

Table 4.1. Ecosystem characteristics at each stage of the Aber model of the development of N saturation. From Aber et al (1989) and Aber (1992).

	Stage 0	**Stage 1**	**Stage 2**	**Stage 3**
Primary form of N	NH_4 - N	NH_4 - N	NH_4 - N > NO_3 - N	NH_4 - N = NO_3 - N
Availability of inorganic N	low	low	high	High
Limitation of vegetation primary production	N	N	N?	Other nutrients (i.e. P, Ca, water)
Limitation of microbial activity	N	N	labile C	Labile C
Net nitrification	~0	~0	low	High (approaching 100%)
Nitrate loss into groundwater	0	0	low	High (approaching inputs of N)

Table 4.2. Retention of added ^{15}N in measured ecosystem components.

Study	Application (kg N ha^{-1} yr^{-1})	Foliage	Wood	Fine roots	Litter/organic layer	Soil	Microbes	Total retained in ecosystem
Tietema et al. 1998 (Klosterhede)	20 ($^{15}NH_4{}^{15}NO_3$)		45%^{b}	N/A	26%	16%	N/A	100%
Tietema et al. 1998 (Klosterhede)	55 ($^{15}NH_4{}^{15}NO_3$)		45%^{b}	N/A	12%	17%	N/A	96%
Tietema et al. 1998 (Aber)	51 ($^{15}NH_4{}^{15}NO_3$, $Na^{15}NO_3$)		32^b	N/A	17-47	1-15	N/A	65-75%
Tietema et al. 1998 (Aber)	91 ($Na^{15}NO_3$)		20^b	N/A	11	15	N/A	50%
Magill et al. 1997	48 (NH_4NO_3)	27-44%	63-83	4-17	N/A	N/A	0	100%
Magill et al. 1997	324 (NH_4NO_3)	7-8%	9-14	5-18	N/A	N/A	0	60-66%
Magill et al. 1997	874 (NH_4NO_3)	5-6%	2-7%	5-9%	N/A	N/A	0	78-83%
Koopmans et al. 1996	4-6 ($^{15}NH_4$-N)	8-26%	2-4%	N/A	N/A	46-65%	N/A	76-79%
Koopmans et al. 1996	44-53 ($^{15}NH_4$-N)	13-21%	3-8%	N/A	N/A	37%	N/A	53-66%
Christ et al. 1995	40 (NH_4-N)		-8%^{b}	N/A	N/A	N/A	N/A	100%
Christ et al. 1995	160 (NH_4-N)		7%^{b}	N/A	N/A	N/A	N/A	100%
Christ et al. 1995	520 (NH_4-N)		9%^{b}	N/A	N/A	N/A	N/A	96%
Nadelhoffer et al. 1995	26 ($^{15}NO_3$-N)	8%	3%	N/A	2%	14%	N/A	28%
Nadelhoffer et al. 1995	56 ($^{15}NO_3$-N)	9%	4%	N/A	2%	10%	N/A	24%

Table 4.2. CONTINUED.

Study	Application (kg N ha^{-1} yr^{-1})	Foliage	Wood	Fine roots	Litter/organic layer	Soil	Microbes	Total retained in ecosystem
Mugasha and Pluth 1994	53	6.94% [a]	5.78%	N/A	N/A	47.2%	N/A	68.9%
Emmett and Quarmby 1991	0.5	N/A	1.5-7.7% [b]	N/A	N/A	31.5-51.6%	6.7-17.5% [c]	67.6-99.0%
Preston et al. 1990	100	N/A	5.3-12.5% [b]	N/A	N/A	39.1-87.0%	N/A	44.4-95.2%
Corbin, Chapter 3	305 (NH_4NO_3)	N/A	N/A	0	N/A	0	0	100%

[a] 15 months after application
[b] Measurements of both foliage and woody structures
[c] 60 days after application
[d] Vegetation excluded from samples

IMAGE EVALUATION
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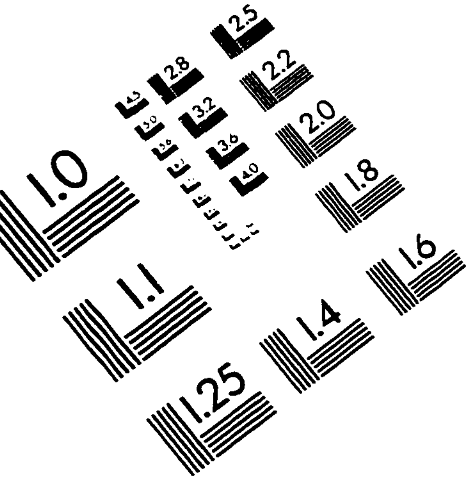

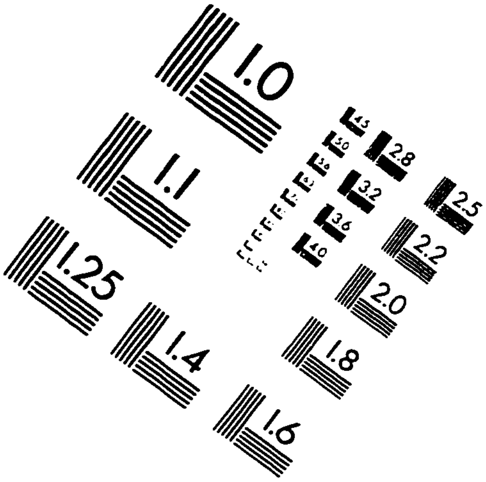

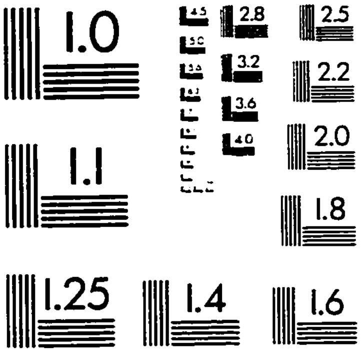

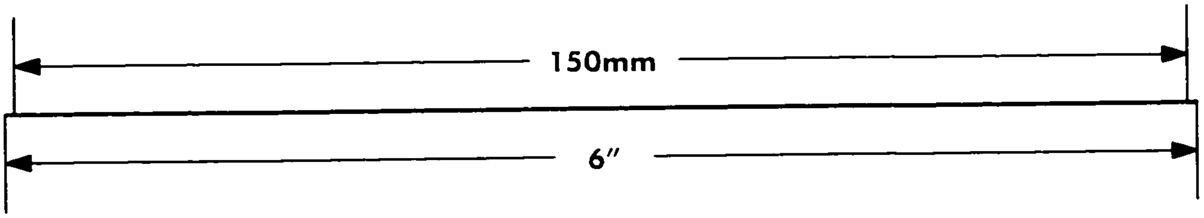

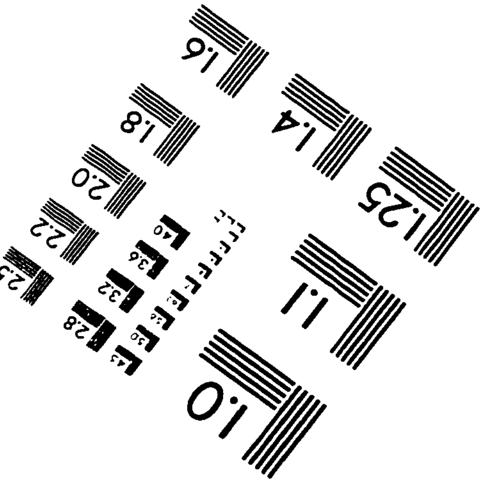

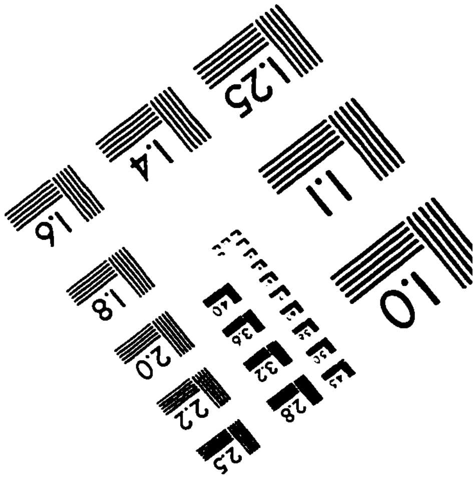

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