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PLANT AND SOIL RESPONSES TO CHRONIC NITROGEN ADDITIONS AT THE HARVARD FOREST, MASSACHUSETTS[1]

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Abstract. Data are presented on changes in plant and soil processes in two forest types (red pine plantation and oak–maple forest) at the Harvard Forest, Petersham, Massachusetts, in response to 3 yr of chronic N fertilization. The hardwood stand exhibited greater N limitation on biological function than the pine stand prior to fertilization as evidenced by a lower net N mineralization rate, nearly undetectable rates of net nitrification, and very low foliar N content.

N additions were made in six equal applications throughout the growing season, and consisted of 5 and 15 $g \cdot m^{-2} \cdot yr^{-1}$ of N as ammonium nitrate. The pine stand showed larger changes than the hardwood stand for extractable N, foliar N, nitrification, and N leaching loss. Retention of added N was essentially 100% for all but the high application pine plot from which significant N leaching occurred in the 3rd yr of application. From 75 to 92% of N added to fertilized plots was retained in the soil, with larger fractions retained in the hardwood stand than the pine stand for all treatments.

As hypothesized, the stands are exhibiting highly nonlinear patterns of nitrogen output in response to continuous nitrogen inputs. The implications of this nonlinearity for regional eutrophication of surface waters and atmospheric deposition control policy are discussed.

Key words: extractable soil nitrogen; foliar chemistry; nitrification; nitrogen leaching; N mineralization; primary production; soil solution chemistry.

INTRODUCTION

Nitrogen in wet deposition over the northeastern U.S. ranges from 5 to >20 times global, ambient, background levels (Galloway et al. 1984, Lovett and Kinsman 1990). Concerns over the long-term effects of elevated nitrogen deposition on forest health and regional water quality have led to the signing of a protocol between the U.S. and the European Community that will lead to the establishment of "critical loads" of both nitrogen and sulfur to sensitive ecosystems (UN ECE 1988). Although the recently enacted Clean Air Bill proposes significant reductions in NO_x, total N deposition (wet plus dry) will remain elevated over the northeastern U.S., and will exceed current outputs for most forested ecosystems for the foreseeable future (e.g., Likens et al. 1977, Friedland et al. 1991).

In an earlier paper (Aber et al. 1989) we hypothesized an integrated set of plant and soil responses to chronic N additions to N-limited forest ecosystems, and described a long-term chronic N addition experiment to be carried out at the Harvard Forest, Petersham, Massachusetts, as part of the Long-term Ecological Research (LTER) Program of the National Science Foundation (NSF). We have now completed 3 yr of N additions to two stands at the Harvard Forest. Changes in trace gas exchanges, including a significant decrease in methane consumption, occurred during the 1st yr of treatment (Steudler et al. 1989, Bowden et al. 1990, 1991). We hypothesized that increases in other soil and plant processes, such as net primary production (NPP), N uptake, and nitrate leaching, would occur slowly and nonlinearly over several years. In this paper we report the results of the first 3 yr of measurements on N cycling in the control and fertilized stands.

MATERIALS AND METHODS

Study sites

The chronic nitrogen addition plots are located at the Harvard Forest in central Massachusetts. Forest ecosystem research has been ongoing at the Harvard Forest since 1907. In 1988, this site became part of the NSF LTER Network. Historical land use practices are well documented, and most areas were in culti-

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TABLE 1. Initial characteristics of two stands at the Harvard Forest used in this study.

	Stand	
	Red pine	Hardwood
Soils		
Forest floor		
Mass (g/m²)	8428	9471
% carbon	26.8	19.4
% nitrogen	1.11	0.80
Mineral soil (to 10 cm depth)		
% carbon	5.78	7.58
% nitrogen	0.26	0.36
Vegetation		
Stem density (no./ha)	1174	1937
Basal area (m²/ha)	51.9	21.8
Tree biomass (g/m²)	14656	11132
% biomass by species		
Red pine	98	0
Black and red oak	1	78
Red maple	>1	5
Black birch	1	8
Others	>1	9

vation or pasture in the early part of this century. Many of the currently forested areas are single-species plantations. The dominant soil types are stony to sandy loams formed from glacial till.

Two adjacent stands were chosen for the research plots: an even-aged red pine (*P. resinosa*) stand ≈65 yr old and an ≈80-yr-old mixed hardwood stand (Table 1). The hardwood stand is dominated by black and red oak (*Quercus velutina* Lam.; *Q. borealis* Michx. f.) with significant amounts of black birch (*Betula lenta* L.), red maple (*Acer rubrum* L.), and American beech (*Fagus grandifolia* Ehrh.). Soils in both stands are Entic Haplorthods of the Gloucester series.

Treatments

Four treatments (one plot per treatment) were established within each stand: control, low N, high N, and nitrogen plus sulfur (N + S). Each plot measures 30 × 30 m (0.09 ha) and is divided into 36 5 × 5 m subplots. Fertilizer additions of NH_4NO_3 and Na_2SO_4 began in 1988 as six applications over the growing season. Fertilizer is weighed, mixed with 20 L of water, and applied to the plots using a backpack sprayer. Two passes are made across each plot to ensure an even distribution of fertilizer.

Total applications for year 1 (1988) were 38 $kg \cdot ha^{-1} \cdot yr^{-1}$ of N for the low nitrogen treatment and the nitrogen portion of the N + S treatment, 74 $kg \cdot ha^{-1} \cdot yr^{-1}$ of SO_4-S for the N + S treatment; and 113 $kg \cdot ha^{-1} \cdot yr^{-1}$ of N for the high nitrogen treatment. Second- and third-year N applications were at the rate of 50 $kg \cdot ha^{-1} \cdot yr^{-1}$ for the low and N + S plots and 150 $kg \cdot ha^{-1} \cdot yr^{-1}$ for the high addition plots. Sulfur additions remained the same as the 1st yr. In this paper we will present results from the nitrogen addition plots only.

Field sampling

Soils.—Net nitrification and net mineralization were measured on all plots using the in situ buried bag technique (Nadelhoffer et al. 1983, Pastor et al. 1984). Three sets of two adjacent soil cores (<30 cm apart) were taken to a depth of 10 cm in the mineral soil in each of three designated subplots (nine samples per plot). Cores were split into organic and mineral horizons and placed into gas-permeable polyethylene bags. One sample (initial) was taken to the laboratory for extraction of ammonium and nitrate. The second sample (incubated) was put inside a 1-mm mesh fiberglass screen bag (to reduce puncture damage to polyethylene bag) and placed back in the ground. In situ samples were incubated for a period of 4–6 wk during the growing season, with an over-winter incubation from November to April. Retrieved incubated samples were analyzed for extractable nitrate and ammonium. Net N mineralization and nitrification were measured throughout the year in years 1 and 3 of the study. One 6-wk incubation was performed in midsummer of year 2. Soil incubations were initiated at least 2–4 wk after the most recent fertilizer addition.

Lysimeters.—Five porous cup tube lysimeters were installed at 60 cm depth in each plot. Soil solution samples were collected once a month by draining any existing water using a syringe, and then applying a tension of 0.05 MPa with a hand pump. Approximately 24 h later, a sample was collected and the volume recorded. A 20-mL subsample was placed in a scintillation vial and frozen until analysis for NO_3 and NH_4.

Foliage.—Foliar litterfall collections began on 1 September of each year. Weekly collections were made throughout the autumn period of leaf senescence (through November). Additional collections occurred in spring and during the growing season. Nine litter baskets (41.5 × 56.5 cm) were located in each plot. Baskets were removed from the plots during fertilization to avoid potential contamination.

Green foliage was collected once per year during the 1st wk of August. In the pine stand, all trees are nearly the same height (20 m) and are codominant in the canopy. Needle samples were collected throughout the canopies of 20 different trees in each plot. These were composited into five aggregate tree samples/plot. In the hardwood stand, a total of four composite samples/species/plot were collected. Each composite sample was taken from three different trees, and included foliage from each canopy class (codominant, subdominant, and understory) in which a species occurred. Red oak and black oak were treated as a single species in all vegetation sampling. All samples were collected by shooting twigs from trees with foliage attached.

Fine roots.—Soil cores (7.5 cm diameter, separate samples for forest floor and 0–10 cm in the mineral soil) were collected from 27 locations throughout each of the two stands in May 1988, prior to the initiation

of fertilizer additions. Each core was divided into organic and mineral horizons, and samples were stored frozen prior to sorting.

Tree increments.—All trees >5 cm in diameter at 1.5 m above ground level were numbered with aluminum tags. Diameter was measured 2.5 cm above the nail. Diameter measurements were made in October 1988 and November 1990.

Laboratory methods

Extractable ammonium and nitrate.—Soil samples were sieved through a 5.6-mm screen to remove all large roots and rocks, and the soil was homogenized. Total soil mass was recorded and a portion of the soil (10–20 g) was weighed and dried at 105°C for 48 h to determine moisture content. Approximately 15 g of soil was placed in 150 mL of 1 mol/L KCl, hand shaken, and then extracted for 48 h. Samples were then filtered and the filtrate frozen. Extracts were analyzed for NO_3-N and NH_4-N using a Bran & Luebbe (Technicon) Traacs 800 Autoanalyser. Ammonium was analyzed using the Berthelot Reaction (Technicon Method 780-86T); nitrate was determined using a hydrazine sulfate reduction technique (Technicon Method 782-86T). Detection limits for both nitrate-N and ammonium-N are 0.01 mg/L using these techniques. Our laboratory is a regular participant in an Environmental Protection Agency sponsored inter-laboratory, quality assurance/quality control procedure.

Foliage.—Litter and green leaf samples were analyzed for nitrogen, lignin, and cellulose content using near-infrared spectroscopy (McLellan et al. 1991). Litter samples were sorted by species (red and black oak combined), dried (48 h at 70°C), weighed, and ground to pass through a 1-mm mesh. Green leaf samples were dried and ground in the same manner without weighing. All samples were redried overnight at 70°C just prior to analysis.

Lysimeters.—Lysimeter samples were filtered and analyzed on the Traacs Autoanalyser using the same technique as for soil extracts.

Fine roots.—Fine root biomass was determined by sorting roots from a known mass of soil. Samples were placed on a double layer of 1-mm mesh screening and soaked in distilled water to remove most of the soil from the roots. The rinsed sample was then sorted into two categories: tree roots (<2 mm) and forb roots. Roots were oven-dried at 105°C for 48 h and weighed. Samples were ground using a mortar and pestle and analyzed for percent carbon and nitrogen using a Perkin-Elmer Model 240B CHN analyzer.

Fine root biomass data were not normally distributed within a plot and horizon. A logarithmic transformation of the raw data was performed to achieve a normal distribution. Mean values for each stand were derived from transformed data and then back transformed to a linear scale.

Calculations

Net N mineralization and nitrification.—Net mineralization was calculated as extractable nitrate + ammonium in the incubated sample minus extractable nitrate + ammonium in the initial. Net nitrification was calculated as incubated minus initial extractable nitrate.

Tree biomass increment.—Woody biomass increment in all plots between fall 1988 and fall 1990 (two growing seasons) was determined by applying allometric equations (Whittaker et al. 1974, Pastor et al. 1984) to tree diameter measurements.

RESULTS

Net N mineralization and nitrification

Net N mineralization (NH_4 plus NO_3) increased immediately in response to N additions, particularly in the forest floor (Figs. 1 and 2). After 2 mo, differences among treatments remained, although those differences were smaller, and generally not significant. Expressed as annual totals (Table 2), differences in net N mineralization in year 1 were equal to 42 and 49% of total N added in the pine low and high addition plots, and 41 and 40% of total N added in the hardwood low and high addition plots, respectively.

Net annual nitrification in year 1 was 13% of net mineralization in the control pine stand, with most of this occurring in the mineral soil (Fig. 1, Table 2). Nitrification increased in response to N additions in the 1st yr, rising to ≈20% of net annual mineralization in both the low and high N treatments.

During the 3rd yr, nitrification had increased in the pine control plot as well as the treated plots (Fig. 1, Table 2). Most of the nitrification still occurred in the mineral soil, with over half of the mineralized N in this horizon being nitrified in all three plots. For both horizons, net nitrification was 33, 36, and 38% of net N mineralization in the control, low N, and high N plots, respectively.

Net nitrification was apparently stimulated within the forest floor by the first N addition to the hardwood stand (Fig. 2), but returned to near-zero levels thereafter. Net nitrification occurred sporadically and at very low rates in the mineral soil of the hardwood stand. Net nitrification was <3% of net mineralization in all hardwood plots.

Extractable ammonium and nitrate

Extractable ammonium and nitrate in the pine plots increased immediately in the forest floor in response to N additions, and increased after 1 mo in the mineral soil (Fig. 3). Concentrations remained elevated throughout the experiment, with differences in nitrate concentration becoming more pronounced in the 3rd yr.

In the hardwood stand, differences in NH_4 concentrations during the first 2 mo of treatment disappeared

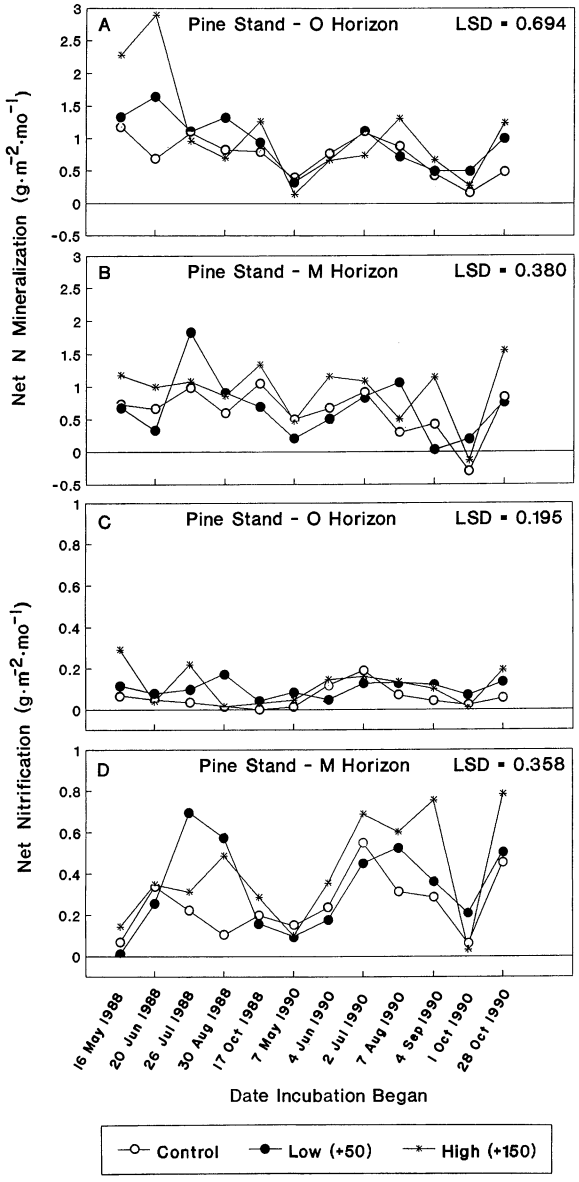

FIG. 1. Changes in net nitrogen mineralization and net nitrification in the red pine stand in response to chronic N additions (0, 50, or 150 kg·ha^{-1}·yr^{-1}): (A) mineralization in the forest floor (O horizon), (B) mineralization in the mineral soil (0–10 cm), (C) nitrification in the forest floor, and (D) nitrification in the mineral soil. LSD (least significant difference) is the value by which any two points must differ for that difference to be significant at the 5% level (Snedecor and Cochran 1967).

by the 3rd mo. By year 3, concentrations were again higher in the N-amended plots, although those differences were smaller than in the pine stand (Fig. 4A, B).

Initial increases in nitrate concentration in the hardwood soils disappeared by July of the 1st yr (Fig. 4C, D). Concentrations remained near or below detection limits throughout the rest of the experiment for all treatments.

Ammonium and nitrate leaching losses

Lysimeter collections began during the 2nd yr of the experiment, 8 mo after installation, in order to minimize the effects of soil disturbance caused by lysimeter installation. Concentrations of both nitrate and ammonium have been near or below detection levels in the hardwood stand throughout the experiment (Fig. 5C, D).

Nitrate concentrations in lysimeter samples from the pine stand were also consistently near detection limits for the control and low N treatment (Fig. 5B). Nitrate concentrations were slightly elevated in the high N treatment in year 2 (1989), and increased substantially

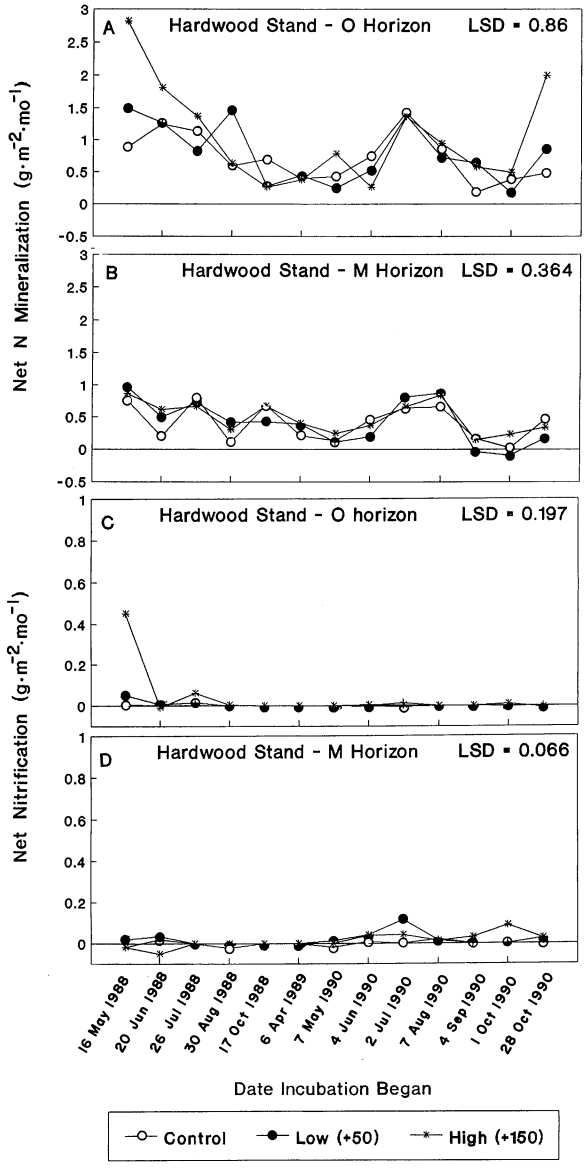

FIG. 2. Changes in net nitrogen mineralization and net nitrification in the hardwood stand in response to chronic N additions. Data presentation in Fig. 1.

TABLE 2. Annual net N mineralization and net nitrification in treated and control plots at the Harvard Forest (all values in $g \cdot m^{-2} \cdot yr^{-1}$).

	Net N mineralization		Net nitrification	
	1988	1990	1988	1990
	Red pine			
Control				
O horizon	4.56	4.20	0.17	0.50
Mineral soil	3.99	3.31	0.94	2.04
Total	8.55	7.51	1.11	2.54
Low N				
O horizon	6.33	4.82	0.51	0.72
Mineral soil	4.43	3.55	1.69	2.32
Total	10.76	8.37	2.20	3.04
High N				
O horizon	8.08	5.03	0.94	0.79
Mineral soil	5.43	5.75	1.57	3.30
Total	13.51	10.78	2.51	3.82
	Hardwood			
Control				
O horizon	4.55	4.49	0.01	−0.01
Mineral soil	2.54	2.51	−0.02	0.01
Total	7.09	7.00	−0.01	0.00
Low N				
O horizon	5.32	4.54	0.07	0.01
Mineral soil	3.02	2.02	0.06	0.21
Total	8.34	6.56	0.13	0.22
High N				
O horizon	6.89	6.40	0.39	0.01
Mineral soil	3.10	2.82	−0.07	0.23
Total	9.99	9.22	0.32	0.24

in November of that year, remaining high through much of 1990 (year 3). Ammonium concentrations (Fig. 5A) remained low for most collections.

Green foliar chemistry

Differences among treatments in green foliar N concentration increased with time in the pine stand (Fig. 6A). The high N plot was higher than the control by 3, 16, and 24% in years 1, 2, and 3, respectively. In years 2 and 3, those differences were statistically significant.

In the hardwood stand, differences in foliar N concentration in the two dominant species, black oak and red maple, were minimal among treatments in the 1st yr and generally increased in years 2 and 3 (Fig. 6B). By year 3, red maple foliar N concentration was 19% higher in the high N plot than in the control, while black oak was only 6% higher. Differences between the control and high N plots were statistically significant in years 2 and 3.

Lignin concentrations of green foliage did not change in response to N additions. Mean lignin concentrations were 22% for red pine, 17% for red maple, and 18% for black oak.

Foliar litter chemistry

Differences in leaf litter N concentrations were consistent with patterns in green foliar N concentrations (Fig. 7). In year 3, concentrations for the pine high N addition plot were 43% higher than the control plot. In the hardwood stand, red maple and oak N concentrations in litter were 16 and 12% higher than controls,

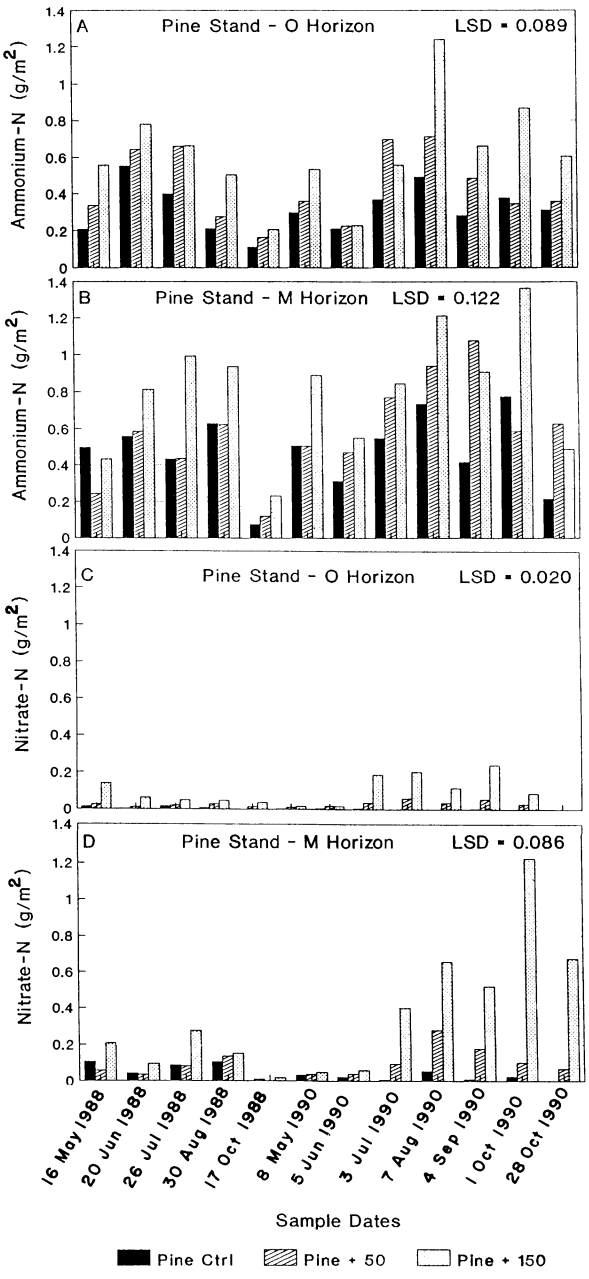

FIG. 3. Extractable ammonium and nitrate concentration in soils of the pine stand in response to chronic N additions (0, 50, or 150 $kg \cdot ha^{-1} \cdot yr^{-1}$): (A) ammonium in the O horizon, (B) ammonium in the mineral soil, (C) nitrate in the O horizon, (D) nitrate in the mineral soil. LSD (least significant difference) is the value by which any two points must differ for that difference to be significant at the 5% level.

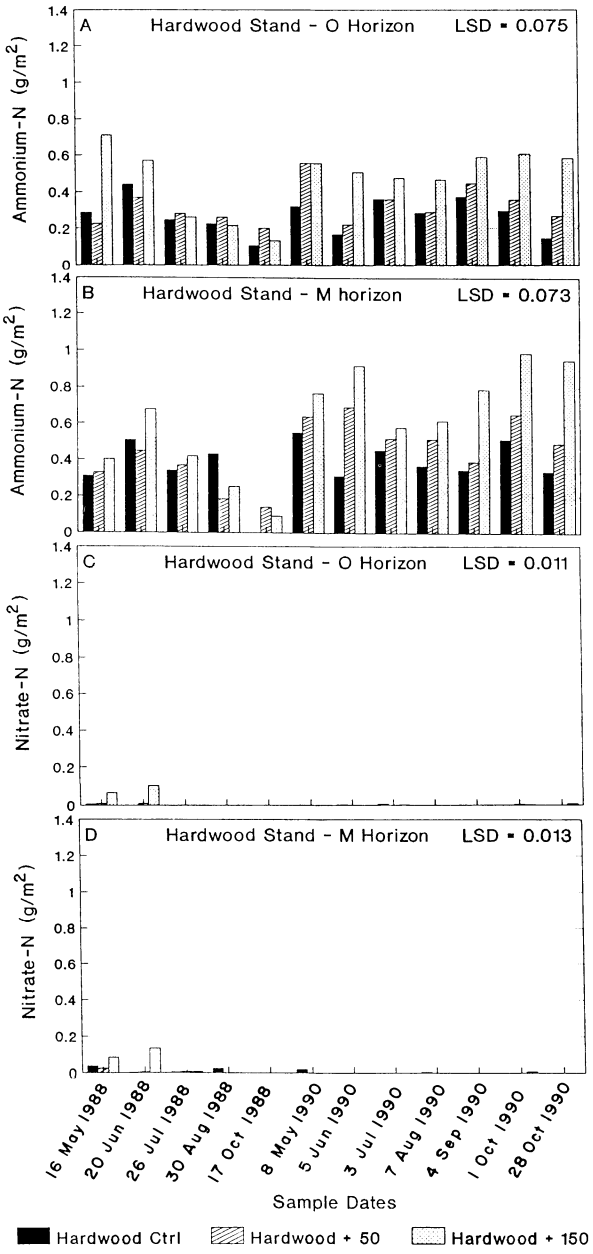

FIG. 4. Extractable ammonium and nitrate concentration in soils of the hardwood stand. Data presentation as in Fig. 3.

respectively, with both differences being statistically significant. Lignin values were unchanged with treatment. The low fraction of N retranslocated before leaf fall in the high N plot in years 2 and 3 is due in part to an increase in the fraction of pine needle litter that was still green when shed. This fraction averaged 13% of total needle fall in the control stand, and 25 and 21% in the low and high N treatments, respectively.

Fine root biomass and chemistry

Initial fine root biomass values (Table 3) are similar to spring values reported by McClaugherty et al. (1982) for two similar stands at the Harvard Forest (250 and 360 g/m^2 for pines and hardwoods, respectively). Nitrogen concentrations in fine root biomass were slightly higher in the pine stand than in the hardwoods. This is the reverse of the pattern shown by McClaugherty et al. (1982). These data yield nearly equal total N

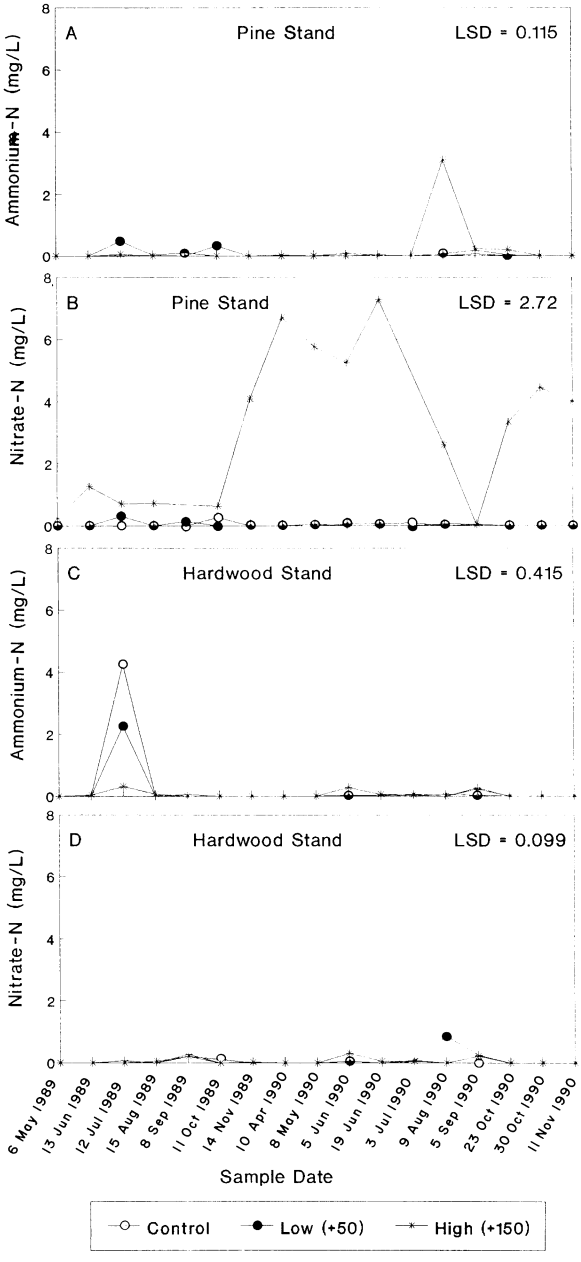

FIG. 5. Concentration of ammonium and nitrate in the lysimeter samples collected at 60 cm depth, in response to chronic N additions (0, 50, or 150 kg·ha^{-1}·yr^{-1}): (A) ammonium in the pine stand, (B) nitrate in the pine stand, (C) ammonium in the hardwood stand, (D) nitrate in the hardwood stand. LSD (least significant difference) is the value by which any two points must differ for that difference to be significant at the 5% level.

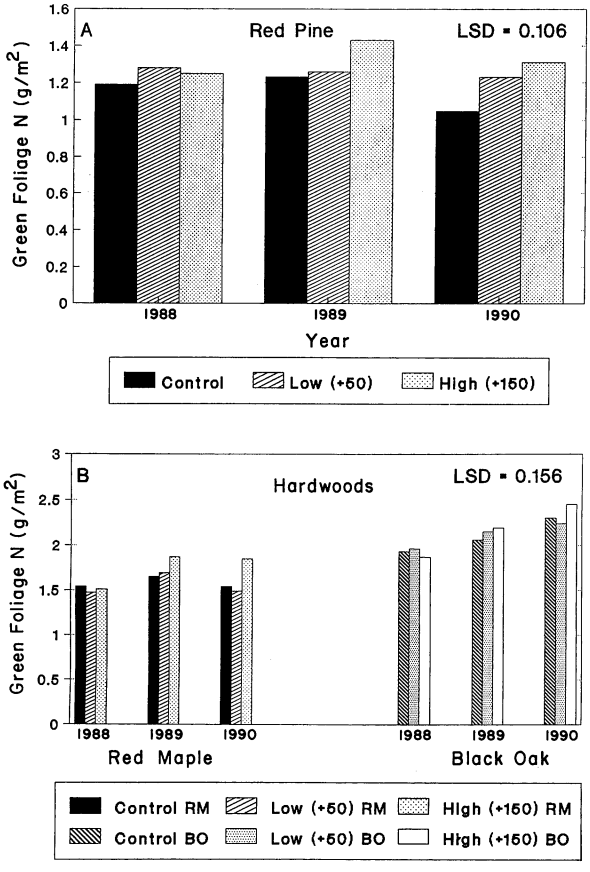

FIG. 6. Effect of N additions (at 0, 50, or 150 kg·ha^{-1}·yr^{-1}) on N concentrations in green foliage collected in August: (A) red pine foliage in the pine stand, (B) major hardwood species in the hardwood stand. LSD as in Fig. 5.

contents in fine root biomass in the two stands (Table 3).

Net primary production above ground

Net primary production (NPP) above ground was calculated as the sum of litterfall and annual woody increment. In the pine stand, woody increment did not vary between treatments, while foliage production (as estimated by litterfall) increased with N additions (Table 4). The reverse occurred in the hardwood stand, with foliar production remaining constant while wood production increased.

TABLE 3. Fine root biomass and nitrogen content in the pine and hardwood stands before initiation of the nitrogen amendments (all values in g/m^2).

	Stand			
	Red pine		Hardwood	
Horizon	Biomass	Nitrogen	Biomass	Nitrogen
Organic	110	1.46	163	1.94
Mineral	121	1.55	113	0.96
Total	231	3.01	276	2.90

TABLE 4. Net primary production above ground by treatment (N additions at 0, 50, or 150 kg·ha^{-1}·yr^{-1}) and stand. Woody increment is the mean of values for years 2 and 3 (all values in g·m^2·yr^{-1}).

	Stand					
	Red pine			Hardwood		
	Control	Low N	High N	Control	Low N	High N
Woody increment	420	450	379	556	652	755
Litterfall	337	389	402	287	288	299
Total	757	834	781	843	940	1054

Retention of added N over the first 3 yr

Total nitrogen retention by each stand was calculated as the difference between total N inputs over the 3-yr study and measured losses (Table 5). N inputs totaled 0.8, 14.6, and 42.1 g·m^{-2}·yr^{-1} in the control, low N and high N plots, respectively. Gaseous N losses as N_2O were <0.1 g·m^{-2}·yr^{-1} for all treatments (R. Bowden et al. 1991, *unpublished data*). N leaching losses were also <0.1 g·m^{-2}·yr^{-1} in all but the high N pine plot. Retention of added N thus equaled 100% in all

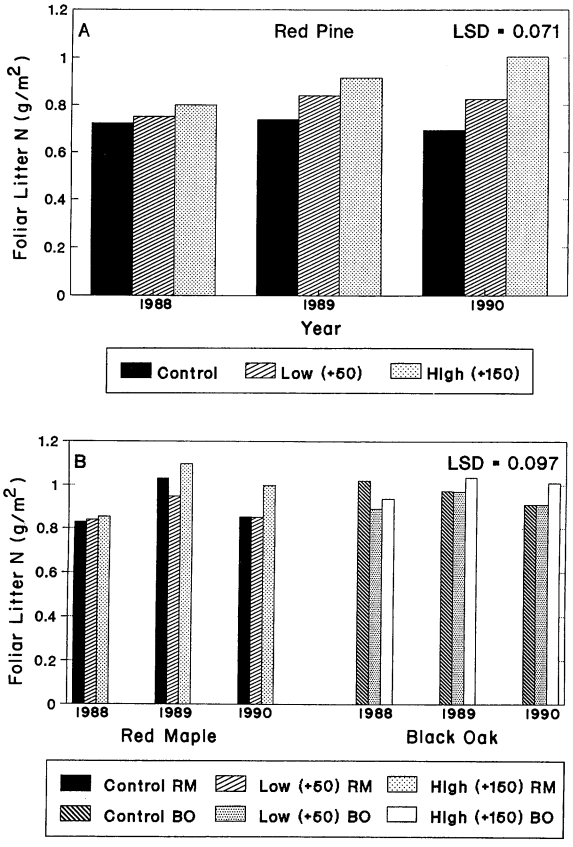

FIG. 7. Effect of N additions (at 0, 50, or 150 kg·ha^{-1}·yr^{-1}) on N concentration in foliar litter: (A) red pine in the red pine stand, and (B) major hardwood species in the hardwood stand. LSD as in Fig. 5.

TABLE 5. Inputs, outputs, retention, and estimated distribution of nitrogen added (as 0, 50, or 150 $kg \cdot m^{-2} \cdot yr^{-1}$) after the first 3 yr of treatment.

	Stand					
	Pine			Hardwood		
Treatment	Control	Low N	High N	Control	Low N	High N
N inputs (g/m^2)						
Atmosph. deposition*	0.8	0.8	0.8	0.8	0.8	0.8
Fertilization	0	13.8	41.3	0	13.8	41.3
Total	0.7	14.6	42.1	0.7	14.6	42.1
N losses (g/m^2)						
Gaseous	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Leaching†	<0.1	<0.1	2.2	<0.1	<0.1	<0.1
Total	<0.1	<0.1	2.2	<0.1	<0.1	<0.1
N retention						
(% of N inputs)	100	100	95	100	100	100
Distribution (change in N storage over 3 yr, g/m^2)						
Soil extractable N	0	0.6	1.3	0	0.4	1.0
Woody biomass	0.8	0.8	0.8	1.1	1.3	1.6
Foliage	0	2.6	3.2	0	0.1	0.9
Fine roots‡	...	...	...	...	...	...
Soil organic matter	0	10.5	34.5	−0.3	12.7	38.5
(% of total addition) (by difference)	...	72	82	...	88	92

* Estimated sum of wet plus dry deposition of nitrogen (NADP regional data and estimated dry deposition; Ollinger et al. 1993).

† Leaching losses estimated as lysimeter concentrations times volume of water leaching below the rooting zone, as calculated by a monthly water balance/photosynthesis/transpiration model (Aber and Federer 1993).

‡ We have assumed that total N in fine roots will not increase substantially due to fertilization (see *Results*).

year and plot combinations, with the exception of year 3 in the high N pine plot.

Distribution of added N within the ecosystem

Allocation of nitrogen to aboveground biomass differed among treatments, but these changes account for only a small fraction of added N (Table 5). Similarly, changes in soil extractable pools of N, though significant (Figs. 3 and 4), cannot account for a substantial fraction of added N (Table 5). Between 72 and 92% of N added in treatments is not in a measured pool, and that fraction increases with increasing addition rate.

The only two significant pools for which direct measures of change in N content with time have not been made are fine root biomass and soil organic matter. Initial results from a resampling of fine roots in 1991 suggest no significant change in biomass. If mass remained constant, changes in N concentration have to be unreasonably large for this pool to contribute significantly to total N retention. Therefore, we assume that most of the unaccounted-for N has been transferred to soil organic matter.

DISCUSSION

Initial differences in the degree of N limitation

Net N mineralization is lower in the control hardwood soil than in the control pine soil, and net nitrification is detectable in the control pine soil, and nearly absent in the control hardwood soil. Together, these suggest a stronger N limitation on biotic activity in the hardwood stand prior to fertilization. The hardwood mineralization rate is particularly low compared with maximum measured rates for mixed-oak stands at mid-latitudes (N up to 14 $g \cdot m^{-2} \cdot yr^{-1}$, Nadelhoffer et al. 1983). In contrast, the N values of 7.0 to 8.0 $g \cdot m^{-2} \cdot yr^{-1}$ measured in the control pine stand are among the highest for this forest type in a northern temperate area (Pastor et al. 1984, Aber et al. 1985, Nadelhoffer et al. 1983).

The presence of significant net nitrification in the pine stand, and its absence in the hardwood stand, also suggests stronger microbial and microbe–plant competition for ammonium in the hardwoods (Robertson and Vitousek 1981, Robertson 1982). The same conclusion is supported by the rapid removal of nearly all added nitrate in the hardwood stand, after the first 2 mo of treatment. The temporal patterns of extractable nitrate in the hardwood stand suggests that nitrate reduction rates were initially quite low, and were stimulated by nitrate additions (as discussed below, increased plant uptake of added N is a small fraction of the total).

Near-zero loss rates of N in either dissolved or gaseous (Bowden et al. 1990) form from both control stands suggest that in neither stand was N availability greater than biological demand, and that the differences described above are relative rather than absolute.

Integrated effects on N cycling

We hypothesized that several internal processes would change in response to N additions before shifts in input-output balances occurred (Aber et al. 1989). We found that foliar N content, aboveground NPP, N mineralization, and pool sizes of extractable ammonium have increased in both stands. In each case, the response has been greater in the pine stand than in the hardwoods. In addition, the high N pine plot has, in the 3rd yr, shown increases in extractable nitrate, net nitrification, and nitrate leaching (Fig. 5). Increases in the fraction of needle fall that is still green in the treated pine plots may also reflect decreased internal demand for N (Turner 1977, Vitousek 1982, Flanagan and Van Cleve 1983).

We conclude from these responses that N limitation on biological activity was stronger in the hardwood stand than in the pine stand before treatment, and that the pine stand is progressing more rapidly to a "nitrogen-saturated" condition (Agren and Bosatta 1988, Aber et al. 1989). We anticipate that continued applications of N fertilizer to the hardwood stand will induce significant rates of net nitrification and the loss of nitrate and N_2O from that stand as well. Increases in extractable ammonium pools in the hardwood stand in year 3 may be a first sign of reduced microbial demand for mineral N.

N retention and distribution of added nitrogen

Despite differences in the initial degree of N limitation, both stands showed very high N retention capacities (Table 5). These values are higher than the retention of fertilizer N reported by Melin et al. (1983) for scots pine (79%), but this may be due in part to a trenching treatment that was applied around the roots of sampled trees. Our results are very similar to those of Fernandez and Rustad (1990), who measured very small (on the order of 10 μmol/L, cf. Fig. 5) increases in nitrate leaching from a mature pine-hemlock stand in response to similar application rates and frequencies. Raison et al. (1990) showed N losses up to 70 kg/ha in the 1st yr after N additions to a water-limited *Pinus radiata* stand, but N application rates were much higher (400 kg/ha) and occurred in only two, rather than six, applications. The degree of water limitation on plant growth and N uptake potential may have limited N retention in that study.

We have not been able to show that the soil organic matter pool is the repository for the majority of added N. However, we have measured all other major potential N sinks and can account for only a small proportion of fertilizer inputs. Because of the large size and spatial variability of the soil organic matter and nitrogen pools, it is not possible to measure small increments in N content. For example, the hardwood control stand soil contains N at ≈ 318 g/m^2 to a depth of 10 cm in the mineral soil. The high N addition treatment has added N at only 41.3 g/m^2, $\approx 13\%$ of the total pool from 0 to 10 cm. In addition, lysimeter samples were collected at a depth of 60 cm, so some fraction of the added N may be diluted by the much larger pool of organic N between 10 and 60 cm depth in the mineral soil.

Direct measurements of the fate of added N began in the 4th yr of the study (1991). Natural abundance quantities of ^{15}N are being added with each application. Sub-fertilizer quantities of highly enriched ^{15}N are also being added to the control plots (K. J. Nadelhoffer, *personal communication*).

Net N mineralization

The most surprising result presented here is the large increase in net N mineralization during the first 1–3 mo of N additions. We had hypothesized (Aber et al. 1989) a slow, continuous increase in net N mineralization as added N was incorporated into the cycle through uptake, litterfall, and decomposition.

We suggest that rapid immobilization/remineralization of added mineral N is the dominant cause of measured increases in net N mineralization in the incubation bags. Extractable soil N pools (Figs. 3 and 4) are similar to control levels within 1 mo after N additions. This could only occur through increased plant uptake, net immobilization, or N losses by leaching or gaseous emissions. Measured loss rates were very low (Fig. 5 and Bowden et al. 1991), and plant N storage above ground was also a very small portion of N added (Table 5), leaving only rapid N immobilization as the major process by which added nitrate and ammonium were removed from the soil solution. Recent studies have suggested that soils in native ecosystems have a very high ratio of gross to net mineralization, indicating that nitrogen turnover through the microbial pool may be 10–20 times the rate of net N mineralization (Schimel et al. 1989, Davidson et al. 1990). Such high rates of N uptake by microbes would provide a mechanism for incorporating the quantities of N added here into microbial biomass.

High rates of immobilization of nitrate as well as ammonium suggest a very strong N limitation on microbial growth, and an abundance of available carbon to drive both the nitrate reduction and N assimilation processes. The rate of remineralization of immobilized N is very high in the first 1–3 mo, and then appears to decline. This result poses some interesting questions regarding possible changes in the mass or composition of the microbial community, and the mass and dynamics of labile carbon pools within these soils.

Implications for nitrogen saturation and N deposition policy

The data presented here support the concept of highly nonlinear responses of nitrate leaching to chronic additions of N to N-limited forests. This nonlinearity results from the need to "saturate" biological demand

for nitrogen within the system before significant rates of nitrification and N loss begin.

There are important policy implications associated with this nonlinear response. The models used by the U.S. Environmental Protection Agency to assess the effects of atmospheric deposition on ecosystems and surface waters (Schnoor et al. 1984, Cosby et al. 1985, Gherini et al. 1985) largely ignore the effects of long-term N deposition and retention, concentrating instead on sulfur. When modified to deal with N balances, these models generally assume a constant retention efficiency. Our results suggest that retention will vary nonlinearly depending on the internal state of the system, and will not be predictable from time-sequence deposition data alone.

The earliest indications of increasing nitrate concentrations in stream water have been identified and documented only recently in northeastern U.S. streams, and only in areas receiving high levels of N deposition (Driscoll et al. 1987, Murdoch and Stoddard 1992). However, increases in soil nitrogen storage and nitrification have been reported for at least one forest type over a much larger part of the northeast (McNulty et al. 1991). It is very likely that these are the first signs of a general eutrophication of traditionally N-limited forest ecosystems in this region. The implications of region-wide nitrogen saturation for soil acidification and changes in surface water quality suggest that long-term responses of forest ecosystems to increased N deposition deserve further study.

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