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# Forest ecosystem response to four years of chronic nitrate and sulfate additions at Bear Brooks Watershed, Maine, USA

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## Abstract

Nitrogen deposition to forest ecosystems is of growing concern, as total N emissions to the atmosphere continue to increase globally. Potential negative effects on forests and surface waters include soil and water acidification, mobilization and leaching of aluminum and heavy metals, and nutrient imbalances in trees. In this paper we report the results of a chronic nitrogen amendment experiment at the Bear Brooks Watersheds in northern Maine (BBWM), and compare them with results from similar studies conducted in Massachusetts and Vermont. Treatments included low and high nitrogen (2000 and 4000 eq ha<sup>-1</sup> year<sup>-1</sup> as HNO<sub>3</sub>), low and high sulfur (2000 and 4000 eq ha<sup>-1</sup> year<sup>-1</sup> as H<sub>2</sub>SO<sub>4</sub>), and nitrogen plus sulfur (2000 eq ha<sup>-1</sup> year<sup>-1</sup> each), with three replicates per treatment. Initial net N mineralization rates were similar in all plots, and net nitrification rates varied between 4 and 9 kg ha<sup>-1</sup> year<sup>-1</sup> in the control plots over the 3 years of measurement (4-12% of net annual mineralization). In 1989, net N mineralization rates in treated plots were equal to or higher than control plot rates for all but the low S treatment while in 1990, measured rates for all treatments were lower than controls. Net nitrification increased in all but the control and low S plots by 1990, representing from 8 to 25% of net annual N mineralization across treatments in that year. Foliar N concentration in nitrogen treated plots was consistently higher than in the controls, and those differences generally increased with time. Differences in woody biomass increment and foliar litterfall were not statistically significant, although tree mortality did increase substantially in all but the low S treatment. In general, N leaching losses increased with increasing N additions. Nitrogen retention ranged from 93 to 97% of inputs in the control and N amended sites. Measurement of ecosystem pools shows that 70-92% of inputs to the N treated plots were retained in the soil pool, similar to estimates obtained by <sup>15</sup>N analyses. Results from the external plots at Bear Brooks are similar to those from other nitrogen manipulation experiments at the Harvard Forest, MA and Mt. Ascutney, VT in several ways, but N retention was less than expected. We hypothesize that differences in previous land use history have had a greater effect on current N cycling rates than have differences in cumulative N deposition.

Keywords: Foliar chemistry; Net mineralization; Net nitrification; Nitrogen additions; Nitrogen deposition

## 1. Introduction

The importance of atmospheric nitrogen deposition in forest nutrient cycling has increased with

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increasing anthropogenic emissions to the atmosphere over the last 50 + years (Galloway, 1995). Deposition of both NH<sub>4</sub>-N and NO<sub>3</sub>-N are of growing concern as total N emissions continue to increase globally, particularly in Asia (Galloway, 1995). While N deposition at low rates may produce a fertilizer effect in N-poor sites, the potential for forest ecosystems to incorporate and retain added N is limited. The long-term removal of N limitations on biotic activity, accompanied by a decrease in N retention capacity, has been termed nitrogen saturation (Agren and Bosatta, 1988; Aber et al., 1989; Aber, 1992). Both increased nitrification in soils and nitrate leaching below the rooting zone are considered key indicators of increasing N saturation (Stoddard, 1994). Nitrate leaching is linked to both soil and stream acidification and the mobilization of aluminum from soils to streams (Driscoll et al., 1987).

Previous papers (McNulty and Aber, 1993; Aber et al., 1993; Magill et al., 1996; McNulty et al., 1996) reported the results of experimental, long-term, chronic N additions in two stands (oak-maple and red pine plantation) at the Harvard Forest, Petersham, MA, USA and one high-elevation spruce-fir stand on Mt. Ascutney, Vermont, USA. Both stands at the Harvard Forest have histories of intensive land use for agriculture and/or logging. The red pine plantation had higher net N mineralization and nitrification rates, and lower plant N demand than the oak-maple stand. Long-term increases in net nitrification rates and nitrate leaching with N additions were also seen in the pine stand. The oak-maple stand exhibited extremely low initial N availability and retained all of the 900 kg N ha<sup>-1</sup> added over 6 years (six equal applications per year of 150 kg N ha<sup>-1</sup> as NH<sub>4</sub>NO<sub>3</sub>) with no significant increase in net nitrification. On Mt. Ascutney, very low levels of N additions (under 32 kg N ha-1 year-1) resulted in significant changes in N cycling and forest growth.

In this paper, the results of a similar study conducted at the Bear Brooks Watershed in northern Maine (BBWM) are reported. The BBWM study is located in an area with total annual N deposition estimated at about 60% of that at the Harvard Forest (total wet + dry N deposition 4.6 vs. 7.5 kg N ha<sup>-1</sup> year<sup>-1</sup>, Ollinger et al., 1995). Therefore, it is hypothesized that, because of lower N deposition rates, the BBWM plots should be farther from N saturation

and exhibit greater N retention and lower nitrification rates than the Harvard Forest sites. As part of a multi-investigator experiment, this portion of the study reports the effects of nitric and sulfuric acid additions on biomass accumulation and nitrogen status; sulfur dynamics are not included in this paper.

#### 2. Materials and methods

# 2.1. Study site

The Bear Brooks watersheds are located on the southeastern slope of Lead Mountain in Beddington, Maine (44°51′55″N, 68°6′25″W). Two simultaneous experiments were conducted as part of the Watershed Manipulation Project: a whole catchment manipulation and a plot level study known as the External Plot Experiment (EPE). Results from the EPE are reported here; results from the watershed manipulation are reported elsewhere (Kahl et al., 1993; Nadelhoffer et al., 1995).

The site for the external plots lies at the bottom and slightly outside the two watersheds used in the manipulation experiment. The dominant tree species (Table 1) is American beech (Fagus grandifolia Ehrh.) with lesser amounts of red spruce (Picea rubens Sarg.), yellow birch (Betula alleghaniensis Britt.), red maple (Acer rubrum L.), sugar maple (Acer saccharum Marsh.) and striped maple (Acer

Table 1 External plot initial stand characteristics (data collected in 1987)

	Control	N + S	Low N	High N	Low S	High S
Basal area a	26.55	25.87	27.42	30.36	25	24.81
Tree biomass b	189	158	171	213	187	192
Stem density c						
Amer. Beech	874.1	1007	1140	1111	1511	888.9
Red Spruce	88.89	222.2	251.9	266.7	118.5	148.2
Yellow Birch	59.26	44.44	133.3	14.81	29.63	59.26
Red Maple	148.2	177.8	14.81	103.7	74.07	59.26
Sugar Maple	14.81	74.07	44.44	103.7	29.63	14.81
Striped Maple	281.5	474.1	622.2	266.7	88.89	177.8
Other	59.26	0.00	0.00	0.00	0.00	29.63

 $a m^2 ha^{-1}$ .

b Mg ha-1.

c stems ha<sup>-1</sup>; includes all stems > 7.5 DBH.

Table 2
External plot nitric acid additions (kg N ha<sup>-1</sup> year<sup>-1</sup>), 1988–1991

				-		
Year	Control	N + S	Low N	High N	Low S	High S
1988	0	18	18	61	0	0
1989	0	25	25	50	0	0
1990	0	28	28	56	0	0
1991	0	28	28	56	0	0
Total	0	99	99	223	0	0

pensylvanicum L.) also present. The stand originated following a commercial cutting in the late 1940s and has no known history of agricultural use or fire. Soils are coarse-loamy, mixed, frigid Typic Haplorthods formed from basal till. Initial C:N ratio averaged 23 for the O horizon and 19 for the mineral soil (David et al., 1990). Soils were very acidic with average pH values (in CaCl<sub>2</sub>) of 3.6 for the forest floor and 3.9 for the upper mineral soil (David et al., 1990).

In 1987, eighteen 15 m × 15 m (0.023 ha) plots were established in three elevational bands (245, 260 and 275 ms) in three rows of six plots each. Treatments were randomly assigned to one plot in each row. Additions of H<sub>2</sub>SO<sub>4</sub> and HNO<sub>3</sub> began in August of 1988 and consisted of a control (water), low and high nitrogen (2000 and 4000 eq N ha<sup>-1</sup> year<sup>-1</sup>), low and high sulfur (2000 and 4000 eq S ha<sup>-1</sup> year<sup>-1</sup>), and nitrogen plus sulfur (2000 eq ha<sup>-1</sup> year<sup>-1</sup>) are given in Table 2. Amendments were carried out using a gravity fed irrigation system with 90° arc sprinklers placed in the four corners of each plot (see Rustad et al., 1993 for a more complete description of experimental design).

## 2.2. Soil sampling and analysis

Net N mineralization and nitrification were measured on all plots using the in situ buried bag technique (Nadelhoffer et al., 1983; Pastor et al., 1984). Three pairs of adjacent soil cores (less than 30 cm apart) were collected from each plot, split into organic and mineral horizons and placed into gas-permeable plastic bags. One sample of each pair (initial) was taken to the laboratory for KCl extraction, and the second sample (incubated) was put inside a 1-mm mesh fiberglass screen bag and placed back in

the ground. In situ samples were incubated for 6-8 weeks; three collections were made over the growing season (approximate dates each year: 20 May-10 July, 10 July-20 August, 20 August-10 October) and one set of bags was incubated over winter.

Once collected, the soils were returned to the laboratory for processing. Each core was homogenized by sieving with a 5.6 mm sieve and removing all large roots and rocks. Total soil core weight 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 N KCl, hand shaken and allowed to sit for 48 h. Samples were then filtered and the filtrate frozen. Extracts were analyzed for NO<sub>3</sub>-N and NH<sub>4</sub>-N using a Bran & Luebbe (Technicon) TrAAcs 800 Autoanalyzer. Ammonium was determined using the Berthelot Reaction chemistry (Technicon Method 780-86T): nitrate was determined using a hydrazine sulfate reduction technique (Technicon Method 782-86T). Limit of detection for both methods was  $0.2 \text{ mg N } 1^{-1}$ .

## 2.3. Foliage sampling and analysis

Litterfall was collected twice a year in December and June. Four permanent litter traps were located in each plot. Each trap measured  $32 \text{ cm} \times 35 \text{ cm}$  at the opening. Baskets sat in rebar holders, approximately 1 m above the forest floor, and were removed from the plots during fertilizer applications to avoid potential contamination.

Green leaf collections (fresh foliage) of American beech, yellow birch and red spruce were made in August of each year. When present, three trees of each species were sampled in each plot, and the same tree re-sampled each year. Red spruce needles were not separated by age class in 1988 or 1989 but were separated into 1, 2 and 3 + year classes in 1990 and 1991. Values reported here are for all age classes combined.

Litter samples were sorted by species, then dried for 48 h at 70°C, weighed and ground using a Wiley Mill with a 1-mm mesh screen. Green leaf samples were dried and ground in the same manner without weighing. Green leaf samples from all years, and litter from 1988 only, were analyzed for percent N using a Perkin Elmer 240 or 2400 CHN analyzer.

Litter collected in 1989, 1990 and 1991 was analyzed for percent nitrogen, lignin and cellulose using near-infrared spectroscopy (McLellan et al., 1991; Bolster et al., 1996). All samples were dried overnight at 70°C prior to analysis.

## 2.4. Tree increments

Tree growth was measured using permanently installed aluminum tree bands with a vernier scale (Cattelino et al., 1986). All trees greater than 7.5 cm DBH were banded in May 1988 and an initial reading taken. DBH data were collected in the fall of each year thereafter. Unusually low increments recorded for 1988 suggested that the bands required a year to achieve a tight fit around the stems. Because of this, only 3 years of data were used to determine biomass accumulation (1989–1991). Aboveground woody biomass was calculated using allometric equations for each species (Whittaker et al., 1974). These equations use DBH only

## $\ln AGB = a + b \ln DBH$

where AGB is the aboveground woody biomass, DBH is the tree diameter at breast height and a and b are constants specific to the tree species (Table 3). Annual increases in woody biomass per tree were determined using initial and final DBH for each year to calculate total woody biomass change and subtracting the initial from the final value.

## 2.5. Statistical calculations

For time series data, which included foliar chemistry and extractable ammonium and nitrate measure-

Table 3
Constants for allometric equation a used in calculating above-ground tree biomass. From Whittaker et al. (1974)

Allometric Constants	····	
Species	a	b
Am. Beech	2.3	2.39
Sugar maple	2.22	2.42
Yellow Birch	2.23	2.42
Striped Maple	2.31	2.25
Red Spruce	2.32	2.18
Red Maple	See Sugar Maple	
Other (i.e. Hemlock)	See Red Spruce	

<sup>&</sup>lt;sup>a</sup>  $\ln AGB = a + b \ln DBH$ .

ments, we used the least significant difference (LSD) calculation (Snedecor and Cochran, 1967; Miller and Miller, 1988), given as

$$LSD = (2s^2/n) \times t_{h(n-1)}$$

where s is the within-sample estimate of  $\sigma_0$  and h(n-1) is the number of degrees of freedom of this estimate; t is the critical value of Student's t at the 95% confidence level. One-way analysis of variance was used to test for differences between treatments, and the P=0.05 rejection level was used to determine significance.

## 2.6. Budget calculations

An estimated nitrogen budget was calculated for all plots. Total 4 year fertilizer inputs were added to estimated N inputs from wet and dry deposition (NADP regional data and estimated dry deposition; Ollinger et al., 1993; Ollinger et al., 1995). Leaching losses were estimated using lysimeter nitrogen concentrations (L. Rustad, personal communication, 1995) multiplied by the volume of water leaching below the rooting zone, as calculated by the PnEt-II monthly water balance/photosynthesis/transpiration model (Aber et al., 1995). Annual losses were summed for the 4 year period. Gaseous losses were not measured; however, data from the Harvard Forest study (Bowden et al., 1990; Bowden et al., 1991; Magill et al., 1996) showed no substantial gaseous N losses after 6 years of nitrogen fertilizer applications. Total losses (trace gas plus leaching) were subtracted from total inputs, yielding the total amount of nitrogen retained by the ecosystem.

Net change in N storage was calculated for several measured ecosystem components by taking the difference in total N content between year 4 (1991) and year 1 (1988). Foliar N storage for a given year was calculated as percent N in green foliage times litterfall biomass. Since green foliage was not collected for any maple species (combined maple litterfall was less than 25% of total on any plot), percent N values from maples on the adjacent, untreated watershed were used to calculate maple N storage on the external plots. The conservative assumption was made that N concentration in maple foliage was the same in control and treated plots.

Woody biomass increment data were available for only 3 of the 4 years of the study (as discussed above). Total N increment in woody biomass for the 4 year period was calculated using the 3 year total (1989–1991) woody increment multiplied by 1.33 to estimate the 4 year total, and then multiplied by mean percent N in wood (0.19%). Fine roots were not collected on these plots and are therefore not included in the budget table. Results from the Harvard Forest study suggest that increases in fine root N concentration may account for as much as 15% of added N (Magill et al., 1996).

#### 3. Results and discussion

## 3.1. Net mineralization and nitrification

Initial net N mineralization rates were similar in all plots for 1988 (100 kg ha<sup>-1</sup> year<sup>-1</sup>). In 1989, the control plot rates dropped 30% to 70 kg ha<sup>-1</sup> year<sup>-1</sup>

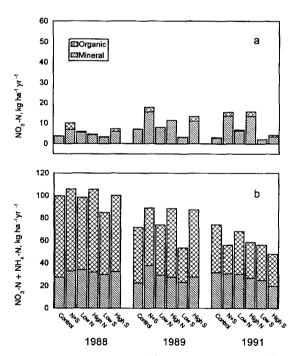


Fig. 1. Annual net nitrification (a) and net mineralization (b) for organic and mineral soil horizons in 1988, 1989 and 1991. Values are the sums of 6–8 week buried bag incubations for the 12 month period from May of the indicated year to May of the following year.

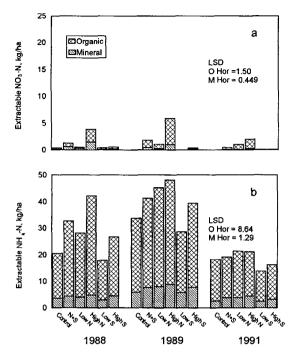


Fig. 2. Mean annual extractable nitrate-N (a) and ammonium-N (b) for organic and mineral soil horizons in 1988, 1989 and 1991. Values are the means of initial buried bag samples collected during the 12 month period from May of the indicated year to May of the following year.

and the other treatments also decreased from 1988 values (Fig. 1). Control plot rates did not change substantially between 1989 and 1991. This large change in N mineralization rate in consecutive years is unusual, and has not been seen in other studies using similar methods (Nadelhoffer et al., 1984; Pastor et al., 1984; Aber et al., 1993; Magill et al., 1996). Initial comparison of mineralization rates with heat sums and water balance models for all 3 years do not explain these differences. Net nitrification rates vary between 4 and 9 kg ha<sup>-1</sup> year<sup>-1</sup> in the control plots over the 3 years of measurement, or from 4 to 12% of net annual mineralization.

In 1988, net N mineralization rates were equal to or higher than control rates for all treatments except low S, while in 1991, control plot measured rates were higher than all other treatments. This same pattern of initial increases and eventual decreases in net mineralization rate has been seen at both the Harvard Forest and at the Mt. Ascutney spruce-fir site (Aber et al., 1995; Magill et al., 1996; McNulty

et al., 1996) and in older studies as well (Baath et al., 1981; Soderstrom et al., 1983). Net nitrification increased in all but the low S stand by 1991 (Fig. 1), representing 8–25% of net annual N mineralization across treatments in that year. Again, similar trends are reported for the Harvard Forest and Mt. Ascutney sites.

Mean annual extractable ammonium pools increased with N additions in 1988 and 1989 (Fig. 2). All values were lower in 1991 and differences between treatments were no longer significant. Extractable nitrate was four to five times higher in the high N plot than the other treatments in 1989, and those differences decreased between 1989 and 1991 in parallel with extractable ammonium (Fig. 2).

# 3.2. Foliar chemistry

Some significant differences in foliar N content were found both between years for a given treatment and between treatments for a given year using one-way analysis of variance with a P=0.05 rejection level. Comparing N treated plots only, foliar N concentration was consistently higher than the control for both American beech and yellow birch (Fig. 3), and those differences generally increased with time, especially the high N plots. Although similar results were reported for the Harvard Forest and Mt. Ascutney studies (Aber et al., 1995; Magill et al., 1996; McNulty et al., 1996), the differences here were smaller than in those stands.

## 3.3. Aboveground biomass

Woody biomass increment and foliar litterfall (equivalent to foliar production for this dominantly

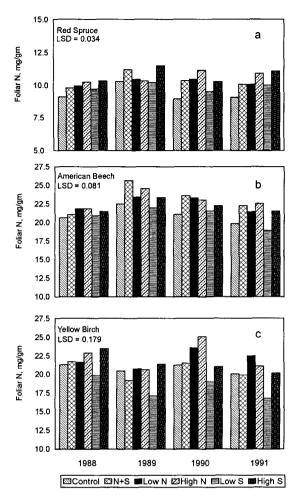


Fig. 3. Green foliage nitrogen concentration (mg g<sup>-1</sup>), by treatment, for the three dominant species on the external plots: (a) red spruce; (b) american beech; (c) yellow birch.

Table 4 Summary of mean annual and cumulative aboveground wood growth, litterfall and NPP for 1989–1991. All mean values in kg ha<sup>-1</sup> year<sup>-1</sup> with standard errors in parentheses, n = 3 plots; cumulative values in kg ha<sup>-1</sup>

			-			
	Control	N + S	Low N	High N	Low S	High S
Mean wood production	5385	5478	5625	6132	6988	4657
	(737.3)	(31.84)	(490.1)	(772.5)	(1055)	(314.3)
Mean litter production	3543	3598	3804	4014	3295	3768
-	(287.2)	(221.7)	(204.7)	(272.4)	(195.6)	(144.2)
Mean aboveground NPP	8928	9075	9430	10146	10282	8425
Cumulative wood production	16154	16434	16875	18395	20963	13970
Cumulative tree mortality	1625	6372	3847	5659	2758	5363
Cumulative biomass change	14529	10062	13029	12736	18205	8607

deciduous forest) were both higher in the N amended and low S plots than in the controls (Table 4), although overall differences were not statistically significant. Tree mortality did increase substantially in all but the low S treatment, although no significant treatment differences were seen. Beech bark disease (Shigo, 1972; Houston, 1975; Houston et al., 1979) occurred on most beech trees in all plots, but whether or not N or S additions might accelerate mortality from this disease is unknown. Beech comprised 70% of total tree mortality in 1989, and 31% in 1991; no beech mortality was observed in 1990. Once again, either declining tree growth or increased tree mortality, or both, were reported at the Harvard Forest and Mt. Ascutney sites.

#### 3.4. Nitrogen budget

An ecosystem budget was calculated in order to determine the retention capacity of the system (Table 5). In general, N leaching losses increased with increasing N additions. Only the low S plot showed no detectable nitrate leaching. Nitrogen retention ranged from 93 to 97% of inputs in the control and

N amended sites. Similarly, both stands at the Harvard Forest retained 100% of the added N under control and low N amendment conditions (inputs were up to three times higher than in this study), and only the high N addition in the pine stand had any significant nitrate leaching over the first 6 years.

Measurement of ecosystem pools (Table 5) revealed that extractable and perhaps organic soil N reserves were depleted to supplement woody biomass growth in the control and low sulfur plots, where inputs of N were only from atmospheric sources. N amended plots retained 57-82% of N inputs, presumably accumulating in the soil pool. Results from a <sup>15</sup>N tracer study on the same plots (Nadelhoffer et al., 1995) identified 14% of added <sup>15</sup>N to be in green leaf and wood pools, and 15% of added <sup>15</sup>N to be in litter, forest floor and the top 10 cm of mineral soil. With 4-7% of inputs lost in leaching (Table 5), it is assumed that the remaining 65% is in deeper mineral soil horizons. Using bulk density and soil percent N data, the total organic N pool was estimated as 1493 kg N ha<sup>-1</sup> in the organic horizon and 4927 kg N ha<sup>-1</sup> in the mineral soil (top 25 cm), for a total of 6420 kg N ha<sup>-1</sup>. Additions of 243 kg N ha<sup>-1</sup> (high

Table 5
Nitrogen inputs, outputs, retention and changes in nitrogen pools after 4 years of treatment. See text for calculations

	Treatment						
	Control	N + S	Low N	High N	Low S	High S	
N inputs (kg NO <sub>3</sub> -N ha <sup>-1</sup> )							
Atmospheric dep.	20	20	20	20	20	20	
Fertilization	0	99	99	223	0	0	
Total	20	119	119	243	20	20	
N losses (kg ha <sup>-1</sup> )							
Gaseous	< 1	< 1	< 1	< 1	< 1	< 1	
Leaching	1	5	3	18	< 1	1	
Total	1	5	3	18	< 1	1	
N retention							
% of N inputs	95	96	97	93	100	95	
Change in N storage (kg ha <sup>-1</sup> )							
Soil extractable N	-3	-14	-6	-23	-4	-11	
Woody biomass	41	42	43	46	53	35	
Foliage	-1	8	3	3	0	-6	
Soil organic matter or long term pool (by difference)	- 18	68	76	199	- 29	1	
Retention by soil pool							
% of N retained	_	60	66	88	_	-	
% of N added	_	57	64	82	_	_	

N treatment) represent less than 4% of this total and would not be detectable in altered total soil N or C:N ratios.

## 4. Conclusions

Results from the external plots at Bear Brooks are similar to those from the Harvard Forest and Mt. Ascutney sites in several ways. N mineralization rates (expressed as a ratio of treated to control sites) increased initially and then decreased as the experiment continued. Nitrification increased substantially with N amendments throughout the experiment. Data in all three studies suggest that either woody production declined or tree mortality increased with increasing, long-term N additions.

Differences between studies may be significant however, and contradict the initial hypothesis that N retention would be greater at the Maine site because of a history of lower N deposition rates. Instead, the Harvard Forest sites, particularly the oak-maple stand which had the lowest, control plot net N mineralization rate of the three, exhibited tighter N cycling (higher retention rates) and lower rates of nitrification. Both Harvard Forest stands had a significant history of land use and/or fire, while the Bear Brooks watersheds were harvested heavily perhaps only once. It appears then, that the difference in previous land use history has had a greater effect on current N cycling rates than differences in cumulative N deposition. This suggests that increased effort should be given to understanding the effects of different land use practices on soil N status and N mineralization and nitrification rates. The results of such studies could add to our understanding and prediction of the spatial and temporal variability of nitrogen saturation in forest ecosystems in industrialized and developing regions.

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