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Watershed liming effects on the forest floor N cycle

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Abstract. The forest floor was expected to play a major role in determining the total ecosystem response to watershed liming because of its high concentration of nutrients and its high level of activity. Net N mineralization and net nitrification were estimated in a field survey using the buried-bag approach. In a laboratory incubation experiment, forest floor humus was mixed with 6 doses of lime to determine the sensitivity of N mineralization and nitrification to lime dose. Forest floor microcosms with and without live tree roots were used to calculate a N budget for the system.

The pH of the forest floor increased from 3.6 to 4.9 in the Oe and to 4.0 in the Oa two years after liming. The extractable ammonium pool in both the field survey and microcosm study was substantially smaller after liming and was probably a result of the 36% to 55% lower net N mineralization rate in limed plots than in reference plots. The laboratory incubation results agreed with the field survey results and further demonstrated that at higher lime doses (pH 5 to 6), N mineralization increased above controls. Net nitrification in limed humus in both the buried bags and laboratory incubation was as much as three times higher than controls, which could explain why nitrate leaching in limed microcosms was greater than in control microcosms. However, nitrate leaching from microcosms with live roots was not affected by liming, suggesting that roots in the forest floor may prevent excess nitrate leaching. Reductions in N mineralization had no effect on N leaching or N uptake, but reduced the extractable ammonium pool.

Introduction

The forest floor of northern hardwood forests, although comprising just 10% of total ecosystem organic matter mass, has a major influence on ecosystem properties and processes, such as nutrient retention (Wood et al. 1984), stream chemistry (Vitousek et al. 1979), aluminum leaching (Rutherford et al. 1985; Evans 1986) and CO_2 production (Edwards & Harris 1977). Mineralization of nitrogen and other nutrients on a unit mass basis is greater in the forest floor than in mineral soil (Federer 1983; Boone 1992). Furthermore, root densities are usually highest in the forest floor, probably as a result of the high nutrient availability, making it a primary site of nutrient uptake by plants (Safford 1974; Yanai 1992; Burke & Raynal 1994). Thus, the response of forest floor dynamics to lime were expected to play a major role in determining the total ecosystem response to watershed liming. The principal objective of this study was to quantify the effects of liming on N transformations and fluxes in a northern hardwood forest floor.

Numerous acid deposition-related studies have demonstrated liming effects on net N mineralization in forest soils (Nyborg & Hoyt 1978; Francis 1982; Sahrawat et al. 1985; Persson et al. 1989). In general, liming increases net N mineralization in the short term (weeks to months), although the mechanism behind this increase is unknown. One hypothesis is that liming makes a portion of the soil organic matter more susceptible to mineralization and that this leads to a flush of microbial activity (Persson 1988). In contrast, two studies using coniferous forest soils reported a decline or no change in net N mineralization after liming, which was attributed to the high C:N ratio (C:N > 32) of coniferous litter (Nommik 1977; Persson 1988; Persson et al. 1989). Because Woods Lake was a predominantly hardwood forest site, we expected net N mineralization to increase in response to liming, at least in the short term.

Net nitrification is strongly correlated with pH in a wide range of soils and was expected to increase with pH (Dancer et al. 1973; Heilman 1974; Nyborg & Hoyt 1978). A major concern of investigators in the present study was that increased nitrification would generate sufficient acidity to partially counteract liming (Driscoll et al. 1996, this issue).

Other factors that could be affected indirectly by liming through its effect on inorganic N concentrations included N uptake and leaching as well as litter N concentration (Smallidge et al. 1993). Both uptake and leaching rates of inorganic N depend upon inorganic N concentration in soil as well as the relative amounts in ammonium and nitrate forms. Increased nitrate leaching into streams was a water quality concern especially during spring snowmelt (Rascher et al. 1987; Driscoll et al. 1996, this issue). Litter N concentrations were expected to increase if N uptake by plants was stimulated by the greater N supply from increased N mineralization (Nadelhoffer et al. 1983). Furthermore, a positive feedback could result in which higher litter N concentrations in response to liming would in turn stimulate N mineralization in the long-term (years).

Methods

Field survey

This investigation was part of the Experimental Watershed Liming Study (Driscoll et al. 1996, this issue). A one time application of 8 tons ha^{-1} of crushed limestone (primarily calcite) was applied to catchments II and IV

Table 1. Means and standard deviations of key forest floor characteristics in study plots at Woods Lake, NY. The number of samples were 80 for forest floor thickness, 32 for bulk density, 32 for percent carbon and 40 for percent N.

Horizon	Thickness (cm)	Bulk Density (kg m^{-3})	%C	%N
Oi	3 (0.4)	nd	59 (3)	1.21 (0.18)
Oe	2 (1.3)	0.08 (0.02)	57 (6)	2.07 (0.34)
Oa	6 (3.6)	0.12 (0.03)	57 (8)	2.36 (0.37)

of the Woods Lake watershed in the Adirondack Park, NY. The response of these two forested catchments was compared to the reference catchments, I and V. Pretreatment measurements were made in the summer of 1989. Lime application took place in early October, 1989 during leaf fall. The lime dissolved slowly, so that after the two-year study approximately 48% of the applied calcite remained undissolved at the surface of the Oe horizon.

Two study plots (10 $\times$ 20 m) were established in the summer of 1989 at random locations within each of the reference and limed catchments. Forest cover of the plots was representative of the upland area of the watershed. The dominant tree species were red maple (*Acer rubrum* L.), American beech (*Fagus grandifolia* Ehrh.) and yellow birch (*Betula allegheniensis* Britt.). The soil in the study plots was a well-developed spodosol derived from granitic till less than 3 meters in depth. It was classified as Tunbridge-Lyman (coarse-loamy, mixed, frigid Typic Haplorthod) and consisted of Oi/Oe/Oa (−12–0 cm), Ae (0–4 cm), Bs (4–10 cm), B (10–50 cm) and C (>50 cm) horizons. Site and soil characteristics are thoroughly described by Cronan (1985), April & Newton (1985) and Smallidge & Leopold (1994). Key forest floor characteristics are summarized in Table 1.

Net N mineralization and net nitrification were measured within each plot using *in situ* buried bags three times during the summer months (Hart et al. 1994). A forest floor block, approximately 200 cm^2 and 8 cm deep, was cut and removed after brushing off the Oi layer. The block was cut in half and each half placed in a polyethylene bag. One of the halves was replaced in the ground, covered with litter and incubated for three weeks while the other was transported under ice to the laboratory for extraction the following day. On each sampling date five blocks were removed from each of the plots for a total of 40 samples. An additional set of five forest floor blocks from each plot were removed monthly from April through October to determine the pool sizes of ammonium and nitrate.

In 1989 each forest floor block was mixed to create one sample. However, in 1990 and 1991 we first removed the layer of undissolved lime from the surface of the Oe and then subdivided each block into Oe and Oa horizon material before processing. Soil material from each horizon was mixed and subsamples were removed for determination of water content and pH (in a 10:1 water:soil mix) and extraction of nitrogen. Ammonium, nitrate and nitrite were extracted for 36 hours in 100 ml of 1N KCl from 8 g (dry equivalent) of soil material and their concentrations determined colorimetrically (Driscoll et al. 1996, this issue, Table 3). Nitrite concentrations were assessed regularly, but were always below the detection limit (4 μmol/L). Net N mineralization was calculated as the sum of net ammonium and net nitrate production during the incubation.

Five litterfall collectors (0.14 m^2) were deployed at 10 m intervals along transects that were centered on each of the study plots. They were set out in late August of each year and collected in late October. In 1989 the lime application occurred in the middle of leaf fall, so litter was collected just before liming and then again at the end of October. Leaves collected after the liming operation in 1989 were coated with lime and were excluded from chemical analysis. Air-dry litterfall (which included woody litter) was sorted into red maple, beech, yellow birch and "other" categories, then oven-dried (60 °C) and weighed. Samples of each of the three species from the same transect were pooled, ground in a Wiley mill and analyzed for nitrogen by micro-Kjeldahl digestion (Bremner & Mulvaney 1982).

Nitrogen data from each year were analyzed separately for two reasons: 1) comparing the pooled Oe/Oa samples from 1989 with the separated Oe and Oa samples in 1990–91 was not appropriate and 2) sampling occurred within different time periods and with differing intensities each year. A repeated measures analysis of variance (RMANOVA) was performed for each year for each horizon using lime treatment and date as classifications (Lindsey 1993). Litter data were analyzed by a RMANOVA where year, lime treatment and species were used as classifications (Lindsey 1993). pH data were classified by year, lime treatment and horizon. If a significant year $\times$ treatment interaction was detected then Fisher's PLSD was used to separate the means. The significance level for all tests was 0.05.

Laboratory incubation

In July 1990, we collected forest floor material to study the effect of different lime doses on N dynamics. Approximately 0.25 m^2 of Oe and Oa material was collected from the reference watershed I, sieved through 2mm mesh and stored overnight at 4 °C. The equivalent of 3 g dry Oe horizon material and 6 g dry Oa horizon material was placed in flasks along with powdered, reagent-grade $CaCO_3$ at the following rates: 0, 20, 40, 70 and 200 mg $CaCO_3$ per gram of dry Oe and 0, 10, 30, 50 and 150 mg g^{-1} dry Oa. Preliminary tests showed that these lime doses would yield a range of pH values (3.3 to 7.0) that encompassed the observed pH *in situ*. The flasks were covered with a plastic film that allowed gas exchange while preventing water loss and were incubated in the dark at 20 °C for 6 weeks. Extractable ammonium, nitrate and nitrite were determined initially and at the end of the incubation as described in the field study. An analysis of variance was performed to test for differences among treatment means and between soil horizons. Fisher's PLSD was used to separate the means at a significance level of 0.05.

Field microcosms

In July 1989 we installed field microcosms that provided a closed system for which a nitrogen budget could be calculated. This permitted estimation of liming effects on soil solution chemistry and N uptake by the root system of mature trees. Four randomly-located trenches ($1 \times 10 \times 0.5$ m deep) were excavated within both reference catchment I and limed catchment II. Eight forest floor microcosms were installed about 0.5 m uphill from the edge of each trench for a total of 64 microcosms. Microcosms consisted of a 17 cm diameter by 10 cm tall polyethylene cylinder with a bead-filled funnel attached to the bottom for drainage. A 17 cm diameter intact forest floor core (Oi/Oe/Oa) was removed and placed in each cylinder. Each microcosm was placed in the hole left by the core so that the surface of the core was level with the surface of the surrounding soil. Collection bottles were set in niches dug out of the wall of the trench beneath each microcosm. Polyethylene tubing connected the funnel to the collection bottle. Finally, the trenches were backfilled. With this system, the intact cores in the microcosms were exposed to ambient temperatures and precipitation. Forest floor leachate drained into the collection bottle and was removed through tubing that led to the soil surface by a hand pump.

We attempted to mitigate some of the disturbance effects created by the coring process by introducing live fine roots into half of the microcosms (Willison et al. 1990). Two small holes were drilled in the sides of four, randomly-chosen microcosms at each trench. Then fine roots were carefully teased from the Oe horizon near the microcosms and 2 cm of a live root tip of beech or maple was inserted into the hole in the microcosm which was then sealed with silicon glue. Roots were misted every few minutes during this process to prevent desiccation. Roots were able to grow into the forest floor core yet microcosm water was contained.

The lime equivalent of 0.8 kg $CaCO_3$ m^{-2} was added to the microcosms in limed catchment II, in October, 1989 (helicopter-applied lime was excluded).

This was the same lime that was applied to the whole catchment. This resulted in a 2×2 factorial experiment with the four treatments being designated as Control, Root, Lime and Root + Lime.

One rain event per month was sampled in 1989 and 1991, whereas 95% of the 1990 rain events were sampled (29 events in 1990 plus spring snowmelt). Collection bottles were emptied prior to these rain events and leachate was retrieved within 24 hours of the event. Volumes were recorded and subsamples transported under ice to the laboratory for analysis the next day. Ammonium was analyzed colorimetrically as described above and nitrate was measured using ion chromatography. In between sampling events, only the volume of leachate was determined. Total water flux (including snowmelt) from each microcosm was determined from December 1989 to December 1990. The volume-weighted annual mean concentration of ammonium and nitrate in each microcosm was calculated during the same period. Total inorganic N flux in leachate was estimated by multiplying the mean water flux by the mean inorganic N concentration.

A total of eight microcosms from the Root and Root + Lime treatments (one randomly-selected from each trench) were harvested in September 1990 to determine the extent of root growth within microcosms. Fine roots (< 2 mm) were hand sorted from 3 pie-shaped subsamples (randomly selected from eight possible pieces) cut from each microcosm horizon. At the same time, fine roots (< 2 mm) were sorted from three randomly-located forest floor blocks (64 cm^2 by 8 cm deep) from each of the plots. Blocks were collected in September of 1990 and stored at 4 °C until processed. After sorting roots were rinsed, dried and weighed. Live roots were distinguished from dead roots by their resiliency, color and whether or not the cortex separated from the pericycle (Burke & Raynal 1994).

Root, ammonium and nitrate data were analyzed using ANOVA with horizon and treatment as classifications (Snedecor & Cochran 1980). Fisher's PLSD was then used to separate means if the ANOVA detected significant differences. Soil solution data were divided into two time periods: pre-lime (4 collection dates) and 1990/91 (34 collection dates). Because the pre-lime samples were taken only in the summer months, it is not appropriate to compare these data with those from 1990/91, which were sampled throughout the year. Therefore, data from these two time periods were analyzed separately. A repeated measures analysis of variance (RMANOVA) was conducted for each element to detect significant effects of treatment and date within each time period (Lindsey 1993). If significant treatment effects were detected then Fisher's PLSD was used to distinguish treatment effects for each time period. The significance level was 0.05 for all tests.

Table 2. Mean soil pH of Woods Lake forest floor in reference and limed catchments. The 1989 samples were taken prior to liming. Each value is the mean of 96 samples collected on three dates during each year.

Year	Oe horizon Reference	Oe horizon Lime	Oa horizon Reference	Oa horizon Lime
1989	3.65	3.63	3.68	3.61
1990	3.71	4.51***	3.68	3.92***
1991	3.56	4.87***	3.52	4.02***

*** Significantly different from reference ($p < 0.001$; Fisher's PLSD)

Results

Field survey

Mean forest floor pH (in water) ranged from 3.61 to 3.68 in the study plots prior to liming and did not change significantly in reference plots during the three-year study period (Table 2). One year after liming, the pH in limed plots was higher than in reference plots by 0.80 units in the Oe horizon and 0.24 units in the Oa horizon. After two years the pH differences increased to 1.31 units in the Oe and 0.50 units in the Oa. Thus, the effects of liming were greatest in the Oe and increased through time.

Extractable ammonium content of the forest floor was extremely variable through time, ranging from 24 to 272 mol ha^{-1} (Fig. 1). In combined Oe/Oa samples prior to liming, there was a significant treatment effect indicating that the ammonium pool was significantly larger on average in treatment catchments than in reference catchments (Table 3). In contrast, in 1990 and 1991 the extractable ammonium pool in the Oe material was significantly smaller in limed areas than in reference areas (by 34% and 31%, respectively). Similarly, in the Oa horizon, ammonium generally was less abundant in limed areas than in reference areas, but that effect was not significant until 1991. The RMANOVA also revealed significant date and treatment $\times$ date interactions. These results simply indicate that part of the variability of the data results from seasonal variations in ammonium concentrations. Ammonium concentrations in the Oe horizon were significantly lower in limed than in reference plots in May, July and September, 1990 and June and July 1991 (Fig. 1). In the Oa horizon significantly lower values appeared only twice: in May 1990 and July 1991.

There was no significant treatment effect in nitrate concentrations except in the Oe horizon in 1990 (Table 3). Nitrate concentrations in the Oe were significantly higher in the limed catchments that year. A significant date and

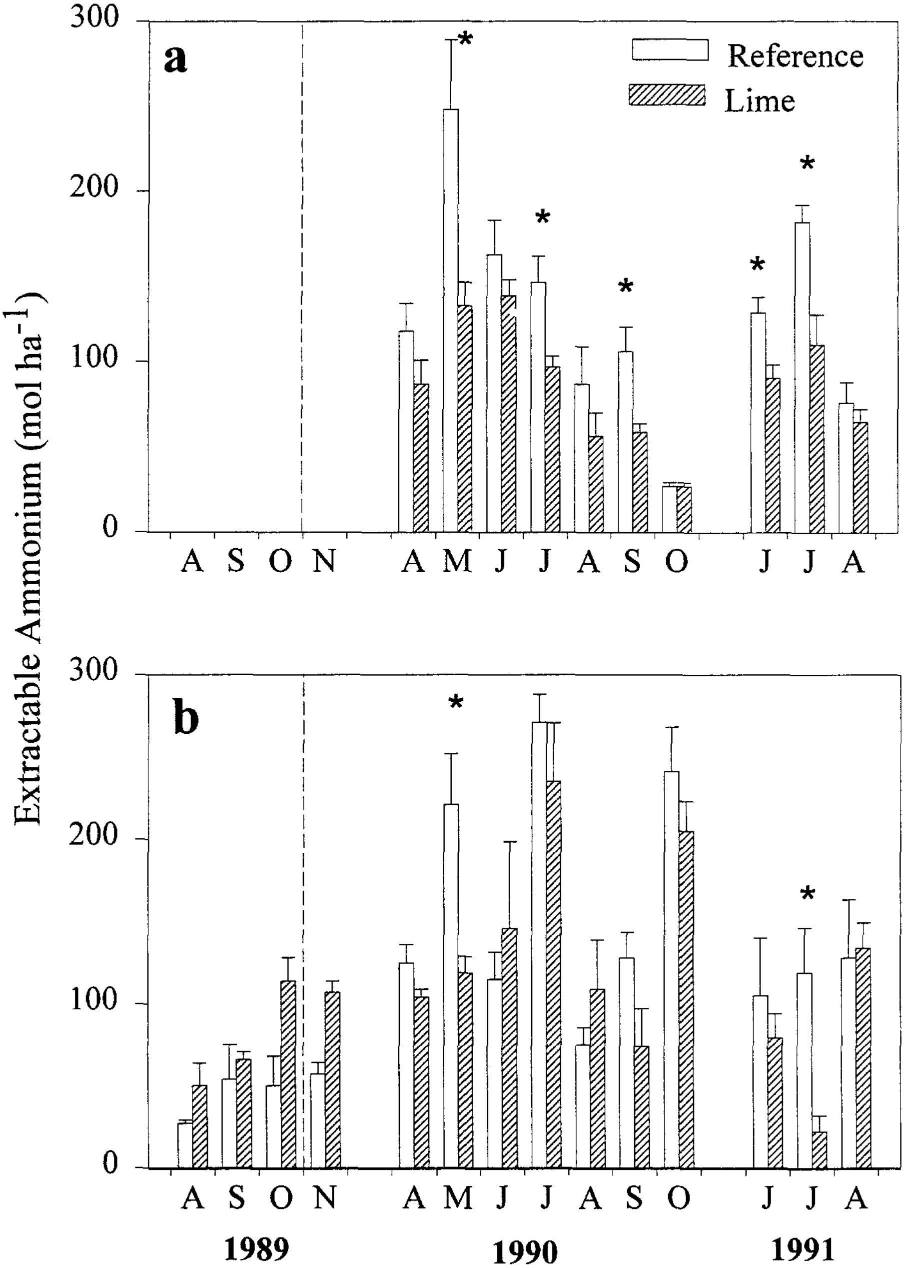

Fig. 1. Extractable ammonium concentration in the Oe (a) and Oa (b) horizons of reference and limed catchments at Woods Lake, NY. The 1989 values represent combined Oe/Oa material. Values are the means of 16 samples with the standard deviation bars. Asterisks indicate that lime values were significantly different from reference values on that date ($p < 0.05$; Fisher's PLSD). The dashed line shows the date of lime application.

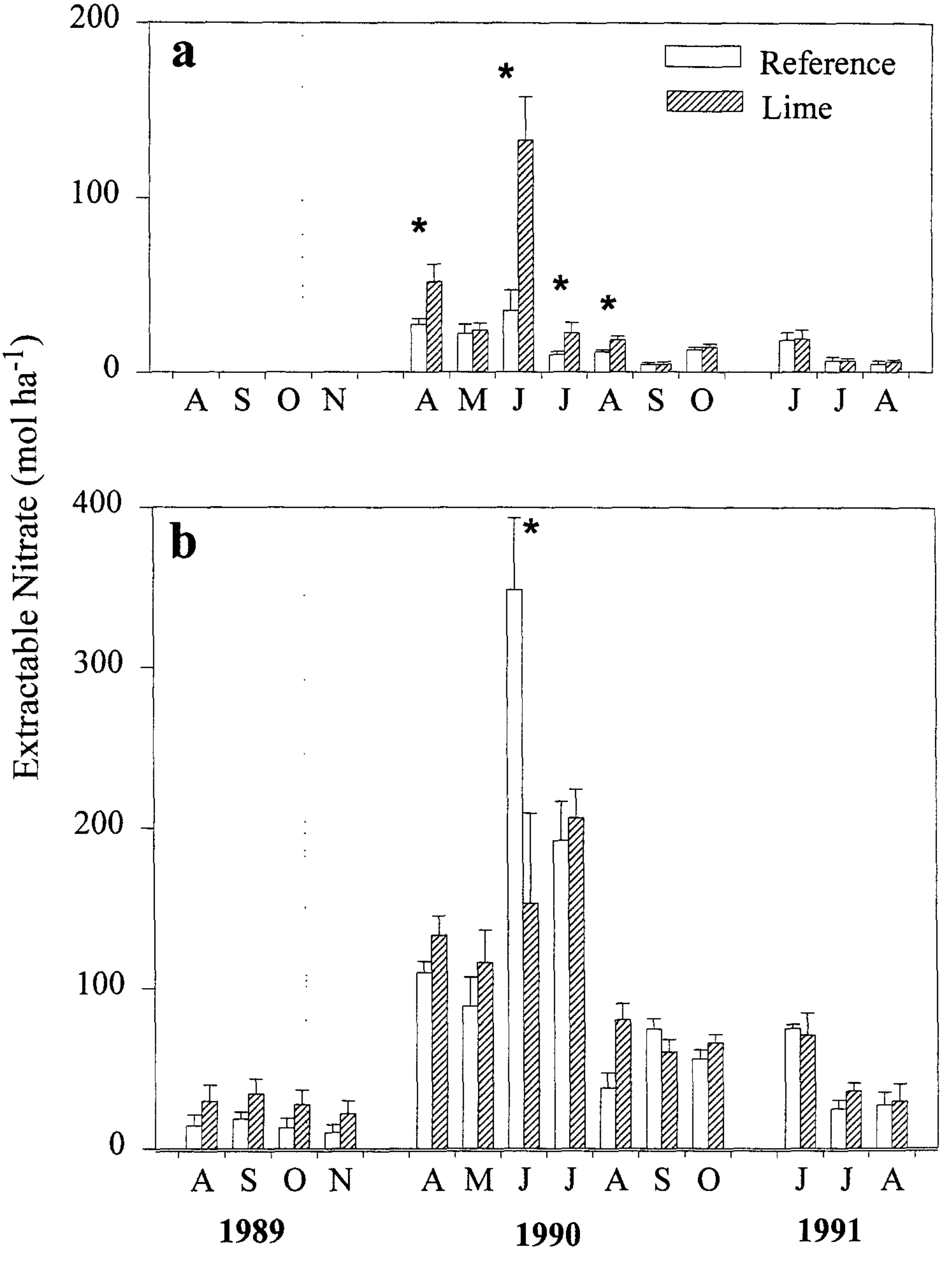

Fig. 2. Extractable nitrate concentration in the Oe (a) and Oa (b) horizons of reference and limed catchments at Woods Lake, NY. The 1989 values represent combined Oe/Oa material. Values are the means of 16 samples with the standard deviation bars. Asterisks indicate that lime values were significantly different from reference values on that date ($p < 0.05$; Fisher's PLSD). The dashed line shows the date of lime application.

Table 3. Differences between limed and reference catchments of mean annual inorganic N concentrations (mol ha^{-1}) and mean annual net N mineralization and nitrification (mol ha^{-1} d^{-1}). Percent change from the reference is shown in parentheses. One or two asterisks indicate that the difference was significant in that year at 0.05 and 0.01, respectively. Positive numbers indicate that the mean annual value in the limed catchment was greater, and negative numbers indicate that the mean annual value in the reference catchment was greater.

Year	Ammonium Concentration		Nitrate Concentration		Net N Mineralization		Net Nitrification	
	Oe/Oa		Oe/Oa		Oe/Oa		Oe/Oa	
1989	38*		13		2.2		−0.7	
	(82)		(93)		(14)		(21)	
	Oe	Oa	Oe	Oa	Oe	Oa	Oe	Oa
1990	−44*	−27	20**	−13	−8*	−1	5.9**	−0.8
	(34)	(16)	(111)	(10)	(36)	(5)	(120)	(7)
1991	−41*	−40*	1	−3	−13*	−15*	6.9**	4.8*
	(31)	(33)	(7)	(7)	(55)	(36)	(164)	(23)

Table 4. Nitrogen concentration (%) of leaf litter for three of the dominant tree species at Woods Lake. Values are means of 4 bulked samples with standard deviations in parentheses. Liming occurred after sampling in 1989.

Year	Red Maple		American Beech		Yellow Birch	
	REF	LIME	REF	LIME	REF	LIME
1989	1.16	1.15	1.26	1.23	1.83	1.87
	(0.11)	(0.05)	(0.05)	(0.03)	(0.05)	(0.10)
1990	1.22	0.89*	1.15	1.10	1.74	1.68
	(0.06)	(0.01)	(0.04)	(0.10)	(0.04)	(0.06)
1991	1.00	0.94	1.03	0.98	1.53	1.51
	(0.05)	(0.05)	(0.03)	(0.02)	(0.11)	(0.09)

* Significantly different from reference ($p < 0.05$; Fisher's PLSD)

treatment × date interaction was apparent in both horizons in 1990 only. Nitrate was significantly greater in limed catchments in April, June, July and August 1990 in the Oe horizon but was significantly lower in June 1990 in the Oa horizon.

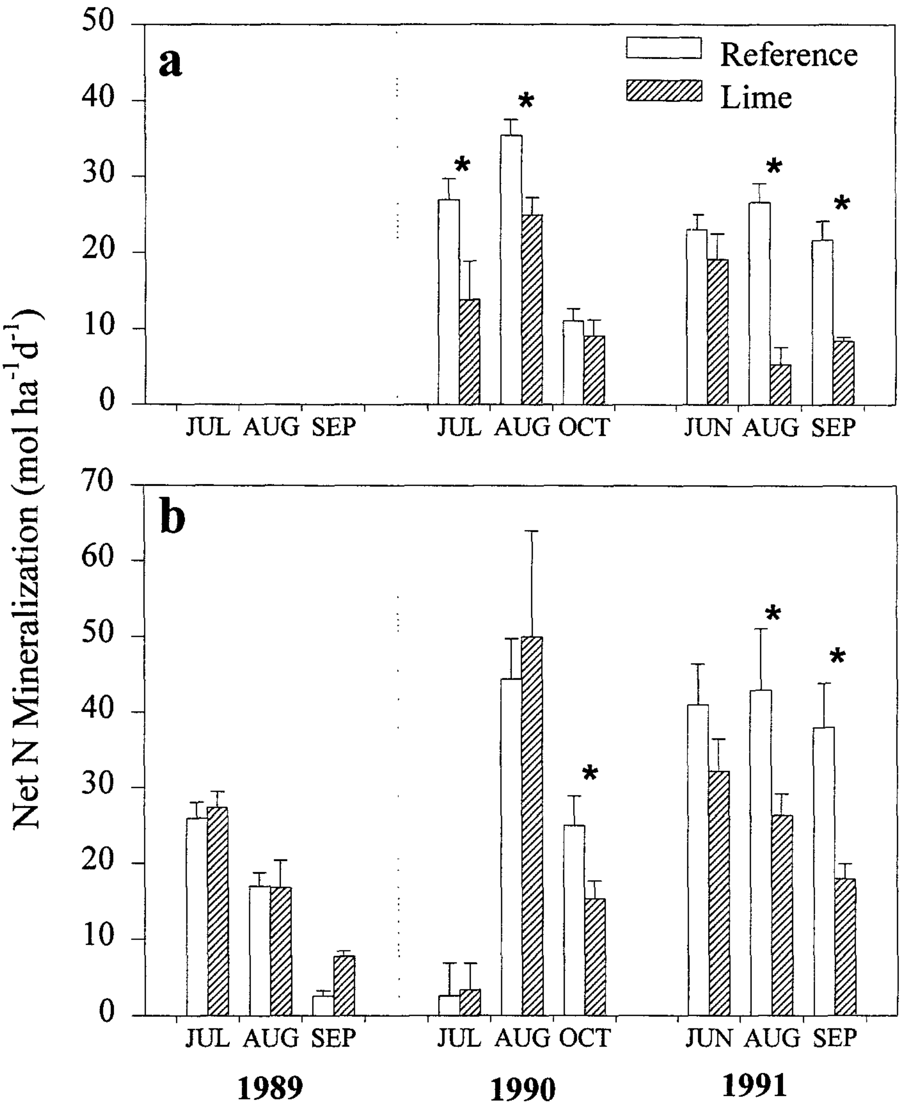

Fig. 3. Net N mineralization in the Oe (a) and Oa (b) horizons of reference and limed catchments at Woods Lake, NY. The 1989 values represent combined Oe/Oa material. Values are the means of 16 samples with the standard deviation bars. Asterisks indicate that lime values were significantly different from reference values on that date ($p < 0.05$; Fisher's PLSD). Lime was applied between the 1989 and 1990 sampling dates.

Net N mineralization estimates in the Oe based upon *in situ* buried bags ranged from 5.7 to 36 mol ha^{-1} d^{-1} (Fig. 3). There was no treatment effect prior to liming, but the RMANOVA revealed significant treatment, date and treatment $\times$ date effects in 1990 and 1991. Mean annual net N mineralization

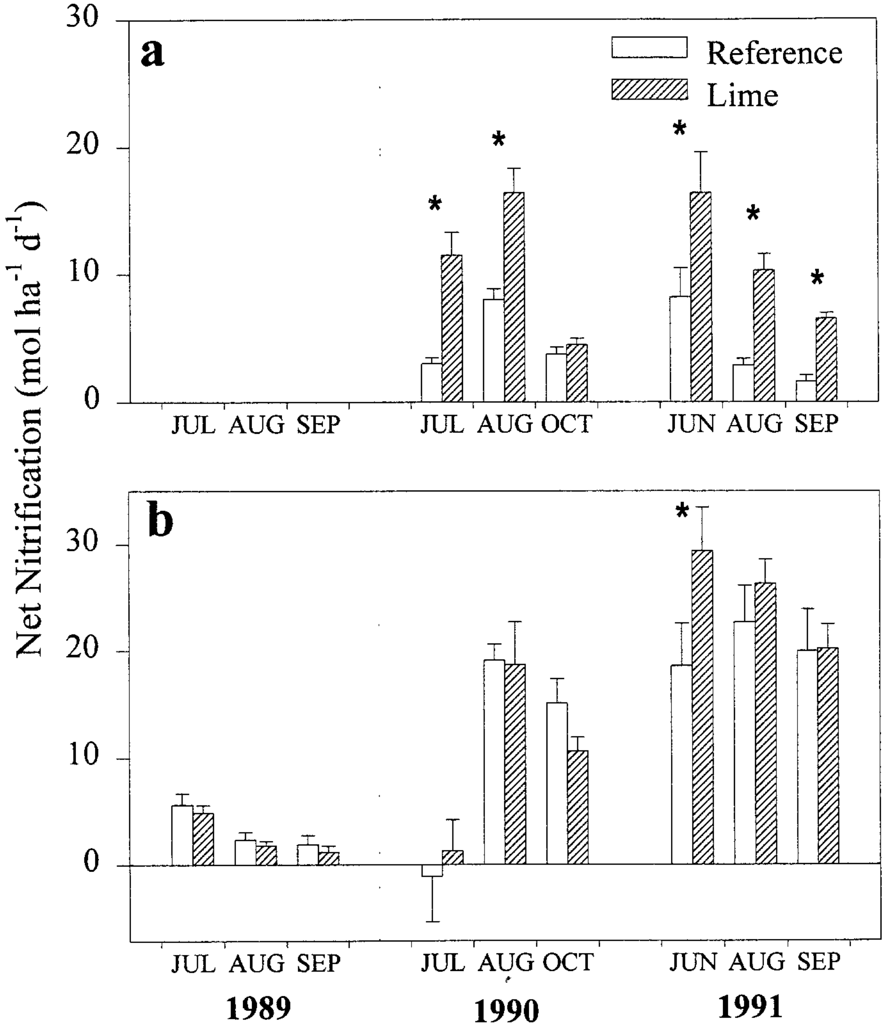

Fig. 4. Net nitrification in the Oe (a) and Oa (b) horizons of reference and limed catchments at Woods Lake, NY. The 1989 values represent combined Oe/Oa material. Values are the means of 16 samples with the standard deviation bars. Asterisks indicate that lime values were significantly different from reference values on that date ($p < 0.05$; Fisher's PLSD). Lime was applied between the 1989 and 1990 sampling dates.

was lower in limed than in reference plots by 36% in 1990 and by 55% in 1991 (Table 3). Net N mineralization rates were significantly lower in July and August 1990 and August and September 1991. In the Oa horizon net N mineralization values were between 2.9 and 49 mol ha^{-1} d^{-1}. Treatment effects were not significant in the Oa horizon until 1991 when mean annual net N mineralization was reduced by 36%. Net N mineralization rates were

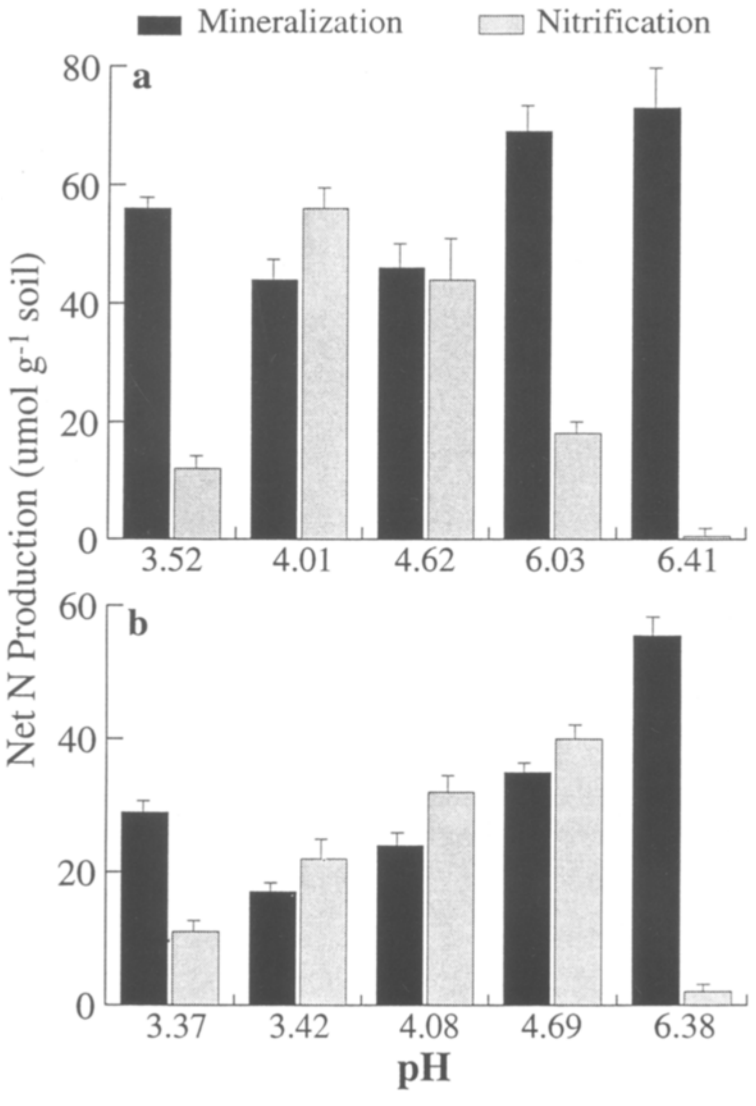

Fig. 5. Net N mineralization and net nitrification during six-week laboratory incubation of Oe (a) and Oa (b) horizon material amended with $CaCO_3$. There were six replicates of each treatment and the standard deviation bars are shown.

significantly lower in limed catchments in October 1990 and August and September 1991.

A large portion of the N mineralized was subsequently nitrified. Net nitrification ranged from 1.4 to 17 mol ha^{-1} d^{-1} for the Oe and -1.2 to 30 mol ha^{-1} d^{-1} for the Oa (Fig. 4). The RMANOVA indicated that it was significantly greater in limed areas than in reference areas in the Oe horizon. On average it was 120% greater than the reference in 1990 and 164% in 1991 (Table 3). Net nitrification was significantly greater in limed areas on all dates except in October 1990. As a percentage of N mineralized, nitrate production in limed catchments increased from 19% in the combined Oe/Oa prior to liming to 92% in the Oe horizon in 1991. In the Oa horizon the differences were not as pronounced. Nitrate was significantly greater in limed areas by 23% in 1991 but in 1990 there was no difference. Focusing on individual dates, nitrate was significantly greater in limed areas only in June 1991.

N concentration of leaf litter was significantly affected by species, year and treatment $\times$ year interaction. Percent N ranged from 0.89 (maple) to 1.87 (birch) and was significantly higher in birch than in maple and beech. N concentrations in leaf litter decreased significantly during the three years of study (Table 4). There was no overall treatment effect, but red maple, the species with the lowest N concentration initially, exhibited a significantly lower N concentration in limed areas one year after liming. N concentration in live red maple foliage was not affected by liming (1.82 $\pm$ 0.008 in reference catchments vs. 1.98 $\pm$ 0.07 in limed catchments in 1990; A. Brach, pers. comm.), suggesting that the significant difference in red maple litter N was a result of greater retranslocation of N before abscission in limed areas.

Laboratory incubation

Adding different amounts of $CaCO_3$ to forest floor material yielded a range of soil pH values from 3.52 to 6.41 for Oe material and from 3.37 to 6.38 for Oa material (Fig. 5). For both horizons net N mineralized during the six week incubation was significantly less under the two lowest $CaCO_3$ additions and significantly greater under the two highest $CaCO_3$ additions compared to the controls. Thus, the response of N mineralization to soil pH varied non-linearly with pH, reversing above pH 4.7 in the Oe and above pH 4.1 in the Oa. Net N mineralization in the laboratory incubation was reduced at pH values similar to those measured in the field (Table 2), confirming the apparent inhibition of mineralization observed in the field survey.

Net nitrification in the Oe showed the greatest increase above controls in the lowest lime treatment (pH 4.01; Fig. 5). Successively higher lime additions led to smaller increases. The highest lime treatment (pH 6.41) exhibited virtually no net nitrification. In the Oa nitrification increased with lime dose except at the highest dose (pH 6.38) where it was significantly less than the control. The stimulation of nitrification at intermediate pH (4.0 to 6.0) is consistent with the field survey results. Few studies have reported a reduction in nitrification with increasing pH in these ranges. Stroo et al. (1986) observed a decline in nitrification above pH 5 in a slurry of Adirondack forest

Table 5. Root mass (g dry wt. m^{-2}) in microcosms and in undisturbed forest floor. The values are means based upon 4 microcosms or 8 cores with standard deviations in parentheses.

	Microcosm		Undisturbed Forest Floor	
Horizon	Root	Root + Lime	Reference	Limed
		1990		
Oe	75 (43)	42 (32)	80 (67)	60 (47)
Oa	89 (130)	62 (55)	310 (220)	250 (190)
Total	164 (137)	104 (64)	390 (230)	310 (196)
		1991		
Oe	98 (61)	62 (37)	99 (81)	77 (38)
Oa	140 (101)	180 (79)	166 (92)	65 (49)
Total	238 (118)	242 (87)	265 (123)	142 (62)

soil. Weier & Gilliam (1986) reported an inhibition of nitrification at pH > 7. It is possible that acid-adapted nitrifiers were not able to function at the highest pH or that immobilization of nitrate by microorganisms increased.

Field microcosms

Live fine roots were abundant in Root and Root + Lime microcosms after just one year of incubation in the forest floor (Table 5). Because of the high spatial variability, there were no statistically significant differences in total fine root density between microcosm and undisturbed forest floor, between soil horizons nor between lime treatments. Nevertheless fine root density in the Oa horizon of microcosms was substantially lower than in undisturbed forest floor in 1990 suggesting that in-growing roots were still in the process of colonizing that horizon. Therefore, we can assume that root uptake estimates based on Root and Root + Lime microcosms in 1990 were underestimates.

The extractable ammonium pool one year after liming was significantly lower in all three treatments compared to the Control, decreasing in the following order: Root $\gg$ Lime > Root + Lime (Table 6). In contrast, the extractable nitrate pool was not affected by the presence of roots. The effect of lime on nitrate was evident in the Oe horizon where Lime microcosms had less nitrate than Controls and Root + Lime had less nitrate than Root microcosms.

Table 6. Mean extractable ammonium and nitrate (mol ha^{-1}) in microcosms one year after liming (standard deviation in parentheses). Amounts of ammonium and nitrate in mixed Oe/Oa material at the time of liming were 230 and 178 mol ha^{-1}, respectively. Values in a row followed by the same letter are not significantly different ($p < 0.05$; Fisher's PLSD).

Horizon	Control	Root	Lime	Root + Lime
		Ammonium		
Oe	370 d (130)	170 c (36)	43 b (9)	20 a (6)
Oa	330 c (110)	140 b (46)	79 a (21)	44 a (15)
		Nitrate		
Oe	160 b (22)	140 b (12)	100 a (86)	79 a (25)
Oa	430 b (100)	290 ab (50)	220 a (45)	210 a (39)

Both lime and root treatments significantly affected leachate chemistry of microcosms (Fig. 6). Roots alone significantly reduced ammonium and nitrate (presumably by absorption) but increased hydrogen ion concentrations compared to those in Controls. In order to maintain charge balance, roots excrete a hydrogen ion for each ammonium ion absorbed (Marschner 1986). This could explain the elevated hydrogen ion levels. Lime alone significantly reduced ammonium and hydrogen ion concentrations, but increased calcium and nitrate concentrations compared to those in controls. The decrease in hydrogen ions and the increase in calcium were expected as the calcium carbonate dissolved, consuming hydrogen ions and liberating calcium ions. The substantial decrease in leachate ammonium was consistent with the reduced extractable ammonium pools in Lime microcosms and limed soil (Table 6 and Fig. 1). The increase in leachate nitrate was consistent with the increase in the extractable nitrate in lab incubations (Fig. 5). Lime additions to microcosms with roots (Root + Lime treatment) resulted in higher calcium concentrations and lower hydrogen ion concentrations than Root microcosms, but no difference in nitrate or ammonium concentrations. Apparently, the presence of roots in the Root + Lime microcosms prevented the increase in leachate nitrate concentration that was observed in the Lime treatment.

Discussion

Contrary to our expectations, liming the forest floor to pH between 4.0 and 5.0 reduced net N mineralization according to the field survey and the laboratory incubation. Lower net mineralization could explain the smaller extractable ammonium pool in limed areas and Lime microcosms (Fig. 1; Table 6). The reduction of net N mineralization after liming was the result of either increased immobilization by microorganisms or decreased gross mineralization. An increase in N immobilization over several years has been documented in other studies using ^{15}N tracer techniques (Lohm et al. 1984; Shah et al. 1990) and a mass balance approach (Marschner et al. 1992). A decrease in gross mineralization could result from the inability of an acid-adapted microbial community to function as efficiently at higher pH.

We have presented data for the first two years after liming. Predicting the longer term response of net N mineralization to watershed liming is complicated by three factors: 1) the response is a non-linear function of pH, 2) the response to a given change in pH generally changes (declines) through time and 3) soil pH itself is variable through time. The pH dependence of liming effects on net N mineralization was noted in studies by Nommik (1977), Weier & Gilliam (1986) and Duggin et al. (1991) as well as in this study. In general, net N mineralization of acidic forest soils declines with slight increases in pH but is enhanced at circumneutral pH. Thus, one would expect that results of studies that employed only a single lime dose would vary depending on the magnitude of the pH change. Indeed both increases and decreases in net N mineralization have been reported in single-dose studies (Nyborg & Hoyt 1977; Francis 1981; Sahrawat et al. 1985; Persson et al. 1989; Marschner et al. 1992).

Changes in rates of N mineralization in response to liming change through time. The addition of lime is a perturbation of the soil system that causes a flush of activity initially until the system can establish a new equilibrium. Long-term studies (several years) by Shah et al. (1990) and Lyngstad (1992) demonstrated that the initial increase in net N mineralization in response to lime additions declined in subsequent growing seasons. Thus, we would not expect the reduced rates of net N mineralization at Woods Lake to be maintained indefinitely.

The forest floor pH in this study increased during the first two years after liming and likely will continue to do so as the remaining 52% of the lime dissolves (Blette & Newton 1996, this issue). Because of the continuously changing pH, the initial perturbation period has been extended to at least two years. All three of these factors (pH dependent response, equilibration and continuing perturbation) make it difficult to predict the long-term effects on net N mineralization.

Liming stimulated net nitrification both in the field and in the laboratory incubation and this result was corroborated by elevated nitrate concentration in Lime microcosm leachate. However, caution must be used in extrapolating

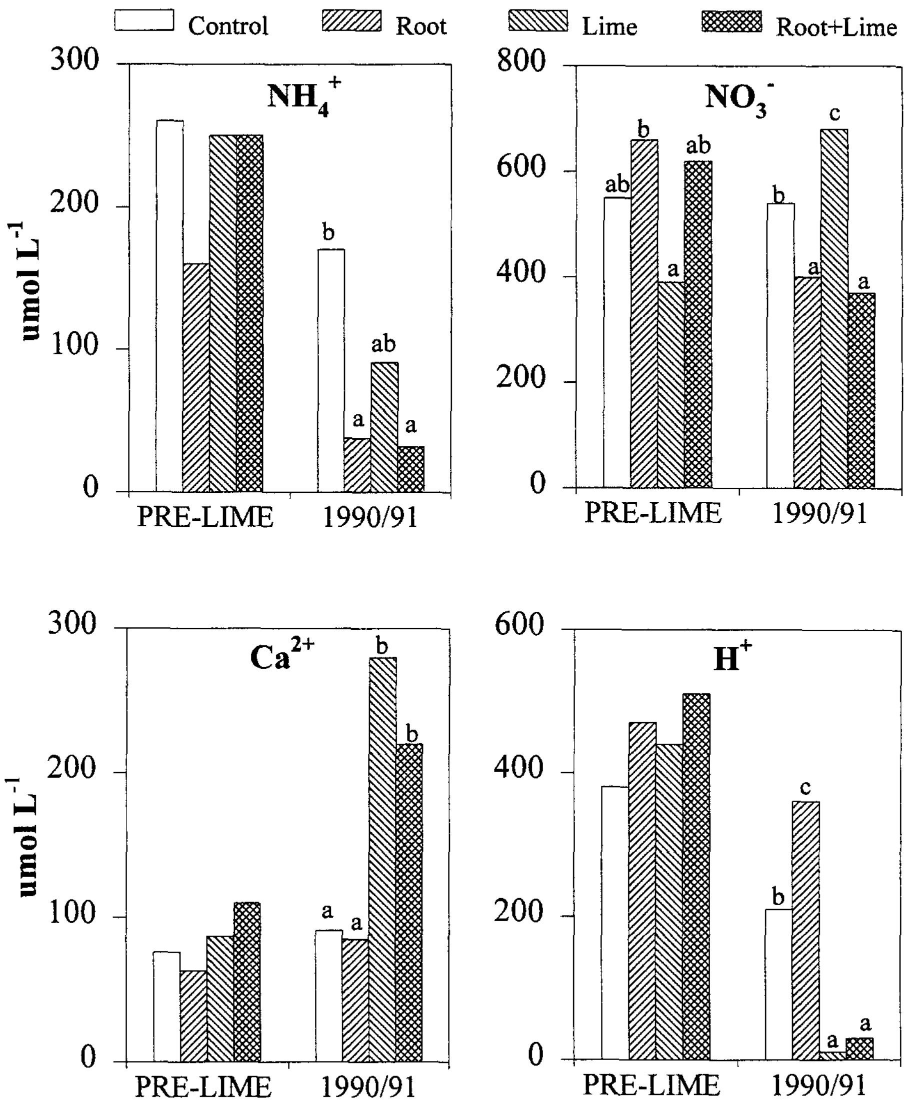

Fig. 6. Mean (volume-weighted) element concentrations in forest floor microcosm leachate. Bars with different letters are significantly different from each other ($p < 0.05$; Fisher's PLSD).

these results to the Woods Lake ecosystem because roots were excluded from these incubations. Tree roots may limit nitrate flux through soil either by absorbing nitrate or by competing with nitrifiers for ammonium (Foster 1989; Anderson et al. 1990; Willison et al. 1990). Indeed, leachate nitrate concentration was not increased by liming in microcosms that contained roots (Fig. 6). The small extractable ammonium pool in microcosms with

Table 7. Annual water and inorganic nitrogen flux from microcosms with standard deviations in parentheses. Water flux was measured from Dec. 1989 to Dec. 1990. Concentration values are volume-weighted mean concentrations for 1990/91. Means in a column followed by the same letter are not significantly different ($p < 0.05$; Fisher's PLSD).

treatment	1990 water flux ($m^3\ ha^{-2}\ yr^{-1}$)	Inorganic N concentration ($\mu mol\ L^{-1}$)	Inorganic N flux ($kmol\ ha^{-2}\ yr^{-1}$)
Control	8800 b (1000)	710 b (200)	6.2 b (1.5)
Root	6700 a (1300)	440 a (130)	2.9 a (0.9)
Lime	8800 b (1000)	770 b (280)	6.8 b (1.3)
Root + Lime	6700 a (1300)	590 a (200)	4.0 a (1.0)

roots demonstrated that there was less ammonium available for nitrifiers, lending support to the idea of competition. Hence, although liming increased the potential for nitrification and nitrate loss, the potential was not realized because of the nutrient conserving mechanisms of the ecosystem.

Geary & Driscoll (1996, this issue) reported a slight, but not significant, elevation in soil solution nitrate in limed catchments the first six months after application. Soil solution concentrations were extremely variable through time, ranging from 10 to 400 $\mu mol\ l^{-1}$. This moderate response to liming is much smaller than would be expected on the basis of our field survey and laboratory incubations, which exhibited a two- to three-fold increase in nitrification. A nitrogen-conserving mechanism such as root uptake is indicated by these data as well. In contrast, stream water nitrate concentrations, which typically range from 0 to 90 $\mu mol\ l^{-1}$, were significantly elevated in limed catchments after liming (Cirmo & Driscoll 1996, this issue). It is possible that excess streamwater nitrate may represent nitrate that has bypassed tree roots by traveling through macropores or nitrate that was produced in riparian zones. Also, it is important to keep in mind that root uptake will only be effective during the growing season.

We used volume-weighted mean ammonium and nitrate leachate concentrations and water flux totals from 1990 to estimate the mean annual inorganic N flux in microcosm leachate. Root uptake was estimated as the difference in N flux between microcosms with roots and their controls. Leachate N flux ranged from 2.9 to 6.8 kmol $ha^{-2}\ yr^{-1}$ (Table 7). Microcosms with roots exhibited significantly lower flux as a result of both lower annual water flux and lower N concentrations, but addition of lime had no effect. Root uptake of N was 3.3 kmol $ha^{-1}\ yr^{-1}$ (SD = 0.9) from Root microcosms and 2.8

kmol ha^{-1} yr^{-1} (SD = 1.1) from Root + Lime microcosms. So, despite the decrease in mineralization and the ammonium pool, nitrogen uptake by trees was not affected by liming. A characteristic of forests that are approaching nitrogen saturation is high nitrate concentrations in streamwater (Aber et al. 1989). Concentrations of nitrate in Woods Lake streams were at the high end of the range of northeastern streams (Gubala & Driscoll 1991). Thus, it is possible that in this relatively nitrogen-rich ecosystem, moderate reductions in N availability will have little effect on plant nutrient status.

Forest floor microcosms have been used to study nutrient leaching, litter decay and the effects of roots and insects on decomposition (Baath 1981; Buldgen 1982; Dighton 1987; Taylor & Parkinson 1988), but have not yet been used to quantify ecosystem element budgets. Estimates of root uptake of N from microcosms at Woods Lake (2.8 to 3.3 kmol ha^{-1} yr^{-1}) were similar to other estimates for northern hardwood forests in New York and Canada (2.3 to 4.2 kmol ha^{-1} yr^{-1}, Mitchell et al. 1992; Foster et al. 1989), but lower than values reported for other temperate deciduous forests (5.4 to 10.2 kmol ha^{-1} yr^{-1}, Likens et al. 1977; Cole 1981; Nadelhoffer et al. 1985). When comparing these values it is important to remember that microcosm estimates represent uptake from the forest floor only, whereas the other studies include uptake from mineral soil. Also, root biomass in microcosms was slightly lower than in undisturbed soil in 1990, so it is likely we underestimated the actual forest floor uptake rates.

Although the N concentration of red maple leaves was significantly lower after liming and red maple tissue constituted 31% of total litter mass, total N fluxes in litterfall in limed and reference areas were not significantly different (data not shown). Thus, the reduced net N mineralization the first year after liming, did not result in decreased uptake or leaching, but rather in smaller pools of inorganic N. Gaseous loss of N from the soil surface was assumed to be negligible in this well-drained, hardwood forest (Bowden et al. 1990). This assumption was supported by the results of periodic sampling of nitrous oxide emission in static chambers which yielded an estimate of 0.2 kmol N ha^{-1} yr^{-1} (SD = 0.3; Simmons 1993).

Conclusions

The well-documented nitrification response to lime was a concern to our research team initially because of the acidifying potential of that process. Liming increased the nitrification potential in that nitrifiers were poised to exploit high ammonium concentrations in disturbed soil without roots. However, in intact soil this potential was not realized probably because root uptake kept ammonium concentrations low. This illustrates an important shortcoming of all liming studies heretofore; the effects of lime on nitrification have only been measured in disturbed soils with severed roots, so that the well-documented nitrification response to lime is actually a response to lime *in the absence of roots*. Thus, we would expect that liming a clearcut forest would increase nitrification tremendously compared to either a clearcut forest without lime or an undisturbed forest with lime.

Although lime application altered the rate of N transformations in the forest floor, it did not have a great impact on other ecosystem processes. Apparently acidity was a controlling factor only for nitrification and this control was overridden by competition with roots for substrate. Changes in nitrogen mineralization as a result of liming had little influence on plants probably because they had sufficient N and were limited by other factors. However, in less fertile ecosystems, such as many coniferous systems or hardwood forests with less available N, liming would be expected to have a greater impact.

This study investigated the lime effects during the first two years after application. It is possible that the longer term effects will be different. As lime continues to penetrate into the rest of the forest floor, forest floor pH should continue to increase leading to more pronounced responses to liming. The field study indicated that the decrease in net N mineralization was becoming greater through time. If N mineralization continues to decrease, then root uptake, leaching and litterfall may eventually be affected.

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