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A field study on the fate of ^{15}N-ammonium to demonstrate nitrification of atmospheric ammonium in an acid forest soil

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Abstract. To demonstrate the contribution of atmospheric ammonium to soil acidification in acid forest soils, a field study with ^{15}N-ammonium as tracer was performed in an oak-birch forest soil. Monitoring and analysis of soil solutions from various depths on the ^{15}N-ammonium and ^{15}N-nitrate contents, showed that about 54% of the applied ^{15}N-ammonium was oxidized to nitrate in the forest floor. Over a period of one year about 20% of the ^{15}N remained as organic nitrogen in this layer. The percentage ^{15}N enrichment in ammonium and nitrate were in the same range in all the forest floor percolates, indicating that even in extremely acid forest soils ($pH < 4$) nitrate formation from ammonium can occur. Clearly, atmospheric ammonium can contribute to soil acidification even at low soil pH.

Introduction

In The Netherlands, forest soils receive high amounts of atmospheric ammonium via dry and wet deposition (Van Breemen et al. 1982; 1988). Input rates of 3 kmoles.ha^{-1}.yr^{-1} (42 kg N.ha^{-1}.yr^{-1}) are common, and in the vicinity of livestock breeding farms input rates of 4—6 kmoles.ha^{-1}.yr^{-1} (56 to 84 kg N.ha^{-1}.yr^{-1}) have been measured. Studies in which ionic fluxes in forest soils were monitored, have shown high nitrate production rates, even in soils with extremely low pH values (Van Breemen et al. 1982; 1987). A rapid soil acidification occurred in these soils as evidenced by a strong decrease in acid neutralizing capacity. This acidification was mainly attributed to nitrate production from atmospheric ammonium.

Nitrate may be formed in the nitrification of organic and inorganic compounds. In most ecosystems oxidation of ammonium to nitrate is catalyzed by autotrophic bacteria (Focht & Verstraete 1977; Belser & Schmidt 1978; Belser 1979; Smith et al. 1968). These nitrifiers are generally not active below pH 4.2 and therefore they are thought to be relatively unimportant in acid soils. There is some evidence that in acid soils nitrate is formed from organic nitrogen compounds (hydroxamates, oximes and nitro- and nitroso compounds) without the intermediate formation of ammonium (Focht & Verstraete 1977; Adams 1986a, b; Killham 1986; Schimel et al. 1984; Lang & Jagnow 1986). This heterotrophic type of nitrification is carried out by bacteria and fungi.

Van Breemen et al. (1982) surmised nitrification on the basis of measured high ammonium inputs and high nitrate levels in soils. Because no distinction was made between heterotrophic and autotrophic nitrification the direct evidence that atmospheric ammonium indeed contributes directly to soil acidification in acid forest soils is lacking. Therefore, a field study was conducted to determine the fate of atmospheric ammonium in an acid forest soil. Deposition of atmospheric ammonium was simulated by application of a solution of ^{15}N-ammonium sulfate. The potential contribution of atmospheric ammonium to soil acidification is discussed.

Materials and methods

Soil site

^{15}N experiments were performed in a 3.2-ha woodland area near the Hackfort estate in the Netherlands (coordinate 215.2-/457.53, map 33H, Zutphen). This area was used earlier for geochemical studies and has been described in detail by Van Breemen et al. (1988). The soil is classified as coarse loamy, mixed, acid, mesic Aeric Haplaquept (Soil Survey Staff 1975). The pH of the upper 30 cm of the soil is 3.5—4.2 and the soil has a well-developed forest floor of about 4 cm thick. Predominant tree species are oak (*Quercus robur* L.) and birch (*Betula pendula* L.). Bracken (*Pteridium aquilinum* L.) is the predominant ground cover. The area has been under forest for at least the last 200 years. Management included regularly cutting the stems sprouting from old stumps (coppicing). The woodland was last coppiced in 1939 and has been left undisturbed since. The groundwater level fluctuates between 0.5 and 1.5 m.

^{15}N-ammonium application

On 27 May 1986, 99.8% enriched ^{15}N-ammonium sulfate was applied at the Hackfort location. The ammonium was prepared from ^{15}N potassium nitrate, purchased from Monsanto Research Cooperation, Miamesburg, Ohio, USA. Reduction and distillation was performed as described below. Over an area of about 4 m^2, 7.8 mmol ammonium per m^{-2} (from a 3.9 mM aqueous solution of ammonium sulfate, pH 4) was applied. Homogeneous application was ensured by adding 0.784 ml aliquots from a dispensette in a 2.8 × 2.8 cm grid supplied by a wire netting template. After adding ammonium sulfate, distilled water was added similarly at a rate of 1 L.m^{-2}. The amount of ammonium added represents the naturally occurring input of about 12 days. This amount allowed the easy quantification of label even after some months of incubation, but it was not unrealistic for the ecosystem. After a dry period much higher amounts of ammonium may enter the ground in a short period of time.

Monitoring

Soil solutions from various depths were collected every two weeks. One month before the addition of ^{15}N-ammonium, high-flow porous ceramic cups (Soil Moisture Equipment[c]) had been installed in triplicate at 10, 30 and 60 cm depth. Distances between cups at any one depth were about 25 cm. The cups were laterally installed from a wood-encased soil pit (about 0.6 × 1 × 1 m) which had been installed in 1985. For monitoring water entering the forest floor, samples were taken from three percolation plates with a diameter of 15.8 cm. The percolation plates were situated about 30 cm sideways from the ceramic cups. Each plate contained a cylindrical piece of high molecular low pressure polyethylene (Supralen RCH 1000), covered with Versapor 0.2 μm membrane filter, and was fitted with a 6 cm high collar of PVC pipe. A cylindrical piece of forest floor (diameter 16 cm) was cut, carefully lifted from the surface of the mineral A horizon, and placed intact on the top of the percolation plate. The collar isolated this piece of forest floor from the surrounding undisturbed forest floor, so that the percolation plate could function as a true lysimeter for measuring drainage fluxes. Sampling flasks were also connected via tubing to the headspace of a closed 2 L bottle, filled largely with water. The water in the bottle was in contact with the groundwater through a siphon tube, thus providing a permanent suction of 5—12 KPa in the headspace for continuous sampling of the soil solution. Samples were removed every two weeks, and stored at 4 °C. Part of each solution was analyzed within two days for ammonium and nitrate. The remainder was frozen at −20 °C and stored for further analysis.

Canopy throughfall and tree litter were collected within 5 to 10 m of the measuring plot. Throughfall water was collected via seven 400 cm^2

high-pressure polythene funnels connected to 5 L opaque polythene bottles, placed at 120 cm above the ground. Volume-averaged bi-weekly samples were analyzed for nitrate and ammonium. Tree litter was collected monthly or bi-weekly from 0.5 m^2 cone-shaped plastic collectors. Further details of the monitoring are given by Van Breemen et al. (1986).

In unsaturated soil profiles, water fluxes cannot be measured directly. We used the SWATRE model (Belmans et al. 1983), as described by Van Grinsven et al. (1987) to calculate soil water fluxes in this study. SWATRE calculates water transport and hydraulic potentials of daily rainfall, (corrected for canopy interception), and daily evapotranspiration estimated by an adapted Penman method. During previous research on the same plot the SWATRE model parameters (evapotranspiration reduction factors, potential root water uptake distribution with depth, hydraulic conductivities) were calibrated by fitting hydraulic potentials to those measured weekly in a field study by tensiometry at 10 cm depth intervals down to 90 cm depth, from April 1981—April 1982. Model results were validated using similar weekly measurements from April 1983—April 1984 (reported by Van Grinsven et al. 1987). Water flux densities from calibrated and validated SWATRE calculations for 4 m^2 study plots were used to calculate solute flux densities. To make a mass balance the bi-weekly water fluxes were multiplied by solute concentrations (both total N and ^{15}N) from individual soil solutions collected over the two-week periods. Occasional missing values of solute concentration were estimated by linear interpolation between earlier and later measurements.

In an earlier study (Van Grinsven et al. 1987), coefficients of variation of solute fluxes for aluminum and nitrate were found to be 10 to 30% between 10 and 90 cm depth, with spatial variability as the most important source of uncertainty. Coefficients of variation of chemical budgets (difference between inputs and outputs of solutes) were 10 to 30% at shallow depth (0—40 cm) and ranged from 40 to 200% for deeper soil layers (60—90 cm).

Preparation of soil cores

On April 28, 1987, the monitoring was terminated and three soil cores were taken. PVC tubes (diameter 6.44 cm) were drilled into the ground and dug out. The forest floor on top of the percolation plates was collected too. Soil cores and forest floor samples were brought to the laboratory and N fractions were extracted the same day as described below.

Preparation of the soil solution samples for ^{15}N analysis

For analysis of the ^{15}N content in ammonium, 10 ml 8 M NaOH was added to a known aliquot (20—300 ml) of soil solution. Ammonia was then steam-distilled as described by Keeney & Nelson (1982) and trapped in 10 ml 0.1 N H_2SO_4. For the analysis of the ^{15}N content in nitrate, ammonium was first removed from the samples by ion exchange. For this purpose a 10 ml syringe was filled with Amberlite IR-120 resin (H+ form) and an appropriate amount of soil solution was percolated slowly through the column. No ammonium could be detected in the eluate. Nitrate was reduced to ammonium with Devarda's Alloy (Merck) and distilled as described above. If ammonium or nitrate concentrations were too low to achieve a concentration of 2 mM in the distillate, a known quantity of a 10 mM ultrapure ammonium sulfate standard (Merck) was added prior to distillation. The percentage ^{15}N in the original sample was calculated from the measured percentage ^{15}N in the mixture and the standard. Before each distillation, the still was cleaned by the distillation of pure ethanol and 0.04 N acetic acid as described by Hauck (1982).

Preparation of soil samples for ^{15}N analysis

The three soil cores were cut into the samples at 10 cm depths and samples of the same depth from the three cores were combined to give single pooled samples. The field moist soil samples were homogenized and sieved through a 2 mm sieve. Organic material which did not pass the sieve was ground and added to the soil samples.

Total nitrogen was analyzed as ammonium after Kjeldahl digestion (Bremner and Mulvaney, 1982). To 0.5 to 2 g soil in digestion tubes, 4 ml 36N H_2SO_4 and 1 g of a digestion mixture (96 g Na_2SO_4, 1.5 g $CuSO_4$ and 0.9 g selenium) was added. The tubes were heated for 30 min at 100 °C (only when wet samples were used) and for 30 min at 250 °C, followed by heating for 90 min at 360 °C. After cooling, 20 ml 8M NaOH was added, ammonia was steam distilled in a Kjeltec (Tecator) apparatus, and trapped into 10 ml of 0.1 N H_2SO_4.

Ammonium and nitrate were extracted with 1N KCl from the separate soil samples (100 g of the field moist soil plus 100 ml 1N KCl, shaken for 3 h). Ammonium was distilled as described above. To remove residual enriched ammonium from the nitrate-containing solution, 10 ml of 10mM NH_4Cl was added twice and distilled off again. Then the nitrate-containing solution was brought into a cleaned still and reduction and distillation was carried out as described for ammonium.

Determination of the percentage ^{15}N

Distillates containing at least 0.07 mmoles of NH_4^+ were dried by vacuum evaporation. Ammonium was converted to N_2 by a modified Rittenberg method. A small tube containing 1 ml of a frozen, degassed solution of 2.4 M lithium hypobromite was placed into a tube containing the dried sample. The tube was evacuated and after thawing the hypobromite was brought into contact with the ammonium. Interfering gasses were trapped cryogenically. The N_2 was then analyzed in a Finnigan MAT 271/251 gas-mass spectrometer. Accurate determination of the percentage ^{15}N was assured as follows: The same reference N_2 gas was used throughout the experiments. A reference ammonium solution with a delta value of 180 was measured before each set of 15 ^{15}N determinations. This solution was also used to check the accuracy of the reduction step with Devarda's alloy. For this purpose, a mineral growth medium with ammonium was prepared according to Schmidt & Belser (1982) and the ammonium was oxidized to nitrate by washed suspensions of autotrophic nitrifying bacteria (*Nitrosomonas europeae*; ATCC 19718 and *Nitrobacter agilis*; ATCC 14123). The nitrate formed was converted again to ammonium with Devarda's alloy as described above, and analyzed on the mass spectrometer. The oxidation and reduction step caused a change in the delta value of less than 1%. In order to check possible changes in the percentage ^{15}N caused by dilution with the ultrapure ammonium standard, a nitrate solution with a known percentage ^{15}N of about 2 was prepared and measured both directly and after tenfold dilution with the standard. The difference in the percentage ^{15}N was less than 2%. To test the reliability of the distillation method to separate ammonium from nitrate in KCl extracts, an equimolar solution of ammonium and nitrate in 1N KCl with a percentage ^{15}N of about 3 and 0.366, respectively, was prepared and analyzed. No increased percentage ^{15}N in the nitrate was measured.

Determination of ammonium, nitrate and organic nitrogen

Ammonium and nitrate concentrations were determined colorimetrically as described by Keeney & Nelson (1982) with a Technicon autoanalyzer. Ammonium was determined with the indophenol-blue method. Nitrate was first reduced with cadmium to nitrite, which was then determined by a modified Griess-Ilosvay method. Total nitrogen was determined by the Kjeldahl method as described above. Organic nitrogen was calculated as the difference between Kjeldahl-N and ammonium.

Results

Fluxes of ammonium and nitrate

The annual fluxes of ammonium and nitrate at various depths in the soil are given in Table 1. Because the 0-cm percolation plates had a known surface area, the 0-cm water fluxes could be measured directly and compared with fluxes estimated by the SWATRE model. This led to values which were about 20% lower than estimated by the model. If, however, corrections are made for some missing values, a difference of 8.8% was found. Though the percolation plates and ceramic cups were placed within an area of about 1 m^2, large differences were found between the individual samplers. This shows that even at short distances large differences in nitrogen transformation rates exist. However, the fluxes given in Table 1 are in the same range as the three-years averaged values reported previously for the same location (Van Breemen et al. 1986).

The calculated cumulative input and output fluxes from the forest floor are given in Fig. 1. Over the year more than 80% of the output of inorganic nitrogen is nitrate. This and the observation that the input of nitrate is much lower than the output of nitrate (143 vs 549 $mmol.m^{-2}.yr^{-1}$) shows the importance of the forest floor in nitrification. Output rates of inorganic nitrogen are much higher than the input rates (about 650 and 400 $mmol.m^{-2}.yr^{-1}$, respectively, Table 1), showing that strong mineralization occurs in the forest floor. If the total amount of inorganic nitrogen in the forest floor was constant, the mineralization rate would be 250 $mmol.m^{-2}.yr^{-1}$, amounting to 43% of the nitrogen in leaffall. Over the

Table 1. Annual fluxes of dissolved ammonium and nitrate in throughfall and at various depths of the soil profiles at Hackfort, measured from April 15 1986 till April 15 1987.

	Ammonium		Nitrate	
Sample	Flux	SD[a]	Flux	SD
	$mmol.m^{-2}.yr^{-1}$			
Throughfall	261		143	
0 cm	105	55	549	160
10 cm	13	14	812	92
30 cm	0	0	613	89
60 cm	0	0	216	182

[a] Standard deviation

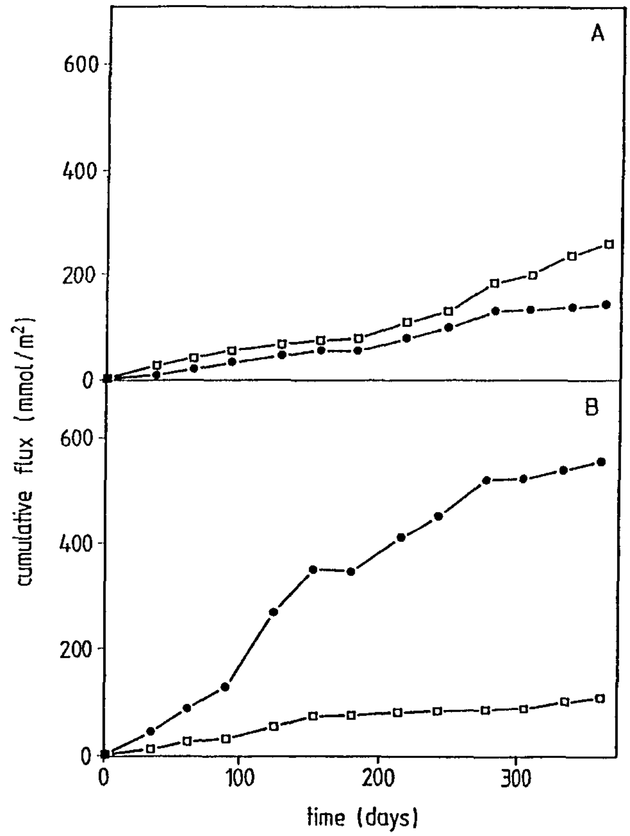

Fig. 1. Cumulative input (A) and output (B) flux of ammonium (□) and (●) for the forest floor at Hackfort A, from 15-4-1986 till 15-4-1987.

same period we measured a leaffall of 670 ± 87 mmol N.m^{-2}.yr^{-1}, a value which corresponds well with the average annual value measured from 1981 till 1987 (Van Breemen et al. 1988). At greater depths most of the nitrate, formed in the upper layers of the soil profile, is presumably taken up by the vegetation. The difference in the downward flux of inorganic nitrogen between 10 and 60 cm (600 mmol.m^{-2}, Table 1) is about equal to the amount of N returned to the soil by litterfall.

Fluxes of ^{15}N-ammonium and nitrate

The percentage enrichment ^{15}N in ammonium and nitrate in the forest floor percolates before and after ^{15}N-ammonium application (7.8 mmol.m^{-2}) was determined. Fourteen days after ^{15}N application the percentage ^{15}N in nitrate had increased significantly and a maximum was observed after one month (Fig. 2). In the first two samplings after the application of ^{15}N the

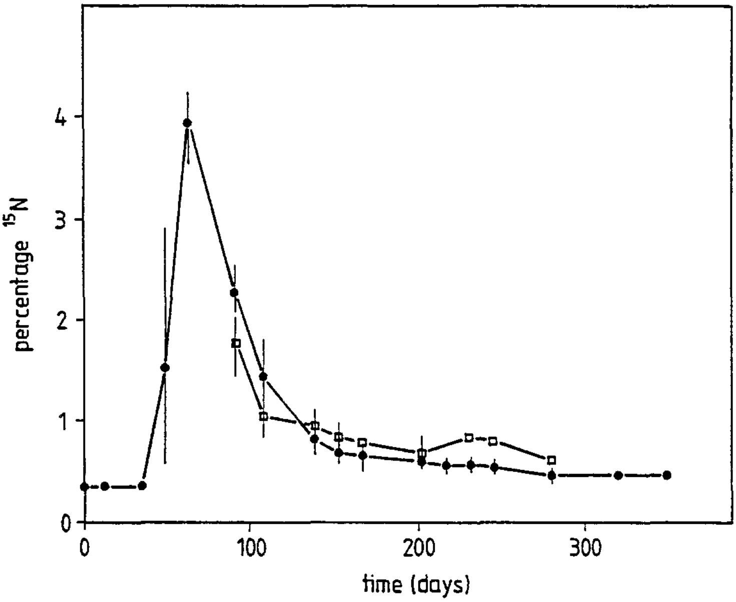

Fig. 2. Measured percentage ^{15}N in ammonium (□) and nitrate (●) in leaf-litter percolates of the Hackfort location. At day 35 (May 20 1986) ^{15}N-ammonium was applied. Values are averages of three percolation plates.

percentage ^{15}N in ammonium was not determined. In later samples the percentage ^{15}N in both ammonium and nitrate were measured. The percentage ^{15}N enrichment in nitrate and ammonium in each sample was in the same range. At days 220 and 250 the percentage ^{15}N in ammonium was higher than that in nitrate. The accuracy of the values of the percentage ^{15}N ammonium at these two days is uncertain. Only one of the percolation plates contained enough sample for analysis of both nitrate and ammonium. These samples, however, had a very low ammonium content. Because the forest floor contains high amounts of KCl extractable ammonium (see Table 3), the close similarity of ^{15}N percentages in NH_4^+ and NO_3^- can be explained only if a major part of the nitrate is formed directly from ammonium. In case of a heterotrophic type of nitrification in which nitrate is directly formed from special organic compounds present in soil as described by Focht and Verstraete (1977) the percentage ^{15}N in nitrate would be the background value or at least be much lower than the percentage ^{15}N in ammonium.

With increasing depth, ^{15}N peaks in soil solution decreased. Peak broadening may be due to a combination of diffusion and of nitrification at greater depths (Fig. 3).

We calculated a ^{15}N mass balance from the percentages ^{15}N in ammonium and nitrate and the fluxes of dissolved ammonium and nitrate,

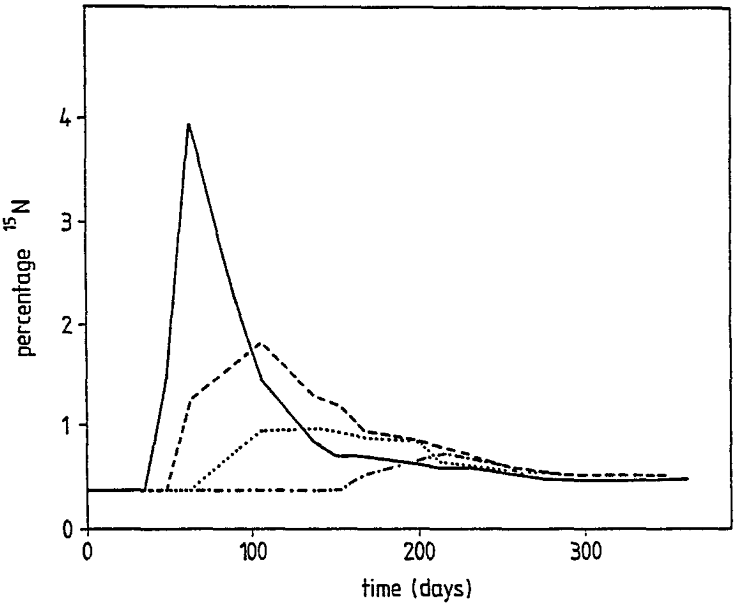

Fig. 3. The percentage enrichment of ^{15}N in nitrate in the soil solution as a function of time at depths of 0 (—), 10 (- - -), 30 (···) and 60 (-·-) cm, below the boundary of the forest floor and the mineral soil. (means of three replicates).

corrected for natural background levels (Table 2). About 54% of the 7.8 mmoles ^{15}N-ammonium applied leached from the forest floor as nitrate, whereas about 11% was leached as ammonium. These results show that most of the surface applied ammonium is oxidized to nitrate in the forest floor. At the end of the experiment, about 20% of the applied ^{15}N was recovered in the organic nitrogen fraction (Table 3). Only a small amount (about 1%) of the applied ^{15}N was still present as inorganic N in the forest floor after 1 year. Except at the 60 cm depth, the cumulative fluxes of ^{15}N at the various depths reached maximum values within 250 days after application (Fig. 4), suggesting that the monitoring period was sufficiently long to make a N budget as based on the data in Table 2.

Soil core analysis

In the core sampling, no separation was made between the F and A layers. The −4—6 cm sample (the F layer was about 4 cm thick) consists of both forest floor and mineral soil. Exact quantification of the amount of applied ^{15}N that is tied up in the organic nitrogen fraction was difficult due to the large quantity of naturally occurring ^{15}N (175 mmoles.m^{-2} over a

Table 2. Flux of the applied 7.8 mmoles.m^{-2} ^{15}N-ammonium at various depths of the Hackfort location.

Depth	Ammonium		Nitrate	
	Flux	SD	Flux	SD
cm	mmol.m^{-2}.yr^{-1}			
0	0.85	0.58	4.18	0.48
10	0	0	4.48	0.46
30	0	0	2.77	0.83
60	0	0	0.71	0.59

Table 3. Distribution of total-N and recovered ^{15}N over organic and inorganic (extracted by N KCl) forms in the forest floor. Material in the percolation plates was quantitative recovered at the end of the experiment (April 28 1987).

N-forms	Total-N		^{15}N	
	Mean	SD	Mean	SD
	mmol.m^{-2}			
Organic nitrogen	5010	450	1.76	0.17
Ammonium	40.9	5.2	0.041	0.016
Nitrate	35.7	8.7	0.034	0.002

depth of 50 cm) compared to the 7.8 mmoles.m^{-2} of applied ^{15}N. Measured ^{15}N in total nitrogen in the upper layer (0.3878% enrichment) was high enough to allow a good estimation of the distribution of ^{15}N in that layer (Table 4). In the 36—46 cm layer, however, measured ^{15}N (0.3682%) was too close to the measured background (0.3678%) to enable a calculation of the ^{15}N distribution in this layer. Results indicate that about 52% of the applied ^{15}N remained in the upper 10 cm of the profile. Only 1% of the applied ^{15}N was still present as ammonium and about 4% was present as nitrate.

Discussion

Although the small number of replicated samples and the appreciable spatial variability do not allow a quantitative extrapolation of our results, a number of general conclusions about the fate of atmospheric ammonium in the soil studied here can be drawn with confidence. This is particularly

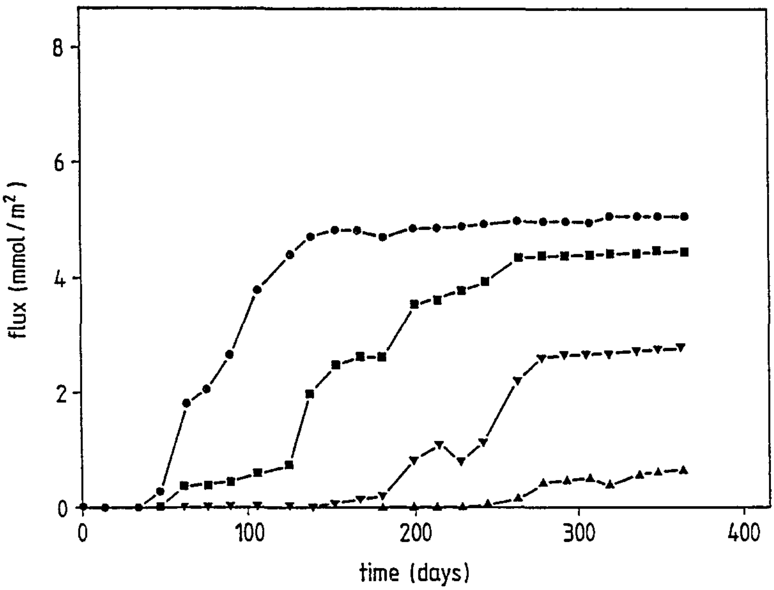

Fig. 4. Cumulative flux of the applied ^{15}N at depths of 0 (●), 10 (■), 30 (▼) and 60 (▲) cm, below the boundary of the organic forest floor and mineral soil. Dips are caused by capillary upward movement.

true because the mean concentrations and fluxes determined at the ^{15}N study plot were similar to those observed in two adjacent plots (i.e. within a distance of 10 m) where N budgets (without the use of ^{15}N) were studied from 1981—1987. Our results clearly show that in the investigated acid forest soil, surface-applied ammonium is oxidized to nitrate directly or at least without a long retention in the organic nitrogen fraction. This implies

Table 4. Contents of total-N and ^{15}N in organic and inorganic forms in soil samples at various depths at the end of the experiment. Cores were taken April 28 1987. The ^{15}N values are corrected for the natural ^{15}N background.

Depth	Organic-N		Ammonium		Nitrate	
	Total	^{15}N	Total	^{15}N	Total	^{15}N
cm	mmol.m^{-2}					
—4— 6	18,200	4.11	72.0	0.06	72.0	0.12
6—16	12,570	0.70	25.7	0.02	32.5	0.05
16—26	8,340	0.47	13.9	0.00	35.6	0.05
26—36	4,810	0.35	10.2	0.0	35.0	0.05
36—46	1,910	0.06	6.1	0.0	24.4	0.05

that a direct relationship exists between atmospherically deposited ammonium and nitrification. Based on results from three-year mean input and output fluxes, it was shown that at the Hackfort location the ammonium deposition flux is in the same range as the nitrate drainage flux (Van Breemen et al. 1987) and that nitrate formation is a major cause of the ongoing soil acidification. Our findings imply that a decrease in the ammonium deposition would result in a reduction of soil acidification.

Nitrification of atmospheric ammonium at the Hackfort location mainly occurs in the forest floor. Two Scots pine locations were also studied (results not shown). At these soil sites the percentage enrichment ^{15}N in ammonium and nitrate in the forest floor percolates were also equal, but only 10—15% and 14—22% of the applied ^{15}N-ammonium was oxidized in the forest floor. From the results obtained with ^{15}N applied at the top of the forest floor we cannot say to what extent nitrification also occurs in the mineral soil. Tietema et al. (1989) observed rates in the mineral horizon of an acid beech forest soil in the same range as in the organic layer.

In a study to identify the nature of the nitrifying microorganisms in acid forest soils, we counted high numbers of autotrophic nitrifying bacteria in the leaf litter layer of the same woodland and were able to show with enrichment cultures of autotrophic nitrifying bacteria that after an activation at a pH of 5.7, nitrification continued while the pH decreased to about 3.8 (Stams et al. 1990). This together with the ^{15}N measurements suggests that the nitrification of the ammonium deposited in the acid woodland soil is largely autotrophic. Nitrification in the forest floor is particularly interesting, because the pH of the forest floor can fluctuate seasonally. The pH of the throughfall water of the Hackfort location varies from 4.2 to 6.5 over the year and is therefore clearly higher than the pH of about 3.5 which is typical for the upper 30 cm of the mineral soil. This throughfall water may cause relatively high pH values locally in the surface (L- or leaf litter) of the forest floor, where mineralization may further help to increase the pH. In this manner mineralization may stimulate nitrification. A relationship between nitrification and mineralization was found by Kreitinger et al. (1985), who showed that NH_4^+ addition to an acid forest soil affected N-mineralization and NO_3^- formation to the same extent. De Boer et al. (1988) reported that autotrophic nitrification in an acid heathland soil was activated by N-mineralization. Since the observed autotrophs were acid sensitive, it was proposed that they live in the close vicinity of heterotrophic microorganisms which are involved in mineralization and may cause a local pH increase.

A large percentage of the applied ^{15}N was incorporated into organic nitrogen compounds. This finding complicates the exact elucidation of the fate of atmospheric ammonium in acid forest soils with ^{15}N tracers. From the flux calculations it is clear that in our oak-birch woodland (Hackfort) 54% of the applied ^{15}N-ammonium is oxidized to nitrate in the forest floor. Analysis of the soil cores, however, showed that the major part of the label (73%) is immobilized into the organic fraction, whereas only about 4% is recovered as nitrate. This discrepancy cannot be explained by the uptake by the vegetation, denitrification or the leaching of nitrate below a depth of 46 cm. The most likely explanation to account for the large differences is that labelled ^{15}N, both in ammonium and nitrate, exchanges with the organic nitrogen fraction of the biomass via mineralization-immobilization processes. The incorporation of labelled ^{15}N in organic nitrogen has two effects. First it retards the movement of ^{15}N through the soil profile. This helps to explain why the percentage ^{15}N in the forest floor percolates, as given in Fig. 2, remains so high in time. Second, the ^{15}N-nitrate formed in the forest floor can be exchanged with ^{14}N organic nitrogen in the mineral soil. This will lead to a removal of ^{15}N label at greater depths and may contribute to the observed ^{15}N decreases with depth (Table 2). For a proper evaluation of the fate of atmospheric ammonium with ^{15}N, the flux calculations were of particular importance; without those the importance of nitrification in this acid forest soil would have been underestimated.

Acknowledgments

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