Chapter 14
Fate, Transport, and Retention of Applied ^{15}N Labelled Nitrogen in Forest Soils

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14.1 Introduction

Forest soils in central Europe have received high atmospheric inputs of protons, sulphate and nitrogen (Chap. 15) since the beginning of industrialisation. As a result, chemical and biological processes in many German forest soils have changed (Godbold and Hüttermann 1994) and soil characteristics like pH, base saturation and C/N ratios have altered (Chap. 21). The mechanisms of the fate and the retention of this high atmospheric input of nitrogen still remain unclear. There are reports indicating that, despite high N-deposition since the beginning of the nineteenth century, most forests continue to retain nitrogen (Ulrich 1994; Johnson et al. 1997; Dise et al. 1998; Borken and Matzner 2004) even when the low C/N ratios in the surface organic layer have become low (Brumme and Khanna 2008). This raises the question of the retention mechanisms of nitrogen in forest soils.

The use of labelled nitrogen makes it easy to follow internal nitrogen cycle of N-inputs in forest ecosystems (Nadelhoffer et al. 2004; Tietema et al. 1998; Feng et al. 2008) and soils (Mochoge and Beese 1983a,b). Often, labelled nitrogen is applied in a single dose which provides information on the nitrogen transformation rates under the current environmental conditions of addition. We applied constant rates of ^{15}N labelled ammonium and nitrate as an artificial crown drip for long periods on undisturbed soil columns (Brumme and Aden 1995) under field and laboratory conditions in order to simulate continuous N-depositions so as to follow N-transformations under close to field conditions.

The aims of this study were to estimate: (1) the leaching losses of deposited ammonium and nitrate from soil of different acidity by adding ^{15}N labelled ammonium and nitrate on undisturbed soil cores from three beech forest ecosystems at the Göttinger Wald, Zierenberg and Solling sites, (2) to compare the transport of added ammonium and nitrate, which are biologically transformed, with chloride as a marker which is not biologically transformed, (3) to determine the retention processes of added ammonium and nitrate involved in forest soils, and (4) to study the temperature effect on N-transformation of added labelled nitrogen.

14.2   Experimental Details

The results presented in this chapter were derived from the same field and laboratory study as already described in the Chap. 13 "Nitrogen and carbon transformations." Undisturbed soil cores of the L–20 cm depth (15 cm diameter) from the Göttinger Wald, Zierenberg and Solling (n =8) sites were placed at the Solling site in such a way that the surface of the soil cores was in level with the surrounding soil (*field study*). The cores were kept covered at the surface to prevent natural rainfall but were open between the soil cores and the lid to enable gas exchange. Every 2 weeks, the soil cores were irrigated with 486 mL of an artificial throughfall (equal to an irrigation of 2.1 mm per day) which contained ^{15}N labelled ammonium sulphate (five of eight soil cores) (8.4 atom % $^{15}N_{ex}$, where ex refers to the ^{15}N in excess of the natural abundance) to achieve a deposition rate of 11 kg N ha^{-1} per year. The columns were closed at the bottom and ceramic suction cups enabled sampling and quantifying of leached water twice a month over a 12-month period, which were combined to a monthly sample and analysed for nitrate, ammonium and the labelled fraction.

In the *laboratory study*, undisturbed soil cores (15 cm diameter) from different soil layers were used to study the vertical distribution of C- and N-transformations at different temperatures and for different form of N-applied: at 3–4°C (Solling: $n = 4$ and Göttinger Wald: $n = 6$), at 7–8°C (Solling:$^{15}NH_4^+$, $n = 8$;$^{15}NO_3^-$, $n = 4$ and Göttinger Wald: $n = 3$), and at 16°C (Solling: $n = 4$ Göttinger Wald: $n = 9$) (Wang 1998). From the Solling site, soil cores of the surface organic layer, 0–10 cm, and 10–20 cm soil depth were collected, and from the Göttinger Wald site mineral soil layers were used (L–10 cm, 10–20 cm, and 20–30 cm soil depth). The L layer was included in the L–10 cm layer at Göttinger Wald. The soil cores were installed in a microcosm system (Hantschel et al. 1994) and automatically irrigated six times per day (Solling: 4 mm per day; Göttinger Wald: 6 mm per day) with an artificially prepared throughfall solution over a 4-month period. ^{15}N labelled nitrogen was applied at the rate of 20 kg N ha^{-1} per year either as ammonium or nitrate but in three different steps. During the first 3 weeks, nitrogen was applied as non-labelled NH_4NO_3 (preliminary period) to achieve a steady state condition in the water flow and in the microbial transformation processes. For the next 60 days, ^{15}N labelled NH_4NO_3 with either ammonium or nitrate ($^{15}N_{ex}$ 49.4 atom%) were applied in separate experiments together with Cl to study the anion transport processes in the soil layers. In the third period, non-labelled NH_4NO_3 was applied to study the remobilisation of ^{15}N from the soils over 4 weeks. Soil solutions were sampled from the bottom of the soil cores where a 0.45 μm nylon membrane (Hybond-N, Amersham, England) was placed, and analysed for ammonium and nitrate and the ^{15}N content to calculate the leaching losses of labelled ammonium and nitrate. Exchangeable ammonium and soluble nitrate were extracted from soil samples with 0.5 M K_2SO_4 at the end of the experiment. The ^{15}N content of ammonium and nitrate was analysed after using the diffusion technique (Brumme and Aden 1995). ^{15}N was measured with an element analyser coupled to an isotope ratio mass spectrometer (EA-IRMS) (Finnigan, Germany).

14.3   Transport and Leaching Losses of Added ^{15}N Labelled Nitrogen

The fate of ammonium and nitrate in plant-free soils depends on several processes such as adsorption and desorption, microbial uptake and remineralisation, fixation, denitrification and the physical transport described by convection–dispersion transport equations (Parker and van Genuchten 1984). In order to study the physical transport processes of anions, we used chloride simultaneous with ^{15}N labelled ammonium and nitrate in undisturbed soil columns at constant water content and steady-state water flow in the laboratory study according to Beese (1982) and Mochoge and Beese (1983a). Chloride does not enter biological and chemical reactions in soils and any difference in the fate of chloride and nitrate is attributed to microbial uptake of N and denitrification. However, for ammonium, there are additional processes such as nitrification, adsorption and fixation which should be considered. Transport studies during steady-state water flow showed that the breakthrough of applied chloride after pulse application culminated after the exchange of 0.5–0.9 pore water volume (which equals the volume of water in soil cores). The C/Co value of 1 was achieved for Cl after 2 pore water volumes had been replaced in soil columns independent of the soils used (Beese and van der Ploeg 1979). This is a typical response for anion transport in soils and is explained by a reduction of mobile soil water by anion exclusion and thereby increased the pore water velocity (i.e. irrigation intensity (cm per day) divided by volumetric water content ($cm^3\ cm^{-3}$)) (Bresler 1973; Beese 1982). Figure 14.1 showed a typical breakthrough of chloride for the Solling soil. After an exchange of 2–3 pore water volumes, the relative concentration C/Co of chloride approached the value of 1, and after that Cl-inputs to the surface of the columns were stopped. That caused the concentration of chloride in the leachates from cores with surface organic layer, 0–10 cm soil and 10–20 cm soils to decline to about zero after additional 2 pore water volume were applied. Labelled nitrate applied to the soil surface showed a small retardation in its transport compared to chloride and approximately 4 pore water volume were required for C/Co to approach the values of >0.8. At day 60 of the leaching experiment, when labelled nitrogen was replaced by unlabelled nitrogen (indicated by crosses in Fig. 14.1), the leaching losses reached between 95 and 99% of Co and indicated that there is nearly no retention of nitrate by microbial uptake or denitrification at the Solling site under these experimental conditions.

However, the leaching of applied ^{15}N labelled ammonium was slow in the Solling soil (not shown). Applied ammonium was partly nitrified and leached as nitrate after nitrification with a large delay in all soil layers (Fig. 14.1). Delayed nitrification resulted in retarded leaching of added ^{15}N labelled ammonium as indicated by a lower values (0.17) of relative concentration C/Co in the mineral soil layers and in the surface organic layer (0.33) than those observed when ^{15}N labelled nitrate (>0.95) was applied after 60 days. Another factor responsible for this retardation in the leaching of added ammonium in acid soils is its adsorption by
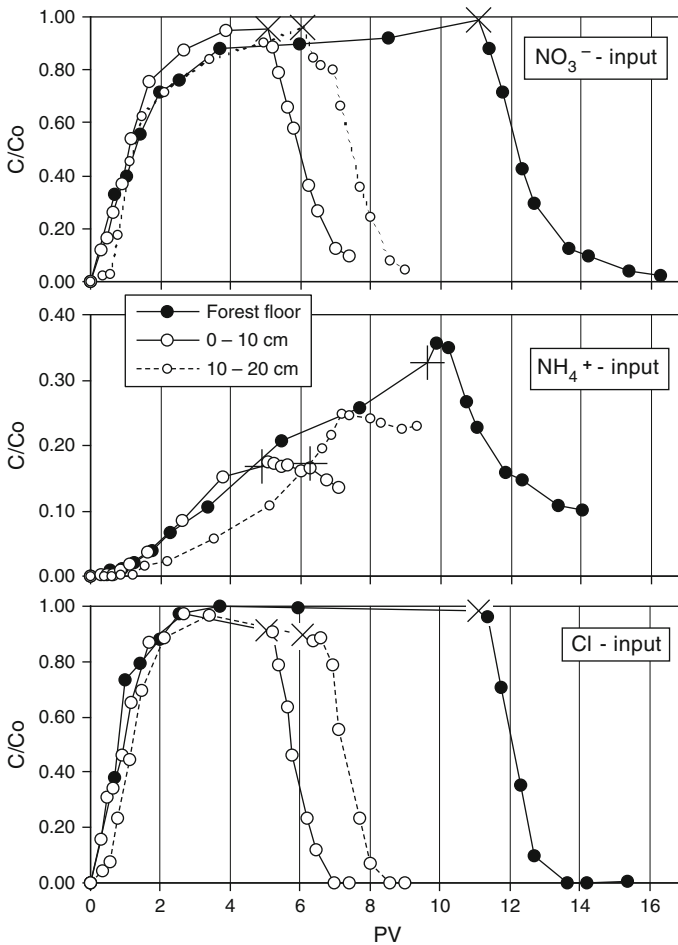
Fig. 14.1 Concentrations (C) of ^{15}N labelled nitrate and chloride in the leachates relative to the input concentration (Co) of ^{15}N labelled nitrate, ^{15}N labelled ammonium, and chloride (chloride is shown for the ^{15}N labelled nitrate treatment), applied to undisturbed soil cores of surface organic layer (forest floor), 0–10 and 10–20 cm depths from Solling at 8°C (laboratory study). Changes from labelled to non-labelled nitrogen and from chloride to sulphate application at day 60 are indicated by (×). (*PV* – Pore water volume in soil cores replaced)

negatively charged clay minerals and humus particles. The proportion of adsorbed ^{15}N labelled ammonium was higher in the mineral soil layers than in the surface organic layer at the end of the experiment (Table 14.1) despite similar nitrification rates (Chap. 13). The high adsorption of ammonium in the mineral soils caused a slow percolation of labelled nitrate after nitrification or even an increase after 60 days as observed in columns of 10–20 cm depth. Fast percolation of labelled nitrate in the surface organic layer is an indication of the lack of adsorption of ammonium in this layer.

Table 14.1 ^{15}N-balance after 60 days of irrigation (4 mm per day) with ^{15}N labelled ammonium at a rate of 20 kg N ha^{-1} per year and 30 days with non-labelled ammonium, on soils from three different soil depths from Solling at 8°C in the "laboratory study," (all values are given as % of ^{15}N applied) ($n = 8$)

Depth	Leachate			Soil				^{15}N-
	NO_3^-	NH_4^+	$\sum$	NO_3^-	NH_4^+	N_{org}[a]	$\sum$	Recovered
Organic layer	29 (13)	18 (4)	47 (20)	2	3	54	59 (17)	106 (29)
0–10	25 (18)	8 (3)	33 (20)	5	24	34	63 (17)	96 (38)
10–20	21 (8)	1 (1)	22 (9)	4	30	34	68 (9)	90 (18)

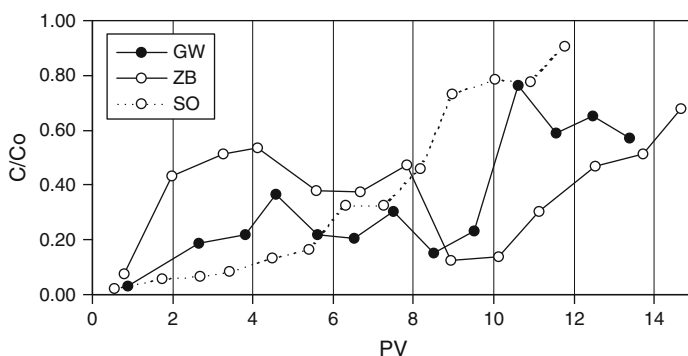[a]Assuming a negligible contribution of N-fixation

Fig. 14.2 Concentration (C) of ^{15}N labelled nitrate in the leachate relative to the concentration of applied ^{15}N labelled ammonium in the input (Co) from undisturbed soil columns (L–20 cm depth) of the Göttinger Wald (GW), Zierenberg (ZB), and Solling (SO) site ($n = 8$) in the field study, related to the water volume in the soil cores (pore water volume, PV) which was replaced by irrigation

In the field study, ^{15}N labelled nitrate and chloride were not added. However, labelled nitrate was produced through nitrification of applied ^{15}N labelled ammonium to the soil. Leaching of labelled ammonium was low (not shown). The behaviour of ^{15}N labelled nitrate in the leachates was different among the three beech forest soils of the Göttinger Wald, Zierenberg, and Solling sites. The concentration of ^{15}N labelled nitrate (C) in the leachate relative to the concentration of added ^{15}N labelled ammonium (Co) increased very quickly at the less acid soil from the Zierenberg site which was followed by those of the Göttinger Wald and Solling sites (Fig. 14.2). The leaching of ^{15}N labelled nitrate from the acid Solling soil was highly retarded as was also observed in the laboratory study involving separate soil layers.

A delay of leached nitrate from added ^{15}N labelled ammonium was also observed in a previous study with undisturbed soil cores from Solling site (Mochoge and Beese 1983a). In their study, leaching of nitrate was delayed by about 1 pore water volume after a pulse application of ^{15}N labelled ammonum sulphate with

continuously applied artificially prepared throughfall solution at rates of 5 mm per day as compared to the case when ^{15}N labelled nitrate was added. The method and the amount of ammonium application will affect the rate of its transport through soil columns. Continuous application compared to pulse application of labelled ammonium may lead to differences in concentration and duration of labelled nitrogen in the soil. A pulse application may increase ammonium fixation in clay minerals if high doses were used, and may also cause some loss of ammonium by leaching. Continuously applied ammonium with rates in low concentrations similar to those in situ conditions in forest ecosystems may increase the biological retention. However, retention of ammonium by cation exchange in acid soils is of a transitory nature as indicated by the steady increase of leached ^{15}N labelled nitrate at the Solling site (Fig. 14.2). At leaching of 9 pore water volumes, a relatively constant nitrate level of $C/Co = 0.8$ was observed.

14.4   Retention of Applied Ammonium and ^{15}N Balance of Forest Soils

A number of factors affect the retention of ammonium by soils. Adsorption by cation exchange in the mineral soils constituted 24% (0–10 cm depth) and 30% (10–20 cm depth) of the applied ammonium in the laboratory study (Table 14.1). The high retention was observed even 30 days after inputs were changed from labelled to non-labelled ammonium. This indicated that the nitrification by heterotrophic organisms was low (see also Chap. 13) and has promoted the adsorption of ammonium in Solling soil. This would explain the retardation in leaching of N as observed in both the field (Fig. 14.2) and the laboratory studies (Fig. 14.1).

The surface organic layer retained only 3% of the applied ammonium on exchange complex and 18% of the applied ammonium was leached as ammonium (Table 14.1). In contrast to this acid soil, a continuous application of labelled ammonium sulphate to the less acid soil from Göttinger Wald resulted in a negligible adsorption of ammonium of 0.3% (Brumme and Beese 1991, Table 14.2). The lower adsorption at Göttinger Wald was related to high nitrification (see also Chap. 13). These results are in accordance with a study on undisturbed soil cores from different European countries. Application of labelled ammonium chloride to six forest soils showed the highest recovery as ammonium from soils with very low nitrification (Coûteaux and Sallih 1994).

Retention of ammonium by cation exchange is of a transitory nature in acid soils. More important for a long-term retention is the transformation of applied nitrogen into stable or meta-stable microbial-mediated organic compounds. Assuming that fixation of ammonium by clay was absent in the surface organic layer, about 54% of the added ammonium was transformed to microbial biomass or soil organic matter

Table 14.2 Balances of ^{15}N-ammonium of the Göttinger Wald soil 160 days after continuous application of 1 kg N ha^{-1} per day in form of $(NH_4)_2SO_4$ to undisturbed soil columns (0.25 m^2, 100 cm length, $n = 1$) irrigated with a rate of 3 mm per day at 8 and 23°C (% of ^{15}N applied) (Brumme and Beese 1991) (ex, exchangeable; fix, fixed; org, organic)

| | Leachate | | | Soil | | | | | ^{15}N- |
	NO_3^-	NH_4^+	$\sum$	NO_3^-	NH_4^+(ex)	NH_4^+(fix)	N (org)	$\sum$	Recovery
8°C	35	0	35	27	0.3	0	25	52	87
23°C	32	0	32	27	0.3	0	29	56	88

Table 14.3 ^{15}N-balances after 1 year of irrigation (2.1 mm per day) with ^{15}N labelled ammonium sulphate at a rate of 11 kg N ha^{-1} per year in the Göttinger Wald (GW), Zierenberg (ZB) and Solling (SO) soil of the field study (% of ^{15}N applied, standard deviation) ($n = 5$)

| | Leachate | | | Soil | | | ^{15}N-recovered |
	NO_3^-	NH_4^+	$\sum$	Organic layer	Mineral soil	$\sum$	
GW	38 (6.3)	0.3 (0.2)	38 (6.4)	4.0 (2.1)	41 (2.1)	45 (2.2)	84 (4.6)
ZB	43 (9.8)	0.5 (0.5)	43 (10)	7.4 (2.9)	26 (2.8)	34 (4.1)	77 (8.5)
SO	31 (8.4)	3.7 (3.3)	35 (11)	20 (3.9)	26 (5.6)	46 (4.8)	81 (7.5)

at the Solling site (Table 14.1). In the mineral soil depths, about 34% of added ammonium was retained by either fixation or transformation into organic compounds. The fixed fraction was not studied in our laboratory experiment, but the study of Mochoge and Beese (1983b) indicated that labelled ammonium could be fixed in clay minerals of the Solling soil to the extent of 17 and 15% at 4° and 23°C, respectively. Mochoge and Beese (1983b) applied 80 kg N ha^{-1} within 2 days which led to a very high ammonium concentration in the soil solution which may have increased the fixation of ammonium. Fixation of ammonium has been generally found to increase with an increase in the amount of ammonium added (Nommik and Vahtras 1982). The soil cores in our laboratory study received a low amount (20 kg N ha^{-1} distributed over 1 year), so the fixation of ammonium might be of minor importance and most of the 34% of the added amount found in the mineral soil depths was assumed to be bound to organic compounds.

The fraction of applied ammonium recovered in the surface organic layer at the end of the field study increased with the amount of organic matter from the less acid Göttinger Wald and Zierenberg sites to the acid Solling site (Table 14.3). The retention of applied ammonium per g Corg increased with an increase in microbial biomass per g Corg (Fig. 14.3) indicating a preferential microbial uptake of applied ammonium. The preferential use of ammonium than nitrate by microorganisms was shown in a ^{15}N-pool-dilution experiment at Solling site. Corre et al. (2003) incubated soil cores of the surface organic layer and the upper soil layer (0–5 cm) with ^{15}N labelled $(NH_4)_2SO_4$ and ^{15}N labelled KNO_3 and found a two times higher uptake of ammonium than nitrate.

Fig. 14.3 Retention of applied ammonium in relation to microbial biomass-C of the L, F, and H layers of Solling, and the L layers and 0–5 cm layers of Göttinger Wald and Zierenberg after 12 months of application $^{15}N–NH_4^+$ in undisturbed soil cores in the field study. Only the surface layers were used in this figure because of a pore water exchange by each irrigation event higher than one (1.6–1.9) to ensure ammonium infiltration into the layers
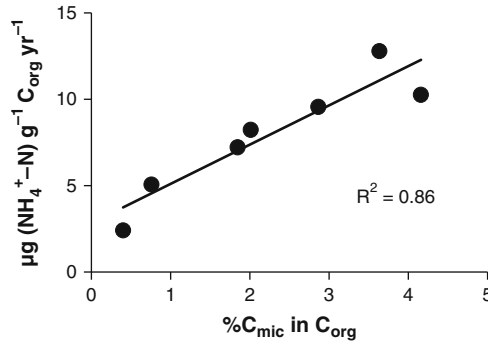

Table 14.4 ^{15}N-balance after 60 days of irrigation (4 mm per day) with ^{15}N labelled nitrate at a rate of 20 kg N ha^{-1} per year and 30 days with nonlabelled nitrate, on the top of three different soil depths from Solling at 8°C in the laboratory study, (% of ^{15}N applied) ($n = 4$)

Depth	Leachate		Soil	^{15}N-
	NO_3^-	NH_4^+	N_{total}	Recovered
Organic layer	100 (3)	3 (1)	3 (2)	106 (5)
0–10	94 (1)	2 (0)	2 (2)	98 (4)
10–20	93 (2)	0 (0)	0 (0)	93 (3)

14.5 Retention of Added Nitrate and ^{15}N Balances of Forest Soils

This study with labelled nitrate in the laboratory showed that the acid Solling soil has no ability to retain significant amount of applied nitrate. About 3% of the applied nitrate was found in the surface organic layer (Table 14.4) as compared to 54% of ammonium (Table 14.1). Among different horizons, L retained the most of all layers, 15- and 60-fold as much of added nitrate per gram matter as the F and H layers of the Solling site.

The soil from 0 to 10 cm depth retained about 2% of the applied nitrate whereas that from the 10 to 20 cm layer did not retain any ^{15}N. A similar fraction of labelled nitrate was recovered after a pulse application of 80 kg N ha^{-1} ^{15}N labelled calcium nitrate to the Solling soil (Mochoge and Beese 1983b). About 6 and 15% of the applied nitrate was found in the organic nitrogen pool in 0–30 soil depth at 4 and 23°C, respectively, and suggested a positive temperature effect on nitrate immobilisation. Generally, micro-organisms prefer ammonium as a nitrogen source and their ability to use nitrate is restricted (Rosswall 1981). A short-time experiment with ^{15}N labelled nitrate has shown that biotic immobilisation of nitrate was about

half that of ammonium in the surface organic layer and 0–5 cm depth of the Solling soil (Corre et al. 2003). Similarly, Puri and Ashman (1999) reported from a temperate woodland soil that ammonium had twice the immobilisation rate of nitrate. These fractions are much higher than found in our laboratory study where nitrate immobilisation in soil organic matter was <6% of ammonium immobilisation. These differences might be related to methodical differences of labelled nitrate application to soils. Whereas a semicontinuous application of small ^{15}N rates with the irrigation simulates field conditions in our field and laboratory study, in other studies the labelled nitrate was injected into soils or added to soil slurries in the ^{15}N-pool dilution technique.

14.6 Temperature Effects on Transformation of Ammonium Applied to the Surface Organic Layer and to Mineral Soil Layers of the Solling Site

Temperature affected the transformation of ammonium when applied continuously to undisturbed soil cores from the Solling site for 2 months in the laboratory study. Leaching of labelled nitrate increased from 10 to 37% of the applied ammonium in the 0–10 cm depth and from 6 to 25% in the 10–20 cm depth at 4 and 16°C, respectively (Fig. 14.4d). Ammonium leaching was low (6–8% and 1–2% of the amount applied in the 0–10 cm and 10–20 cm depths, respectively) and showed no difference in relation to temperature (not shown). A similar increase in nitrate leaching with temperature was observed in the surface organic layer (19 and 39%
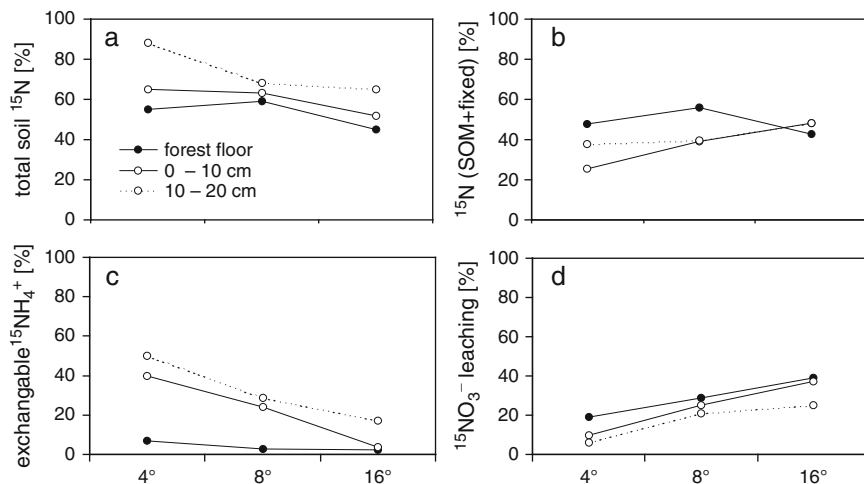

Fig. 14.4 Proportion of applied ammonium that remained as total nitrogen (**a**), organically bound plus fixed ammonium (**b**), exchangeable ammonium (**c**), or leached as nitrate (**d**) from the forest floor, 0–10 cm and 10–20 cm soil depth at Solling at 4, 8 and 16°C in the "laboratory study"

of the amount applied at 4 and 16°C; Fig. 14.4d) but a reduction in ammonium leaching (24 and 19% at 4 and 16°C, respectively, not shown). Increased leaching losses were attributed to adsorbed labelled ammonium which was reduced with increasing temperature. Figure 14.4c showed that about 40 and 50% of the applied ammonium were adsorbed in 0–10 cm and 10–20 cm soil depths at 4°C and were reduced to 4 and 17% at 16°C. The later desorption of ammonium caused leaching to increase (by about one-third in both the mineral soil depths). The moderate increased of retention by organic matter (Fig. 14.4b) could not prevent a general decrease in total retention at higher temperatures (Fig. 14.4a).

14.7 Conclusions

- Applied ^{15}N labelled ammonium was retained longer in an acid soil with moder humus but was quickly nitrified and leached as nitrate in base rich soils with mull humus type of surface organic layer. Longer retention of applied ammonium in acid soils was associated with low nitrification (heterotrophic type) rate. However, the surface organic layer in moder humus adsorbed only a small amount of the applied ammonium whereas most was adsorbed in the mineral soil layers. Retention of ammonium by adsorption depended on soil temperature which was reduced at higher temperature by increasing nitrification, and was increased with decreasing temperature by lowering nitrification. This mechanism potentially delayed leaching losses of applied ammonium as nitrates in acid soils especially at low temperatures in the nongrowing season and probably increased the uptake of N by microbes and plants.
- Retention of ^{15}N labelled ammonium in organic compounds (microbial biomass, soil organic matter) was high when microbial biomass was high. The dominant effect of microbial immobilisation, which was independent of soil layers and humus forms, suggested the importance of the fate of microbial detritus for the long-term retention of deposited ammonium in forest soils. Retention of ^{15}N labelled nitrate in organic compounds was very low in the Solling soil in contrast to other studies with different application procedures of labelled nitrate.

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