Biol Fertil Soils (1994) 17:101–107

ORIGINAL PAPER

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Fate of inorganic ^{15}N in the profile of different coniferous forest soils

Received: 14 December 1992

Abstract The fate of inorganic ^{15}N added to different coniferous forest soils was traced throughout the soil profile (0–25 cm) in a laboratory experiment under controlled conditions of temperature and water content. Six soils with different chemical climates were compared. The sequestration of labelled N was significantly explained by the clay content but the correlation was improved when C and N content were included. The level of acidification, even in soil with a fine texture, reduced the immobilization. For a similar N input, sandy soils with low C content or high acidification showed a reduced N storage capacity, so that N excess would be able to pollute the groundwater.

Key words Nutrient cycle · Coniferous forest · Soil properties · ^{15}N · Acidification

Introduction

N cycling in forest soils has been studied extensively for many years because of the limiting nature of N in many northern coniferous forests (Williams 1972; Popovic 1980). In recent years, the deposition of air-borne N compounds appears to have compensated for this N deficiency and the N nutrition status of coniferous forest is suboptimal over wide areas. N storage capacity is a decisive factor which varies between sites (Zöttl 1990) and contributes to the problem of defining N saturation in ecosystems.

The responses by biochemical processes in coniferous forest soils to changes in the quantity and composition of atmospheric pollutants were investigated in the Core project (CE/STEP-CT91-0118). Undisturbed soil cores were exchanged within European sites with different pollution environments. A site in the Fontainebleau forest hosted cores from Ireland (Kilkenny), United Kingdom (Exeter and Grizedale), The Netherlands (Wekerom), and Germany (Solling), and the chemistry of the leachates was investigated for 21 months (Core et al. 1992). The incident N input (April 1989 to April 1990) ranged from 4.6 to 23.1 kg ha^{-1} $year^{-1}$. This inorganic N may be either directly leached or incorporated into the soil profile by microorganisms or through physicochemical stabilization in organomineral complexes.

In most studies, the effect of inorganic N input has been investigated only in the litter and surface layers (Schimel and Firestone 1989a, b; Verhoef and Brussaard 1990; Blair et al. 1992) but the role of soil properties in determining the degree of immobilization of exogeneous N in the soil profile has not been demonstrated.

As part of the Core experiment (Core et al. 1992), soil columns (so-called cores) from five sites were brought to the Fontainebleau forest and incubated in the field for 21 months. When that experiment concluded, one column (selected from the seven replicates according to the mean N output during the Core experiment) from each site was brought to the laboratory with two aims: (1) to test the conservative capacity of each soil for chloride, and (2) to measure N immobilization and the movement of exogeneous N through the profile. Results from the second study are presented here.

Materials and methods

Unit description

Undisturbed soils were sampled using Plexiglass cylinders (25 cm long by 15 cm wide). The base was capped and sealed with silicone adhesive. A ceramic cup was inserted through a lateral hole and embedded in the bottom of the core. The ceramic cup was connected to a 2-litre glass bottle and pumped down to −0.06 MPa in order to avoid water stagnancy.

Site description

The cores originated either from mature closed-canopy plantations of Norway spruce (*Picea abies*) or from Scots pine (*Pinus*

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Table 1 Location and characteristics of the original sites

Site	Location	Tree species	Soil type	Humus type
Exeter (United Kingdom)	50°38′N, 3°36′W	*Picea abies*	Brown soil	Mull
Grizedale (United Kingdom)	54°19′N, 3°02′W	*Picea abies*	Podzol	Mor
Kilkenny (Ireland)	52°37.5′N, 7°23′W	*Picea abies*	Acid brown soil	Moder
Solling (Germany)	51°8′N, 9°5′E	*Picea abies*	Acid brown soil	Moder
Wekerom (The Netherlands)	52°06′N, 5°41′W	*Pinus sylvestris*	Podzol	Mor

sylvestris) plantations with more open canopies (Table 1). The soil types were either podzol with a mor humus or acid brown soil with moder humus.

This experiment was linked with the field Core experiment. Five soils were compared. A sixth unit was a replicate of the Exeter unit but was capped and watered with distilled water instead of rain during the field experiment. In this paper, the experimental units are called by their original site names and the capped unit is called Exeter 2.

Experimental procedure

After the field Core experiment, the undisturbed cores were brought back into the laboratory and kept at 15 °C. The soils were remoistened with 2000 ml distilled water applied at 100 ml h^{-1} for 5 h, every 2 days for 8 days. After 10 days, when the moisture was stabilized by suction through the ceramic cups, 500 ml of a solution with 100 µl $litre^{-1}$ N as $^{15}NH_4Cl$ at 25% atom excess (100 ml h^{-1}) was added to each unit. This represented 50 mg labelled N for each unit. Distilled water was then added in the same way, 500 ml on days 7, 14, 21, 45, and 56, 1500 ml on day 35. The columns were maintained under suction during the entire period. After 68 days, the cores were divided into sections representing soil layers at depths of 0–1 cm, 1–5 cm, 5–10 cm, 10–15 cm, 15–20 cm, and 20–25 cm, and stored at −18 °C until soil properties were measured and the ^{15}N analysis was carried out.

Soil characteristics

The particle-size distribution of the soils is given in Table 2. Most of the soils had a clay-loam texture but Wekerom was a sandy soil. The cation exchange capacity (Table 3) was low in the Wekerom soil and reached the highest levels in the Grizedale and Solling soils. Nevertheless, the rate of base saturation was low in all three soils.

The C content (Fig. 1) ranged between 40 and 50% in the litter layer (0–1 cm) except for the Exeter 2 soil where there was no 0–1 cm horizon because it had been capped for 21 months and had not received any litter input. In the underlying layer (1–5 cm), the Grizedale and Solling soils were very rich in organic matter, with 40% C, compared to the other soils which ranged from 10 to 20%.

Table 2 Particle-size distribution

	Depth (cm)	Particle-size class				
		<2 µm (g kg^{-1})	20–2 µm (g kg^{-1})	50–20 µm (g kg^{-1})	200–50 µm (g kg^{-1})	2–0.2 mm (g kg^{-1})
Exeter	1–5	338	343	153	71	95
	5–10	267	340	156	88	149
	10–15	263	327	154	89	167
	15–20	261	325	151	89	174
	20–25	260	311	145	92	192
Exeter 2	0–5	296	335	142	86	141
	5–10	256	321	148	90	185
	10–15	258	329	150	93	170
	15–20	255	316	149	91	189
	20–25	231	274	127	85	283
Grizedale	1–5	363	339	144	66	88
	5–10	503	285	165	11	36
	10–15	303	322	261	83	31
	15–20	255	334	242	105	64
	20–25	265	332	245	101	57
Kilkenny	1–5	426	306	130	85	53
	5–10	375	332	131	95	67
	10–15	343	315	132	111	99
	15–20	336	310	147	108	99
	20–25	347	294	141	128	
Solling	1–5	448	336	121	52	43
	5–10	262	326	284	96	32
	10–15	259	314	287	98	42
	15–20	249	318	277	100	56
	20–25	259	316	280	100	45
Wekerom	1–5	151	77	43	452	277
	5–10	46	29	45	573	307
	10–15	28	23	33	511	405
	15–20	31	17	33	538	381
	20–25	29	24	34	557	356

Table 3 Cation exchange capacity (CEC) and base saturation

	Depth (cm)	CEC (cmol kg^{-1})	Exch Ca (cmol kg^{-1})	Exch Na (cmol kg^{-1})	Exch Mg (cmol kg^{-1})	Exch K (cmol kg^{-1})	Exch Al (cmol kg^{-1})	Base saturation
Exeter	1–5	37.8	6.8	0.092	1.30	0.459	3.9	22.88
	5–10	17.4	2.1	0.029	0.39	0.316	4.0	16.29
	10–15	13.7	1.0	0.027	0.29	0.308	4.9	11.86
	15–20	11.9	1.8	0.034	0.54	0.312	3.4	22.57
	20–25	10.4	2.3	0.054	0.87	0.257	2.3	33.47
Exeter 2	0–5	30.7	4.4	0.049	1.04	0.306	5.8	18.88
	5–10	13.2	3.0	0.029	0.21	0.225	5.9	26.24
	10–15	11.3	0.7	0.028	0.21	0.244	5.0	10.46
	15–20	11.3	1.7	0.039	0.59	0.248	3.3	22.80
	20–25	10.4	2.5	0.045	1.00	0.212	1.9	36.12
Grizedale	1–5	67.3	7.4	0.153	2.67	0.675	2.7	16.19
	5–10	99.2	3.3	0.171	1.85	0.671	8.2	6.04
	10–15	45.6	1.3	0.082	0.62	0.191	10.2	4.81
	15–20	35.3	1.0	0.063	0.28	0.106	12.6	4.10
	20–25	37.8	0.8	0.058	0.21	0.119	15.0	3.14
Kilkenny	1–5	34.2	6.2	0.043	1.46	0.420	3.3	23.75
	5–10	19.2	2.1	0.047	0.75	0.321	4.2	16.76
	10–15	14.7	3.5	0.052	0.84	0.227	2.5	31.42
	15–20	12.0	3.7	0.065	1.08	0.183	1.7	41.90
	20–25	9.5	3.6	0.081	1.28	0.130	1.0	53.59
Solling	1–5	78.0	3.0	0.119	0.86	0.603	6.8	5.87
	5–10	26.7	4.7	0.042	0.28	0.236	5.7	19.69
	10–15	21.4	0.6	0.035	0.14	0.178	8.2	4.45
	15–20	17.0	0.6	0.027	0.09	0.157	7.6	5.14
	20–25	13.4	0.3	0.027	0.06	0.127	6.5	3.83
Wekerom	1–5	34.7	2.0	0.028	0.41	0.236	2.9	7.71
	5–10	8.9	0.4	0.016	0.07	0.072	2.0	6.26
	10–15	5.2	0.2	0.016	0.02	0.045	1.9	5.40
	15–20	3.7	0.1	0.017	0.02	0.036	1.6	4.68
	20–25	3.7	0.1	0.013	0.02	0.032	1.4	4.45

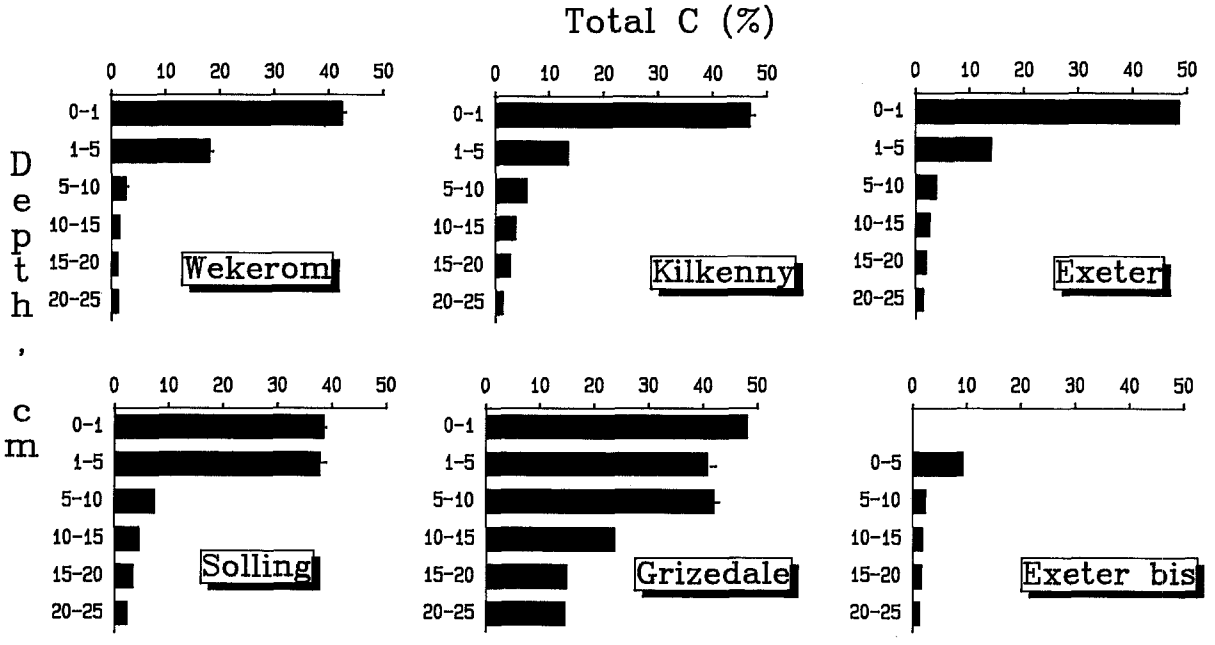

Fig. 1 Total C content versus depth of soil columns, *Exeter bis*, Exeter 2 column

At deeper horizons, the Grizedale soil was still the most organic, with 15% C at 25 cm, while the C content of the other soils did not exceed 2.5%.

The C : N ratio in the surface layer (Fig. 2) ranged from 32 in the Wekerom soil to 48 in the Exeter soil with a N content of 1 – 1.3%. In the 1 – 5 cm layer, the C : N ratio ranged from 20 to 30, with a N content of 0.5%, except in the Grizedale and Solling soils, where it exceeded 1%. In the Wekerom soil, because ot its low N content (from 0.1% at 5 – 10 cm to 0.05% in the deepest horizon), this value decreased very little with depth compared to the Exeter soil, where it reached 12 in the deepest horizon. The Grizedale soil, which was very organic, had the highest N content, ranging from 1.4% at the surface to 0.8% at 25 cm.

There were only small differences between the Exeter soil and the Exeter 2 soil compared to the other soils (Tables 2, 3, 4), indicating that the variability between columns corresponded to the differences between sites. These findings were in agreement with similar measurements made on the soils sampled in their original sites, indicating that incubation of the Core soils did not change the soil properties and therefore the columns can be considered representative of the sites (Raubuch 1992).

Analysis

The total N content of the soils was determined using Kjeldahl digestion and colorimetry on a continuous flow analysis system (Spectrophotometer Pye-Unicam PU 8600 Philips). Inorganic N (exchangeable NH_4^+-N + NO_3^--N) was extracted from soil by shaking with 0.5 M K_2SO_4 (w : v 3 : 1) for 30 min. NO_3^--N was reduced by a Devarda mixture. NH_4^+-N was steam-distilled and colorimetrically determined from an aliquot. To determine the ratio of ^{15}N to ^{14}N, distilled N (total and inorganic N) was acidified to pH 3.0, to prevent loss of NH_3, and evaporated to dryness (70 °C) on a hot plate. N_2 gas was prepared from the resulting dry NH_4^+ salts by oxidation with lithium hypobromite. $^{15}N_2$ enrichments were determined by optical spectrometry. Total C was determined by dry combustion, using a Carmhograph 12A (Bottner and Warembourg 1976).

Statistics

All ^{15}N analyses were performed in triplicate. Statistical analysis: ANOVA, Newman – Keuls tests, and regression were performed with STATITCF software. Statistical differences between the columns for each layer are given in Table 4.

Table 4 Organic matter characteristics

Depth (cm)	Soil	C (%)	N (mg 100 g^{-1})	C : N	^{15}N (mg 100 g^{-1})
1 – 5	Exeter	14.2a	570a	25a	0.65a
	Exeter 2	9.5b	406b	23b	0.64a
	Grizedale	41.0c	1318c	31c	2.23b
	Kilkenny	13.6d	647d	21d	1.14c
	Solling	37.9e	1092e	35e	1.47d
	Wekerom	18.2f	580a	31c	0.76a
5 – 10	Exeter	4.0a	246a	16a	0.33a
	Exeter 2	2.5b	152b	16a	0.14a
	Grizedale	42.1c	1461c	29b	1.2b
	Kilkenny	5.9d	394d	15a	0.56c
	Solling	7.5e	284e	26c	0.22a
	Wekerom	2.8f	102f	17c	0.12a
10 – 15	Exeter	2.7a	193a	14a	0.14a
	Exeter 2	1.9b	118b	16b	0.06b
	Grizedale	23.8c	1180c	20c	1.3c
	Kilkenny	3.9d	240d	16b	0.29d
	Solling	4.6e	206a	22d	0.14a
	Wekerom	1.6f	65e	25e	0.06b
15 – 20	Exeter	2.1a	160a	13a	0.11ab
	Exeter 2	1.8b	112b	16b	0.06b
	Grizedale	15.1c	824c	18c	0.96c
	Kilkenny	2.9d	194d	15b	0.16a
	Solling	3.5e	161a	22d	0.1ab
	Wekerom	1.3f	55e	24e	0.05b
20 – 25	Exeter	1.5a	123a	12a	0.08ab
	Exeter 2	1.4a	95b	15b	0.07ab
	Grizedale	14.7b	770c	19c	0.94c
	Kilkenny	1.5a	129a	11a	0.09a
	Solling	2.4c	124a	19c	0.06ab
	Wekerom	1.3d	57d	23d	0.05b

Values within a layer followed by a different letter are significantly different ($P < 0.05$)

Results

Total and mineral labelled N

The incorporation of ^{15}N into the profile was most efficient in the Kilkenny and Grizedale soils, where 92 and 85% of the initial input was recovered, respectively, in the units. The Wekerom soil, which was the most sandy soil, lost 67.5% of the ^{15}N input. The Exeter, Exeter 2 and Solling soils retained about half of the input. Except for the Grizedale soil, the best incorporation was in the upper layer (0 – 10 cm), which contained 60 – 70% of the initial labelled N (Fig. 3). In the Grizedale soil, the labelled N content was similar in all layers.

The incorporation was mainly in an organic form, which means that the $^{15}NH_4^+$ input was reorganized by the microbial biomass. Inorganic ^{15}N recovery was very low in the Wekerom and Solling soils, which came from highly polluted areas with considerable acidification (Fig. 4). In the Kilkenny and Grizedale soils, the inorganic N was mainly NH_4^+-N while in the Exeter and Exeter 2 soils, NO_3^--N was dominant.

Isotopic excess

The isotopic excess (Fig. 5) was, in most layers, well above the detection limit of the method of measurement. It was highest in the surface layer (0 – 1 cm), especially in the Kilkenny soil. As the labelled N was mostly organic N, reorganization must have been very active in this layer. The isotopic excess decreased with depth, except in the Wekerom soil, where the N content was very low, and the Grizedale soil, which had a deep organic profile.

Proportion of the input of labelled N in each layer

The sequestration of labelled N in a soil horizon depends on the labelled N input into that horizon. Postulating that the flow moves only vertically downwards, we calculated the input of labelled N into each horizon as the difference

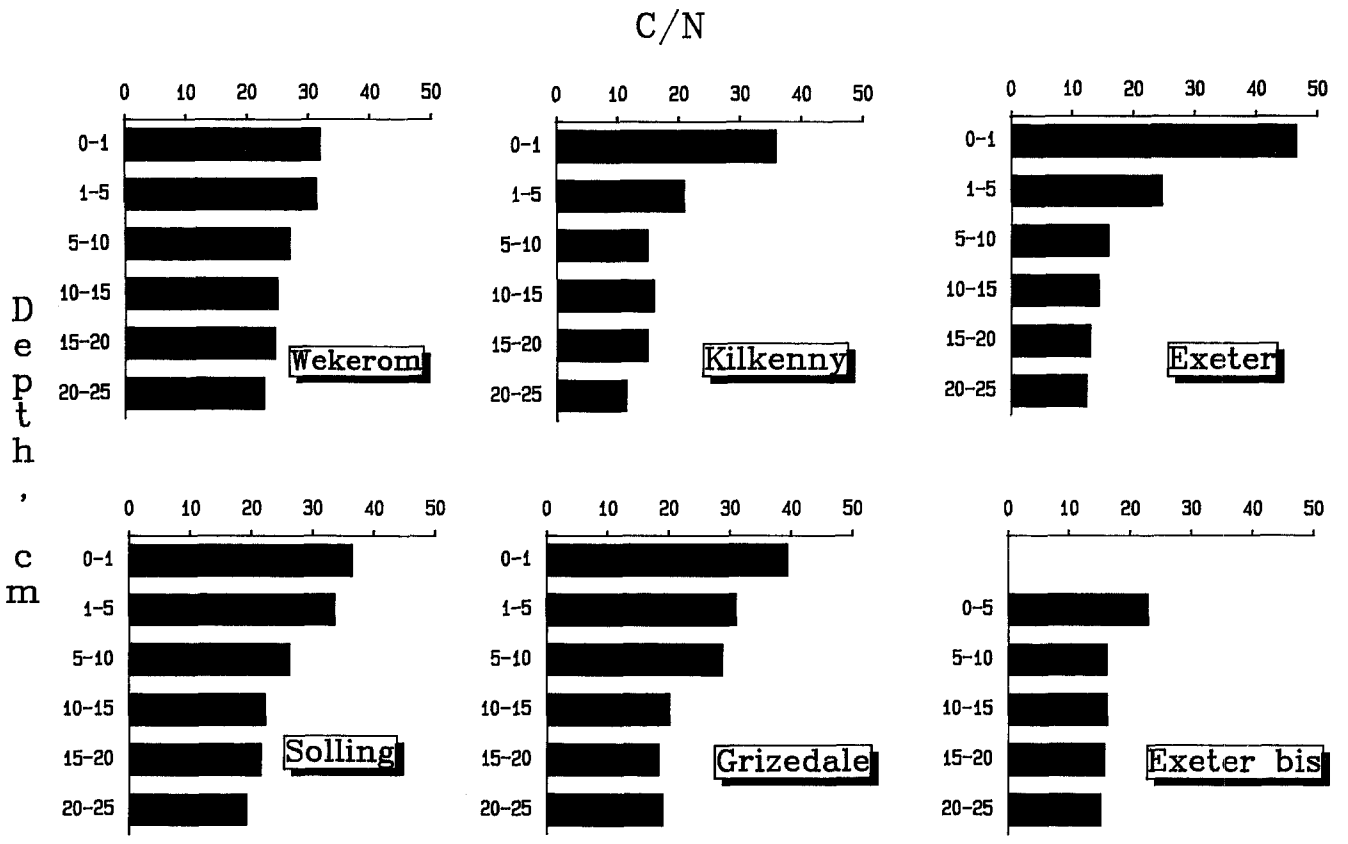

Fig. 2 C:N ratio versus depth of soil columns. *Exeter bis*, Exeter 2 column

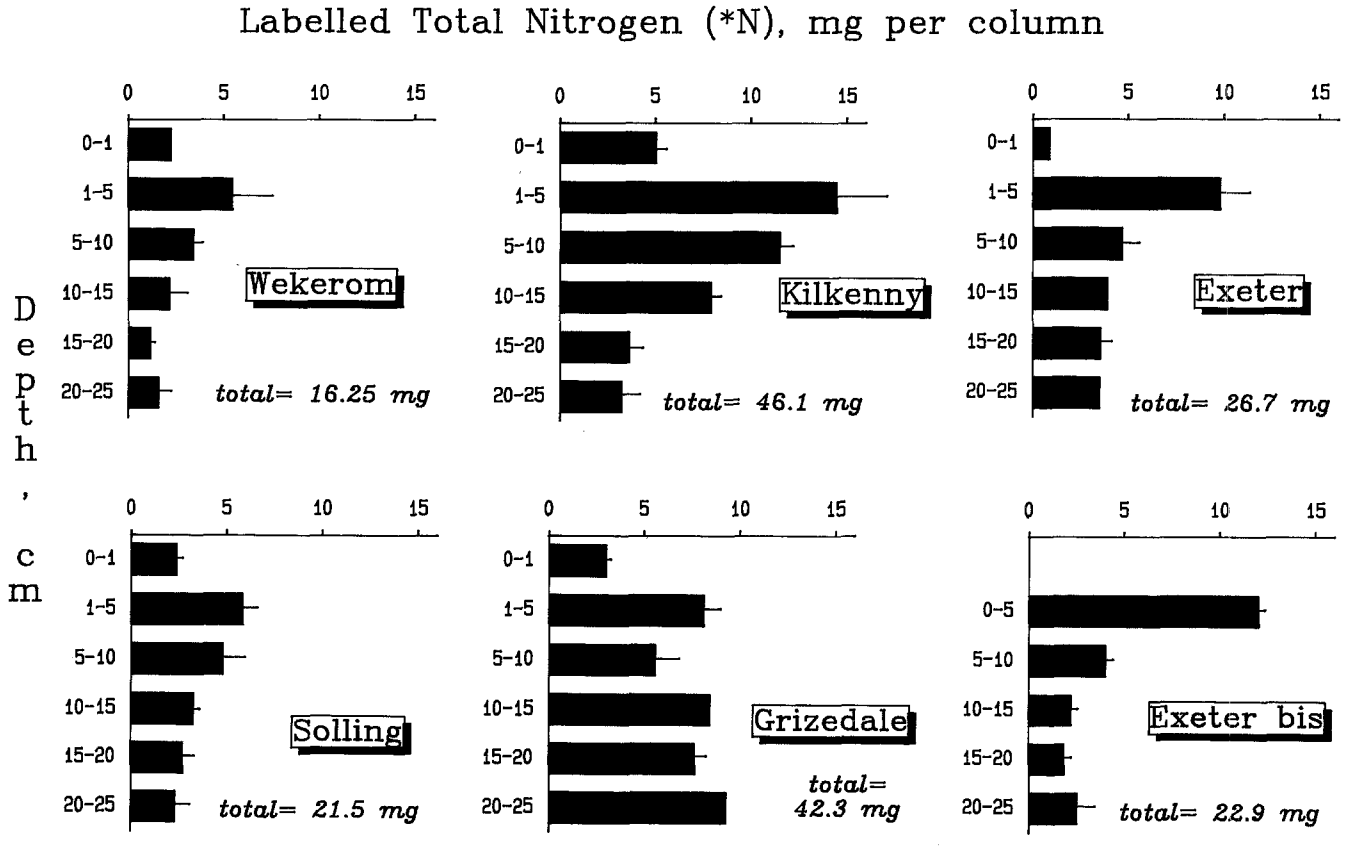

Fig. 3 Total labelled N versus depth of soil columns. *Exeter bis*, Exeter 2 column

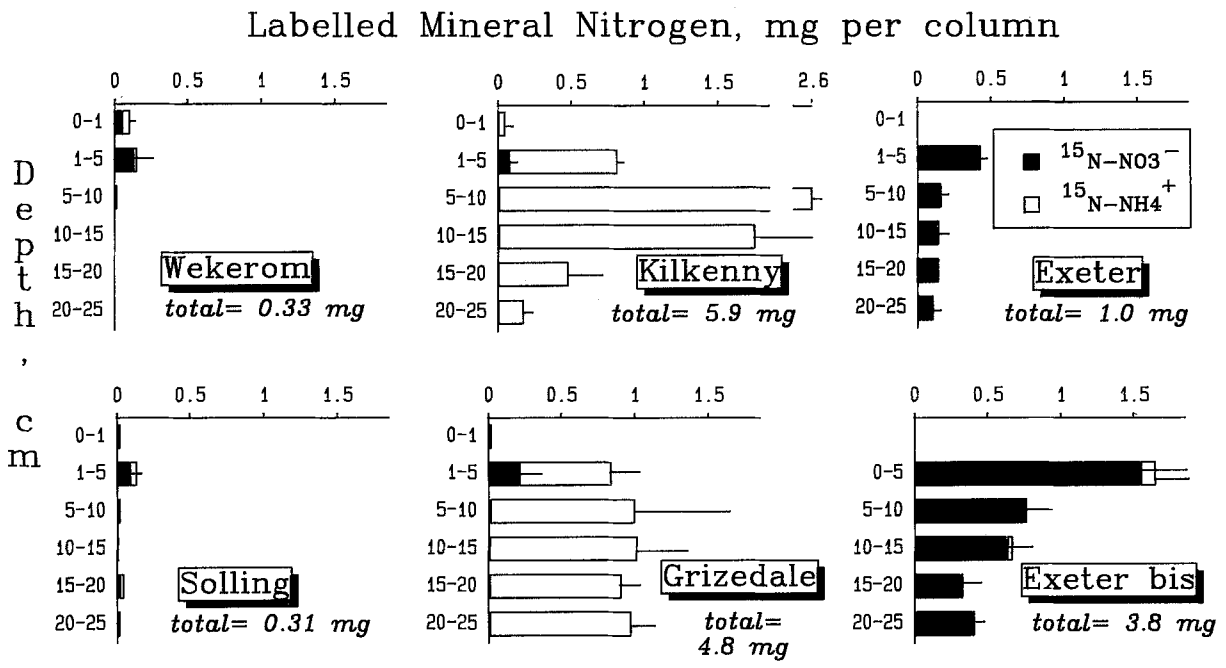

Fig. 4 Mineral labelled N versus depth of soil columns. *Exeter bis*, Exeter 2 column

between the input to the upper layer and the immobilization in that layer. The proportion of the input immobilized in each layer was related by simple correlation to the properties of the horizon (Table 5). Only the relationship with particle-size distribution, especially the clay content, was significantly, but this explained only 22% of the variation. In a multiple linear regression, C and N content increased the significance from 0.05 to 0.001, explaining 50% of the variation. The high recovery from the Kilkenny soil and the low recovery from the Wekerom soil were explained by texture, while the high recovery from the Grizedale soil was linked to organic matter content and the low recovery from the Solling soil to acidification, as indicated by the low base saturation.

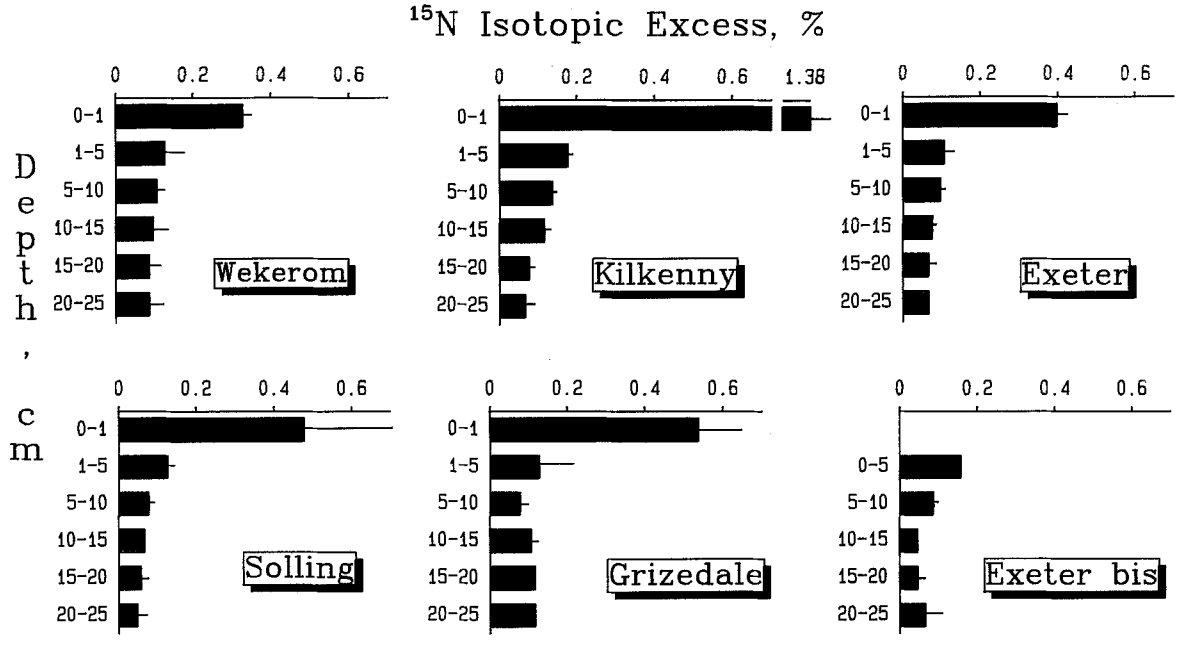

Fig. 5 ^{15}N isotopic excess versus depth of soil columns. *Exeter bis*, Exeter 2 column

Table 5 Regression between the labelled N proportion of the input in each layer and the properties of the layer

	r^2	r	P
Single linear regression			
N	0.078	0.279	NS
C	0.016	0.125	NS
Clay content	0.220	0.469	<0.05
Stepwise multiple linear regression			
Clay content	0.220	0.469	<0.01
Clay + C	0.247	0.497	<0.01
Clay + C + N	0.498	0.706	<0.001

Discussion

The purpose of this experiment was to determine the fate of exogeneous N, for example NH_4^+, from the throughfall input, in soils with different characteristics, and in particular to determine whether the exogeneous $^{15}NH_4^+$ was trapped in the profile or leached directly to the groundwater. A similar experiment performed by He et al. (1988) on one soil and its surface layer, using chemical fractionation, led to the conclusion that a significant fraction of recently immobilized N occurs as insoluble components of the microbial biomass, such as fungal melanins. Our results are in accord with those of He et al. (1988) since, in all soils, we recovered $^{15}NH_4^+$ mainly as organic ^{15}N.

The highest recovery of ^{15}N was from the Kilkenny soil (92%) and the Grizedale soil (85%), which had the highest cation exchange capacities, the highest clay contents and, for Grizedale, the highest C content. Schimel and Firestone (1989b), experimenting on a sandy loam soil, obtained a recovery of 64% after 31 days when inorganic ^{15}N was injected into the O_2 horizon. In the other soils of the present experiment, ^{15}N recovery ranged from 32.5% to 53.4%. The highest loss was from the Wekerom soil, where 67.5% of the input was lost. Denitrification was not measured and the experimental procedure may have enhanced this process, but such a high loss is unlikely to be attributable to this cause. Therefore the losses have to be explained by leaching. The texture of the soil, which controls the stabilization of organic matter, is the main factor allowing N to be trapped in organomineral complexes and the microbial biomass.

Schimel and Firestone (1989b) injected inorganic ^{15}N into O and A horizons and found little transfer through these horizons. After 1 day, the label was incorporated into the microbial biomass, and after 31 days, part of the label had accumulated in coarse root and detritus components. In the present experiment, where the inorganic ^{15}N was added to the top of the soil, most of the ^{15}N was retained in the upper 0–10 cm layers. The transfers occurred mainly through these layers and, except for the Wekerom soil, where leaching was even smaller, only 10% of the total ^{15}N in the profile reached the deepest layer (20–25 cm). In the soil from Grizedale, where the organic matter was distributed throughout the profile, all the layers contained about the same quantity of ^{15}N. Two processes probably acted simultaneously: ^{15}N is probably linked to fungal growth through organic matter and, in soils with a high clay content, ^{15}N is stabilized in organomineral complexes.

In conclusion, this experiment focused on soil properties that control the fate of exogeneous N, in particular N storage capacity and N saturation. Most of the N was stored in organic forms. We conclude that for the same N input, sandy soils with a low C content reach N saturation levels more quickly than loamy or organic soils and allow N excess to pollute the groundwater.

Acknowledgments This experiment was part of the Core project supported by CEC contract no. STEP-CT91-0118 (DTEE) with contributions from P. Ineson, coordinator (Merlewood, United Kingdom), J.M. Anderson (Exeter, United Kingdom), F. Beese (Munchen, Germany), T. Bolger (Dublin, Ireland), and H. Verhoef (Amsterdam, The Netherlands). We thank Dr. L. Palka, National Museum of Natural History, Brunoy, France, for his contribution and the Station Biologique de Foljuif, St Pierre-lez-Nemours, Ecole Normale Supérieure, France, for technical assistance.

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