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Natural ^{15}N abundance in two nitrogen saturated forest ecosystems

Abstract Natural ^{15}N abundance values were measured in needles, twigs, wood, soil, bulk precipitation, throughfall and soil water in a Douglas fir (*Pseudotsuga menziesii* (Mirb.) and a Scots pine (*Pinus sylvestris* L.) stand receiving high loads of nitrogen in throughfall (>50 kg N ha^{-1} year^{-1}). In the Douglas fir stand δ^{15}N values of the vegetation ranged between -5.7 and -4.2% with little variation between different compartments. The vegetation of the Scots pine stand was less depleted in ^{15}N and varied from -3.3 to -1.2% δ^{15}N. At both sites δ^{15}N values increased with soil depth, from -5.7% and -1.2% in the organic layer to $+4.1\%$ and $+4.7\%$ at 70 cm soil depth in the Douglas fir and Scots pine stand, respectively. The δ^{15}N values of inorganic nitrogen in bulk precipitation showed a seasonal variation with a mean in NH$_4^+$-N of -0.6% at the Douglas fir stand and $+10.8\%$ at the Scots pine stand. In soil water below the organic layer NH$_4^+$-N was enriched and NO$_3^-$-N depleted in ^{15}N, which was interpreted as being caused by isotope fractionation accompanying high nitrification rates in the organic layers. Mean δ^{15}N values of NH$_4^+$ and NO$_3^-$ were very similar in the drainage water at 90 cm soil depth at both sites (-7.1 to -3.8%). A dynamic N cycling model was used to test the sensitivity of the natural abundance values for the amount of N deposition, the ^{15}N ratio of atmospheric N deposited and for the intrinsic isotope discrimination factors associated with N transformation processes. Simulated δ^{15}N values for the N saturated ecosystems appeared particularly sensitive to the ^{15}N ratio of atmospheric N inputs and discrimination factors during nitrification and mineralization. The N-saturated coniferous forest ecosystems studied were not characterized by elevated natural ^{15}N abundance values. The results indicated that the natural ^{15}N abundance values can only be used as indicators for the stage of nitrogen saturation of an ecosystem if the δ^{15}N values of the deposited N and isotope fractionation factors are taken into consideration. Combining dynamic isotope models and natural ^{15}N abundance values seems a promising technique for interpreting natural ^{15}N abundance values found in these forest ecosystems.

Key words ^{15}N · N saturation · Natural abundance · *Pinus sylvestris* · *Pseudotsuga menziesii*

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Introduction

Over decades, elevated nitrogen (N) deposition has affected forested areas of Europe and North America. Increased N inputs may cause N saturation when the capacity of the system to retain and use N is exceeded (Aber et al. 1989; Schulze 1989). Nitrogen saturation is associated with increased rates of N cycling and losses of nitrate (NO$_3^-$) to drainage waters (Ågren and Bosatta 1988; Dise and Wright 1995; Lajtha et al. 1995). The use of natural ^{15}N abundance values of ecosystem pools offers possibilities for checking and improving estimates of nitrogen fluxes and nitrogen losses from forest ecosystems (Nadelhoffer and Fry 1994). As nitrogen cycles through the ecosystem, slight fractionation, or discrimination against the heavier isotope ^{15}N, is usually observed (Nadelhoffer and Fry 1994).

The ^{15}N natural abundance technique has recently been used in forest ecosystem health studies by Gebauer and Schulze (1991) and Gebauer et al. (1994). Needles from a healthy Norway spruce stand were more depleted in ^{15}N than those from a declining stand receiving in-

creased N and S depositions. The earlier onset of nitrogen reallocation in needles of the declining stand would have resulted in increased soil $\delta^{15}N$ values. Long-term forest fertilization trials (Högberg 1990, 1991; Högberg and Johannisson 1993; Johannisson and Högberg 1994) showed increased ^{15}N isotope ratios in the vegetation. They attributed these increases to increased N-pool sizes, cycling of nitrogen and to preferential losses of the lighter isotope ^{14}N. Garten (1993) cocluded from topographic patterns in foliar ^{15}N abundance on a catchment scale that foliar ^{15}N values were positively correlated with net nitrification potentials. Garten and Van Miegroet (1994) showed that foliar $\delta^{15}N$ values and enrichment factors ($\delta^{15}N_{leaf} - \delta^{15}N_{soil}$) were positively correlated with net N mineralization and net nitrification potentials in the soil. Due to isotope fractionation, $\delta^{15}N$ values of the output of NO_3^- and denitrification products, being relatively depleted in ^{15}N, may result in relatively enriched ecosystem pools. Several authors (Garten 1993; Garten and Van Miegroet 1994; Nadelhoffer and Fry 1994) predicted that natural abundance of ^{15}N would increase for systems approaching nitrogen saturation. Therefore, natural ^{15}N abundance values might identify the position of forests along a gradient from nitrogen deficiency to nitrogen saturation (Garten 1993; Kjønaas et al. 1993).

This study investigated (1) whether nitrogen-saturated forest ecosystems in the Netherlands are characterized by high natural ^{15}N abundance values in comparison with N-limited ecosystem, as a result of elevated nitrogen inputs and considerable leaching losses of ^{15}N depleted NO_3^-, and (2) whether certain ecosystem pools acting as a net source in N transformations were enriched with ^{15}N, whereas the resulting products in ecosystem pools acting as a net sink were depleted in ^{15}N. To investigate changes in isotope ratios in ecosystem pools resulting from isotope fractionation during N transformations, all major ecosystem compartments were investigated, including vegetation, soil, bulk precipitation, throughfall and soil water. In addition, the dynamic nitrogen and carbon isotope cycling model NICCCE (Van Dam and Van Breemen 1995) was used to determine the sensitivity of simulated ^{15}N abundance values within ecosystem compartments with respect to: (1) the amount of N input; (2) the isotope ratio of N deposited, and (3) intrinsic isotope discrimination factors accompanying N transformation processes.

Methods

Experimental sites

The research was carried out in two forest stands in the Netherlands that have been exposed to elevated nitrogen inputs for approximately 40 years (Van Breemen and Verstraten 1991). The first stand, a 35-year-old Douglas fir [*Pseudotsuga menziesii* (Mirb.) Franco.] stand, is embedded in a forested area in the central part of the Netherlands near the village of Speuld (52°13′N, 5°39′E) at 50 above sea level. Trees are approximately 22 m high (1993)

and stem density is about 800 stems ha^{-1}. In 1994 the vitality of the trees was low but average for Douglas fir tree vitality in the Netherlands, with an average needles loss of 26–60% (Heij and Schneider 1995). The soil, with a 4- to 7-cm-thick organic layer, was classified as a Haplic Podzol (FAO/UNESCO 1988). Soil pH$_{H20}$ ranged from 3.7 in the organic layers to 5.1 in the mineral soil, and the organic C content from 4.5% in the upper part of the mineral soil to 0.3% at 70 cm soil depth. Mean annual nitrogen deposition via throughfall at this site is approximately 50 kg ha^{-1} year^{-1} (Boxman et al. 1995), mainly in the form of NH_4^+-N (74%).

The second stand, dominated by 45-year-old Scots pine (*Pinus sylvestris* L.), is in the southern part of the Netherlands close to the village of Ysselsteyn (51°30′N, 5°55′E), 30 m above sea level. Average stem density is 650 stems ha^{-1} with a tree height in 1995 of 12 m. The stand is surrounded by agricultural areas. A high intensity of animal stock breeding characterizes the surrounding area. The organic layer consists of a 5- to 8-cm-thick L and F horizon. The mineral soil, classified as a Haplic Podzol (FAO/UNESCO 1988), has an organic rich mineral top layer (50 cm) with an organic C content decreasing from 4.7% in the upper 10 cm to 2.4% at 50 cm soil depth and 0.6% up to 70 cm soil depth. The soil is acidic (pH$_{H20}$: 3.7–4.9) and well drained. Current mean annual nitrogen deposition in throughfall amounts to 58 kg ha^{-1} year^{-1} (Boxman et al. 1995), mainly as NH_4^+-N (78%). Houdijk and Roelofs (1991) reported very high (90 kg N ha^{-1} year^{-1}) N depositions in throughfall in the past (1986) for the same area. The climate at both sites is temperate (average mean air temperature 9–9.5°C), with a mean annual precipitation of approximately 750 mm.

Sampling

Samples of foliage and soils were taken in the dormant season (January to February) when photosynthesis and N uptake by trees is low. Samples were taken from 10×10 m plots serving as control plots of large-scale N manipulation experiments (Boxman et al. 1995; Koopmans et al. 1996). Needles and twigs were sampled from the upper sun crown during three successive years (1992–1994). The Scots pine needles were divided in two needle cohorts of current and 1-year-old needles, the only needle cohorts being present on these trees. The Douglas fir needles were sampled from the current-year cohort, while 1-year-old needles and the few 2-year-old needles were pooled. Samples of twigs were taken from current-year twigs and 2-year-old twigs at both stands. Wood cores were taken from stems at breast height and separated into sapwood, heartwood and bark. Samples from three trees were pooled, resulting in three composite samples per plot. Litterfall was sampled in four collectors (1 m^2) in each plot. The samples were bulked quarterly for ^{15}N analysis. Five replicate soil samples (25×25 cm) were taken from the organic layer and divided into a LF1 (top 1 cm) and F2 horizon (5–8 cm below). The mineral soil was sampled down to 70 cm soil depth using a 5-cm internal diameter corer.

Bulk precipitation was collected in forest clearings close to the experimental sites (two replicates), whereas throughfall was collected on the plots (five replicates). Bulk precipitation and throughfall were collected fortnightly in continuously open collectors. Soil water was collected fortnightly from ceramic plates (diameter 135 mm) installed just below the organic layer (0 cm; four replicates) and ceramic cups, installed below the rooting zone (90 cm; eight replicates). Soil water was collected at a continuous pressure of −100 mbar. All liquid samples were pooled on a volume basis for 3-month periods from February 1991 to March 1994.

^{15}N and total N determinations

All vegetation and soil samples were dried at 70°C. Cones and roots were removed from the soil samples. Samples were ground into a very fine powder in a planetary mill. The solid samples were ana-

lyzed for atom% ^{15}N and total N, the liquid samples on atom% ^{15}NH$_4^+$-N and ^{15}NO$_3^-$-N and total NH$_4^+$-N and NO$_3^-$-N. The solid samples (with more than 0.15% N) were analyzed for total N using a Carlo Erba CHN elemental analyzer. Mineral soils and wood samples (with less than 0.15% N) were digested before total N and ^{15}N analysis. A modified version of the regular Kjehldahl method was used (Bremner and Mulvaney 1982; Mulvaney 1993). After heating 0.4 g sample with sulphuric acid, a Se/Cu catalyst and salicylic acid, 40 ml of the 100 ml digest was distilled with NaOH. The liberated NH$_3$ was trapped in 50 ml 0.15 M H$_2$SO$_4$, followed by colorimetric measurement of NH$_4^+$-N. Another 40 ml of the digest was distilled and the NH$_3$ was trapped into 60 ml 0.1 M HCl, which was evaporated (80°C) and prepared for the mass-spectrometer.

Nitrogen isotopes were measured on a Finnigan MAT stable isotope mass spectrometer, equipped with a Hereaus elemental analyzer for conversion of nitrogen into N$_2$, followed by a CN-version CT trapping box for isolation and purification of N$_2$, before entering the dual-inlet system of the mass spectrometer. Samples were measured against N$_2$ gas (>99.99%) which was calibrated against atmospheric dinitrogen.

To prepare water and soil water samples for ^{15}N determinations, the diffusion method according to Sørensen and Jensen (1991) was used. Total N recovery of this method is generally better than 97% (Koopmans et al. 1996). In this method NH$_4^+$-N and NO$_3^-$-N, following reduction to NH$_3$, are allowed to diffuse to an acid-wetted glassfibre filter enclosed in polytetrafluoroethylene tape. Glass fibre filters were handled as solid samples for nitrogen isotope measurements. Total NH$_4^+$-N and NO$_3^-$-N in the liquid samples were measured by continuous flow colorimetry.

Results of ^{15}N natural abundance are expressed in δ values, common in research at the natural abundance level (Shearer and Kohl 1993). The ^{15}N excess is expressed in parts per thousand relative to atmospheric N$_2$ (0.3663 atom% ^{15}N):

$$\delta^{15}N = (R_{sample}/R_{atmosphere} - 1) \times 1000‰$$

in which R_{sample} and $R_{atmosphere}$ are the atom% of the sample and the atmospheric N$_2$ standard, respectively. The analytical precision obtained at the natural ^{15}N abundance level was in general better than 0.2‰ δ-units for the standards KNO$_3$, pine needles and acetanilide. Results of an intercalibration between five laboratories in Europe and the United States indicated differences between laboratories at the natural abundance level of <1.3‰ δ^{15}N. Natural abundance levels presented here were generally in the lower range of values found in this intercalibration procedure.

Results

Vegetation and soil

Natural ^{15}N abundance of the current needles of the Douglas fir trees in Speuld was in the range of −5.4 to −4.4‰. δ^{15}N values in the 1-year old needles were slightly lower (−6.0 to −5.0‰) than values in current needles (Table 1). δ^{15}N values of needle litterfall and twigs were close to values found in the needles. Slightly higher δ^{15}N values were observed in sapwood (−4.6 to −3.4‰) than in heartwood (−6.2 to −5.4‰), the latter ones being close to δ^{15}N values observed in bark in 1992 and 1993. With a few exceptions, variation in δ^{15}N values between successive years was about 1‰. Total N concentration varied considerably between different tree compartments and successive years (Table 1). Although this range in total N concentrations was large, the range in δ^{15}N values within these Douglas fir trees compartments was limited.

Substantially more variation in δ^{15}N values was observed between tree compartments of the Scots pine at Ysselsteyn (Table 2). The needles of the Scots pine trees were less depleted in ^{15}N than the Douglas fir needles of the Speuld site. The current needles had values of −2.9 to −2.1‰ δ^{15}N. Older needles were more depleted (−3.1‰), whereas the mean δ^{15}N value of the needle litter was −1.5‰. Twigs showed slightly higher δ^{15}N values (−1.7 to −1.3‰) than fresh needles and slightly decreasing values with age (−2.5 to −1.6‰). In the Scots pine trees, δ^{15}N values in the bark were slightly more negative (−3.3‰) than in the sapwood and heartwood (−3.0 to −1.4‰) during 1992 and 1993. However, samples of 1994 showed that variation between years of these wood samples can be considerable. As at Speuld, a large variation in nitrogen concentrations was observed in needles and twigs between successive years (Table 2).

Table 1 Average total-N concentrations and δ^{15}N values (1992–1994) of the vegetation ($n = 3$), organic rich upper soil ($n = 5$) and lower mineral soil (composite sample of 5 replicates) in Speuld (Douglas fir vegetation). Standard errors of the mean are shown in parentheses

Compartment	1992		1993		1994	
	N (%)	δ^{15}N (‰)	N (%)	δ^{15}N (‰)	N (%)	δ^{15}N (‰)
Vegetation						
Needles (current)	1.57 (0.06)	−5.37 (0.23)	1.76 (0.02)	−4.84 (0.23)	1.89 (0.03)	−4.37 (0.53)
Needles (1 year)	2.26 (0.10)	−6.01 (0.09)	2.25 (0.01)	−4.95 (0.16)	2.45 (0.02)	−5.15 (0.10)
Litterfall needles	1.50 (0.05)	−4.72 (0.06)	1.78 (0.12)	−4.83 (0.33)	2.01 (0.04)	−5.95 (0.10)
Twigs (current)	0.82 (0.02)	−4.84 (0.47)	1.36 (0.05)	−5.25 (0.15)	1.30 (0.03)	−5.08 (0.17)
Twigs (2 years)	0.64 (0.02)	−5.81 (0.35)	0.42 (0.02)	−5.69 (0.17)	0.50 (0.04)	−5.31 (0.20)
Sapwood	0.05 (0.00)	−4.61 (0.14)	0.06 (0.00)	−3.36 (0.11)	0.08 (0.00)	−4.55 (0.16)
Heartwood	0.05 (0.00)	−5.44 (0.12)	0.04 (0.00)	−5.54 (0.25)	0.05 (0.00)	−6.19 (0.13)
Bark	0.47 (0.01)	−6.34 (0.08)	0.46 (0.00)	−5.60 (0.67)	0.48 (0.02)	−2.26 (0.57)
Soil						
LF1 horizon	2.03 (–)	−6.17 (–)	2.10 (0.08)	−5.43 (0.08)	2.11 (0.04)	−5.46 (0.12)
F2 horizon	1.95 (–)	−6.47 (–)	1.99 (0.05)	−5.31 (0.19)	2.04 (0.03)	−5.51 (0.15)
Mineral soil (0–10)	0.15 (–)	−0.31 (–)	0.18 (0.01)	−0.68 (0.41)	0.17 (0.01)	−1.74 (0.27)
Mineral soil (10–25)	0.05 (–)	1.12 (–)	0.06 (–)	−0.15 (–)	0.04 (–)	1.14 (–)
Mineral soil (25–50)	0.03 (–)	4.17 (–)	0.03 (–)	3.13 (–)	0.03 (–)	3.11 (–)
Mineral soil (50–70)	0.02 (–)	3.70 (–)	0.02 (–)	4.64 (–)	0.02 (–)	4.06 (–)

Table 2 Average total-N concentrations and $\delta^{15}N$ values (1992–1994) of the vegetation ($n = 3$), organic rich upper soil (n = 5) and lower mineral soil (composite sample of 5 replicates) in Ysselsteyn (Scots pine vegetation). Standard errors of the mean are shown in parentheses

Compartment	1992		1993		1994	
	N (%)	$\delta^{15}N$ (‰)	N (%)	$\delta^{15}N$ (‰)	N (%)	$\delta^{15}N$ (‰)
Vegetation						
Needles (current)	2.70 (0.07)	−2.85 (0.13)	2.56 (0.10)	−2.12 (0.12)	2.60 (0.00)	−2.14 (0.18)
Needles (1-year)	2.54 (0.12)	−3.78 (0.27)	2.74 (0.10)	−2.47 (0.34)	2.56 (0.18)	−3.13 (0.26)
Litterfall needles	1.67 (0.13)	−1.04 (0.20)	1.90 (0.17)	−1.09 (0.31)	2.32 (0.28)	−2.25 (0.18)
Litterfall cones	1.08 (0.22)	1.34 (1.91)	0.91 (0.30)	0.75 (0.10)	1.17 (0.00)	1.63 (0.00)
Twigs (current)	1.66 (0.03)	−1.69 (0.11)	1.38 (0.11)	−1.34 (0.24)	1.76 (0.03)	−1.43 (0.18)
Twigs (2 years)	1.39 (0.06)	−2.5 (0.03)	0.79 (0.01)	−1.55 (0.08)	0.64 (0.01)	−1.62 (0.06)
Sapwood	0.09 (0.00)	−2.78 (0.19)	0.08 (0.00)	−2.47 (0.26)	0.10 (0.00)	−2.54 (0.18)
Heartwood	0.05 (0.00)	−2.98 (0.18)	0.05 (0.00)	−1.40 (1.17)	0.05 (0.00)	−2.91 (0.36)
Bark	0.69 (0.01)	−4.96 (0.08)	0.60 (0.01)	−3.01 (0.04)	0.79 (0.01)	−1.93 (0.26)
Soil						
LF1 horizon	1.85 (−)	−1.30 (−)	1.95 (0.06)	−1.07 (0.14)	1.92 (0.08)	−1.18 (0.28)
F2 horizon	2.28 (−)	−4.84 (−)	2.20 (0.03)	−3.10 (0.34)	2.23 (0.06)	−3.03 (0.21)
Mineral soil (0–10)	0.09 (−)	−2.29 (−)	0.24 (0.04)	−1.71 (0.92)	0.17 (0.03)	−1.56 (0.57)
Mineral soil (10–25)	0.06 (−)	1.92 (−)	0.08 (−)	2.07 (−)	0.07 (−)	2.36 (−)
Mineral soil (25–50)	0.04 (−)	3.84 (−)	0.06 (−)	3.62 (−)	0.05 (−)	3.55 (−)
Mineral soil (50–70)	0.01 (−)	4.44 (−)	0.02 (−)	4.97 (−)	0.02 (−)	nd[a]

[a] *Nd* not determined

For the LF1 horizon, $\delta^{15}N$ values were found to be close to the ranges observed in the needle litterfall. At Speuld, a mean of −5.2‰ was observed in the needle litterfall, whereas in the LF1 horizon a 3-year mean of −5.6‰ was found. The $\delta^{15}N$ values of the F2 horizon did not differ from the LF1 horizon in Speuld (Table 1). $\delta^{15}N$ values increased in the upper part of the mineral soil and increased further with soil depth (Table 1).

At the Ysselsteyn site, a mean $\delta^{15}N$ value of −1.5‰ was found in the needle litterfall, compared to a mean of −1.2‰ in the LF1 horizon (Table 2). At this site a lower $\delta^{15}N$ value was observed in the F2 horizon (−3.7‰) than in the LF1 horizon. In the mineral soil horizons, an increase in $\delta^{15}N$ values was found, culminating at +4 to +5‰ in the lower mineral soil (Table 2). Mean $\delta^{15}N$ values of soil organic N were slightly higher at the Ysselsteyn site than at the Speuld site, although this difference was only significant in the organic horizons.

Bulk precipitation, throughfall and soil water

For NH_4^+-N, the most important form of nitrogen deposited at the Speuld site, a mean $\delta^{15}N$ value of −0.6‰ was found in bulk precipitation, but variation during the year was considerable (Figs. 1 and 2). In throughfall, slightly higher $\delta^{15}N$ values were observed. In soil water below the organic layer, the $\delta^{15}N$ values of NH_4^+-N increased strongly to +7.2‰, which may be related to the strong net nitrification taking place in the organic layer at this site. At 90 cm soil depth, NH_4^+-N was found again depleted in ^{15}N with a mean of −3.8‰. Only a small amount of nitrogen in bulk precipitation occurred as NO_3^--N. Its $\delta^{15}N$ value was negative, decreasing strongly in the throughfall to −10.1‰. A fur-

ther decrease was observed in leachate of the organic layer. At 90 cm soil depth, $\delta^{15}N$ values of NO_3^--N (approx. −6.7‰) were close to values observed for NH_4^+-N.

The $\delta^{15}N$ values of the NH_4^+-N deposited in Ysselsteyn (Figs. 1 and 2) were positive (about +10.8‰), whereas the NO_3^--N deposited was depleted in ^{15}N (−12.1‰). In throughfall these values were similar (+11.5‰ and −9.3‰ for NH_4^+-N and NO_3^--N, respectively). The $\delta^{15}N$ values of NH_4^+-N and NO_3^--N in soil water were comparable to the Speuld site. Below the organic layer, $\delta^{15}N$ values remained positive for NH_4^+-N, whereas NO_3^--N was found still depleted in ^{15}N. At 90 cm soil depth both NH_4^+-N and NO_3^--N were found to be depleted, with average $\delta^{15}N$ values of −7.1‰ and −6.1‰, for NH_4^+-N and NO_3^--N, respectively.

Discussion

In this study two sites were examined, representative of many coniferous forest stands on sandy, acid forest soils in the Netherlands. We observed N deposition inputs of >20 kg N ha^{-1} year^{-1} in bulk precipitation, increasing in throughfall to >50 kg N ha^{-1} year^{-1}, and with leaching losses of N matching up 50–70% of the total N input (Boxman et al. 1995), which is not uncommon in Dutch forest stands (Van Breemen and Verstraten 1991). The sites discussed in this study are at the high end of the European N deposition gradient (Wright and Van Breemen 1995) and highest in N deposition and N leaching within the European NITREX project (Tietema and Beier 1995). The internal N cycle is characterized by relatively high net mineralization and nitrification rates (Koopmans et al. 1995; Koopmans and Van Dam 1997). Soil water N chemistry is dependent on the N deposition

Fig. 1 Time series of $\delta^{15}N$ natural abundance values of NH_4^+-N (–+–) and NO_3^--N (--△--) in bulk precipitation, throughfall, in soil water below the organic layer (0 cm soil depth) and at 90 cm soil depth at Speuld and Ysselsteyn
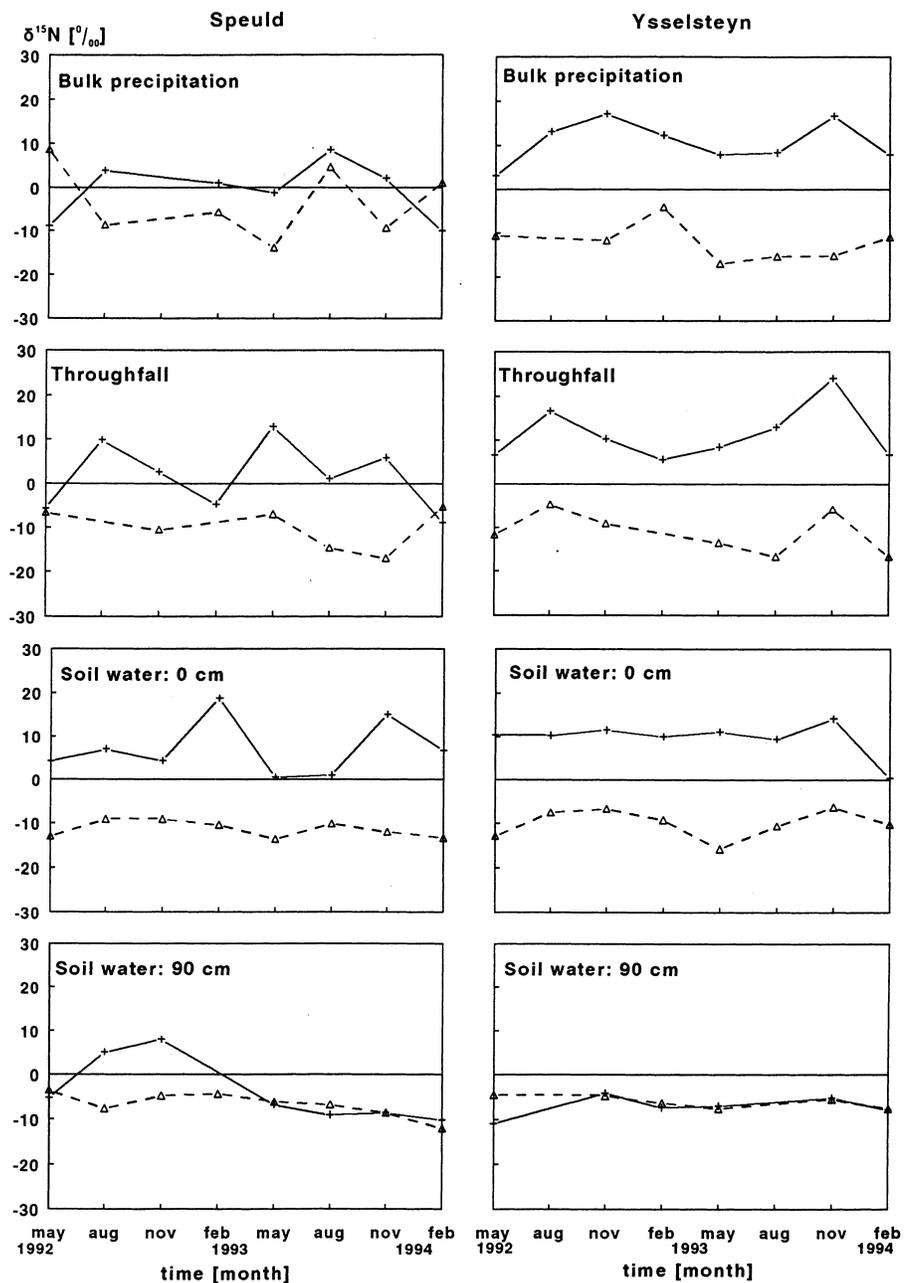
levels, as evidenced by reduced N concentrations in the soil water which were observed within a few months after N deposition was strongly lowered (Boxman et al. 1995).

The differences in ^{15}N natural abundance levels of the vegetation and soils of our ecosystems are consistent with results reported for the various compartments of forest ecosystems (Garten 1993; Gebauer et al. 1994; Nadelhoffer and Fry 1994). At both sites $\delta^{15}N$ values of the needles were negative, decreasing with needle age and increasing again in the litterfall. These findings correspond to results reported by Gebauer and Schulze (1991), Gebauer et al. (1994) and Nasholm (1994), indicating age dependent $\delta^{15}N$ values of the needles. Gebauer and Schulze (1991) attributed the differences

between the healthy and declining stands to earlier onset of nitrogen reallocation in the declining stand. Redistribution of N from the needles must have taken place in our needles before litterfall, as indicated by the increased $\delta^{15}N$ levels observed in the litterfall as compared to the older needles. Twigs showed generally lower $\delta^{15}N$ levels than needles, decreasing somewhat with age. Soils at both sites showed characteristic low (negative) $\delta^{15}N$ values in the organic layers, increasing strongly in the mineral soil to positif $\delta^{15}N$ values ($>+4\%_{oo}$). Fractionation during decomposition of soil organic matter (Nadelhoffer and Fry 1988) followed by losses of ^{15}N-depleted products, e.g. uptake by the vegetation or leaching, is assumed to control this increase in the mineral soil.
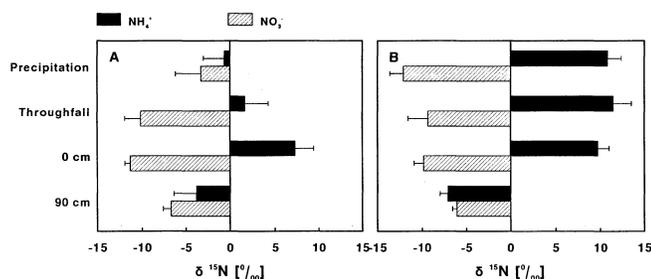
Fig. 2 $\delta^{15}N$ values of NH_4^+-N and NO_3^--N in water samples from **A** Speuld and **B** Ysselsteyn, averaged for the entire period. *Error bars* represent standard error of the mean

Below the organic layer, natural ^{15}N abundance of NH_4^+-N and NO_3^--N in soil water showed positive ($+1\%_o$) and negative ($<-5\%_o$) values, respectively. This may have resulted from the high net mineralization and nitrification rates in the organic layer, resulting in a considerable fractionation. A decrease in $\delta^{15}NH_4^+$-N values was observed deeper in the soil, whereas $\delta^{15}NO_3^-$-N increased somewhat with depth in soil. These values are the sum of processes including N-mineralization, -immobilization, -adsorption and -uptake contributing to these ultimate $\delta^{15}N$ levels. Nadelhoffer and Fry (1994) emphasized that the extent of nitrogen fractionation and the natural abundance of pools, is probably the result of many cumulative small fractionations occurring in the different steps of the nitrogen cycle. The time-integrated ^{15}N leaching from our forested sites indicated that the NO_3^--N leaving the ecosystem is relatively depleted in ^{15}N compared to the soils and corresponded to our model simulations.

The $\delta^{15}N$ values observed in the vegetation and soils of our N saturated sites were in the lower range of values found for non-N-fixing plants and soils (Létolle 1980; Nadelhoffer and Fry 1994; Peterson and Fry 1987). Especially in the Douglas fir stand, $\delta^{15}N$ values of about -6 to $-5\%_o$ were in the lower range of averages generally found in non-saturated forest ecosystems in Europe and the United States (about -6 to $+2\%_o$). Comparison of our $\delta^{15}N$ values within the European NITREX project also indicated that our sites, at the high end of the N deposition gradient, showed $\delta^{15}N$ values of vegetation and soils lower than findings of N limited sites (NITREX et al. 1997). Laboratory differences may have accounted for less than $1.3\%_o$ in this respect.

Garten and Van Miegroet (1994) calculated the "enrichment factor" (Mariotti et al. 1981) to correlate observed $\delta^{15}N$ values found in plant foliage with soil nitrogen dynamics. The enrichment factor ($\varepsilon_{p-s} = \delta^{15}N_{leaf} - \delta^{15}N_{soil}$), indicates the difference between ^{15}N abundance in the substrate (i.e. total soil N) and the product (i.e. foliar N). The enrichment factor accounts for differences in isotope composition of soil-N between sites. It might correlate with the N availability in forests and therefore with the stage of N saturation. The enrichment factor was calculated for the Speuld and Ysselsteyn site (Table 3). At Ysselsteyn, higher N inputs, higher net mineralization and nitrification rates corresponded to a higher enrichment factor in comparison with Speuld. Within NITREX, the enrichment factors of the sites spanning the N deposition range within Europe, highly correlated with the amount of N deposited, net nitrification in the soil and annual N flux in litterfall (NITREX et al. 1997). Speuld and Ysselsteyn accounted for the highest (least negative) enrichment factors found in NITREX and reported by Garten and Van Miegroet (1994). The high correlations suggest that the $\delta^{15}N$ enrichment factor will be useful in identifying sites influenced by nitrogen deposition (NITREX et al. 1997). Major questions remain, however, in using such an approach using an enrichment factor. A closed system was assumed in the derivation of the enrichment factor (Mariotti et al. 1981) and therefore it is questionable whether this approach holds in ecosystems with considerable leaching losses. Simulations suggested a considerable uptake of NO_3^- by the trees (Koopmans and Van Dam 1997) not directly accounted for by using an enrichment factor. In addition, the organic layer contributes considerably to the overall net N mineralization and nitrification at our sites which limits the use of the mineral soil as the only source of N (Koopmans et al. 1995). We preferred a dynamic simulation to evaluate the natural ^{15}N abundance data in these complex ecosystems as the enrichment factor leaves many questions unsolved.

To study the impact of the long-term N deposition level on the ultimate $\delta^{15}N$ values to be expected in ecosystems reaching N saturation, the ^{15}N natural abundance values observed in the field were combined

Table 3 N deposition in throughfall, net mineralization and nitrification and the calculated enrichment factor ($\varepsilon_{p-s} = \delta^{15}N_{leaf} - \delta^{15}N_{soil}$) for the Speuld and Ysselsteyn site

	Throughfall N deposition[a] (kg N ha^{-1} year^{-1})	Net N mineralization[b] (kg N ha^{-1} year^{-1})	Net nitrification[b] (kg N ha^{-1} year^{-1})	$\delta^{15}N$ leaf[c] ($\%_o$)	$\delta^{15}N$ soil[d] ($\%_o$)	Enrichment factor (ε_{p-s}) ($\%_o$)
Speuld	50	51	21	−4.86	−0.91	−3.95
Ysselsteyn	58	171	24	−2.37	−1.85	−0.52

[a] Mean 1990–1994
[b] After Koopmans et al. (1995)
[c] Mean of the current needles
[d] Mean of the mineral soil (0–5 cm)

with a sensitivity analysis of the NICCCE model (Van Dam and Van Breemen 1995).

This process-oriented dynamic simulation model describes the turnover of N and C isotopes in coniferous forest ecosystems. The model includes processes such as heat and water transport, evapotranspiration, primary production, mineralization, decomposition, root uptake, transport of solutes and isotope cycling (^{14}N, ^{15}N, ^{12}C, ^{13}C and ^{14}C), in coniferous forests with a one-dimensional multi-compartment soil profile. The processes giving rise to isotope fractionation are included in the model (Fig. 3).

NICCCE calculates isotope ratios for each ecosystem compartment, with isotope fractionation being treated as a dynamic process accompanying the nitrogen transformations (Van Dam and Van Breemen 1995). In every time-step, first an auxiliary value for the total flux
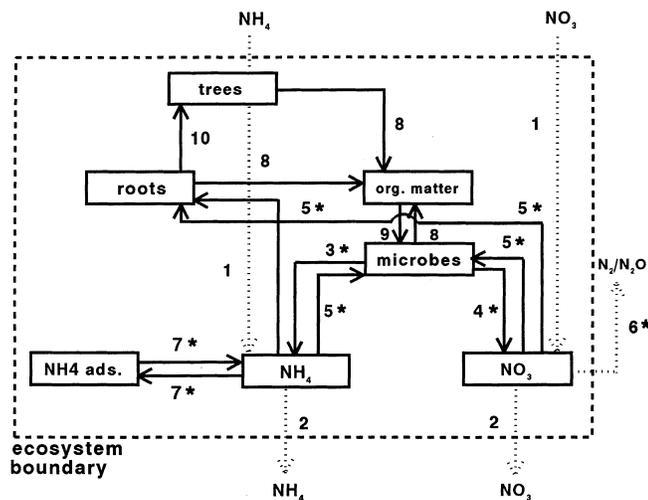
of N (J_A) is calculated, which is subsequently used to calculate the fluxes of ^{14}N and ^{15}N (J_{14N} and J_{15N}). The total flux of ^{14}N + ^{15}N (J_N) is then recalculated as $J_N = J_{14N} + J_{15N}$. Isotope discrimination factors in nitrogen transformation processes were obtained from literature. A summary of the equations for calculating isotope ratios using NICCCE is presented in Table 4.

NICCCE was calibrated for the Speuld site using data from a ^{15}N tracer experiment (Koopmans et al. 1996; Koopmans and Van Dam 1997). The sensitivity of simulated isotope ratios at the natural abundance level was investigated with the calibrated version of NICCCE for three *cases*.

Case 1: N deposition level

In the first case, two N deposition scenarios were compared. In the "business as usual" scenario N deposition remained at a high N deposition (40 kg ha^{-1} year^{-1}) during a period of 80 years. In the second scenario a "pre-industrial N input" was simulated, amounting to 10% of the high N deposition. In these simulations isotope discrimination factors were kept at constant values; δ^{15}N values found nowadays at ambient conditions at the two sites were used as initial input. Fractionation accompanying N transformations resulted in a preferential leaching from the ecosystem at 90 cm, of the lighter isotope in NO$_3^-$-N (Fig. 4A). Simulations indicated, that 80 years of present-day N deposition in the "business as usual" scenario (40 kg ha^{-1} year^{-1} deposition; 30 kg ha^{-1} year^{-1} leaching) and ongoing forest growth would increase δ^{15}N values in the organic layer considerably, whereas δ^{15}N values of the current-year needles and the NO$_3^-$-N would increase more slowly. Values of δ^{15}N of the ecosystem pools remained at nearly constant isotope levels in the "pre-industrial N input" scenario (Fig. 4B). Pre-industrial N depositions (4 kg ha^{-1} year^{-1}) resulted in considerably lower N leaching losses (< 2 kg ha^{-1} year^{-1}) and lower N transformation rates in the soil.

Fig. 3 N-cycling (—>) and N-input and output fluxes (--->) in the NICCCE model. Processes giving rise to isotope fractionation are marked with an *asterisk*. (*1* Input of NH$_4^+$ and NO$_3^-$, *2* Transport and leaching of NH$_4^+$ and NO$_3^-$, *3* mineralization, *4* nitrification, *5* uptake of NH$_4^+$ and NO$_3^-$ by microbes and roots, *6* denitrification, *7* ad- and desorption of NH$_4^+$, *8* Above and belowground litter production, *9* decomposition of litter and organic matter, *10* N allocation in the tree)

Table 4 Calculation of the isotope ratios in the NICCCE model (after Van Dam and Van Breemen, 1995). In this calculation K is the reaction rate constant [1/time], k_1 is the reaction rate for ^{14}N (approximately equal to the overall reaction rate K if isotope ratios are low) and k_2 the reaction rate for ^{15}N. IR is the ^{15}N/(^{15}N + ^{14}N) ratio, J_N the flux of total N, and TOTN is the pool size of total-N. J_A is an auxilliary value, β is the intrinsic isotope fractionation factor ($\beta = k_2/k_1$) whereas α ($\alpha = 1 - \beta$) is the intrinsic isotope discrimination factor

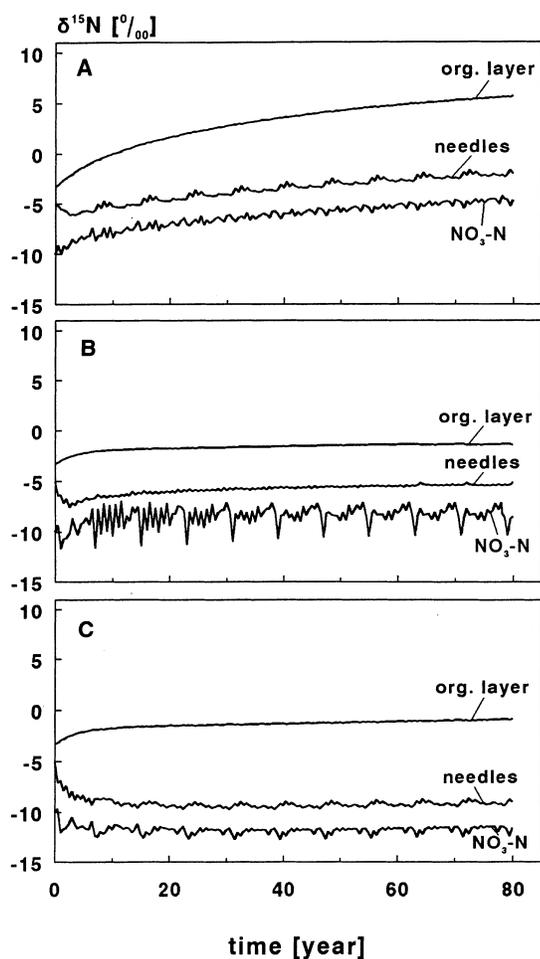| Pool	Rate constant	Flux
1. TOTN = ^{14}N + ^{15}N	K	$J_A = K \times$ TOTN
2. ^{14}N = (1/IR − 1) × ^{15}N	$k_1 = K$	$J_{14N} = K \times (1/\text{IR} − 1) \times {}^{15}\text{N}$
		$= K \times (1/\text{IR} − 1) \times \text{IR} \times \text{TOTN}$
		$= J_A \times (1 − \text{IR})$
3. ^{15}N = IR × (^{14}N + ^{15}N)	$k_2 = k_1 \times (1 − \alpha) = K \times \beta$	$J_{15N} = K \times \beta \times \text{IR} \times ({}^{14}\text{N} + {}^{15}\text{N})$
		$= K \times \beta \times \text{IR} \times \text{TOTN}$
		$= J_A \times \beta \times \text{IR}$
2 + 3. TOTN		$J_N = K \times \text{IR} \times (1/\text{IR} − 1 + \beta) \times \text{TOTN}$
		$= K \times \text{IR} \times (1/\text{IR} − \alpha) \times \text{TOTN}$
		$= J_A \times (1 − \alpha \times \text{IR})$

$\delta^{15}N\ [^o/_{oo}]$

Fig. 4A–C Simulated $\delta^{15}N$-values of the organic layer, current-year needles and NO_3-N in soil water at 90 cm for **A** a "business as usual" scenario and **B** a pre-industrial N input scenario. In **C**, $\delta^{15}NH_4^+$-N ($-1\%_{oo}$) and $\delta15NO_3^-$-N ($-3\%_{oo}$) of the input were lowered to $-10\%_{oo}$ at present-day N deposition. Isotope discrimination factors used were 0.010 for nitrification, 0.002 for uptake of NH_4^+ and NO_3^-, 0.005 for mineralization of organic N and 0.020 for denitrification. $\delta^{15}N$ observations were used for initial conditions

Case 2: $\delta^{15}N$ values of the N deposited

The $\delta^{15}N$ level of deposited N also obscures changes in $\delta^{15}N$ values due to a "N deposition history". In the second case, we varied $\delta^{15}N$ values of the $\delta^{15}N$ input. A ^{15}N depletion of the N input of $\delta^{15}NH_4^+ = -3\%_{oo}$ and $\delta^{15}NO_3^- = -1\%_{oo}$ was compared to a depleted N input of $-10\%_{oo}$ for [15]-N and [15]NO_3^--N. After 80 years at present day N deposition, a lower $\delta^{15}N$ of the N input ($-10\%_{oo}$) would result in considerably lower $\delta^{15}N$ levels of major ecosystem pools (Fig. 4C). $\delta^{15}N$ values in the organic layer would be $6.6\%_{oo}$ lower, whereas $\delta^{15}N$ values of current-year needles and NO_3^--N would be respectively $7.0\%_{oo}$ and $6.9\%_{oo}$ lower (Fig. 4A and C). In these simulations $\delta^{15}N$ values of the deposited N were kept at a constant level, although field observations indicated a seasonal variation in $\delta^{15}N$ values that may arise from local N emission sources.

Case 3: isotope fractionation during N transformations

In the third case, the sensitivity of predicted $\delta^{15}N$ values in ecosystem compartments for intrinsic isotope discrimination factors accompanying N transformations was investigated. $\delta^{15}N$ values of the organic layer appeared particularly sensitive to the isotope fractionation during nitrification, mineralization and N uptake processes, as indicated by changes in the $\delta^{15}N$ values of more than $2\%_{oo}$ if isotope discrimination factors were varied within the range reported in literature (Table 5). Changes in $\delta^{15}N$ values of the needles and soil water NO_3^--N are smaller and generally less than $2\%_{oo}$. The results indicate that the natural ^{15}N abundance values of our N-saturated sites are very sensitive to isotope fractionation during nitrification and mineralization. The highest isotope discrimination factors reported in literature for nitrification and mineralization seem unrealistic in our ecosystems as they would have resulted in

Table 5 Range of isotope discrimination factors reported in literature (after van Dam and van Breemen 1995) and the values used in the NICCCE simulations. In a sensitivity analysis one fractionation factor was changed at the time. Presented are the changes in $\delta^{15}N$ values after 80 years due to the change in the discrimination factor (repeating the period 1987–1995). Observed $\delta^{15}N$ values for the organic layer, current-year needles and NO_3^--N in the soil water were $-5.7\%_{oo}$, $-4.9\%_{oo}$ and $-6.7\%_{oo}$, respectively

Process	Isotope discrimination factor			Change in $\delta^{15}N$		
	Range reported	Standard in NICCCE	Sensitivity analysis	Organic layer (‰)	Needles current (‰)	[15]NO_3^--N 90 cm (‰)
Nitrification	0.000–0.040	0.010	0.005	−2.97	−0.87	+1.72
			0.015	+2.97	+0.86	−1.73
Mineralization	0.002–0.020	0.003	0.000	−2.93	+1.70	+2.08
			0.001	−2.06	+1.23	+1.32
			0.006	+3.09	−1.86	−1.99
Plant uptake	0.000–0.027	0.002	0.000	+2.58	+1.22	+1.36
			0.004	−2.26	−1.36	−1.48
Denitrification	0.020–0.030	0.020	0.030	+0.04	+0.06	+0.21
Ad/Desorption of NH_4	0.001	0.005	0.001	+0.24	−0.18	+0.10

considerable increases of natural ^{15}N abundance values in the organic layer and tree compartments of these N-saturated forest ecosystems.

The results of the sensitivity analysis indicate that in addition to the amount of N deposited and N transformations in the soil, also the ^{15}N natural abundance level of the deposited N contributes considerably to the natural abundance levels observed in ecosystem compartments reaching N saturation, as can be concluded from our second model case (Fig. 4A and C). The δ^{15}N value of the N deposited depends on the N emission source and formation of NH_4^+ and NO_3^- in precipitation and was shown to vary seasonally (Freyer 1978; Heaton 1986).

Higher overall δ^{15}N values in the ecosystem at Ysselsteyn in comparison with Speuld (Fig. 5) might therefore arise from tree species and soil type differences (memory effect), from differences in the δ^{15}N values of the N input and the stage of N saturation. Our field approach did not account for direct uptake of gaseous N depositions (NH_3, NO_3) by the vegetation, which could be important for the isotopic composition of our overall N input. Our approach also does not allow us to distinguish between preferential ammonium or nitrate uptake by the vegetation. Preferential ammonium or nitrate uptake by the trees might account for difference in δ^{15}N values of the vegetation. Higher N transformations found at Ysselsteyn (Table 3) must have contributed to higher natural ^{15}N values of the vegetation and the soil, as compared to the Speuld site. Although we found lower leaching losses at Ysselsteyn in comparison with Speuld, higher N depositions and N leaching losses in the past (Houdijk and Roelofs 1991) might have contributed to the higher δ^{15}N values observed at the "more N saturated" Ysselsteyn site. At Speuld, negative δ^{15}N values were found for NH_4^+-N in bulk precipitation, whereas at Ysselsteyn positive δ^{15}N values for NH_4^+-N in bulk precipitation contributed to higher δ^{15}N values. ^{15}N abundance values in bulk precipitation are still largely unexplained. Hoering (1957), Moore (1977), Freyer (1978), Heaton (1986, 1987) and Garten (1992) reported $\delta^{15}NH_4^+$-N and $^{15}NO_3^-$-N values in precipitation in the range of −18 to +10‰. Results of Hoering (1957) and Moore (1977) corresponded to our findings with respect to δ^{15}N values of NH_4^+ being enriched or less depleted than NO_3^- in precipitation. For aerosol and dry deposition of NH_4^+ and NO_3^- compounds, positive δ^{15}N values (up to 15‰) were reported (Moore 1977; Heaton 1986). The use of open collectors did not allow us to distinguish between wet and dry deposition. The unexpected positive δ^{15}N values for NH_4^+ in bulk precipitation at Ysselsteyn might result from a considerable contribution of dry deposition or from the high intensity of animal stock breeding in the immediate surrounding of Ysselsteyn. Moore (1977) reported δ^{15}N values for NH_3-gas, sampled close to the emission source in barnyards, of more than 20‰. At Speuld, the distance to any emission source is much further than in Ysselsteyn which might result in an other N deposition fraction with much lower δ^{15}N values but close to values observed by
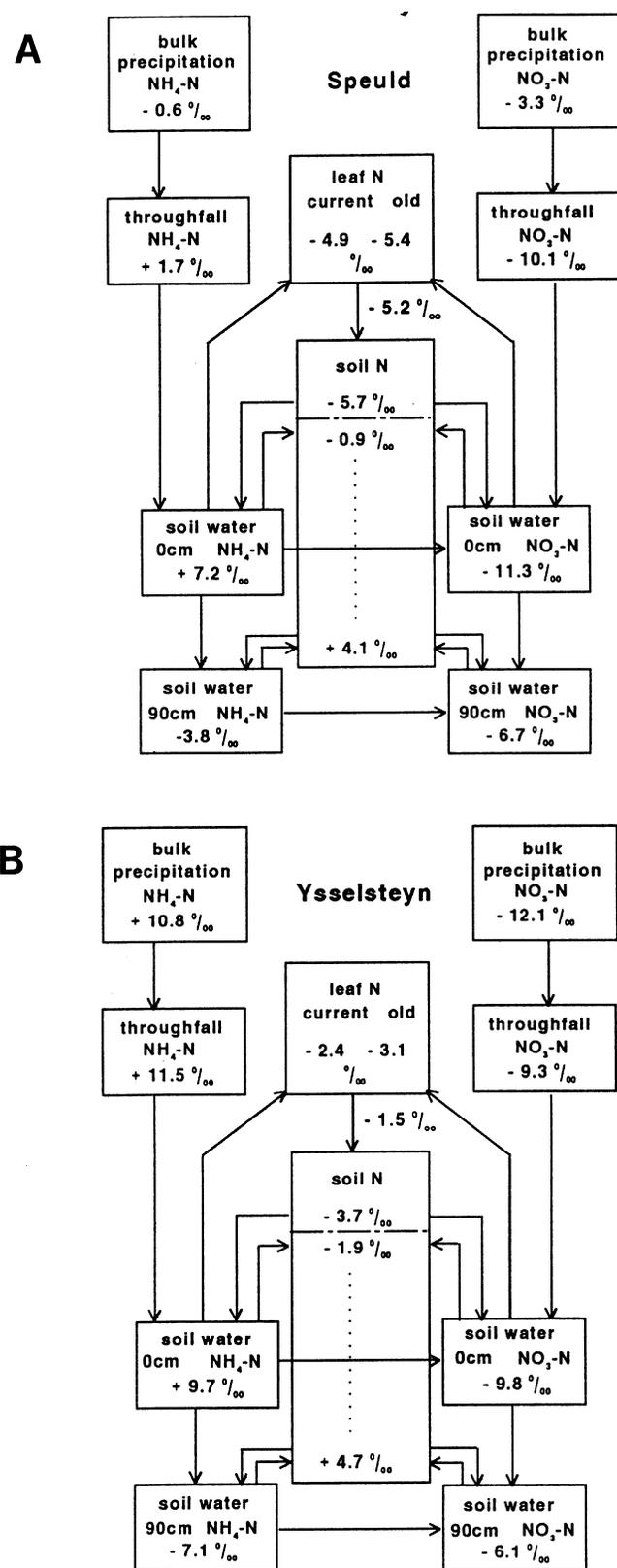

Fig. 5A–B Diagram of natural δ^{15}N abundance values of various ecosystem compartments at A Speuld and B Ysselsteyn; (− - − indicates the boundary between organic and mineral soil)

Freyer (1978), Heaton (1987) and Garten (1992). More detailed information on the nature of reactions between gaseous, particulate and dissolved nitrogen compounds in the atmosphere would be necessary to explain the observed $\delta^{15}N$ values (Heaton 1986).

The inconsistency of patterns in ^{15}N natural abundance observed by some authors (Binkley et al. 1985), emphasizes the importance of combining findings on the natural abundance level with information from conventional N studies and model studies. It was shown that natural ^{15}N values of the vegetation and soils only, are inadequate indicators of the stage of nitrogen saturation of these ecosystems. Low ^{15}N abundance levels of vegetation and soils may indicate tightly closed N cycles in N limited forest ecosystems (Garten 1993; Högberg and Johanisson 1993; Kjønaas et al. 1993), but are also found in these nitrogen saturated ecosystems. The integration of data on ^{15}N natural abundance of the vegetation and soil, with data on ^{15}N in bulk precipitation, throughfall, and soil water increases the potential of the ^{15}N natural abundance method. Further, the dynamic simulation models including isotopes, are a valuable tool in interpreting data on ^{15}N natural abundance levels. Our modelling approach is certainly not complete on the process-level in the ecosystem, and in particular time series of ^{15}N natural abundance would be useful to improve process formulations and model performance. In addition, isotope fractionation factors may not be constant for biologically mediated reactions (Mariotti et al. 1981) and are probably more complex than fractionations occurring during simple chemical conversion of substrates to products (Nadelhoffer and Fry 1994).

This study indicated that the natural ^{15}N abundance levels of certain ecosystem compartments may be used as a tool and an indicator for the nitrogen cycling patterns and the stage of nitrogen saturation of an ecosystem, but only so if the nitrogen inputs and their natural abundance values are taken into consideration. These $\delta^{15}N$ values should therefore be investigated when comparing ecosystems receiving different loads of nitrogen deposition. The natural ^{15}N abundance values of compartments within ecosystems indicated important differences that are closely related to N transformations. The simultaneous use of dynamic isotope models and natural abundance data therefore provides a technique to determine N transformations in the ecosystem. However, more reliable isotope discrimination factors and long-term series of ^{15}N natural abundance values within ecosystems would improve simulations considerably.

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