# The fate of $^{15}$N enriched throughfall in two coniferous forest stands at different nitrogen deposition levels

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**Abstract.** The stable isotope $^{15}$N was added as ($^{15}$NH$_4$)$_2$SO$_4$ to throughfall water for one year, to study the fate of the deposited nitrogen at different levels of N deposition in two "N saturated" coniferous forests ecosystems in the Netherlands. The fate of the $^{15}$N was followed at high-N (44–55 kg N ha$^{-1}$ yr$^{-1}$) and low-N (4–6 kg N ha$^{-1}$ yr$^{-1}$) deposition in plots established under transparent roofs build under the canopy in a Douglas fir (*Pseudotsuga menziesii* (Mirb.) Franco.) and Scots pine (*Pinus sylvestris* L.) forest.

The applied $^{15}$N was detectable in needles and twigs, the soil and soil water leaching below the rooting zone (90 cm depth). Total $^{15}$N recovery in major ecosystem compartments was 71–100% during two successive growing seasons after the start of a year-round $^{15}$N application to throughfall-N. Nine months after the year-round $^{15}$N application, the $^{15}$N assimilated into tree biomass was 29–33% of the $^{15}$N added in the Douglas fir stand and less than 17% in the Scots pine stand. At the same time total $^{15}$N retention in the soil (down to 70 cm) of the high-N plots was about 37% of the deposited $^{15}$NH$_4$-N, whereas 46% and 65% of the $^{15}$N was found in the soil of the low-N deposition plots at the Douglas fir and Scots pine stand, respectively. The organic layers accounted for 60% of the $^{15}$N retained in the soil. The total N deposition exceeded the demand of the vegetation and microbial immobilization. Total $^{15}$N leaching losses within a year (below 90 cm) were 10–20% in the high-N deposition plots in comparison to 2–6% in the lowered nitrogen input plots. Relative retention in the soil and vegetation increased at lower N-input levels.

Species differences in uptake and tree health seem to contribute to lower $^{15}$N recoveries in the Scots pine trees compared to the Douglas fir trees. The excessive N deposition and resulting "N saturation" lead to conditions were the health and functioning of biota were negatively influenced. At decreased N deposition, lower leaching losses together with increased soil and plant retention indicated a change in the fate of the $^{15}$N deposited. This may have resulted from changes in ecosystem processes, and thus a shift along the continuum of N saturation to N limitation.

## Introduction

Forests in large areas of Europe and North America are subjected to increased nitrogen inputs which may influence the health of the vegetation and func-

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tioning of these ecosystems (Schulze 1989; Van Breemen & Verstraten 1991; Aber 1993). If nitrogen inputs remain high for prolonged periods, the ecosystems may become "nitrogen saturated". This situation may be characterized by increased leaching of nitrate from the soil compartment (Aber et al. 1989).

At present, the problem of increased nitrogen inputs is acute in the Netherlands; most of the ecosystems can already be characterized as nitrogen saturated (Van Breemen & Van Dijk 1988). To address the question as to whether decreased nitrogen inputs can reverse nitrogen saturation and its accompanying effects, field scale manipulation experiments have been carried out in two nitrogen saturated coniferous forests in the Netherlands (Boxman et al. 1995). These experiments are part of the European project NITREX (Nitrogen Saturation Experiments), in which nitrogen deposition to whole catchments or large forest stands is changed drastically across a present-day gradient of nitrogen deposition across Europe (Dise & Wright 1992; Wright & Van Breemen 1995).

Fluctuations in the internal N fluxes due to higher, or lower, nitrogen deposition levels are difficult to detect. The large pool of nitrogen already present in the ecosystem obscures any changes in the nitrogen cycle resulting from experimental treatments (Kjønaas et al. 1993). Our objective in this study was to examine the effects of lowered N deposition on the forest nitrogen cycle and on losses of nitrogen from the ecosystems. The stable isotope $^{15}$N was used as a tracer to determine the fate of the incoming nitrogen. During one year $^{15}$N tracer was applied to the various plots exposed to different N deposition levels. Similar $^{15}$N tracer studies have been carried out across several of the NITREX sites (Kjønaas et al. 1993). The use of the isotope $^{15}$N allows us to examine the fate of the deposited ammonium, its distribution, transformation, retention and leaching, in more detail compared to conventional studies (Nadelhoffer & Fry 1994).

## Materials and methods

### Site description

This study was carried out in two forest stands in the Netherlands. The first stand is a 35-year-old Douglas fir stand (*Pseudotsuga menziesii* (Mirb.) Franco.) located in the central part of the Netherlands near the village of Speuld (52°13′ N, 5°39′ E). The sandy soil, a Haplic Podzol (FAO/UNESCO 1988) is well-drained. The humus form, classified according to Green et al. (1993), is a Mormoder. Mean annual N inputs with bulk depositions were 22 kg N ha$^{-1}$ (mean 1990–1995) at this site. In throughfall a mean deposition of 50 kg N ha$^{-1}$ yr$^{-1}$ was found, mainly in the form of $NH_4$-N (74%).

The second stand, located in the southern part of the Netherlands near Ysselsteyn (51°30′ N, 5°55′ E), is dominated by 45-year old Scots pine (*Pinus sylvestris* L.) with a undergrowth of ferns (*Dryopteris dilata*). The humus form was classified as a Mormoder (Green et al. 1993), the soil as an Haplic Podzol (FAO/UNESCO 1988). The mineral top layer is organic rich up to 50 cm depth due to ploughing before tree planting. A 5-years-mean annual nitrogen deposition of 32 kg N ha$^{-1}$ yr$^{-1}$ was observed in bulk deposition increasing in throughfall to 58 kg ha$^{-1}$ yr$^{-1}$ mainly as $NH_4$-N (78%). Both sites have a similar temperate climate with a mean annual precipitation of approximately 750 mm.

*Experimental procedures*

During the winter of 1988–1989, transparent roofs (14 by 28 meters each) were built under the canopies of both stands, approximately 3 meters above the ground in order to lower nitrogen and sulphur deposition onto the forest floor. Two plots (10 × 10m) were established under each roof with buffer zones of 2 meters around each plot. A third plot, the ambient plot, was established outside the roof. The low-N deposition plot under the roof received nitrogen depositions at pre-industrial levels (4–6 kg N ha$^{-1}$ yr$^{-1}$). Artificial throughfall, consisting of demineralized water with salts added in the same quantity as in ambient throughfall, except for N and S, was applied to this low-N deposition plot. The high-N deposition plot under the roof received natural throughfall collected on the roof. The ambient plot received natural throughfall directly. The addition of the water to the plots under the roof occurred in a fully automated, real time watering regime by 360° sprinklers, installed about 60 cm above the forest floor. More details on the general experimental design, the soils, vegetation and climate can be found in Van Dijk et al. (1992), Boxman et al. (1995) and Dise & Wright (1992).

*Application of the $^{15}N$*

Because of the relatively low enrichments in the $^{15}N$ manipulations within NITREX, we use the $\delta^{15}N$ notation common to studies at the natural abundance level (Shearer & Kohl 1993). The per mil $^{15}N$ excess (‰) is defined by:

$$\delta^{15}N = (R_{sample}/R_{standard} - 1)*1000 \text{‰}$$

in which $R_{sample}$ and $R_{standard}$ are the atom% of the sample and standard, respectively. The standard used in this study is atmospheric $N_2$, with 0.3663 atom% $^{15}N$.

For one year (May 1992–May 1993), the $^{15}$N tracer was applied onto the manipulated plots under the roof as 99.4% enriched $(NH_4)_2SO_4$ coupled on-line to the automated sprinkling system. We aimed to add the same absolute amount of $^{15}$N to all plots under the roof, however, due to small perturbations of the sprinkling system small differences in the water application occurred, leading to small differences in the amount of $^{15}$N added to each plot (Table 1). The $\delta^{15}$N values of the throughfall in the low and high deposition plot were different, and depended on the nitrogen concentration already present in the throughfall solution.

*Collection of samples*

To determine whether a significant increase in $\delta^{15}$N values was observed in the manipulated plots, $^{15}$N natural abundance levels and their variations were determined for the ambient-N plots (Koopmans, in prep.). Needles and twigs from all trees within the plots (9–11 trees per plot) were collected from the upper sun crown in the dormant season of 1992, 1993 and 1994, respectively 3 months before and 9 and 21 months after the start of the $^{15}$N application in May 1992. Harvested needles and twigs were stored at 2 °C until further processing. Douglas fir needles were divided into current and older needles (1-years old and 2-year old needles). The Scots pine foliage was divided into current and 1-year old needles; these trees did not have older needles. Twigs were divided into current and 2-year-old twigs ($>1^{st}$ order twigs). Needles and twigs were quickly rinsed with demineralized water before drying.

Wood cores (0.5 cm internal diameter) were taken from trees at breast height and divided into sapwood and heartwood. A 16 cm$^2$ piece of bark was sampled from each trees. In 1991 wood cores were sampled only in the ambient plot. Needles, twigs, wood and bark samples of three trees were pooled, resulting in three pseudo-replications per plot.

Five replicate soil samples (25 × 25 cm) from the organic layer (5–0 cm) and mineral soil (5 cm diameter corer, 0–10, 10–25, 25–50, 50–70 cm depth) were taken in each plot. The organic layer was divided into a L horizon (approximately first cm) and a F horizon. Lower mineral soil samples (deeper than 10 cm) were pooled per horizon. Cones, twigs and roots were removed from all soil samples. All samples were dried at 70 °C for 48 hours. Needles, twigs and soil samples were ground to a very fine powder in a planetary mill before $^{15}$N analysis.

Throughfall and soil moisture below the root zone at 90 cm soil depth were sampled fortnightly. Throughfall was sampled using five random installed, continuously open collectors, per plot. Soil moisture was collected from eight replicate ceramic lysimeter cups per plot at a continuous tension of 100 mbar.

Table 1. The amounts of NH$_4$-N, NO$_3$-N, total-N and calculated $\delta^{15}$NH$_4$-N values in the throughfall onto the plots in Speuld and Ysselsteyn in the period of $^{15}$N application (May 1992–May 1993). In February 1992, 1993 and 1994 ecosystem compartments were sampled for $^{15}$N analysis.

| Plot | May 1992–February 1993 | | | | | May 1992–May 1993 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | NH$_4$-N (kg.ha$^{-1}$) | NO$_3$-N (kg.ha$^{-1}$) | Total N (kg.ha$^{-1}$) | $^{15}$N (g.ha$^{-1}$) | $\delta^{15}$NH$_4$-N (‰) | NH$_4$-N (kg.ha$^{-1}$) | NO$_3$-N (kg.ha$^{-1}$) | Total N (kg.ha$^{-1}$) | $^{15}$N (g.ha$^{-1}$) | $\delta^{15}$NH$_4$-N (‰) |
| **Speuld** | | | | | | | | | | |
| low-N | 2.0 | 1.5 | 3.5 | 5.65 | 39339 | 2.7 | 1.7 | 4.4 | 6.44 | 33224 |
| high-N | 26.7 | 7.8 | 34.5 | 5.51 | 2874 | 33.5 | 10.1 | 43.6 | 6.92 | 2876 |
| ambient-N | 28.7 | 12.4 | 41.1 | –[1] | –[1] | 36.6 | 15.4 | 52 | –[1] | –[1] |
| **Ysselsteyn** | | | | | | | | | | |
| low-N | 2.4 | 1.3 | 3.7 | 6.88 | 43002 | 3.5 | 2.6 | 6.1 | 8.29 | 35535 |
| high-N | 30.5 | 6.6 | 37.1 | 6.84 | 3363 | 42.3 | 10.4 | 52.7 | 8.08 | 2864 |
| ambient-N | 36.1 | 7.8 | 43.9 | –[1] | –[1] | 51.5 | 14.1 | 65.6 | –[1] | –[1] |

[1] = no $^{15}$N addition.

Samples were pooled on a volume-weight-basis for three months intervals from February 1992 until January 1994.

*Nitrogen concentrations and $^{15}N$ analysis*

Vegetation and soil samples up to 10 cm soil depth were analyzed directly for total N using a Carlo Erba CHN elemental analyzer. Nitrogen isotopes at the natural abundance level 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 enriched in $^{15}N$ were measured using a continuous-flow combustion system consisting of a Carlo Erba elemental analyzer and a VG-SIRA stable isotope mass-spectrometer. Samples were measured against $N_2$-gas (>99.99%) which has been calibrated against atmospheric dinitrogen. The $\delta^{15}N$ value of atmospheric dinitrogen is by definition zero. The analytical precision in $\delta^{15}N$ is in general better than 0.2‰ at the natural abundance level (Finnigan) and about 2‰ for the VG at 500‰. Wood samples and mineral soil samples (10–70 cm) were digested before total N and $^{15}N$ analysis. A modified version of the regular Kjeldahl method was used (Bremner & Mulvaney 1982; Mulvaney 1993). After heating about 0.4 g sample with sulphuric acid, a Se/Cu catalyst and salicylic acid, 40 ml of the 100 ml destruate was distilled with NaOH and the liberated $NH_3$ trapped in 50 ml 0.15 M $H_2SO_4$, followed by colorimetric measurement of $NH_4$-N. Another 40 ml of destruate 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. In the liquid samples, $NH_4^+$ and $NO_3^+$ were separated prior to $^{15}N$ analysis using the diffusion method described by Sørensen & Jensen (1991). Total N recovery of this method is higher than 97%. Total $NH_4^+$ and $NO_3^-$ were analyzed on an autoanalyzer.

It was not always possible to analyze each ecosystem compartment with a statistically sufficient replication. If appropriate, differences between the manipulated plots were tested through a one-way ANOVA, using pseudo-replications within the plots.

*Total pool sizes and $^{15}N$ distribution*

The soil nitrogen pools were calculated from 3-years average nitrogen concentrations and bulk density data (Tiktak et al. 1988 for mineral soil of Speuld; Jenniskens 1993 for mineral soil of Ysselsteyn). For calculations of the $^{15}N$ distributions, the same biomass of tree compartments was assumed in the high-N and low-N deposition plots. From diameter at breast height

(DBH) measurements and litter production data (Boxman et al. 1995) no indications were found for significant differences in tree biomass or annual biomass increments between the manipulated plots at the Speuld site. Large discrepancies were observed in calculating needle biomass for the Speuld site. Litter production, calculated for the Speuld site using litter traps (Boxman et al. 1995; Van der Maas & Pape 1990) differed considerably from litterfall production derived indirectly from needle counts and occupancy (Steingröver et al. 1995). These latter high needle biomass data cannot be used in combination with the low litter production rates (Tiktak et al. 1995). In this study, data on total N pool sizes in the needles discussed by Van der Maas et al. (1991) were combined with the annual averaged nitrogen pool increase in the needles (12 kg N ha$^{-1}$ yr$^{-1}$; Steingröver 1995). For the low-N plot a correction was made for the lower N concentration observed in this plot. Biomass of twigs and stems was calculated from biomass studies during successive years (Swart et al. 1991; Jans et al. 1994; Steingröver 1995) combined with nitrogen concentrations observed in this study.

Scots pine biomass was calculated from allometric relationships between tree biomass compartments and DBH measurements determined for different age classes of Scots pine (*Pinus sylvestris* L.) trees in the Netherlands (Dik 1984; Bartelink & Van Hees, in press). Current-year biomass of the twigs was calculated from the annual biomass increments of the twigs, while 2-year-old twigs represented a fifth of total twig biomass.

Nitrogen leaching was calculated from water fluxes, calculated by the NICCCE model (Van Dam & Van Breemen 1995) and the volume-weighed averaged nitrogen concentrations of the soil water samples measured in this study.

The $^{15}$N distribution within the plots was calculated for February 1993 (9 months after the start of the one-year-long $^{15}$N application) and February 1994 (9 months after the end of the $^{15}$N application) by using the mass balance equation discussed by Nadelhoffer & Fry (1994):

$$m_{lab} = m_f * (\delta^{15}N_f - \delta^{15}N_i)/ (\delta^{15}N_{lab} - \delta^{15}N_i)$$

where:

| | |
|---|---|
| $m_{lab}$ | = mass of $^{15}$N-label incorporated into the labelled N pool; |
| $m_f$ | = final mass of the ecosystem N pool; |
| $\delta^{15}N_f$ | = final $^{15}$N abundance of the ecosystem N pool; |
| $\delta^{15}N_i$ | = initial $^{15}$N abundance in the ecosystem N pool; |
| $\delta^{15}N_{lab}$ | = $^{15}$N abundance of added N |

The increase in a $\delta^{15}$N value of an ecosystem compartment will depend on the size of the total N pool: a tracer flux into a large pool results in lower $\delta^{15}$N values compared to the same tracer flux into a relatively smaller pool.

## Results

*Vegetation*

Prior to the $^{15}$N manipulation, natural $\delta^{15}$N ratios of all investigated plant compartments of Douglas fir were negative ($-4$ to $-6\%o$). After the start of the $^{15}$N application, the observed $\delta^{15}$N values were always above the year-to-year variation in natural $^{15}$N abundance measured in the ambient plot (Figure 1) except in heartwood (not shown). A steady increase in $\delta^{15}$N values was observed in the two successive years except for the current needles which showed a higher increase in $\delta^{15}$N values in the second year. This may be partly due to the time at which the $^{15}$N application started, in May 1992 when the current foliage was already partially emerged. In 1993, $\delta^{15}$N values of the current twigs were found to be higher than the $\delta^{15}$N values of the needles at that time, but remained at lower levels in 1994. In needles and twigs, the low-N deposition treatment resulted in higher $\delta^{15}$N values than the high-N treatment. However, these differences were not always significant. In sapwood significant lower $\delta^{15}$N values were observed in the low-N plot (Figure 1) than in the high-N plot. $\delta^{15}$N values of bark increased from $-6.3\%o$ to values of $+13-30\%o$ in 1994 in the manipulated plots which was above average natural abundance values at the ambient plot.

At Ysselsteyn, the mean $\delta^{15}$N values of needles and twigs increased from slightly negative values ($-1$ to $-3\%o$) to $+10-40\%o$ in 1993 and to $+60-145\%o$ in 1994 (Figure 2). The increase in $\delta^{15}$N values was more pronounced in the second year. As with the Speuld site, the 1993 $\delta^{15}$N values of the current twigs were higher than the values of the current needles (Figure 2). Comparing the two N deposition treatments, the opposite trend was observed in needles and twigs of Scots pine compared to Douglas fir: Significantly higher $\delta^{15}$N values were observed in the high-deposition treatment, compared to the low-deposition treatment (Figures 1 and 2). $\delta^{15}$N values of Scots pine wood increased significantly in sapwood (from $-2.6$ to $+30-43\%o$ in 1994) and heartwood (not shown). In bark (natural abundance of $-3.3\%o$) no significant increase in $\delta^{15}$N was found.

Nitrogen concentrations of Douglas fir foliage differed significantly between the two N deposition treatments for the current needles in 1993 and 1994 (Table 2). The 1-year-old needles always had higher nitrogen concentrations than the current needles. Differences between the plots observed
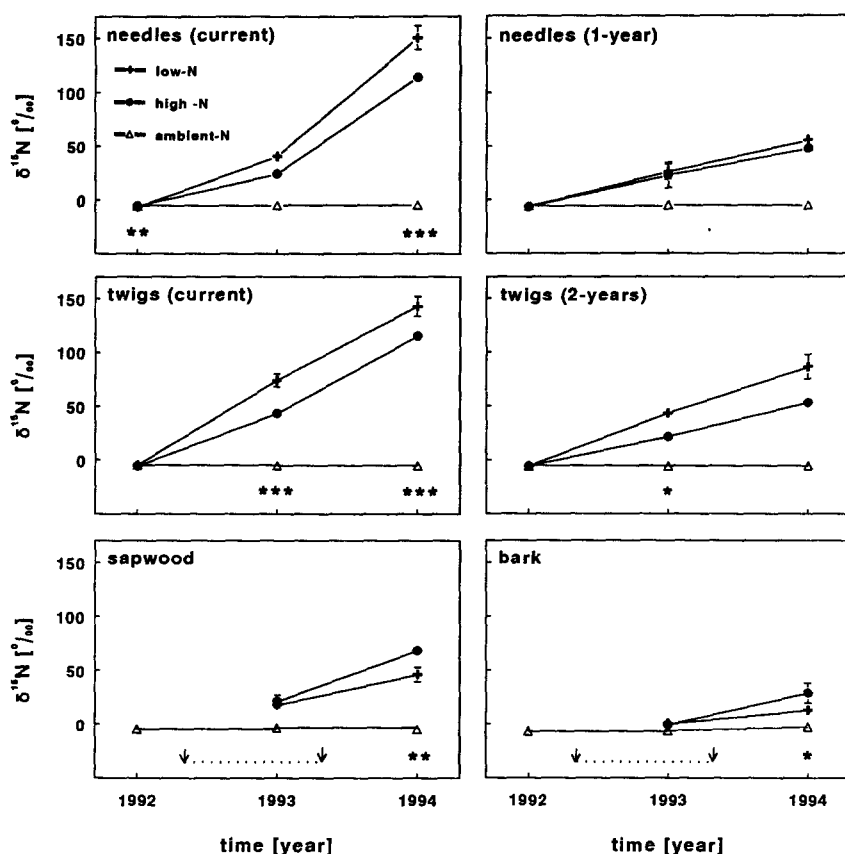
*Figure 1.* $\delta^{15}$N ratios of needles, twigs, sapwood and bark of Douglas fir trees 9 months (1993) and 21 months (1994) after the start of the one-year $^{15}$N manipulation to the plots at the Speuld site. Means and standard errors are presented ($n = 3$). The dashed line indicates the period of $^{15}$N application. Significant differences between the low-N and high-N plots are indicated by asterisks (***$p < 0.001$; **$p < 0.05$; *$p < 0.1$).

in 1994 were mainly due to an increase in nitrogen concentrations in the high deposition plot. Nitrogen concentrations of wood and bark did not differ between plots and were presented as means of all plots (Table 2).

Total N concentrations in Scots pine needles and twigs (Table 3) at Ysselsteyn were higher than Douglas fir needles and twigs of the Speuld site. Foliage nitrogen concentrations remained more or less constant with needle age. During all years N concentration of the current needles were significantly lower under low deposition regime than under high deposition. In 1994, nitrogen concentrations in all foliage 2 years and younger were lower in low than high deposition plots. However, great variations in nitrogen
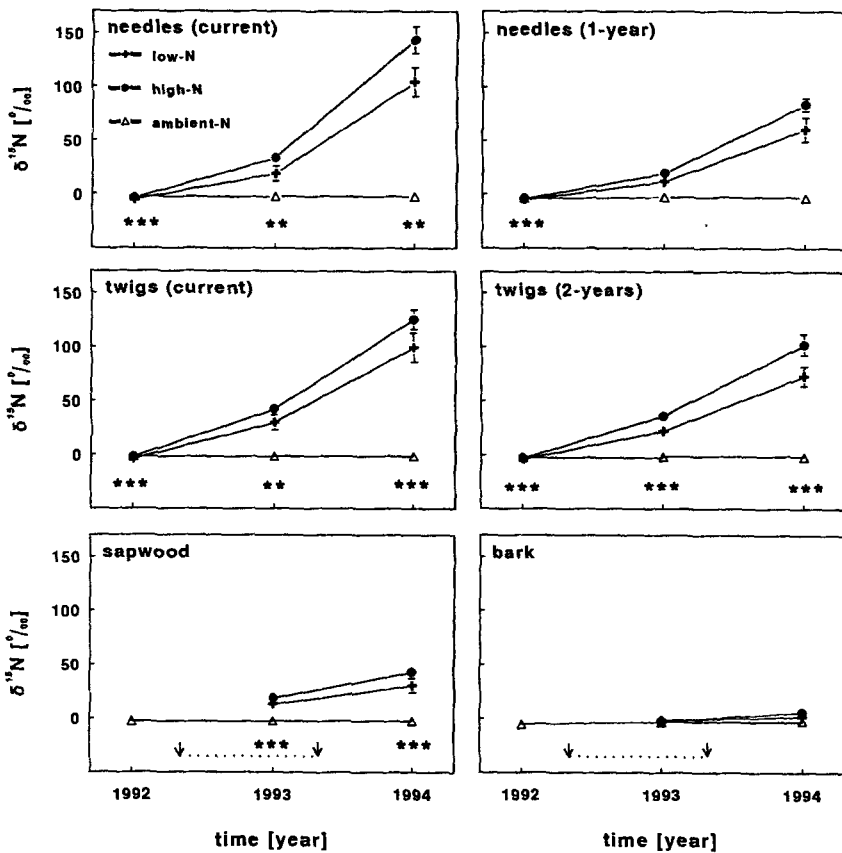
*Figure 2.* $\delta^{15}$N ratios of needles, twigs, sapwood and bark of Scots pine trees 9 months (1993) and 21 months (1994) after the start of the one-year $^{15}$N manipulation to the plots at the Ysselsteyn site. Means and standard errors are presented ($n = 3$). The dashed line indicates the period of $^{15}$N application. Significant differences between the low-N and high-N plots are indicated by asterisks (***$p < 0.001$; **$p < 0.05$; *$p < 0.1$).

concentration were observed between successive years. As for the Douglas fir, N concentrations of Scots pine wood and bark was averaged for all plots.

*Soil*

Natural $\delta^{15}$N values at the Speuld site were predominantly negative in the organic layer ($-5$ to $-7‰$) (Figure 3a) increasing in the mineral soil to $+4‰$ at 70 cm (Figure 3b). The organic layer at Ysselsteyn was slightly more positive ($-1$ to $-5‰$) than at Speuld and values increased also with depth to $+6‰$ in the lower mineral soil. In Ysselsteyn higher natural $\delta^{15}$N values

*Table 2.* Average N concentrations (1992–1994) as a percentage of sample dry weight for needles, twigs and wood of Douglas fir (Speuld). Standard errors of the mean (*n* = 3) are shown in parentheses. Wood samples refer to means of all plots (*n* = 9). Significant differences between the low-N and high-N plots are indicated (***$p < 0.001$; **$p < 0.05$; *$p < 0.1$).

| Plant tissue | 1992 N (%) | 1993 N (%) | 1994 N (%) |
|---|---|---|---|
| Needles (current) | | | |
| Low-N | 1.48 (0.06) | 1.61 (0.02) * | 1.62 (0.07) * |
| High-N | 1.51 (0.03) | 1.69 (0.03) | 1.75 (0.02) |
| Ambient-N | 1.57 (0.06) | 1.76 (0.02) | 1.89 (0.03) |
| Needles (1-year) | | | |
| Low-N | 1.71 (0.06) | 1.92 (0.02) | 1.91 (0.04) * |
| High-N | 1.84 (0.04) | 1.93 (0.03) | 2.26 (0.02) |
| Ambient-N | 2.26 (0.10) | 2.25 (0.01) | 2.45 (0.02) |
| Twigs (current) | | | |
| Low-N | 1.06 (0.02) ** | 1.08 (0.07) ** | 1.21 (0.06) |
| High-N | 0.85 (0.07) | 1.39 (0.12) | 1.35 (0.08) |
| Ambient-N | 0.82 (0.02) | 1.36 (0.05) | 1.30 (0.03) |
| Twigs (2-years) | | | |
| Low-N | nd | 0.47 (0.02) | 0.38 (0.01) |
| High-N | nd | 0.44 (0.02) | 0.46 (0.03) |
| Ambient-N | nd | 0.42 (0.02) | 0.50 (0.04) |
| | | | |
| Sapwood | 0.05 (0.01) | 0.06 (0.01) | 0.07 (0.01) |
| Heartwood | 0.05 (0.01) | 0.05 (0.01) | 0.05 (0.01) |
| Bark | 0.47 (0.01) | 0.51 (0.06) | 0.47 (0.02) |

nd: not determined.

were observed in the mineral soil of the plots under the roof in comparison to the mineral soil in the ambient plot (Figure 3b). After the $^{15}$N applications, $\delta^{15}$N values in the manipulated plots increased at both sites. This increase was highest in the organic layers. In 1994, $\delta^{15}$N values decreased again in the L horizon at both sites. $\delta^{15}$N values increased more in the low-deposition plots than in the high-deposition plot in the organic layer. However, differences were not always significant (Figure 3a). After the $^{15}$N additions $\delta^{15}$N values seem to increase also in the mineral soil of the treated plots at both sites. Due to pooling of samples it could not be tested whether this increase was significant (Figure 3b).

Average nitrogen concentrations of the soil (means of 1992–1994) showed only small differences between the plots. In Speuld and Ysselsteyn nitrogen concentration decreased with depth. In Ysselsteyn a nitrogen concentration increasing from the L to the F horizon was found (Table 4).

*Table 3*. Average N concentrations (1992–1994) as a percentage of sample dry weight for needles, twigs and wood of Scots pine (Ysselsteyn). Standard errors of the mean ($n = 3$) are shown in parentheses. Wood samples refer to means of all plots ($n = 9$). Significant differences between the low-N and high-N plots are indicated (***$p <$ 0.001; **$p < 0.05$; *$p < 0.1$).

| Plant tissue | 1992 N (%) | 1993 N (%) | 1994 N (%) |
|---|---|---|---|
| Needles (current) | | | |
| Low-N | 2.20 (0.04) *** | 2.22 (0.01) ** | 2.28 (0.03) *** |
| High-N | 2.54 (0.06) | 2.52 (0.07) | 2.80 (0.03) |
| Ambient-N | 2.70 (0.07) | 2.56 (0.10) | 2.60 (0.00) |
| Needles (1-year) | | | |
| Low-N | 2.31 (0.01) *** | 2.42 (0.07) | 2.23 (0.03) *** |
| High-N | 2.59 (0.05) | 2.60 (0.04) | 2.82 (0.05) |
| Ambient N | 2.54 (0.12) | 2.74 (0.10) | 2.56 (0.18) |
| Twigs (current) | | | |
| Low-N | 1.39 (0.07) ** | 1.37 (0.05) | 1.48 (0.02) *** |
| High-N | 1.61 (0.03) | 1.52 (0.05) | 1.93 (0.05) |
| Ambient-N | 1.66 (0.03) | 1.38 (0.11) | 1.76 (0.03) |
| Twigs (2-years) | | | |
| Low-N | 1.33 (0.05) | 0.84 (0.04) *** | 0.70 (0.02) *** |
| High-N | 1.45 (0.03) | 1.24 (0.17) | 0.93 (0.03) |
| Ambient-N | 1.39 (0.06) | 0.79 (0.01) | 0.64 (0.01) |
| Sapwood | 0.09 (0.01) | 0.08 (0.01) | 0.09 (0.01) |
| Heartwood | 0.05 (0.01) | 0.06 (0.01) | 0.05 (0.01) |
| Bark | 0.69 (0.01) | 0.62 (0.04) | 0.63 (0.01) |

*Soil water*

Ammonium concentrations in soil water at 90 cm depth were low in both N-manipulated plots at Speuld (Figure 4) while the experimental decrease in N deposition resulted in a considerable decrease in $NO_3$-N concentrations in the low deposition plot. In the high-N plot, $\delta^{15}N$ values of $NH_4$ and $NO_3$ increased in the first three months after the $^{15}N$ application. $\delta^{15}N$ values remained high in both plots from November 1992 until $^{15}N$ additions stopped in May 1993. Thereafter, a decrease in $\delta^{15}N$ values was observed. The enrichment levels of both $NH_4$-N and $NO_3$-N were similar.

In both manipulated plots at Ysselsteyn low $NH_4$-N concentrations were measured in the soil water at 90 cm (Figure 5). $NO_3$-N concentrations were reduced by about one third in the low-N plot in comparison to the high-N plot. A response in soil water at Ysselsteyn was observed for the first three months
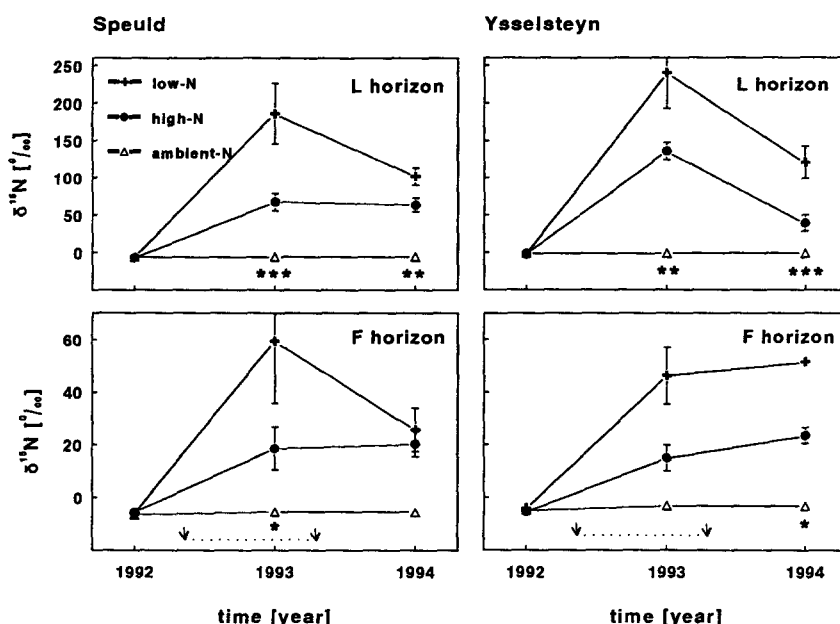
*Figure 3a.* $\delta^{15}N$ ratios of the organic layers, 9 months (1993) and 21 months (1994) after the start of the one-year $^{15}N$ manipulation to the plots at Speuld and Ysselsteyn. Means and standard errors are presented ($n = 5$). The dashed line indicates the period of $^{15}N$ application. Significant differences between the low-N and high-N plots are indicated by asterisks (*** $p <$ 0.001; ** $p < 0.05$; * $p < 0.1$).

after the start of the $^{15}N$ addition in both plots. Almost stable $\delta^{15}N$ values were observed during the $^{15}N$ addition (Figure 5). After the $^{15}N$ addition stopped, $\delta^{15}N$ values decreased more slowly than at Speuld. $NO_3$-N concentrations in the soil water leaving the ecosystem at 90 cm were high at the Ysselsteyn site, especially in the low-N deposition plot, in comparison to the Speuld site.

## $^{15}N$ distribution

Nine months after the start of the $^{15}N$ application, 100% of the applied $^{15}N$ could be found in the investigated compartments and fluxes in the low and 74% in the high-deposition plot in Speuld (Table 5). At that time about 13–15% of the tracer was taken up by the vegetation. 83% of the $^{15}N$ was retained in the soil in the low-deposition plot whereas only 42% was found in the high-deposition plot. At both plots, the organic layer accounted for more than 60% of the $^{15}N$ retained in the soil. In the high-N plot 19% of the applied $^{15}N$ leached out of the system during those nine months, compared to only 2% in the low deposition plot. In the winter of 1994, nine months after the end of the $^{15}N$ application, the total amount of $^{15}N$ as a percentage of the $^{15}N$ applied,

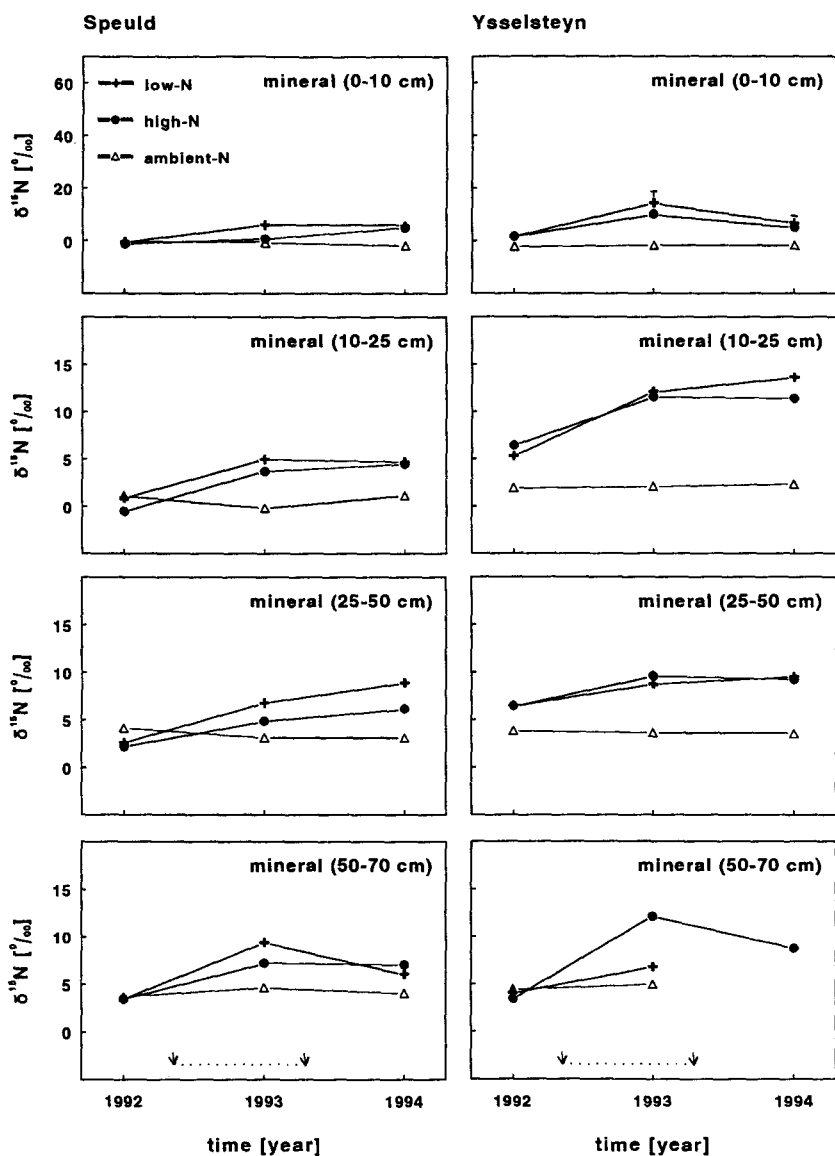*Figure 3b.* $\delta^{15}$N ratios of the mineral soil, 9 months (1993) and 21 months (1994) after the start of the one-year $^{15}$N manipulation to the plots at Speuld and Ysselsteyn. Means and standard errors are presented for the 0–10 cm horizon ($n = 5$). For the lower horizons symbols from a pooled sample. The dashed line indicates the period of $^{15}$N application.

*Table 4.* Total-N concentrations (average of 1992–1994) as a percentage of dry weight (70 °C) in the organic layer and the mineral soil in Speuld (Douglas fir) and Ysselsteyn (Scots pine). Standard errors are given in parentheses (*n* = 3).

|  | Low-N plot N (%) | High-N N (%) | Ambient-N N (%) |
|---|---|---|---|
| **Speuld** | | | |
| L horizon | 2.04 (0.08) | 2.06 (0.02) | 2.10 (0.03) |
| F horizon | 1.83 (0.04) | 1.92 (0.03) | 2.00 (0.02) |
| Mineral soil (0–10 cm) | 0.15 (0.01) | 0.16 (0.01) | 0.16 (0.01) |
| Mineral soil (10–25 cm) | 0.04 (0.01) | 0.04 (0.01) | 0.02 (0.00) |
| Mineral soil (25–50 cm) | 0.03 (0.00) | 0.03 (0.01) | 0.03 (0.00) |
| Mineral soil (50–70 cm) | 0.02 (0.00) | 0.02 (0.01) | 0.05 (0.01) |
| **Ysselsteyn** | | | |
| L horizon | 1.76 (0.10) | 1.85 (0.08) | 1.78 (0.03) |
| F horizon | 2.17 (0.07) | 2.19 (0.06) | 2.26 (0.01) |
| Mineral soil (0–10 cm) | 0.14 (0.01) | 0.15 (0.01) | 0.14 (0.02) |
| Mineral soil (10–25 cm) | 0.07 (0.01) | 0.08 (0.01) | 0.07 (0.01) |
| Mineral soil (25–50 cm) | 0.06 (0.01) | 0.06 (0.01) | 0.05 (0.01) |
| Mineral soil (50–70 cm) | 0.02 (0.01) | 0.02 (0.01) | 0.02 (0.01) |

found in these compartments and fluxes was 81% in the low-N and 99% in the high-N plot. In the low-deposition plot a higher percentage of the $^{15}$N applied was retained in the vegetation (33%) and in the soil (46%) compared to the high-deposition plot (29 and 37%). 33% of the $^{15}$N was leached out in the high-deposition plot mainly as $NO_3$-N (95%), compared to only 2% leaching out of the low-N plot.

Similar patterns were found at Ysselsteyn (Table 6), with highest total $^{15}$N recovery in the low deposition plot. In contrast to Speuld a smaller proportion of the applied $^{15}$N was retained in the trees (10–17% in Ysselsteyn in comparison to 29–33% in Speuld in 1994) and more $^{15}$N was retained in the vegetation of the high than in the low deposition plot. As at the Speuld site, a large portion of the $^{15}$N was retained in the soil, especially in the low deposition plot (82% in 1993; 65% in 1994) compared to the high deposition plot (58% in 1993; 37% in 1994). Most of the $^{15}$N recovered was found in the F-horizon. The difference between the plots in $^{15}$N leaching losses, was smaller at Ysselsteyn than at Speuld. As at Speuld, leaching was mainly in the form of $NO_3$-N.

*Table 5.* Calculated $^{15}$N recovery, 9 months (February 1993) and 21 months (1994) after the start of the $^{15}$N tracer application (May 1992–May 1993) for the Douglas fir site at Speuld. Leaching term refers to total period since start of the $^{15}$N tracer application.

| Ecosystem component | Low-N plot | | | | High-N plot | | | |
|---|---|---|---|---|---|---|---|---|
| | N pool/flux 1993 (kg ha$^{-1}$) | N pool/flux 1994 (kg ha$^{-1}$) | Recovery 1993 (%) | Recovery 1994 (%) | N pool/flux 1993 (kg ha$^{-1}$) | N pool/flux 1994 (kg ha$^{-1}$) | Recovery 1993 (%) | Recovery 1994 (%) |
| **Vegetation** | | | | | | | | |
| Needles (current) | 76.9 | 80.6 | 4.6 | 14.1 | 81.0 | 84.8 | 3.2 | 10.5 |
| Needles (1-year) | 158.7 | 166.2 | 6.6 | 11.5 | 172.9 | 181.1 | 6.6 | 10.2 |
| Twigs (current) | 6.8 | 7.2 | 0.7 | 1.2 | 7.2 | 7.7 | 0.5 | 1.0 |
| Twigs (2-years) | 17.6 | 18.6 | 1.1 | 1.9 | 18.6 | 19.8 | 0.7 | 1.2 |
| Wood | 141 | 145 | 1.8 | 4.0 | 141 | 145 | 1.9 | 5.9 |
| *Subtotal* | | | *14.8* | *32.7* | | | *12.9* | *28.8* |
| **Soil** | | | | | | | | |
| L horizon | 93.7 | 46.6 | 23.0 | 5.7 | 141.2 | 40.6 | 13.7 | 3.0 |
| F horizon | 374.6 | 465.0 | 31.2 | 16.5 | 388.8 | 447.0 | 12.2 | 12.0 |
| 0–10 cm | 1691 | 1691 | 13.9 | 11.3 | 1750 | 1750 | 4.6 | 11.3 |
| 10–25 cm | 789 | 789 | 5.6 | 3.3 | 789 | 789 | 4.4 | 4.1 |
| 25–50 cm | 1052 | 1052 | 4.8 | 7.4 | 1052 | 1052 | 3.7 | 4.4 |
| 50–70 cm | 634 | 634 | 4.6 | 1.9 | 634 | 634 | 3.1 | 2.4 |
| *Subtotal* | | | *83.1* | *46.1* | | | *41.7* | *37.2* |
| **Leaching** | | | | | | | | |
| NH$_4$-N | 0.1 | 0.5 | 0 | 0.1 | 1.1 | 1.1 | 0.7 | 0.8 |
| NO$_3$-N | 0.9 | 4.1 | 1.6 | 2.0 | 32.8 | 60.0 | 18.3 | 32.3 |
| *Subtotal* | *1.0* | *4.6* | *1.6* | *2.1* | *33.9* | *61.1* | *19.0* | *33.1* |
| **Total recovery** | | | **99.5** | **80.9** | | | **73.6** | **99.1** |

*Table 6.* Calculated $^{15}$N recovery, 9 months (February 1993) and 21 months (1994) after the start of the whole-year $^{15}$N tracer application (May 1992–May 1993) for the Scots pine site at Ysselsteyn. Leaching term refers to total period since start of the $^{15}$N tracer application.

| Ecosystem component | Low-N plot | | | | High-N plot | | | |
|---|---|---|---|---|---|---|---|---|
| | N pool/flux 1993 (kg ha$^{-1}$) | N pool/flux 1994 (kg ha$^{-1}$) | Recovery 1993 (%) | Recovery 1994 (%) | N pool/flux 1993 (kg ha$^{-1}$) | N pool/flux 1994 (kg ha$^{-1}$) | Recovery 1993 (%) | Recovery 1994 (%) |
| **Vegetation** | | | | | | | | |
| Needles (current) | 76.9 | 78.3 | 1.8 | 6.9 | 90.4 | 92.0 | 3.3 | 11.2 |
| Needles (1-year) | 22.2 | 22.6 | 0.4 | 1.2 | 25.6 | 26.0 | 0.6 | 1.9 |
| Twigs (current) | 2.2 | 2.3 | 0.1 | 0.2 | 2.7 | 2.7 | 0.1 | 0.3 |
| Twigs (2-years) | 17.6 | 17.9 | 0.5 | 1.1 | 22.0 | 22.4 | 0.7 | 1.9 |
| Wood | 62.8 | 66.8 | 0.6 | 1.0 | 62.8 | 66.8 | 0.7 | 1.4 |
| *Subtotal* | | | *3.4* | *10.4* | | | *5.5* | *16.7* |
| **Soil** | | | | | | | | |
| L horizon | 78.1 | 38.5 | 18.4 | 1.5 | 76.8 | 49.1 | 10.3 | 1.7 |
| F horizon | 626.4 | 987.4 | 30.5 | 44.0 | 898.4 | 828.2 | 17.8 | 19.7 |
| 0–10 cm | 1494 | 1494 | 18.5 | 6.3 | 1523 | 1523 | 12.4 | 4.5 |
| 10–25 cm | 1276 | 1276 | 8.4 | 8.6 | 1429 | 1429 | 7.2 | 5.9 |
| 25–50 cm | 2032 | 2032 | 4.5 | 5.0 | 2032 | 2032 | 6.3 | 4.8 |
| 50–70 cm | 516 | 516 | 1.4 | – | 516 | 516 | 4.4 | – |
| *Subtotal* | | | *81.7* | *65.4* | | | *58.4* | *36.6* |
| **Leaching** | | | | | | | | |
| NH$_4$-N | 2.9 | 3.3 | 1.1 | 1.2 | 2.1 | 3.2 | 1.1 | 1.5 |
| NO$_3$-N | 20.2 | 34.9 | 5.2 | 8.9 | 34.0 | 56.2 | 10.4 | 15.8 |
| *Subtotal* | 23.1 | 38.2 | *6.3* | *10.1* | 36.1 | 59.4 | *11.5* | *17.3* |
| **Total recovery** | | | **91.4** | **85.9** | | | **75.4** | **70.6** |
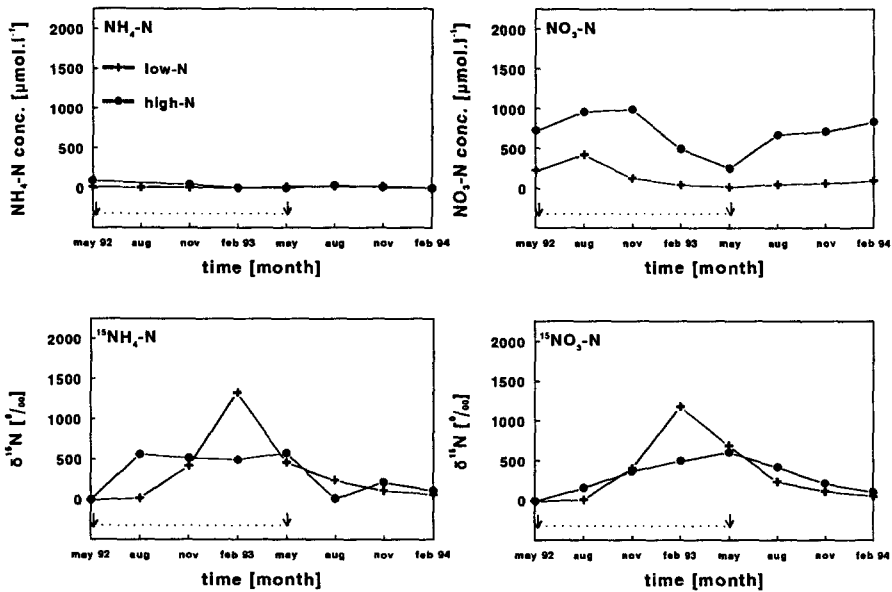
*Figure 4.* Total NH₄-N and NO₃-N concentration and corresponding $\delta^{15}$NH₄ and $\delta^{15}$NO₃ values in the soil water at 90 cm depth at the Speuld site. Symbols represent means of eight volume-weighed replicates, sampled fortnightly during three-months periods. The dashed line indicates the period of $^{15}$N application.

## Discussion

### $^{15}$N recovery

The fate of nitrogen in forest ecosystems has been studied using the tracer $^{15}$N in studies on forest decline (Buchman et al. 1995; Eilers & Brumme 1993) and several forest fertilization studies (Nambiar & Bowen 1986; Hulm & Killham 1990; Mugasha & Pluth 1994a,b; Preston & Mead 1994). However, in all these studies the nitrogen was applied to the forest sites in a single dose (Buchman et al. 1995) or split applications (Nason 1989; Thomas & Mead 1992a,b). Most studies concern small plots (Stams et al. 1991), single trees (Eilers & Brumme 1993) and frequently young trees (Buchman et al. 1995; Heilman 1982a,b; Thomas & Mead 1992a,b). A comparable study was presented by Nadelhoffer et al. (1995). In their study the tracer $^{15}$N was applied as NO₃-N during four growing seasons to study the changes in the nitrogen cycle within the ecosystem. In contrast to Nadelhoffer et al. (1995) we applied $^{15}$N as NH₄-N to the throughfall for one full year, because NH₄ is the most important form of nitrogen deposited in the Netherlands. Nadelhoffer
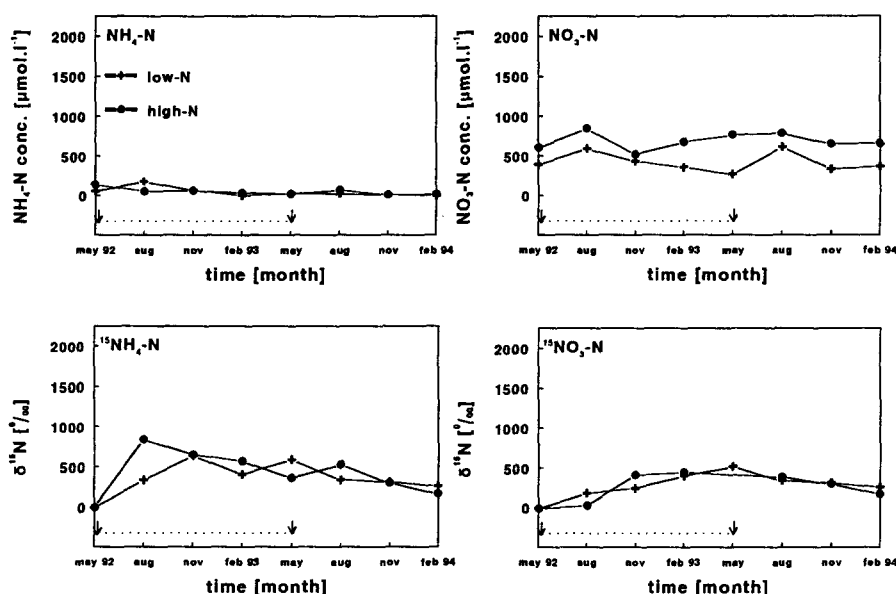
*Figure 5.* Total $NH_4$-N and $NO_3$-N concentration and corresponding $\delta^{15}NH_4$ and $\delta^{15}NO_3$ values in the soil water at 90 cm depth at the Ysselsteyn site. Symbols represent means of eight volume-weighed replicates sampled fortnightly during three-months periods. The dashed line indicates the period of $^{15}N$ application.

et al. (1995) studied nitrogen cycling in a non-nitrogen saturated ecosystem, whereas our ecosystems may be characterized as nitrogen saturated.

This study did not account for $^{15}N$ in all ecosystem compartments. However, recoveries were comparable to findings of other $^{15}N$ tracer studies in forests (Mugasha & Pluth 1991b; Preston & Mead 1994; Buchman et al. 1995). The undergrowth of ferns (*Dryopteris dilata*) was not accounted for in the $^{15}N$ budget for Ysselsteyn due to a neglectible aboveground biomass at the time of sampling in February. Investigation of the enrichment of ferns in August 1993 revealed that the aboveground part accounted for 1 to 2% of the applied $^{15}N$. At the time of sampling most of this $^{15}N$ had reached the soil as litter. Roots were removed from our soil samples. Estimates of $^{15}N$ retention by the roots range between <1% to 11% (Mugasha & Pluth 1994; Buchman et al. 1995). Gaseous nitrogen losses were expected of minor importance in these well-aerated sandy forest soils. A part of the $^{15}N$ not accounted for in this study might be leached out of the soil as dissolved organic N (DON). Unpublished DON measurements indicate DON leaching losses of up to 14% of the total inorganic nitrogen leaching losses at the Ysselsteyn plots and about 1% at the Speuld site.

*Uptake of the $^{15}N$ by the vegetation*

A significant part of the $^{15}N$ deposited was already taken up by the vegetation during the first growing season of 1992 (Tables 5 and 6). A large part of that $^{15}N$ was allocated to current foliage. This finding agreed with most studies where tissues with high metabolic activity, responded quickly (Buchman et al. 1995; Eilers & Brumme 1993; Mugasha & Pluth 1994; Nason 1989). The enrichment of the 1-year old needles during the first year of $^{15}N$ application indicated a distribution of the $^{15}N$ into these older needles either directly with the synthesis of a new batch of photosynthetic enzymes or as a result of translocation of N from other plant parts.

$^{15}N$ taken up by the Douglas fir trees at the Speuld site accounted for up to 33% while not more than 17% was found in the Scots pine trees at the Ysselsteyn site (high-deposition plot). Besides the lower biomass of the Scots pine trees, species differences in uptake, tree health and the stage of nitrogen saturation of the ecosystems may have resulted in lower N recoveries in the Scots pine trees. Signs of poorer tree health of the Scots pine trees are supported by only 2 needle age classes in the Scots pine trees at Ysselsteyn. A significant response in the nitrogen concentration of the needles due to a decrease in nitrogen deposition was observed in the Scots pine stand. This response was even more pronounced in the arginine level of the needles, a sink for an excess of nitrogen (Boxman et al. 1995). Foliar N concentrations were much lower in the Douglas fir and did not change much with N exclusion. In addition the rates of N leaching declined much more in Speuld than in Ysselsteyn. This suggests that when N availability reaches such high levels that plants are storing free amino acids in foliage, that this pool will have to be reduced before further reductions in N leaching will occur. This is supported by the lower recovery on $^{15}N$ in vegetation at the Ysselsteyn site and the differences in recovery between the low-N and high-N plots at both sites. Whereas in Ysselsteyn lower N additions result in lower $^{15}N$ recovery in the vegetation (N in free amino acids reduced), in Speuld lower N additions increase $^{15}N$ recovery in the vegetation due to the strong dependency on throughfall-N of the vegetation at this site.

*$^{15}N$ retention of the soil*

A significant part of the deposited $^{15}N$ was found in the soil, especially in the organic layer (Tables 5 and 6). At Speuld, soil and plant compartments were approximately of equal importance in retaining the $^{15}NH_4$-N additions in the long term (21 months), whereas at Ysselsteyn the soil was more important than the vegetation. Nadelhoffer et al. (1995) reported that plant and soil retention were approximately of equal importance in retaining the $^{15}N$-nitrate. They

observed that the absolute nitrate retention in the organic layer at high (56 kg $NO_3$-N $ha^{-1}$ $yr^{-1}$) and low (28 kg $NO_3$-N $ha^{-1}$ $yr^{-1}$) deposition rates was comparable whereas retention in the mineral soil increased slightly with increasing nitrogen application.

The retention of $^{15}N$ in the soil showed very fast dynamics in the organic layers compared to the mineral soil. The $^{15}N$ was quickly retained in the L-horizon in 1993 but after the $^{15}N$ additions stopped released again. Lower values in 1994 might also result from litterfall and a transfer of L material to the F horizon. In the mineral soil, the $^{15}N$ concentration was more stable and changes between years difficult to detect. Lower $\delta^{15}N$ values observed in 1994 in comparison to 1993 might result from a translocation of $^{15}N$ from the soil to roots and aboveground tissues. Perhaps it represents also transfers of $^{15}N$ to deeper soil horizons.

Our results show that the retention of throughfall-N is relatively more important at low-N deposition levels than at high-N deposition. In absolute terms (kg N $ha^{-1}$ $yr^{-1}$) however, less of the throughfall N is retained in the soil of the low-N deposition plots. In the organic layer of Speuld, about 5 kg N $ha^{-1}$ $yr^{-1}$ was retained in the high-N deposition plot in 1994, whereas less than 1 kg N $ha^{-1}$ $yr^{-1}$ was found to be retained in the low-N deposition plot. The same pattern was found in the mineral soil at reduced N deposition levels. In Ysselsteyn, N retention in the organic layer reduced from about 9 kg N $ha^{-1}$ $yr^{-1}$ in the high deposition plot to 1.7 kg N $ha^{-1}$ $yr^{-1}$ in the low-N plot.

Coûteaux & Sallih (1994) found in their laboratory study on the $^{15}N$ immobilization and the movement of exogenous N through various soil cores from all over Europe that the level of acidification, as indicated by low base saturation, correlated with lower net immobilization of the added $^{15}N$. For a similar $^{15}NH_4$-N input, sandy soils with low organic C content or a high level of acidification showed low N storage capacities, so that the excess N was lost with leaching water.

Our results indicated a large proportion of the N deposited was retained in the soil, either immobilized or at the adsorption complex. Preliminary results on soil extracted before and after chloroform-fumigation indicate that about 10% of the $^{15}N$ can be accounted for by the soil microbial biomass in both plots of Speuld in 1993. Zak et al. (1990) and Emmett & Quarmby (1991) showed microbial biomass can indeed be an important sink for added $^{15}N$ with a high immobilization capacity and a quick turnover. The lower N recovery, found in the soil at both sites at high-N deposition levels suggest saturation of N retention mechanisms in these N saturated ecosystems. In Ysselsteyn the low N retention in the upper mineral soil might be associated with the excess uptake of N and free amino acid content of the foliage.

In addition, the question arises whether less throughfall-N availability in the low-N plots is compensated by an increased net mineralization. Results from long-term field incubation studies showed nitrogen transformations (net nitrification and net mineralization) in the organic layer and upper part of the mineral soil decreased with the reduced N input level at the Ysselsteyn site (Koopmans et al. 1995). In the low-N plot at Ysselsteyn therefore, a low absolute retention of throughfall-N was not compensated by increased N mineralization. Less N availability for trees in the low-N deposition plot was confirmed by lower N concentrations in the needles of the Scots pine.

At Speuld no effect of the nitrogen deposition on net nitrogen transformations was found (Koopmans et al. 1995). The differences in N retention between the two manipulated plots were small compared to the total N uptake of the vegetation (approx. $>30$ kg N ha$^{-1}$). This $^{15}$N experiment showed that after 5 years of N manipulation, N retention from throughfall in the soil and N uptake patterns seemed to be changed due to the level of N deposition at Speuld. However, due to the stage of nitrogen saturation at this site this did not result in significant differences in uptake and N concentrations in the needles.

*Leaching losses of the $^{15}$N*

In these nitrogen saturated forest stands the total N deposition exceeded the nitrogen retention capacity of the vegetation and soil. A substantial part of the ammonium inputs leached out of the soil as nitrate within a short time period (1 year), especially in the highly nitrogen saturated plots. Under lower N deposition, a larger percentage of the $^{15}$N deposited was retained, and nitrogen leaching was drastically reduced.

The response of the soil water was much quicker than the response observed by Eilers & Brumme (1993), who observed the $^{15}$N at a depth of 1 m, after 9 months. This may be due to soil type differences and the stage of nitrogen saturation of this ecosystem.

Durka et al. (1994) studied the effects of forest decline on uptake and leaching of deposited $NO_3$-N from $^{15}$N and $^{18}$O measurements at the natural abundance level. The $NO_3$ leaching ranged between 16% and 114% of the $NO_3$-N input. Although some evidence was presented indicating that this amount originated from the atmospheric $NO_3$-N deposition their study did not give any insight into the residence time of the $NO_3$-N in the soil. At our sites, $NH_4$ was the most important nitrogen form deposited. Our $^{15}$N study provides some insight into the residence time of $NH_4$-N inputs to the ecosystem (Tables 5 and 6). A significant amount of the $^{15}NH_4$ (25–35%) leached from the system either during the year of application or in the subsequent 9 months. This N leached predominantly in the form of $NO_3$-

N although some leaching of $^{15}NH_4$ was observed, probably due to direct hydrological flow and vertically installed lysimeters. The contribution of N deposition to leaching losses may be quickly reduced by a decrease in the $NH_4$-N deposition. This implies important implications for policy with regard to air pollution standards.

The enrichment of the leached $NO_3$-N is about the same for low and high-N plots, whereas the concentrations of nitrate are different. The correspondence of $\delta^{15}N$ values of $NH_4$ and $NO_3$ in the soil solution leaving the ecosystem at 90 cm soil depth pointed to autotrophic nitrification. Heterotrophic nitrification would have resulted in lower $\delta^{15}N$ values of $NO_3$-N compared to the $NH_4$-N. This confirmed findings of Stams et al. (1991) and De Boer et al. (1992) who suggested that the nitrification in these acid forest soils is largely autotrophic.

## Conclusions

Although, not all of the $^{15}N$ could be accounted for, the $^{15}N$ tracer methodology has provided further insights as to the fate of the one-year round N deposition.

The large amounts of $^{15}N$ retained in the soil stress the importance of the soil processes and the microbial population in retaining the nitrogen deposited. The retention of the soil and nitrogen leaching losses seem to be rather complementary. At high deposition levels, leaching of the $^{15}N$ as $NO_3$-N is high, whereas the retention by the soil is relatively low. At lowered deposition levels most of the nitrogen is retained by the soil, leaving only small amounts of nitrogen to be leached. These results might indicate that the lowered N deposition has indeed influenced the biota and the soil chemical status, leading to increased soil retention capacity. The higher retention of the N deposited, indicates a change towards a more conservative strategy of the biota concerning deposited N in the low deposition plots and thus a shift along the continuum of N saturation to N limitation. The results for the Ysselsteyn site suggest that when N availability reaches such high levels that plants are storing free amino acids in foliage, that this pool will have to be reduced before further reductions in N leaching occur.

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