Nitrogen biogeochemistry of a mature Scots pine forest subjected to high nitrogen loads

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Abstract Nitrogen (N) biogeochemistry of a mature Scots pine (*Pinus sylvestris* L.) stand subjected to an average total atmospheric N deposition of 48 kg ha^{-1} year^{-1} was studied during the period 1992–2007. The annual amount of dissolved inorganic nitrogen (DIN) in throughfall (TF) averaged 34 kg ha^{-1} year^{-1} over the 16-year monitoring period. The throughfall fluxes contained also considerable amounts of dissolved organic nitrogen (DON) (5–8.5 kg N ha^{-1} year^{-1}), which should be incorporated in the estimate of N flux using throughfall collectors. Throughfall DIN fluxes declined at a rate of −0.9 kg N ha^{-1} year^{-1}, mainly due to the decreasing TF fluxes of ammonium (NH$_4$), which accounted for 70% to TF DIN. The decrease in TF DIN was accompanied by a decrease in DIN leaching in the seepage water (−1.6 kg N ha^{-1} year^{-1}), which occurred exclusively as nitrate (NO$_3^-$). Nitrate losses in the leachate of the forest floor (LFH) equalled the TF NO$_3^-$ delivered to the LFH-layer. On the contrary, about half of the TF NH$_4^+$ was retained within the LFH-layer. Approximately 60% of the TF DIN fluxes were leached

indicating that N inputs were far in excess of the N requirements of the forest. For DON, losses were only substantial from the LFH-layer, but no DON was leached in the seepage water. Despite the high N losses through nitrate leaching and NO$_x$ emission, the forest was still accumulating N, especially in the aggrading LFH-layer. The forest stand, on the contrary, was found to be a poor N sink.

Keywords Ammonium · Dry deposition · Dissolved organic nitrogen · Forest floor · Nitrogen retention · Nitrogen cycling · Nitrate leaching · Throughfall

Introduction

Elevated nitrogen (N) inputs into terrestrial ecosystems are causing major changes in the composition and functioning of ecosystems. The chronic input of atmospheric N has led to an increased availability of N in forests and a disruption of the closed N cycle in temperate forests (Gundersen et al. 2006). Nitrogen saturation of forest ecosystems is characterised by increased soil acidification and aluminium mobility, elevated nitrate and cation loss, high N/nutrient ratios in foliage, high N pools and gaseous N losses (Aber et al. 1989).

High emission of ammonia due to agricultural activities combined with high NO$_x$ emission from

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fossil fuel combustion has lead to high N loads in northern Belgium. The coexistence of intensive animal husbandry close to efficient air scavenging coniferous forests on coarse sandy soils has caused substantial exceedances of critical loads regarding eutrophication and acidification (Neirynck et al. 2004). Despite decreases in N loads in recent years, inorganic N deposition is generally still larger than 25 kg N ha^{-1} year^{-1}. Beyond this deposition level the decoupling of ecosystem demand and atmospheric N deposition leads to nitrate leaching (Dise and Wright 1995).

Implementation of the Gothenburg protocol (http://www.unece.org/env/lrtap/multi_h1.htm), the National Emission Ceilings directive, their recent revisions and the broader thematic CAFE strategy towards air pollution abatement, must lead to further emission reductions and increased ecosystem protection against adverse effects of air pollution. Long-term monitoring of N fluxes and their effects on total N cycling is imperative to assess ecosystem control on N losses and allow early detection of ecosystem recovery from historical long-term N deposition. Decreased nitrate losses, resulting from declining excessive nitrogen deposition are also likely to be accompanied by diminished cation losses and a decreased rate in the ongoing soil acidification. Temporal analysis of N cycling at long-term monitoring plots also increases the understanding of the fate of N in terrestrial ecosystems and is helpful in adjusting emission targets (Dise et al. 1998). In addition, it will enable researchers to further examine how resilient ecosystems are to decreases in N input in a long-term perspective, which could lead to improved critical load calculations for nitrogen rich areas.

The magnitude of N storage and its temporal change in forest ecosystems are also important when analysing global change. Cycles of carbon (C) and N are closely linked and retention of N affects the global C sink (Norby 1998). Alterations in the N cycle have therefore also implications for soil C response and ecosystem C dynamics (Magnani et al. 2007).

In view of the importance of studying long-term dynamics of N biogeochemistry, we studied N cycling in a N-impacted, mature coniferous forest. Therefore, fluxes and pools of N within the stand were quantified. We also tried to determine whether atmospheric ammonium and nitrate behaved differently in this forest ecosystem. Another objective was to examine the response of the forest to decreased N loads over a 16-year monitoring period.

Material and methods

Site description

The site under consideration is a 2-ha first generation plantation of Scots pine (*Pinus sylvestris* L.) stand on former heathland, planted in 1929, which belongs to a mixed coniferous/deciduous forest located at Brasschaat, near Antwerpen (51°18′N, 4°31′E; Neirynck et al. 2002). In 2001, the mean height of the stand was 21 m, with a mean DBH of 0.29 m and a mean canopy depth of 3.7 m (Xiao et al. 2003). The stem number in 1980 was very high and amounted to 1,390 trees ha^{-1}. Frequent thinnings reduced the stem number to 899 ha^{-1} in 1987 and 560 ha^{-1} in 1997. In November 1999, the last thinning was performed reducing the stocking density to 377 trees ha^{-1} leading to a more open overstorey canopy (Xiao et al. 2003). The standing volume, measured on a 2,500 m^2 large UN/ECE observation plot, amounted to 252 m^3 ha^{-1} in 2004. Between 1987 and 2004, 120 m^3 stem boles per ha were removed through thinning (1993, 1997, 1999). Volume increment between 1987 and 1995 amounted to 8.1 m^3 ha^{-1} year^{-1}. Between 1995 and 2004, volume increment dropped to 4.3 m^3 ha^{-1} year^{-1}.

The understorey was initially occupied by *Prunus serotina* Ehrh. that was combated in 1993. Afterwards, a scant but steady recolonisation of the shrub layer by *Betula* species and *Sorbus aucuparia* L. took place. Also a dense herbaceous vegetation mainly consisting of *Molinea caerulea* L. Moench. emerged along with *Rubus* species and *Dryopteris* ferns. Extensive moss cover on the ground characterises some patches.

The soil is covered with a modern organic surface layer of 7.5 cm depth. A deep (1.20–2.25 m) Aeolian cover sand layer (Dryas III) rests on a substratum of Clay of the Campine (>40% of clay) (Tiglian) at variable depth, between 1.2 and 2.5 m and more. The soil is moist, but rarely saturated, because of rapid hydraulic conductivity in the upper horizons. A perched water table is present at variable depth ranging between 0.8 and 2.5 m. According to the World Reference Base for Soil Resources version 2006 (WRB 2006), the soil is classified as an Albic

Hypoluvic Arenosol. pH (CaCl$_2$) values vary from 2.7 in the forest floor, 3.1 in the top soil to 3.6 deeper in the soil profile (Table 1). Base saturation is lower than 10% throughout the soil column. The C:N ratio of the forest floor was 31 whereas values in the mineral soil fell below 20.

Nitrogen fluxes and pools

Air

Measurements of gaseous nitrogen oxides (NO$_x$) (chemiluminescence, Ecophysics CLD 700 AL, Switzerland, detection limit 1 ppb) and ammonia (NH$_3$) (Conductivity, AMANDA, ECN, the Netherlands, detection limit 0.1 µg m^{-3}) were conducted at two levels above the canopy every half hour. The AMANDA analyser was operated during the period June 1999 till November 2001. Gases of nitrous acid (HONO), nitric acid (HNO$_3$) and fine particles of ammonium ($_p$NH$_4$) and nitrate ($_p$NO$_3$) were measured at one height on a daily basis, in three separate sampling campaigns, during the time when the AMANDA analyser was operational (Neirynck et al. 2007). A combination of the gradient method (NH$_3$, NO$_x$) and resistance-modelling techniques (NO$_2$, HNO$_3$, HONO, $_p$NH$_4$, $_p$NO$_3$) were used to calculate dry deposition (DD) of measured N species. Gradients of NH$_3$ and NO$_x$ at 24 and 40 m were used to calculate net fluxes using the gradient method (Dyer and Hicks 1970):

$$F = -K \frac{\partial [\mathrm{NH_3}]}{\partial z} \tag{1}$$

where F = flux (negative sign implies deposition, in ng m^{-2} s^{-1}), K = turbulent diffusivity (m^2 s^{-1}).

Dry deposition fluxes of other gaseous species HONO and HNO$_3$ and particles, sampled on daily basis, were calculated using a modelled deposition velocity (fore more detailed information see Neirynck et al. 2007).

Water

Throughfall (TF) has been measured from 1992 onwards and has been sampled with ten systematically distributed bulk collectors in a 0.25 ha large plot. They consisted of a polyethylene funnel (14 cm ∅) placed at a standard height of 1 m, which was connected to a subterranean 2-l polyethylene bottle. A nylon mesh was placed in the funnel to avoid contamination by large particles and debris. Throughfall was sampled monthly in 1992 and fortnightly from 1993 onwards. At every sampling, TF volumes from the collectors were recorded in the field and a pooled sample was taken as a weighted average from all collectors. Bulk precipitation (BP) was collected with four bulk collectors on an adjacent pasture from 1993 onwards. Funnels and bottles were replaced at every sampling event. Water samples were kept cool during transport and stored in darkness at 4°C after filtration (0.45 µm). Fractions of ammonium (NH$_4^+$) and nitrate (NO$_3^-$) were analysed using ion chromatography (Dionex DX-100). Nitrite (NO$_2^-$) was mostly below the detection limits. When measurable, its concentration was less than 5% of the sum of NH$_4^+$ and NO$_3^-$. Dissolved organic nitrogen (DON) was determined as the difference between total dissolved nitrogen (TDN) and dissolved inorganic nitrogen (DIN = NH$_4^+$ + NO$_3^-$). During the period 2005–2007 TDN was measured using Flow Injection Analysis (Lachat 8500 Quick Chem Autoanalyzer, module Total nitrogen, Lachat Instruments, Milwaukee, WI). Earlier measurements of TDN using Forster Kjeldahl analysis were available for the period 1998–2000.

Table 1 Soil characteristics of the LFH-layer and soil column

Layer	C (%)	N (%)	C/N	pH-CaCl$_2$	BC (cmol$_c$ kg^{-1})	CEC (cmol$_c$ kg^{-1})	Bs (%)
LFH	47.6	1.52	31	2.7	–	–	–
0–5 cm	1.89	0.11	17	3.1	0.25	1.76	14
5–10 cm	1.08	0.06	18	3.2	0.10	1.40	7
10–20 cm	0.85	0.06	15	3.3	0.11	1.22	9
20–40 cm	0.71	0.05	15	3.4	0.08	1.24	6
40–80 cm	0.32	0.03	13	3.6	0.07	1.06	7

BC base cation concentration, *CEC* cation exchange capacity, *Bs* base saturation

Soil water was sampled using lysimeter candles equipped with ceramic cups installed in the Ap horizon (± 10 cm), A/E/Cg (± 30–40 cm) and Cg horizon (± 70–80 cm). The lysimeters were operated at a transient vacuum during 2 days before sampling, using an initial tension of about -60 cbar. Afterwards, samples were collected and pooled into one composite sample per layer for analysis. Sampling started in 1992 with one set of lysimeters at two locations, later being extended to 3 locations in 1996, which were eventually duplicated in 2000. Soil water was sampled on a monthly basis in 1992, but from 1993 onwards sampling was carried out fortnightly. Forest floor solution was collected fortnightly with 4 zero tension collectors since mid-1993.

Soil

The most recent sampling of the soil compartment dated from August 2004. The forest floor (LFH) consisting of a litter (L), a fragmented litter (F) and a humus (H) layer was subdivided into L- and FH-layer and weighed separately over a frame of 0.25×0.25 m. Samples were oven-dried and pulverized in a grinder (Pulverisette 14, Fritsch, Idar-Oberstein, Germany). The mineral soil was sampled at fixed depths (0–5, 5–10, 10–20, 20–40 and 40–80 cm). Three composite samples were taken from the forest floor and the mineral soil each consisting of eight systematically chosen subsamples. Soil samples were passed through a 2 mm sieve and thoroughly mixed. Living macroscopic roots and all particles, mineral and organic, with a diameter larger than 2 mm, were removed from the samples by dry sieving as a preparation for analysis. Intact soil samples were taken from the same depths using a Kopecky core (volume $= 100$ cm^3) for determination of bulk density. Total N was determined according to the modified Kjeldahl method.

Soil emission of NO, typically occurring in N-impacted coniferous stands (Pilegaard et al. 2006), was estimated by measuring the above-canopy emission fluxes of NO_x (NO + NO_2) using the gradient method (see Sect. "Air"). Although this micrometeorological method cannot be used to measure denitrification directly, it is an appealing method to measure NO fluxes over relatively large areas (Kroeze et al. 2003). It must be considered as an underestimate since it also takes into account the

ingoing flux of ambient NO_2 and does not include the whole amount of soil emitted NO. The soil emitted NO enters the trunk and crown space where it is turned into NO_2 formed through reaction with ozone. This newly formed NO_2 can be emitted above the canopy but it can also be recovered partly by the canopy (Duyzer et al. 2004).

Plant biomass

Four sample trees were selected based on the basal area distribution of the stand. Two sample trees were representative of the stand average diameter; the two remaining trees represented the lower and the upper quartile of the diameter distribution. After felling in 1999, all branches from the tree crown were weighed immediately after harvest. In addition, branch diameter (at 3 cm from the branch base) along with branch length, determined by the longest axes, were measured. One randomly selected branch from each whorl was sampled completely: the needles were stripped from the branches and separated according to their age. The branches were chopped into four size classes (<1, 1–2, 2–5 and >5 cm) and freshly weighed. Subsamples were taken from each fraction for dry weight determination and chemical analysis. The boles of the sample trees were sawn into 1 m logs, which were measured and weighed. Every 3 m a disc of about 3 cm thickness was sawn from the log and was taken to the laboratory for chemical analysis. All samples were dried at 80°C to constant weight, ground and analysed on N using the modified Kjeldahl method. Allometric relationships between dry weight from the selected branch/needle fractions and measured branch length and/or diameter were established to estimate the total branch or needle dry weight per tree. Multiplying the dry masses from branches, needles and logs with the N concentrations from the corresponding whorl or bole section yielded total N content of the branches and needles. Data on N content of branches, needles and logs were combined to yield the total N content per sample tree. Scaling up to stand mass was done by multiplying the average sample tree N content by the ratio of the standing volume to the average sample tree volume. Repeated forest surveys conducted in 1995 and 2004 provided the volume class distribution of the 2,500 m^2 large observation plot.

Fine root biomass was determined by core sampling in 1997, 2001 and 2003 (resp. Janssens et al. 2002; Xiao et al. 2003; Curiel Yuste et al. 2005). Fine roots were subdivided and partitioned into three size classes (<1, 1–2 and 2–5 mm).

Litterfall has been collected continuously since 1998 using ten randomly distributed litter traps in the forest. Litter was collected monthly but during autumn a fortnightly collection was made. Each trap had an opening size of 0.28 m^2 (60 cm Ø). The litter from the traps were individually dried and weighed, subdivided into needles, twigs and branches. Biyearly sampling of current year and older needles from the upper crown were made according to the guidelines of the ECE/UN manual for analysis and sampling of needles and leaves (http://www.icp-forests.org/Manual.htm). From 1995 onwards, needles obtained from five sample trees were dried and ground to pass a 0.5 mm sieve. Earlier measurements comprising a higher number of sample trees were provided by Van Den Berge et al. (1992).

Calculations and statistical analysis

Annual or monthly fluxes in BP or TF were obtained by multiplying the precipitation volumes from every sampling event with the accompanying concentrations and subsequent summing of fortnightly fluxes to a monthly or yearly value. Total deposition (TD) was calculated as the sum of dry deposition (DD) and wet deposition (WD). The latter was derived from BP measurements (Neirynck et al. 2007). Annual or monthly fluxes of NO_3^- and NH_4^+ leaving the forest floor (LFH leachate) and the soil compartment (seepage) were determined by multiplying the ratio of yearly or resp. monthly chloride (Cl^-) deposition to yearly or monthly averaged Cl^- concentration with the yearly or monthly average of NH_4^+ and NO_3^- from the LFH collector and deepest lysimeter, respectively. We assumed that there was only negligible immobilization and sorption of Cl^- onto the soil matrix and that the collected water at 70 cm of depth reflected the leaching below the main part of the root profile.

The amount of N returned to the soil in aboveground litter (needles, branches, twigs) was calculated using the measured litterfall multiplied by the N concentrations of each fraction. Nitrogen returning via fine root turnover was estimated as the average N concentration of fine roots multiplied by the fine root turnover. The latter was estimated by multiplying the fine root biomass by the estimated turnover rates of fine roots. Turnover rates, determined at the site, were 0.8 year^{-1} for roots <1 mm and 0.3 year^{-1} for roots 1–2 mm (Janssens et al. 2002). For the 2–5 mm size class, a turnover rate of 0.25 year^{-1} was applied (Xiao et al. 2003). The mortality of larger roots was assumed to be negligible.

The amount of N required for the formation of new branches, wood increment, new foliage and newly formed rootlets was estimated by multiplying the net primary productivity (NPP) from each compartment with the weighted mean N concentration of stems, twigs, branches, needles and rootlets. Net change in N storage was calculated for each ecosystem component by taking the difference between the values of a recent and an older survey.

Trends of monthly DIN concentrations in the soil water were studied using a generalised least squares method, which allowed for correcting the variance (homogeneity) and correlation structure (autocorrelation) in the time series (Zuur et al. 2007). An exponential variance and correlation structure was added to the linear regression in order to correct for heterogeneity and independence, respectively. Variables tested in the full model comprised besides the time step also monthly air temperature, water fluxes, number of lysimeters contributing to the sample, mean water volume collected per lysimeter and their first order interactions. When the residual variance structure was optimised, a model with significant explanatory variables was fitted in order to examine whether the time trend was still significant after factoring in possible dilution or concentrating effects.

Comparison of two samples (e.g. seasonal effect, retention percentage) was conducted using a Wilcoxon rank sum test. Summer half-year was defined as the period April–September.

Results

Atmospheric nitrogen deposition: total deposition versus throughfall

The forest was subject to various mechanisms of N deposition (Neirynck et al. 2007). In terms of concentration, nitrogen oxides (NO_x) were the dominant N compounds, which reached the highest levels

during winter (Fig. 1a). Ammonia levels were the highest during the summer period. Other N species were smaller in magnitude, but still substantial (Fig. 1a). Despite the ubiquitous presence and high concentrations of oxidised N, reduced N (NH_3 + $_pNH_4$) fluxes dominated the dry deposition (DD) of N at the site (Fig. 1b). Measured net-NO_x fluxes, on the contrary, were upward, indicating that the outgoing (likely soil-derived) flux from the canopy exceeded the ingoing NO_x flow. Flux calculations using inferential models resulted in a downward flux for ambient NO_2. Other oxidised N compounds as $_pNO_3$, HNO_3 and HONO were contributors to the downward flux of NO_y. Reduced N comprised 79% of the total deposition (TD) measured during the 1999–2001

measuring campaign (48 kg N ha^{-1} $year^{-1}$; Table 2). Total deposition of NH_x amounted to 38 kg N ha^{-1} $year^{-1}$, which was mainly delivered through DD. Throughfall (TF) NH_4^+ deposition amounted to 23 kg N ha^{-1} $year^{-1}$ implying a canopy uptake of 15 kg N ha^{-1} $year^{-1}$ over that period. With regard to NO_y, no substantial differences between TD and TF existed.

The discrepancy between N fluxes in TF and TD could partly be explained by the fact that TF DON fluxes were not added to the TF DIN fluxes. During the period 1999–2000, TF DON fluxes amounted to 8.1 kg N ha^{-1} $year^{-1}$, which approached the amounts measured in 2005–2007 (Fig. 2). Dissolved organic nitrogen accounted for 13–23% (5–8.5 kg DON ha^{-1})

Fig. 1 Concentrations (a) and dry deposition fluxes (b) of oxidised and reduced nitrogen species measured above the forest during 1999–2001 monitoring campaigns (means ± SE) subdivided into summer and winter half-year (positive fluxes denote deposition)
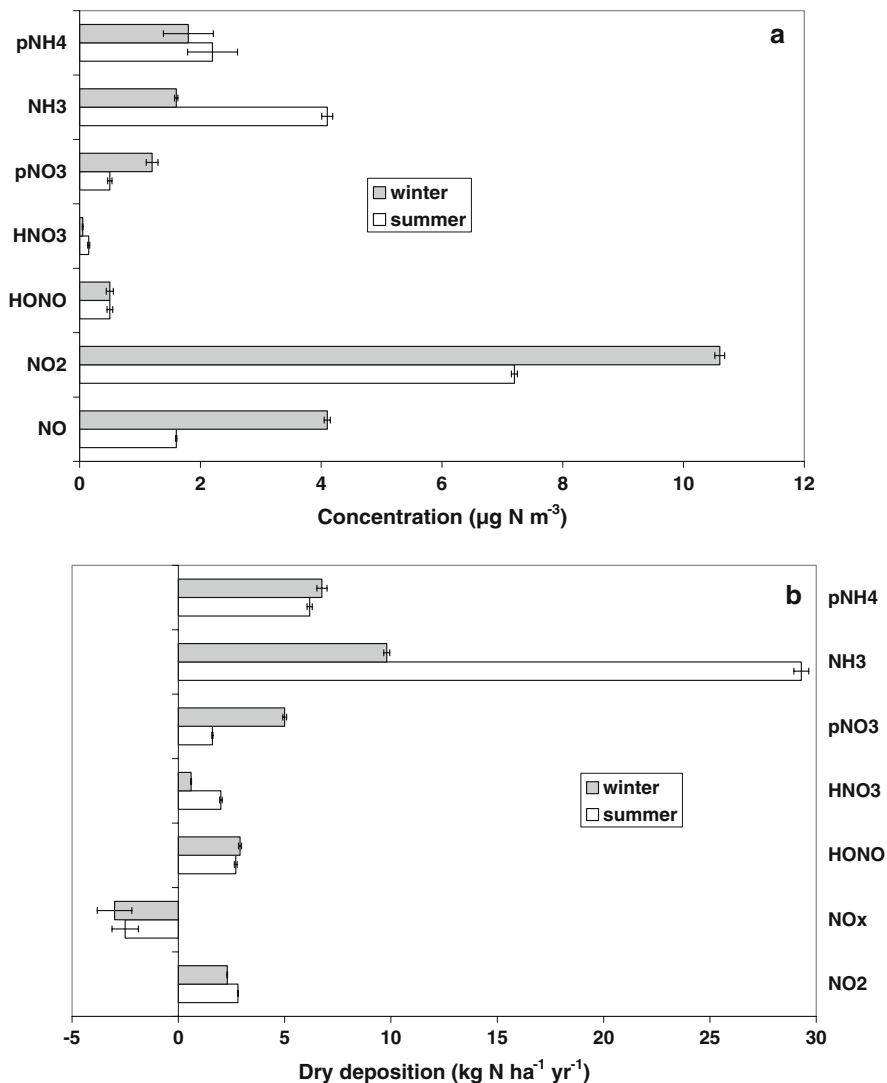
Table 2 Total deposition (TD) comprising wet deposition (WD) of NH_4^+ and NO_3^- and dry deposition (DD) of NH_x and NO_y versus annual throughfall (TF) of NH_4^+ and NO_3^- measured during the period 1999–2001 (in kg N ha^{-1} year^{-1})

		NH_x			NO_y			$NH_x + NO_y$	
		Flux	% TD		Flux	% TD		Flux	% TD
WD	NH_4^+	12.0	25	NO_3^-	5.5	11	$NH_4^+ + NO_3^-$	17.5	36
DD	NH_x	26.0	54	NO_y	4.7	10	$NH_x + NO_y$	30.7	64
TD (WD + DD)	NH_x	38.0	79	NO_y	10.2	21	$NH_x + NO_y$	48.2	100
TF	NH_4^+	23.0	48	NO_3^-	10.1	21	$NH_4^+ + NO_3^-$	33.1	69
Canopy uptake[a]		15.0	31		0.1	0		15.2	31

Percent contribution in total nitrogen deposition given in additional column

[a] Canopy uptake is calculated as TD − TF

Fig. 2 Amounts of DIN (dissolved inorganic nitrogen) and DON (dissolved organic nitrogen) in open field and throughfall for the years 2005, 2006 and 2007 (in kg N ha^{-1} year^{-1})
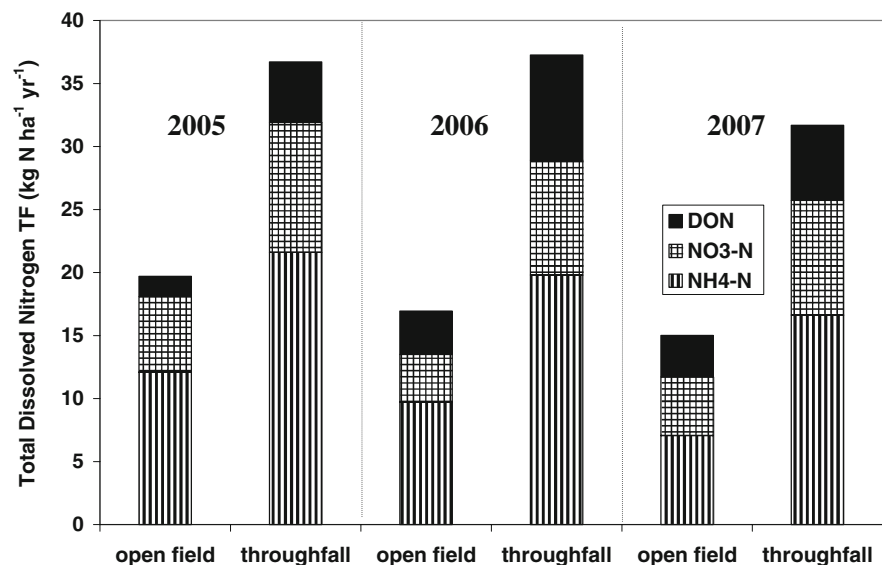
of the yearly total dissolved N amounts in TF, which varied between 32 and 37 kg N ha^{-1} year^{-1}. DON amounts collected in bulk collectors at an open place (BP) were 2–3 kg N ha^{-1} year^{-1}.

Trends of DIN in throughfall, forest floor leachate and soil seepage 1992–2007

Throughfall DIN averaged 34 kg N ha^{-1} year^{-1} over the 16-year monitoring period. Recorded TF volume averaged 785 mm year^{-1}, but varied strongly with over 20–30% lower TF volumes during the years 1996, 1997 and 2003 as compared to the average TF volume. During the period 1998–2002 up to 20% higher TF volumes were recorded. Ammonium accounted for about 70% of the TF DIN. TF DIN fluxes exceeded 40 kg ha^{-1} year^{-1} at the beginning of the

measurements (Fig. 3) but declined at a rate of -0.86 ± 0.16 kg N ha^{-1} year^{-1} ($p < 0.001$). TF depositions of NH_4^+ decreased at a rate of -0.57 ± 0.12 kg N ha^{-1} year^{-1} ($p < 0.01$) from 28 kg ha^{-1} year^{-1} to values below 20 kg ha^{-1} year^{-1}, with the main decline taking place in the second part of the measuring period. TF NO_3^- decreased by -0.29 ± 0.10 kg N ha^{-1} year^{-1} ($p < 0.05$) although this drop was mainly achieved at the beginning of the measuring period. Later on, TF NO_3^- depositions fluctuated around 10 kg N ha^{-1} year^{-1}.

Only about half of the TF NH_4^+ (± 11.5 kg N from 23.1 kg N ha^{-1}) passed through the forest floor (LFH-layer) over the period 1994–2007 (Fig. 3). In contrast to NH_4^+, NO_3^- losses from the LFH-layer seemed to be unaltered compared with the TF NO_3^- fluxes. Between 1994 and 2007, cumulated TF NO_3^-

Fig. 3 Annual course of nitrate and ammonium throughfall fluxes versus yearly ammonium and nitrate leaching in forest floor leachate and seepage (in kg N ha^{-1} year^{-1}) for the period 1992–2007. *TF* throughfall, *LFH losses* leaching losses from organic layers, *seepage* leaching losses at the depth of the deepest lysimeter
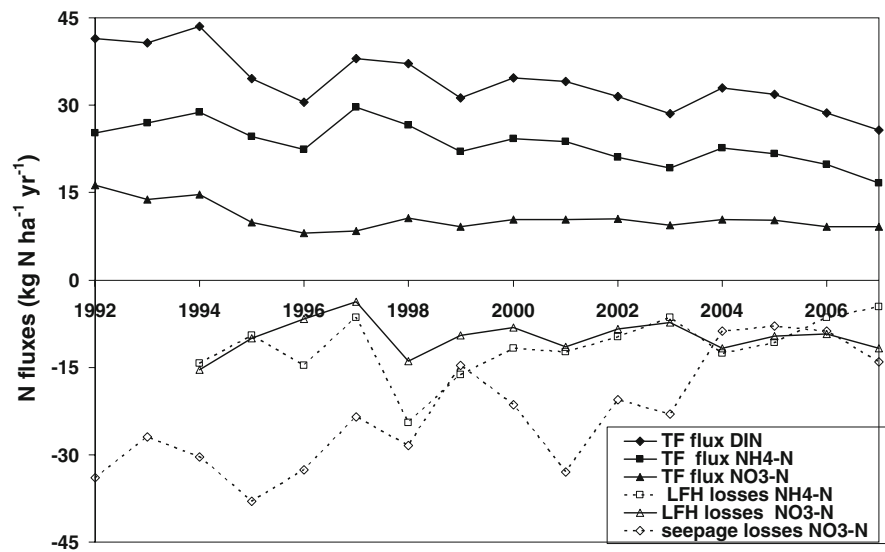
input to the LFH-layer amounted to 140 kg N ha^{-1}, whereas the NO$_3^-$ output from the LFH-layer totalled 137 kg NO$_3$-N ha^{-1}. As a whole, 21.2 ± 1.9 from the 33 kg ha^{-1} year^{-1} TF DIN passed through the LFH-layer, but because of the differing retention rates among N forms, DIN output became equally partitioned between NH$_4^+$ and NO$_3^-$.

The seepage DIN fluxes, calculated from the soil solution chemistry in the deepest horizon, did not contain any NH$_4^+$. Nitrate seepage losses averaged 22.9 ± 2.7 kg N ha^{-1} year^{-1}, which did not differ from the amounts of DIN leached from the LFH-layer, except that NH$_4^+$ no longer appeared in the deepest soil water. Over the study period, nitrate seepage fluxes followed a decreasing trend from 35 to 15 kg N ha^{-1} year^{-1} (Fig. 3), reflected in an annual decrease of −1.62 ± 0.33 kg N ha^{-1} year^{-1} from 1992 onwards ($p < 0.001$). Nitrate concentrations in the deeper horizons dropped from values above 20 to 5 mg NO$_3$-N L^{-1} although concentrations raised again above 10 mg NO$_3$-N L^{-1} in 2007 (Fig. 4a). A conspicuous drop was noticed from the autumn of 1998 onwards when large rainfall caused dilution effects in the soil solution chemistry, enhancing the declining trends in the NO$_3^-$ concentrations. Dilution effects might have systematically depressed the NO$_3^-$-concentrations over the rainy period 1998–2002 in the soil solution. The effect on nitrate leaching of this drop in concentrations was, however, largely outweighed by a simultaneous increase in the seepage water fluxes (Fig. 4c). Ammonium concentrations

were only important in the Ap and A/E/Cg-horizon (not in the Cg), and even there became hardly detectable after the autumnal rains of 1998 (Fig. 4b). A time series analysis was performed on the DIN concentrations in the soil solution in order to account for hydrological effects due to varying temperature, seepage water fluxes or lysimeter effects (variable number and collected volume among season and across years). At all depths the long-term trend in the DIN levels remained significant when keeping the latter effects constant (Table 3). High hydrological fluxes or sample volumes decreased the DIN concentrations in the soil solution (dilution) at all depths whereas high temperatures led to increased DIN concentrations (A/E/Cg). The number of lysimeters was used to correct the model in the Ap-horizon.

The drop in NO$_3^-$-concentrations in the seepage water was also accompanied by a marked raise in the pH. This resulted in a highly significant ($p < 0.001$) inverse linear relationship between pH and logarithmically transformed NO$_3^-$-concentrations (Fig. 5).

Seasonality of total dissolved N in throughfall, forest floor leachate and soil seepage

A marked seasonality was observed in the TF input and output of DIN (Fig. 6a, b). Throughfall fluxes of NH$_4^+$ and NO$_3^-$ reached their highest levels during the months May–September with fluxes being resp. 30 and 60% higher (resp. $p < 0.05$ and $p < 0.001$). The same pattern was observed for the NO$_3^-$ losses

Fig. 4 a Average monthly
nitrate concentrations in Ap,
A/E/Cg and Cg-horizon
from 1992 till 2007 (in
mg N L^{-1}). **b** Average
monthly ammonium
concentrations in Ap and
A/E/Cg horizon (in
mg N L^{-1}). **c** Monthly
throughfall precipitation
and water seepage fluxes (in
mm month^{-1})
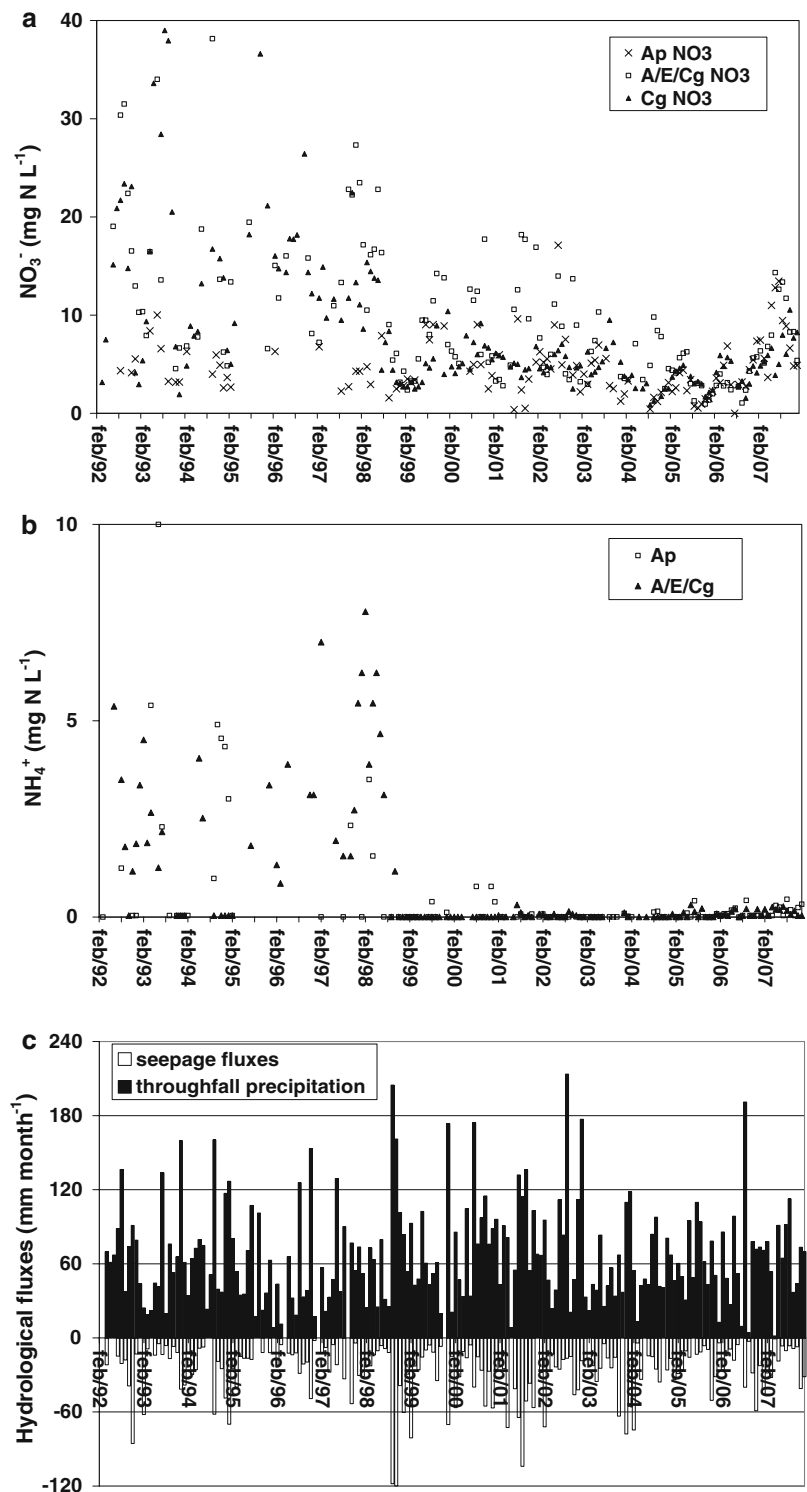
from the LFH-layer, which mirrored the TF NO$_3^-$
(Fig. 6a). On the other hand, an inverse pattern was
found for the NO$_3^-$ seepage flux and the NH$_4^+$

output from the LFH-layer (Fig. 6b). The latter was
minimal during the months May–August when
almost 75% from the TF NH$_4^+$ was retained within

Table 3 Model results of a generalized least squares method applied to log-transformed monthly DIN concentrations using time step (time), number of lysimeters (Lnumber), mean sample volume (Lvolume), monthly water fluxes (WF) and temperature (temp) as explanatory variables

Horizon	Ap			A/E/Cg			Cg		
	Value	se	t	Value	se	t	Value	se	t
Intercept	0.7087	0.0492	14.4***[a]	0.9261	0.0630	14.7***	0.9515	0.0525	18.9***
Time	−0.0292	0.0086	−3.4***	−0.0401	0.0083	−4.8***	−0.0311	0.0097	−3.2**
Lnumber	0.0225	0.0059	3.8***						
Lvolume				−0.0002	0.0001	−2.6*			
WF	−0.0021	0.0009	−2.3*				−0.0014	0.0005	−3.1**
Temp				0.0118	0.0041	2.9***			

[a] Regression coefficients are statistically significant at the * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ level

R^2 was 0.88 ($n = 106$); 0.59 ($n = 137$) and 0.58 ($n = 161$) for Ap, A/E/Cg and Cg horizon resp
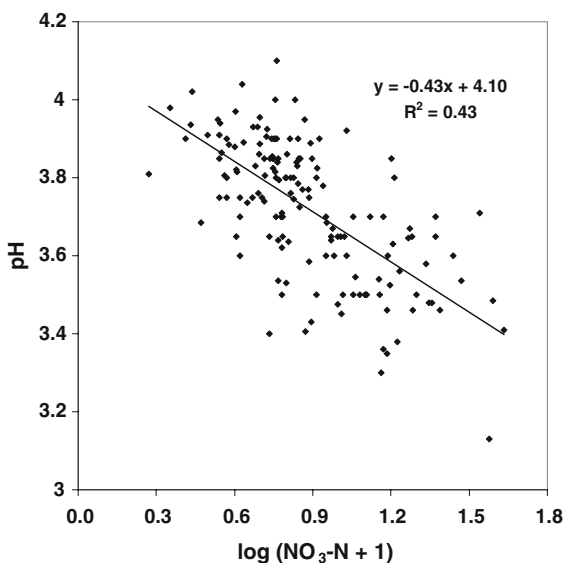

$$y = -0.43x + 4.10$$
$$R^2 = 0.43$$

Fig. 5 Relationship between pH and logarithmically transformed NO₃ concentrations (NO₃-N +1) in the seepage water

the LFH-layer. Outside this period only 35% TF NH_4^+ was retained within the LFH-layer. Similarly, NO_3^- losses in the soil seepage were the highest during the dormant period, reaching their maximum in October ($p < 0.01$). During the months May–July, NO_3^- leaching in seepage water was minimal and hardly different from the NO_3^- leached from the LFH-layer. Losses were limited to 30% from incoming TF DIN. During the winter half-year, retention capacity dropped to 20% from TF DIN and even less (Fig. 6c, $p < 0.001$). The retention capacity of TF DIN within the whole soil compartment did not differ from that of the LFH-layer alone. Differences in

retention capacity for all months were found not to be significant.

Monthly losses of DON from the LFH-layer or in the seepage, averaged for the period 2004–2007, did not exhibit any obvious seasonal fluctuation in contrast to the TF DON fluxes (Fig. 7). Losses of DON in seepage water were negligible compared to DON losses from the LFH-layer, implying that DON losses from the LFH-layer were not necessarily losses to the ecosystem.

Above-canopy losses of NO_x 1996–2007

Net-flux of NO_x compounds, measured using the gradient method, give insight in the above-canopy fluxes of NO_x. Over a 12-year period, net ecosystem losses of NO_x amounted to 3.7 ± 0.3 kg N ha^{-1} year^{-1}, representing about 12% of the annual TF. Net NO_x losses in the summer half-year (1.3 kg N ha^{-1} 6 months^{-1}) were significantly lower than NO_x losses in the winter half-year (2.4 kg N ha^{-1} 6 months^{-1}) ($p < 0.001$). Emissions of NO_x were highest in the morning and the evening. At noon, net NO_x flux tended towards zero or turned even into deposition (Fig. 8). There was no distinct trend in the yearly aboveground canopy emission fluxes of NO_x ($p = 0.456$). Nitrate leaching and above-canopy losses of NO_x accounted together for about 75% of the TF DIN fluxes over the period 1996–2007.

Nitrogen pools and change in N storage

In 2004, the aboveground standing biomass (179 Mg DW ha^{-1}) contained in total 237 kg N ha^{-1}

Fig. 6 a Seasonal pattern
of monthly nitrate
throughfall fluxes (TF flux
NO$_3$-N) versus monthly
nitrate losses in forest floor
leachate (LFH losses NO$_3$-
N) and seepage (seepage
losses NO$_3$-N) averaged for
the period 1994–2007 (in
kg N ha^{-1} month^{-1}).
b Seasonal pattern of
ammonium throughfall
fluxes (TF flux NH$_4$-N)
versus monthly ammonium
leaching in forest floor
(LFH losses NH$_4$-N)
averaged for the period
1994–2007 (in
kg N ha^{-1} month^{-1}).
c Monthly retention of DIN
(dissolved inorganic
nitrogen) throughfall within
the forest floor
compartment and forest
floor + mineral soil (in %)
averaged for the period
1994–2007. Error bars
indicate 1 standard error
about the mean
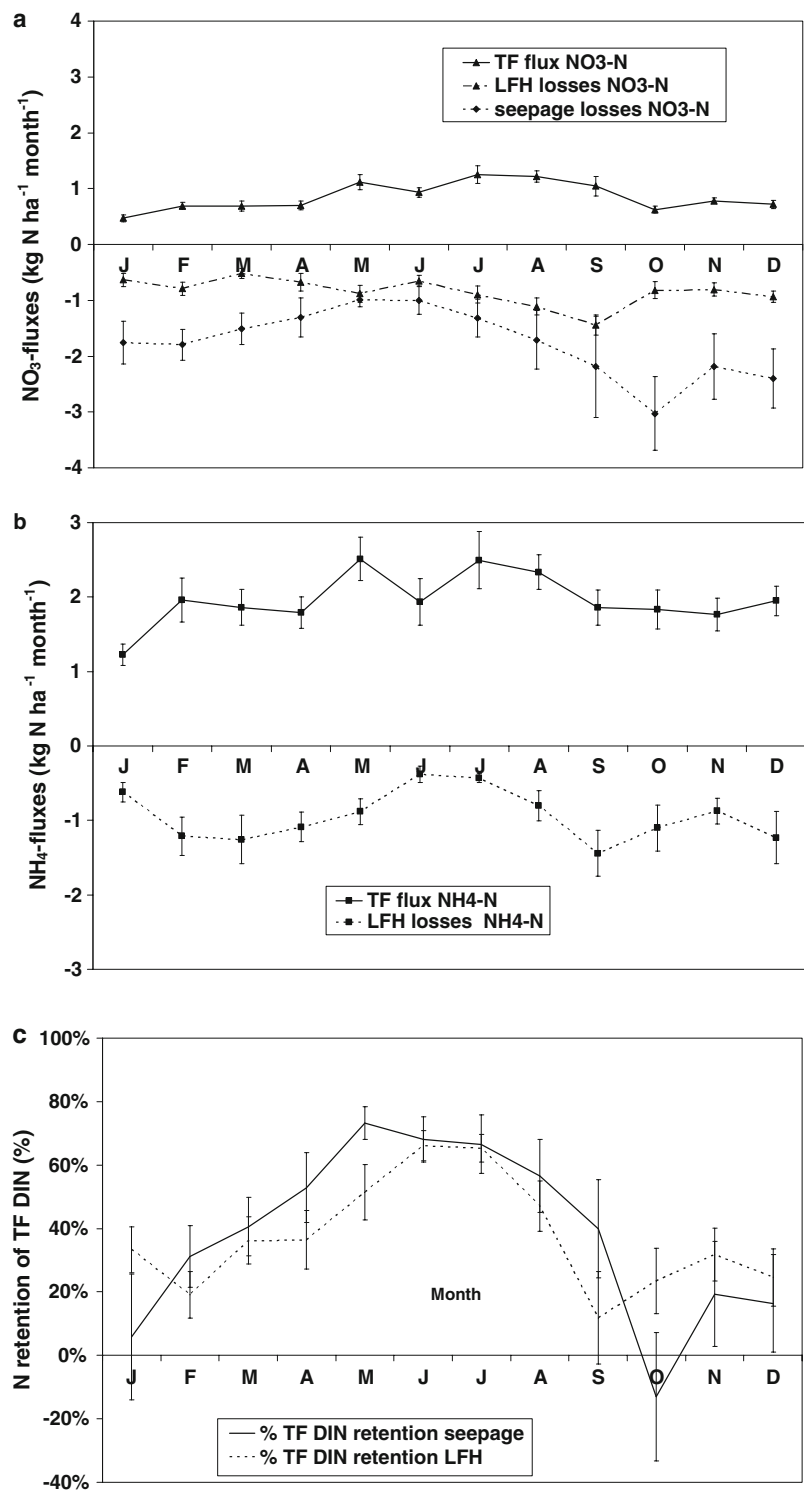
(Table 4). Over 120 kg N ha^{-1} was stored in stem
boles, which accounted for over 50% of the total
aboveground N pool. The remainder was almost

equally partitioned into branches and needles. New
foliage contained almost 50 kg N ha^{-1}, whereas
the N content of older needles only comprised

Fig. 7 Seasonal course of dissolved organic nitrogen (DON) in monthly throughfall fluxes (TF flux DON) versus monthly forest floor leachate (LFH losses DON) and seepage (seepage losses DON) for the period 2005–2007 (in kg N ha^{-1} month^{-1}). *Error bars* indicate 1 standard error about the mean
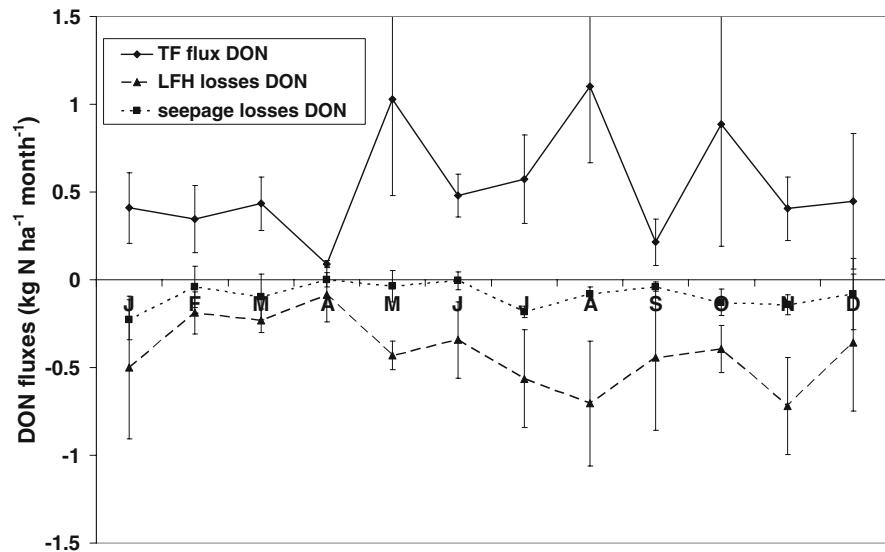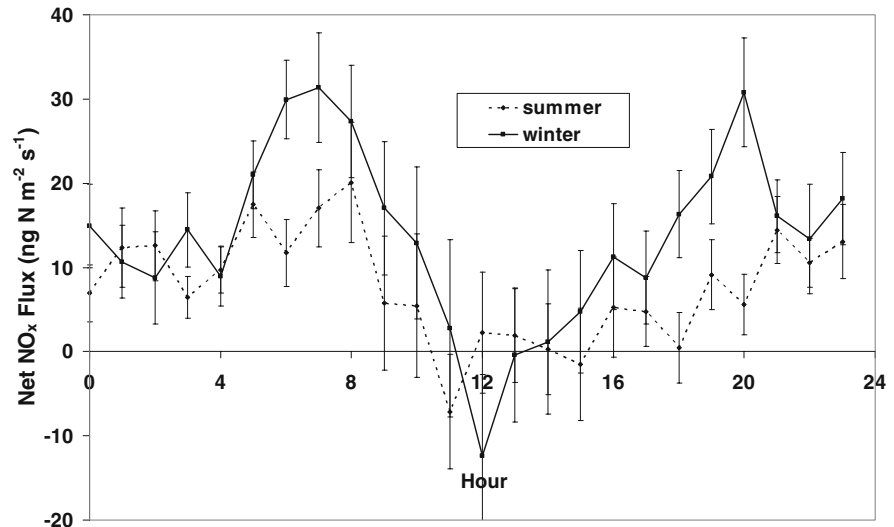

Fig. 8 Diurnal course of net-NO$_x$ fluxes considered over the summer and winter half-year for the period 1996–2007. *Error bars* indicate 1 standard error about the mean

10 kg N ha^{-1} year^{-1}. During the growing period 1995–2004, 2.7 kg N ha^{-1} year^{-1} accumulated in the aboveground biomass, which was especially due to accretion of N in stem boles.

The finer roots (<5 mm), which represented a total biomass of 5.2 Mg DW ha^{-1} in 1997 (Janssens et al. 1999) contained approximately 60 kg N ha^{-1}, mainly in the rootlets (<1 mm). The biomass of coarse roots was estimated at 22.5 Mg ha^{-1} (Janssens et al. 2002). Although they also make up a considerable compartment, detailed information about size distribution was not available to make reliable estimates of their N contents. A rough estimate of

the N incorporation due to the increase in standing coarse root biomass amounts to 0.2 kg N ha^{-1} year^{-1}. In total, 2.9 kg N ha^{-1} year^{-1} accumulated in the standing biomass in association with almost 1 Mg C ha^{-1} year^{-1} (derived from Tables 4, 5).

According to the last soil analysis conducted in 2004; 1,230 kg N ha^{-1} had accumulated in the L-, F- and H-layers (mass = 80.7 Mg DW ha^{-1}). This corresponded to an average annual N accumulation rate of 16.5 kg N ha^{-1} since the establishment of the forest on heathland in 1929. For the period 1997–2004, the current N accretion within the LFH-layer amounted to 24 kg N ha^{-1} year^{-1}. The mass of the LFH-layer

Table 4 Nitrogen content and net annual change in storage of different aboveground tree compartments between 1995 and 2004

Tree part	Stand N 1995 (kg ha^{-1})	Stand N 2004 (kg ha^{-1})	N change[a] 1995–2004 (kg ha^{-1} year^{-1})
Current year needles	44.3	49.3	0.56
Older needles	8.7	9.7	0.11
Branch Ø <1 cm	26.9	29.9	0.34
Branch Ø 1–2 cm	5.9	6.6	0.08
Branch Ø 2–5 cm	9.4	10.5	0.12
Branch Ø >5 cm	1.6	1.8	0.02
Boles (wood + bark)	111.6	124.3	1.41
Cones	4.6	4.9	0.03
Total	213.0	237.0	2.67

[a] N change includes the amount included in thinning and dead wood

Table 5 Net primary productivity of tree parts (Mg ha^{-1} year^{-1}), their mean N concentration (%) and annual N fluxes required for formation of different tree parts (kg N ha^{-1} year^{-1})

	NPP[a]	% N[b]	N flux
Stems	2.40	0.08	1.8
Branches <1 cm	0.36	0.74	2.6
Branches 1–2 cm	0.17	0.34	0.6
Branches 2–5 cm	0.46	0.20	0.9
Branches >5 cm	0.12	0.13	0.2
Branches total[c]	1.10		4.3
Foliage	2.50	1.88	47.0
Cones	1.70	0.48	8.1
Aboveground total	7.70		61.2
Roots <1 mm	2.70	1.33	35.9
Roots 1–2 mm	0.30	1.13	3.4
Roots 2–5 mm	0.30	0.43	1.3
Belowground total	3.30		40.6
Total	11.0		101.8

[a] NPP was determined in 1995–2001 by Janssens and others (2002)

[b] N concentrations of aboveground tree parts are biomass weighted means

[c] Branch size class distribution was based on the 1999 felling: 32% for branches <1 mm, 15% for branches 1–2 mm, 42% for branches 2–5 mm and 11% for branches >5 mm

increased since 1997 by ±1 Mg DW ha^{-1} year^{-1} (Fig. 9a). This increase was also observed in an increased thickness from 6 to 7.5 cm between 1991 and 2008. Previous N analysis revealed statistically significantly lower N concentrations in the LFH layer during the 1990s than in 2004 (Fig. 9b). Because the C concentration remained quasi constant, the N accumulation had implications for the C:N ratio (Fig. 9c),

which decreased by one unit, although the ratio remained elevated (±30). The decrease in C:N was, furthermore, statistically not detectable at $p < 0.05$. The retention of atmospheric-derived and canopy-derived N within the forest floor was associated with a yearly carbon sequestration of 480 kg C ha^{-1} in the forest floor.

The total N pool in the mineral soil, to a soil depth of 80 cm, amounted in 2004 to 4,970 kg N ha^{-1}. Comparison with previous soil surveys was not solid given sampling was conducted according to different depths or based upon morfogenetic features. Current estimates on soil N and C sequestration are therefore not sufficiently robust for publication in peer-reviewed literature.

Integrated over biomass and LFH (and thus excluding the mineral soil), the estimated N accumulation at the site thus amounts to 27 kg N ha^{-1} year^{-1}, most of which occurring in the surface litter layer.

Nitrogen fluxes involved in net primary production and returned via litterfall

Nitrogen fluxes required for yearly formation of aboveground biomass amounted to 60 kg N ha^{-1} year^{-1} (Table 5). N requirement for new foliage comprised about 80% of this N flux. More N was required for the formation of cones compared to the N required for the wood increment of stems and branches. Also N required for formation of fine roots was considerable. Using root measurements of Janssens et al. (1999, 2002), about 40 kg N ha^{-1} year^{-1} was needed, especially in the rootlets <1 mm.

The yearly N amount returned in aboveground litterfall varied between 27 and 44 kg ha^{-1} since the
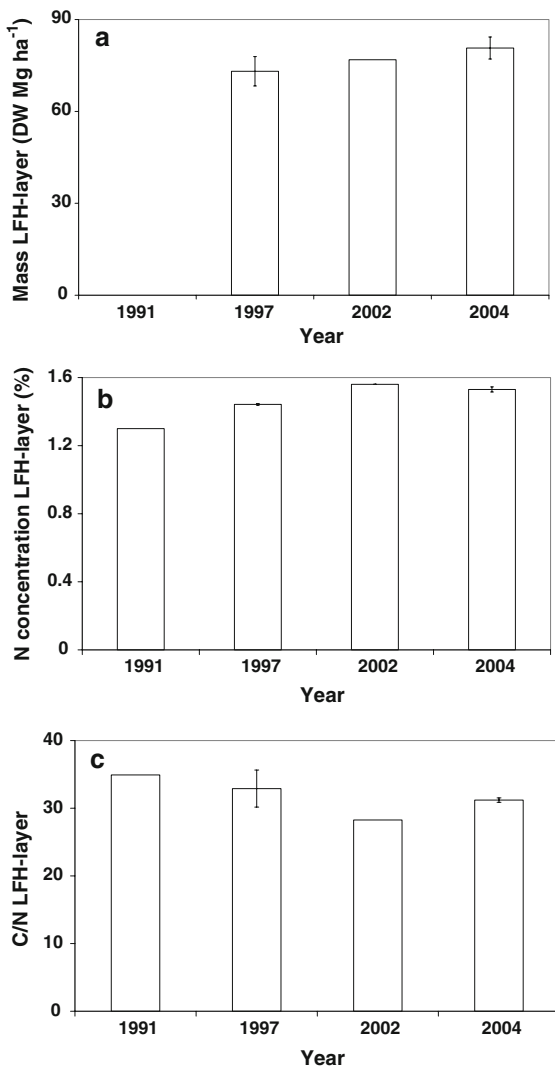
Fig. 9 a Evolution of mass (Mg DW ha^{-1}), **b** N concentration (%) and **c** C/N ratio of LFH-layer in 1991 ($n = 36$, no LFH mass measured; Neirynck et al. 2002), 1997 ($n = 15 \times 2$; Janssens et al. 1999), 2002 ($n = 25$; Curiel Yuste et al. 2005) and 2004 ($n = 3 \times 8$ subsamples). Standard errors available for sampling year 1997 and 2004

start of the measurements in 1998 (Table 6). During the first litterfall sampling in 1988, the amounts of N collected in litterfall were estimated at 47 kg ha^{-1}. Annual litterfall averaged 4.1 Mg DW ha^{-1} and consisted for about the half of needle litter with a N concentration ranging between 0.9 and 1.1% DW. Needle litterfall comprised about 55% of total N returned in litterfall. Nitrogen concentrations sampled from current and older needles in the upper crown were above 2% and remained constant throughout the

years (Table 6) despite the declining N deposition. The resorption index (ratio of fresh litter to litterfall N levels) ranged between 2.0 and 2.7 during the measuring period, suggesting the presence of efficient retranslocation patterns. Nitrogen amounts in below-ground litter were estimated at 41 kg N ha^{-1} year^{-1}. Litter originating from sloughed rootlets <1 mm accounted for 90% of the N returned from the belowground parts.

Discussion

Total nitrogen deposition versus throughfall nitrogen: impact of canopy processes

Monitoring of TF fluxes since 1992 revealed that the pine stand was subjected to large DIN inputs of on average 34 kg N ha^{-1} year^{-1}, with reduced N as dominating N form (70% of TF N). The high inorganic TF fluxes of reduced N reflected the high DD fluxes of NH_3 at our site (Fig. 1b). Both TF and DD of reduced N peaked in the summer period as a consequence of increased ammonia levels during this period (Neirynck et al. 2007). The difference between TD and TF N observed during the measuring period 1999–2001, suggested a canopy uptake of ±15 kg N ha^{-1} year^{-1}, which took mainly place for the reduced N form (Table 2). The TF water contained, however, substantial amounts of DON which helped to explain the mismatch between TF-DIN measurements and TD calculations based on WD and DD measurements. Measured TF fluxes of DON measured during the period 1999–2000 and 2005–2007 averaged 8.1 kg N ha^{-1} year^{-1} and thus explained only half of the difference between TD and TF inorganic N fluxes. The missing 7 kg N ha^{-1} year^{-1} then was absorbed by the foliage, converted to gaseous N compounds, or reflects uncertainties in each of the previous N fluxes. The measured DON fluxes fell well within the range from 1.2 to 11.5 kg N ha^{-1} year^{-1} for temperate forests, as reviewed by Michalzik et al. (2001). The latter authors also found a positive relationship between DON fluxes from the canopy and NH_4 and NO_3 in TF fluxes. Carlisle et al. (1966) and Stadler et al. (2001) suggested that DON could have been formed at the expense of DIN, which was extracted from the precipitation. The inorganic nitrogen was believed to

Table 6 Nitrogen concentrations of needles from upper crown (in % DW) and litterfall and N inputs returned in litterfall (kg N ha^{-1} year^{-1})

Year	N (%) needles		N flux litterfall		
	N current needles (%)	N older needles (%)	Weighted N needles litterfall (%)	Needle litterfall (kg N ha^{-1})	Total litterfall (kg N ha^{-1})
1988	2.11 (0.06)	2.20 (0.09)	0.99	26.6	46.6
1991	2.06 (0.05)	2.19 (0.06)			
1995	2.34 (0.07)	2.38 (0.08)			
1996					
1997	2.17 (0.09)	2.32 (0.09)			
1998			0.96	19.3	34.8
1999	2.44 (0.08)	2.52 (0.07)	0.89	17.2	26.9
2000			1.13	19.4	29.6
2001	2.18 (0.07)	2.01 (0.19)	1.06	19.3	41.2
2002			1.11	22.0	43.8
2003	2.48 (0.05)	2.29 (0.05)	1.00	12.0	19.2
2004			1.02	19.0	33.0
2005	2.31 (0.06)	2.43 (0.06)	0.97	14.7	27.8
2006			0.93	19.6	36.8
2007	2.27 (0.06)	2.28 (0.08)	0.97	19.9	34.1

Standard errors of upper crown needle concentrations between brackets

be adsorbed on the leaf surfaces, and immobilized by epiphytic microorganisms (bacteria, yeasts, fungi) dwelling on the leaf surfaces. After supporting the growth of microflora, microbial dieback could lead to the generation of DON, retrieved in the throughfall collectors. Gaige et al. (2007) observed high retention (70–80%) of fertilizer DIN by a forest canopy during a whole canopy fertilization of a mature coniferous forest in central Maine (USA). It was found from isotope analysis that both NH_4^+ and NO_3^- were rapidly converted to soluble organic N in the canopy. The authors speculated that the rapid incorporation into DON might have arisen from lichen and/or microbial N cycling rather than assimilation and re-release by tree tissues in this forest.

Stomatal uptake of NH_3 could be another mechanism involved in canopy uptake but the analysis of bidirectional exchange of NH_3 revealed that mostly stomatal emission occurred at our site (Neirynck and Ceulemans 2008). Inorganic nitrogen (NH_4^+) could also been absorbed by the needles through ion exchange with base cations (Ca^{2+}, Mg^{2+}, K^+), which were released from the leaves (Draaijers and Erisman 1995). The canopy exchange losses of base cations, obtained by subtracting the base cation dry deposited

flux (Ulrich 1983) from the net-throughfall, were, however, too low to explain this ion exchange process as plausible mechanism for nitrogen uptake (Neirynck et al. 2002).

The decline in TF DIN fluxes was primarily due to a reduction in reduced nitrogen. Measures to abate ammonia emissions frame within the 'nitrates' directive (91/676/EEC) and the National Emission Ceilings directive (NEC) (2001/81/EC). The reduction of ammonia emission in Flanders was enforced through a regional manure legislation (Manure Action Plan or abbreviated to MAP). The main reduction in the ammonia emission was recorded from 2000 onwards after the enforcement of the second Manure Action Plan (MAP II). Measures included emission-poor application of manure and later also reduction in cattle numbers. Current ammonia emission densities in adjacent rural communities to our site are 50% lower compared to emission densities registered at the beginning of 1990.

Drivers of inorganic nitrogen leaching

Nitrate seepage losses over the 16-year monitoring period averaged 23 kg NO_3-N ha^{-1} year^{-1}, which

indicated that N availability was far in excess of biotic and abiotic retention capacity. The low N uptake of the forest and the coarse textured soil (low hydrological residence times and low cation exchange capacity due to low clay content) might have predisposed the forest to low N retention and excessive NO_3^- losses (Fenn et al. 1998). Also soil acidity could exacerbate N losses in different ways (Venterea et al. 2004). At our site, nitrate concentrations in the seepage water were inversely related to pH (Fig. 5) but the decreased pH could also be the consequence of a nitrification-induced acidification (Tietema et al. 1992).

Findings of studies encountered in the literature are conflicting about the origin of the DIN found in the forest floor leachate and the seepage flux. Throughfall N may be directly leached without being incorporated in soil or plant organic matter (deposition-related). On the other hand, elevated atmospheric deposition may stimulate nitrification also indirectly, as long-term ecosystem response, through the production of N-rich foliage, litter and soil organic matter with a decreased C:N ratio (Melillo et al. 1982; Fenn et al. 2005). The C:N ratio of the forest floor at our site remained, however, high despite the large sustained N deposition. Dise et al. (1998) reported that N enrichment of soil organic matter and current N deposition levels both affected nitrification and nitrate leaching. Evans et al. (2006) predicted that NO_3 breakthrough was most rapid at sites where N deposition was high and the soil C pool was small. Rowe et al. (2006) also emphasized the vegetation-mediated proportion of recalcitrant carbon as an influencing factor of nitrate leaching. Nitrate leaching was likely to start at a higher C:N ratio in soils with a large proportion of recalcitrant carbon.

Features of inorganic nitrogen retention: role of N form and season

There was a striking difference in retention of both nitrogen forms. In contrast to NH_4^+, NO_3^- fluxes were unaffected when passing through the LFH-layer, indicating unsuitable conditions for nitrification and/or nitrate uptake. Considering the entire monitoring period, NO_3^- output from the LFH-layer almost equalled that of TF NO_3^- fluxes (Figs. 3, 6a). The absence of NO_3^- uptake indicated the preference of plants and microbes for NH_4^+ as a N source instead

of the more mobile NO_3^- (Emmet et al. 1998) or unsuitable conditions for N immobilisation. According to KjØnaas et al. (1998), plants and microbes exposed to increased N deposition have a reduced ability to absorb NO_3^-. Also Tietema et al. (1998) concluded that there was less immobilization of NO_3^- by soil microbes at coniferous forest sites with substantial N deposition in north-western Europe. Decreased litter sink strength for NO_3^- under elevated N inputs was also mentioned by Micks et al. (2004) and Nadelhoffer et al. (2004).

Besides the absence of NO_3^- absorption within the LFH-layer, there was also never a net-gain of NO_3^- in the forest floor leachate, suggesting that nitrification did not occur in the organic layers. Measurements of potential net mineralization and nitrification conducted at our site confirmed the low nitrification rate in the forest floor (Vande Walle and Lemeur 2001). They found a relative nitrification (potential net nitrification/ potential net N mineralisation) of 28% in the forest floor compared to 52% in the mineral soil, although net mineralization was stronger in the forest floor. The poor nitrification potential of the forest floor could be attributed to the high competition for NH_4^+ (both for TF NH_4^+ as mineralized NH_4^+) in the forest floor given its high C:N ratio (Fenn et al. 2005), the presence of inhibitory organic compounds in the organic horizon (White 1994) or the low pH of the forest floor (here: pH = 2.7) (Nugroho et al. 2007), impeding the activity of autotrophic nitrifiers. In fact, NO_3^--losses seemed to be only enhanced in the mineral soil where C:N ratios dropped below 20 and more favourable conditions for nitrification occurred (smaller N demand by soil organisms). Although the C:N ratio of organic horizons is generally believed to be a good integrating variable of the propensity for nitrate leaching in coniferous ecosystems (Gundersen et al. 1998; Nadelhoffer et al. 1999; Rueth and Baron 2002), it was found to be a weak indicator of nitrate leaching at German forest sites (Borken and Matzner 2004). According to Dise et al. (1998), wider confidence intervals on estimated nitrate leaching in response to C:N were obtained for high N deposition levels and therefore other variables, besides the C:N ratio, should be considered to refine leaching estimates.

Our measurements demonstrated that the retention of TF NH_4^+ took place mainly in the LFH-layer, where about half of the TF NH_4^+ was retained. Experiments with labelled N elsewhere confirmed

that added ammonium was especially retained within the organic layer and upper mineral soil where microbial activity was the highest (Gundersen 1998; Nadelhoffer et al. 1999, 2004; Feng et al. 2008). The soil organic N pool was also identified as the major sink of deposited DIN in several other studies (Magill et al. 1997; Fenn et al. 1998; Rueth and Baron 2002). It is also likely that chemical exchange of NH_4^+ for base cations or Al^{3+} contributed to the retention of TF NH_4^+ in the LFH-layer.

In the dormant period, DIN retention capacity of the forest floor was generally low and decreased to 20–30%, compared to 50–70% in the growing season (Fig. 6c). Nitrate losses, occurring consistently outside the growing season, were symptomatic for more progressed stages of N saturation (Stoddard 1994). According to KjØnaas et al. (1998) such a seasonal trend of nitrate leaching depended both on biological and hydrological factors. On one hand, this could indicate a temporal reduction of the capacity of microbes and vegetation to immobilize the incoming N. On the other hand, the higher seepage of nitrate at our site could also be due to the twofold higher water seepage fluxes during the dormant season. Biotic immobilisation may further decrease with increasing grade of N saturation (Johnson et al. 2000).

NO_x emissions

Besides NO_3^- leaching, above-canopy NO_x emission fluxes indicated that NO emission is occurring at the site. Nitric oxide emission could arise from deposited N or the large organic N pool present in the forest floor and mineral soil. Both high NO_3^- seepage losses and NO emission are diagnostic for N saturation of forest stands (Aber et al. 1989; Fenn et al. 1996, 1998). There were less NO_x losses during the summer half-year, although soil emission of NO is expected to be higher during the growing season as a result of increased soil temperature (Beier et al. 2001). The lower NO_x losses might be due to increased uptake through stomata of NO_2 deposited from the atmosphere or produced from biogenic NO after photochemical reactions in the trunk space (Duyzer et al. 2004). Part of the NO_2 absorbed by the canopy during the summer half-year, could be later excreted and retrieved by subsequent washing in the throughfall collectors. This could explain the higher TF NO_3^- fluxes measured during the summer half-year.

Forest response to declining inorganic nitrogen depositions

Nitrate seepage fluxes showed strong declining trends, which took place commensurately with the drop in TF-DIN fluxes. Sub-canopy roof experiments, carried out to evaluate the impact of clean rain on chemical recovery of the soil solution, revealed that efforts to turn down N deposition in coniferous forests rapidly led to declining nitrate levels (Emmet et al. 1998). Time series analysis of DIN levels in the soil solution revealed a significant downward trend of DIN concentrations in the soil solution from all horizons. Nevertheless, besides N deposition, other unconsidered short or long term processes could be convoluted in the observed time trend: (1) Increased N uptake in the deciduous understory outweighing the decreasing wood uptake of the pine trees (2) Stimulation of belowground biomass production without observable effect on aboveground production (Goodale et al. 2005). At our site, a shift in C allocation toward fine roots (decrease in shoot/root NPP) was noticed by Curiel Yuste et al. (2005) and Janssens et al. (2002). According to their measurements, C has accumulated in soil at a rate of 3–5 Mg ha^{-1} year^{-1}. (3) Inter-annual climatic variation (in hydrology, temperature, frost, soil moisture) might affect mineralization, photosynthesis and mortality of microbes or fine root turnover, thus adding noise to the time series. Changes in microbial composition due to climatic variability (possibly triggered by autumnal rains of 1998; alternation of wet periods after a relatively dry period) over the 16-year period might have led to increased consumption by heterotrophic microbes or mycorrhizae that subsequently outcompetied the nitrifiers. This could explain the remarkable disappearance of NH_4^+ in the soil solution from 1998 onwards. (4) The drop in DIN levels in the seepage water took place with a concomitant rise in the pH of the soil solution (Fig. 5). Although this could have been the result of reduced nitrification-induced acidification, the higher soil water pH could conversely also have contributed to (1) decreased nitrification by stimulating heterotrophic competition for NH_4^+ or (2) decreased NO_3^- leaching by stimulating heterotrophic NO_3^- assimilation (Venterea et al. 2004).

In contrast to NO_3^- leaching, no trend in above-canopy NO_x losses through NO emission could be

discerned at our site. It is, however, possible that soil emission fluxes of NO did respond to the declining N fluxes, but that it was not seen in the aboveground fluxes of NO_x. There were, unfortunately, no soil emission fluxes from dynamic chambers available to underpin this finding. Neither litterfall N concentrations nor the mineral N nutrition as judged by N concentrations in the upper crown part, showed any response the declining N fluxes. Possibly the rate of response of these indicators is slower than that of nitrate fluxes.

Nitrogen pools and sinks in the ecosystem

The aboveground biomass (0.2 Mg N ha^{-1}) was found to be a small N reservoir compared with the N stored in the forest floor (1.2 Mg N ha^{-1}) and in the mineral soil (5.0 Mg N ha^{-1}). Changes in N storage in tree biomass were less than 3 kg N ha^{-1}-year^{-1}, which was much lower than the current N increments in the LFH-layer. The current accumulation of N in the LFH-layer, measured over the period 1997–2004, was estimated at 24 kg ha^{-1} year^{-1}, which was higher than the long-term estimate of 16.5 kg N ha^{-1} year^{-1} since the plantation in 1929. Over the period 1997–2004, about 50% of total N deposition was retained in the forest ecosystem (compared to a TD of 48 kg N ha^{-1} year^{-1}). During the same period about 22 kg NO$_3$-N was found to be leached.

Total C accumulation in the biomass and LFH-layer was 1.5 Mg C ha^{-1} year^{-1}. Hence, per unit N deposited into the ecosystem, about 30–35 g of C are being sequestered, values that are not a-typical for coniferous forests (e.g. de Vries et al. 2008; Pregitzer et al. 2008). This N-induced C sequestration rate, however, excludes N accumulation in the mineral soil and is thus an underestimation. Because our C and N sequestration estimates in the mineral soil are not sufficiently robust, we can, at this moment, not assess the magnitude of this underestimation and hence cannot conclude whether or not this high N-affected forest exhibits C sequestration rates in the order of magnitude as reported by Magnani et al. (2007).

Our estimate of N accumulation in the surface litter layer (24 kg ha^{-1} year^{-1}) of this N saturated forest exposed to almost 50 kg ha^{-1} year^{-1} atmospheric deposition was substantially higher than the N accumulation of 13 and 18 kg N ha^{-1} year^{-1}

observed in the topsoil (forest floor plus uppermost 30 cm mineral soil) from two Scots pine ecosystems in Southern Germany that received resp. 24 and 30 kg ha^{-1} year^{-1} of N deposition (Prietzel et al. 2006). Our estimate of N accumulation in the LFH-layer was of similar magnitude as that in a beech forest in central Germany (19 kg ha^{-1} year^{-1} with TD = 19.3 kg ha^{-1} year^{-1}), but below the sequestration rate of 41 kg N ha^{-1} year^{-1} in the forest floor of a spruce forest in the same region, with TD = 45 kg ha^{-1} year^{-1} (Berg 2004). According to Magill and Aber (1998), increasing N contents due to N additions are often associated with an apparent lack of change in N concentrations, because the latter can be outweighed by changes in total organic horizon mass. At our study site, there was indeed an aggrading forest floor since the beginning of the measurements (increase in both forest floor mass and thickness), which was responsible for most of the observed increase in N content of the forest floor at our site. Nonetheless, we were able to detect a significant increase in N concentration as well, which contributed for about one-third to the increasing N content of the forest floor. In contrast, we could not statistically detect a decrease in the C:N ratio, despite a decrease from 32.9 to 31.2 between 1997 and 2004. The main reason why we could not detect changes in C:N ratio despite statistically significant changes in N, lies in the fact that trends in element ratio's are more difficult to detect than changes in single variables (Sulkava et al. 2007). In order to detect significant changes in C:N, a larger number of replicates or a longer observation time will be required.

According to Berg et al. (1995), a N-induced retardation of decomposition takes place in the later stages of the litter decay process as a result from a combination of lignolytic enzyme suppression and formation of chemically stable compounds by the reactions of N with lignin and phenolic compounds. Waldrop and Zak (2006) hypothesized that increasing soil solution NO_3^- reduced lignolytic enzyme activity especially in forest ecosystems with high abundance of white-rot fungi and lignin-rich leaf litter. Aber et al. (1995, 1998) explained declining mineralization rates as a longer-term response to N additions, although DIN supply might initially stimulate N mineralization (McNulty et al. 1990). Meiwes et al. (2002) attributed the reduced microbial humus mineralization rate to increased soil acidity, increased

availability of toxic heavy metals and increased N content of the organic matter, or a combination of these factors. All of these mechanisms may have contributed to the large LFH-layer build-up at this site and to the disproportionate increase in its N concentration.

Conclusions

We studied N biogeochemistry in a Scots pine forest subjected to very high N deposition between 1992 and 2007. The annual amount of dissolved inorganic nitrogen (DIN) in throughfall (TF) averaged 34 kg ha^{-1} year^{-1} over the 16-year monitoring period. There were, however, also important TF fluxes of dissolved organic nitrogen (DON), which should be incorporated in the estimate of the N flux using throughfall collectors. Inclusion of DON fluxes in the throughfall will therefore yield better estimates of actual soil N input in N rich areas.

The high N status of the forest was characterised by excessive nitrate leaching and the occurrence of canopy NO$_x$ emissions to the atmosphere. The elevated losses of N to the atmosphere and the nitrate leaching to the soil solution indicated that the atmospheric N inputs were far in excess of the N requirement of the forest ecosystem. Despite the fact that a lot of atmospheric N remained unprocessed, the forest was still accumulating N, especially through N retention in the forest floor. Throughfall N was mainly retained during the growing season, because of higher microbial retention, plant uptake or lower water losses. Only TF NH$_4^+$ was retained within the forest floor, in contrast to TF NO$_3^-$, which seemed not to take part in forest floor processes.

The decline in the TF DIN fluxes during the course of this study period was especially due to the declining TF NH$_4^+$, which was especially marked during the second part of the measuring period, as a result of nitrogen abatement strategies. The declining TF fluxes have led to a concomitant decrease in the nitrate fluxes in the seepage. In 2007, nitrate concentrations and fluxes increased again, despite the fact that the lowest TF DIN flux was measured since the beginning of the measurements. Other unaccounted variables related to climate, root and microbial activity and their interactions therefore also determine the long term or short term variation.

Despite the declining depositions over the 16-year period, there was no change in the foliar and litterfall-N. Likewise, above-canopy NO$_x$ losses, indicative for NO emission at our site, did not show any significant time trend.

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