

# Acid Rain Research

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## NITREX: Phase 2 Final Report



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Abstract: NITREX-2 comprises the second phase of the European NITREX project (Nitrogen Saturation Experiments). This report deals with results obtained during phase 2, which ran from 1 November 1993 - 31 March 1995. NITREX continues phase 3 through March 1996.
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for Bjørn Olav Rosseland

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## TABLE OF CONTENTS

	Page
<b>PREFACE</b>	<b>3</b>
<b>1. EFFECTS OF REDUCED NITROGEN AND SULPHUR INPUTS ON TWO CONIFEROUS ECOSYSTEMS IN THE NETHERLANDS</b> Andries W. Boxman, Harrie F.G. van Dijk, Paul J.M. van der Ven and Jan G.M. Roelofs	<b>5</b>
<b>2. <sup>15</sup>N POOL DILUTION EXPERIMENTS</b> Albert Tietema	<b>19</b>
<b>3. EFFECTS OF MANIPULATION OF NITROGEN DEPOSITION ON LITTER DECOMPOSITION AND SOIL BIOTA: A CROSS-SITE COMPARISON</b> René F. Hogervorst & Hermann A. Verhoef	<b>25</b>
<b>4. EFFECTS ON ENHANCED N DEPOSITION IN A SPRUCE FOREST AT KLOSTERHEDE, DENMARK, EXAMINED BY MODERATE NH<sub>4</sub>NO<sub>3</sub> ADDITION</b> Per Gundersen	<b>31</b>
<b>5. EFFECTS OF ENHANCED NITROGEN DEPOSITION IN A SITKA SPRUCE STAND IN UPLAND WALES</b> B.A. Emmett, S.A. Brittain, S. Hughes, V. Kennedy, D. Norris, B. Reynolds, M. Silgram and P.A. Stevens	<b>35</b>
<b>6. RESPONSE OF SOIL WATER CHEMISTRY TO EXPERIMENTAL "CLEAN RAIN" IN THE NITREX ROOF EXPERIMENT AT SOLLING, GERMANY</b> Bredemeier, M., Blank, K., Lamersdorf, N., and Wiedey, G.A.	<b>39</b>
<b>7. NITREX project: ecosystem response to chronic additions of nitrogen to a spruce-forested catchment at Gårdsjön, Sweden</b> Richard F. Wright, Tor-Erik Brandrud, Anna Clemensson-Lindell, Hans Hultberg, O. Janne Kjønås, Filip Moldan, Hans Persson and Arne O. Stuanes	<b>43</b>
<b>8. Alptal, Switzerland: Activity Report 1994</b> Schleppi P., Muller, N., Feyen, H., Bucher, J.B., Papritz, A., & Flühler, H.	<b>54</b>

## PREFACE

This report comprises the final report to the Commission of European Communities for NITREX phase 2.

NITREX (Nitrogen Saturation Experiments) began with national funding as parallel large-scale experiments with nitrogen deposition at 8 sites in Europe. These joined to form NITREX with partial funding from the Commission of European Communities, STEP programme, contract number CT90-0056. NITREX phase 1 ran for 24 months from 1 August 1991 to 31 July 1993.

NITREX phase 2 was supported in part by the Commission of European Communities, Environment research programme, contract CT93-0264. NITREX phase 2 ran for 17 months from 1 November 1993 to 31 March 1995.

NITREX phase 3 is currently in progress, also supported in part by the Commission of European Communities, Environment research programme, contract CT94-0436. NITREX phase 3 runs for 12 months from 1 April 1995 to 31 March 1996.

Previous official reports to the Commission from NITREX include:

1). *Project description and background data.*

Dise, N.B. and Wright, R.F. (eds.). 1992. The NITREX project (Nitrogen saturation experiments). Ecosystem Research Report 2. Commission of the European Communities, Brussels, 101 pp.

2) *Final report for NITREX phase 1.* Special issue of the journal *Forest Ecology and Management*, R.F. Wright and A. Tietema, editors.

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Oslo, May 1995

Richard F. Wright

## 1. EFFECTS OF REDUCED NITROGEN AND SULPHUR INPUTS ON TWO CONIFEROUS ECOSYSTEMS IN THE NETHERLANDS

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### ABSTRACT

Atmospheric deposition of nitrogen and sulphur was reduced to pre-industrial levels in nitrogen and sulphur saturated *Pinus sylvestris* and *Pseudotsuga menziesii* stand. Within a few months after reduction of the input, output to the groundwater was also strongly reduced, implying a tight input-output coupling. As a result, leaching of aluminium and base cations (to counteract nitrate and sulphate leaching) decreased and the mineral balance in the soil solution improved. Consequently, tree health improved as shown by increased root and shoot growth and by an improved mineral balance of the needles.

### INTRODUCTION

During the past five years research has been conducted at two locations in the Netherlands, where atmospheric deposition of nitrogen and sulphur has been very high during the past decades. The research has been conducted within the NITREX framework, a consortium of European experiments on ecosystem scale, in which the nitrogen and sulphur input was manipulated in catchments or forest stands at 8 sites spanning a nitrogen gradient across Europe (Dise and Wright 1992). Both Dutch sites are nitrogen saturated i.e. nitrogen availability has increased to levels that exceed biotic demand (i.e.  $>10 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ ; Encke, 1986), and nitrogen has appeared in the leachate below the rooting zone (Aber *et al.*, 1989).

In a *Pseudotsuga menziesii* (Mirb.) Franco and a *Pinus sylvestris* L. stand atmospheric deposition of nitrogen and sulphur was reduced to pre-industrial levels by means of a roof. The central question was whether nitrogen saturation is reversible. If it is reversible, in what way does it proceed and on what time scale? Does a reduction of atmospheric deposition lead to increased tree health or changes in understorey vegetation?

Here the main results are described for the *Pinus sylvestris* stand. Although both ecosystems reacted similarly, the most significant results were obtained in this stand.

### MATERIALS AND METHODS

In 1989 two research sites were established in which ambient throughfall water was intercepted by means of a roof, and replaced by demineralized water to which all components were added in the same amount as present in the throughfall, except acid, nitrogen and sulphur. At Ysselsteyn, southeast Netherlands, a research site was established in a Scots pine (*Pinus sylvestris* L.) stand. This stand is classified as vitality class 3, indicating a needle loss

of 26-60% (Smits, 1992). The other site was established at Speuld, central Netherlands, in a Douglas fir (*Pseudotsuga menziesii* (Mirb.) Franco) stand. This stand is classified as vitality class 1, indicating a needle loss of 0-10%. Underneath the roof, two plots (10x10m) were designed to receive either clean water (roof-clean plot) or ambient throughfall (roof-control plot). Outside the roof a second control plot was established, receiving ambient throughfall (control plot).

A detailed description of the sites and of the methods has been given elsewhere (Dise and Wright, 1992; Van Dijk *et al.*, 1992a,b; Boxman *et al.*, 1994; Boxman *et al.*, 1995).

## RESULTS AND DISCUSSION

### Throughfall fluxes to the forest floor

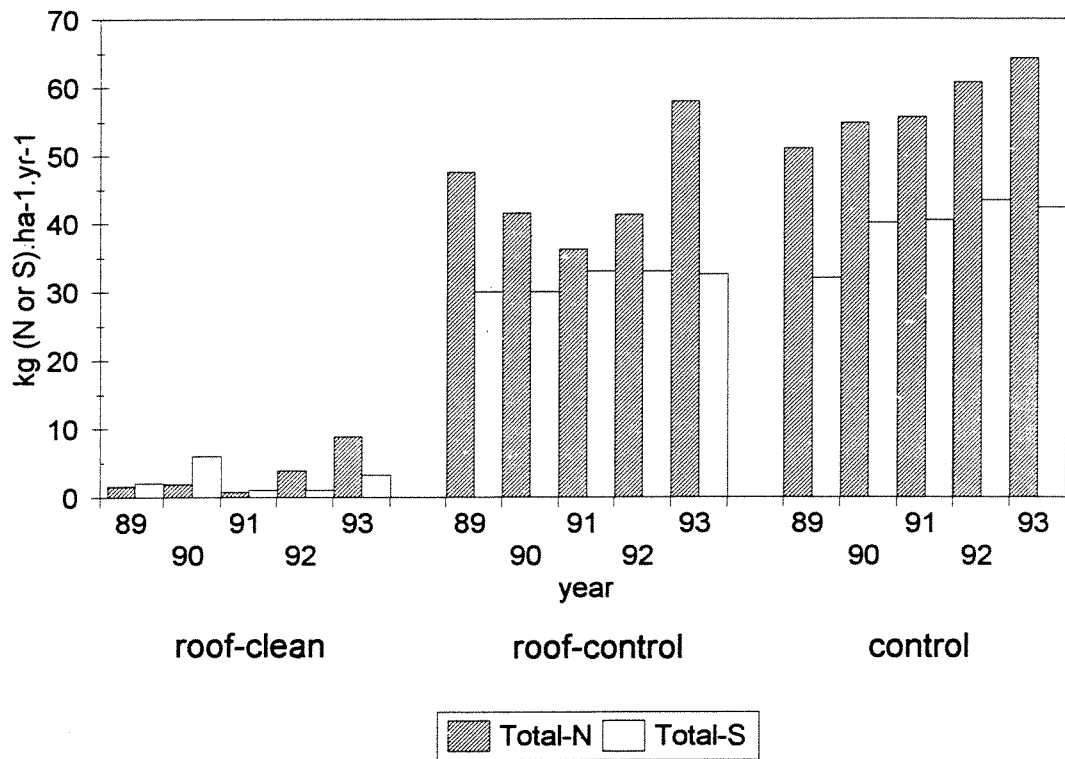
Interception of throughfall in the roof-clean plot reduced atmospheric input of nitrogen and sulphur to  $<2 \text{ kg ha}^{-1}\text{yr}^{-1}$  (Figure 1). During the experimental period the deposition to the ambient control plots was high ( $55 - 70 \text{ kg N ha}^{-1}\text{yr}^{-1}$  and  $35 - 40 \text{ kg S ha}^{-1}\text{yr}^{-1}$ ) and exceeded the critical load value of  $15 - 20 \text{ kg N ha}^{-1}\text{yr}^{-1}$  (Bobbink *et al.*, 1992).

Atmospheric deposition to the roof-control plot was somewhat lower than to the control plot due to the technical method watering and storage of the water, and some water losses during heavy showers. Input of the other nutrients was approximately equal in all plots (Boxman *et al.*, 1995).

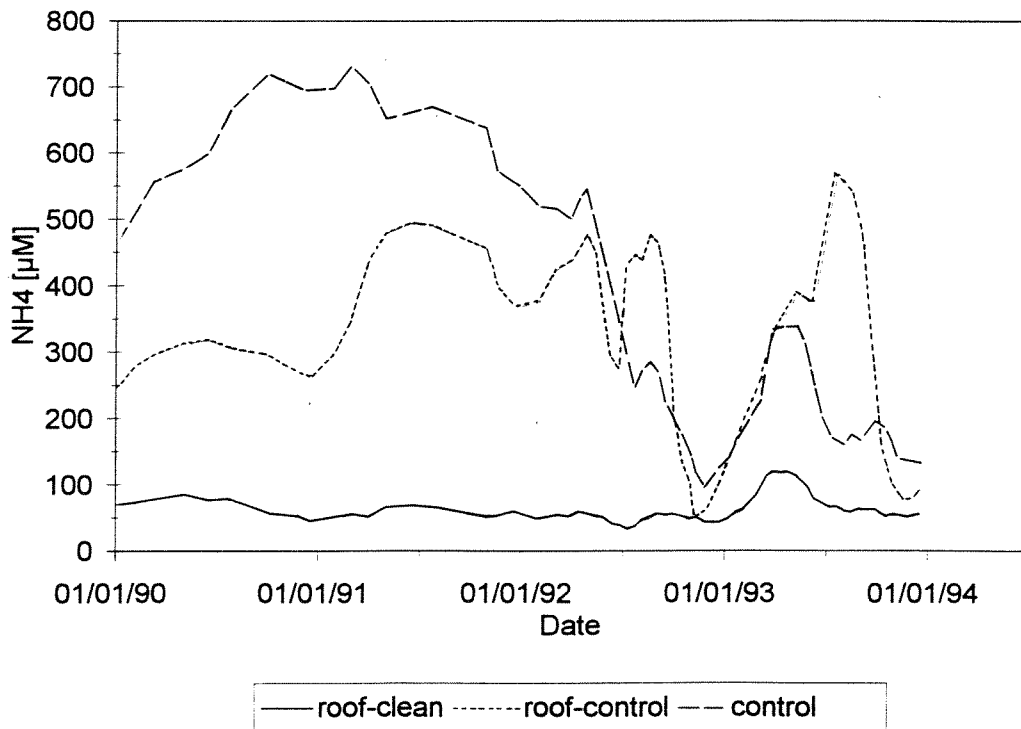
### Soil solution chemistry

At both locations the inorganic nitrogen and sulphur concentrations in the soil solution of the roof-clean plot responded within six months to a reduced input of nitrogen and sulphur (Figures 2 - 4). Unfortunately, no pretreatment soil solution data are available, but soil solution data (including pretreatment data) from the Solling NITREX site (Germany) show the same rapid response (Bredemeier *et al.*, 1995).

Soil extracts taken before the start of the treatment revealed no significant differences between the plots in availability of ammonium, nitrate or sulphur. During the first two years the watering procedure influenced differences between the control plots, but automation of the watering regime in 1992 converged the data of these plots (Figure 2 - 7).  $\text{NH}_4^+$  availability in the -upper soil layer of control plots was strongly determined by meteorological conditions. The ammonium concentration in the soil solution decreased rapidly in the very wet autumns of 1992 and 1993, which clearly stimulated nitrification and leaching to deeper soil layers. The same seasonality was observed for nitrate, which showed an increase every autumn. After an initial decrease of  $\text{NO}_3^-$  concentrations in the soil solution of the roof-clean plot a tendency was observed to increasing levels (Figures 1 and 3), which is related to increased dry-deposition to the plots (data not shown). While in throughfall a dominance of ammonium over nitrate was found, in the soil solution the reverse situation was the case due to nitrification, preferential uptake of ammonium by the vegetation or immobilization. As a result, the  $\text{NH}_4/\text{K}$  ratio in the soil solution of the roof-clean plot decreased to levels below 5 (Figure 5), considered favourable for a balanced nutrient uptake (Roelofs *et al.*, 1985).

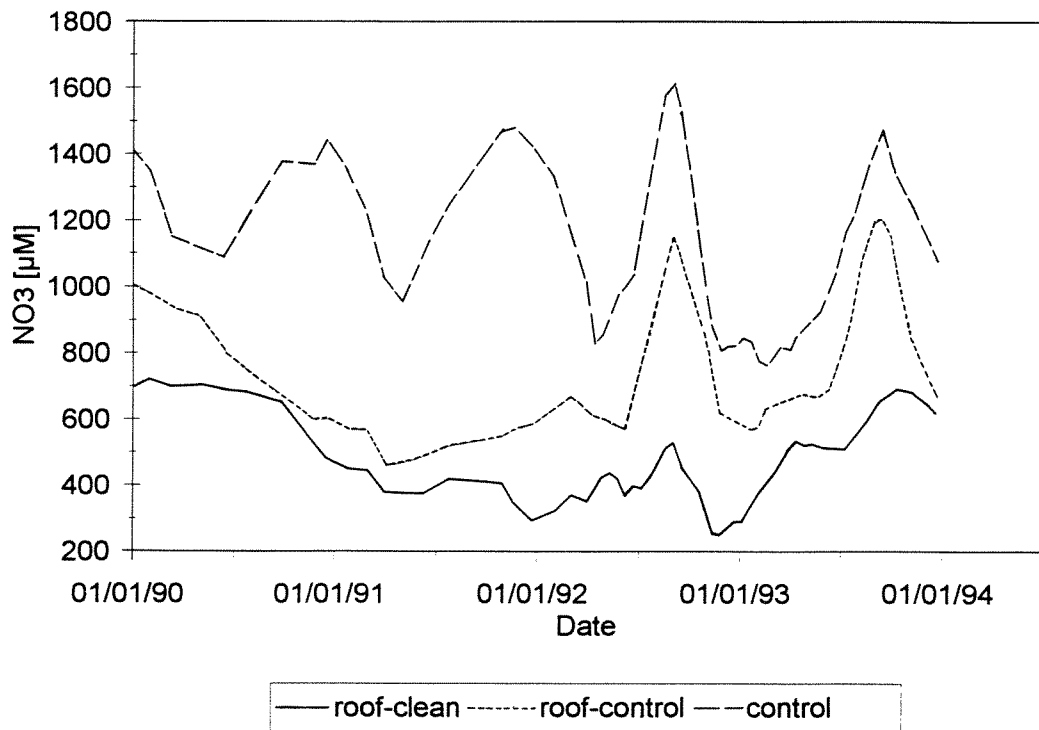


**Figure 1.** Deposition ( $\text{kg ha}^{-1} \text{ yr}^{-1}$ ) of total-N and total-S to the forest floor of the plots at Ysselsteyn.

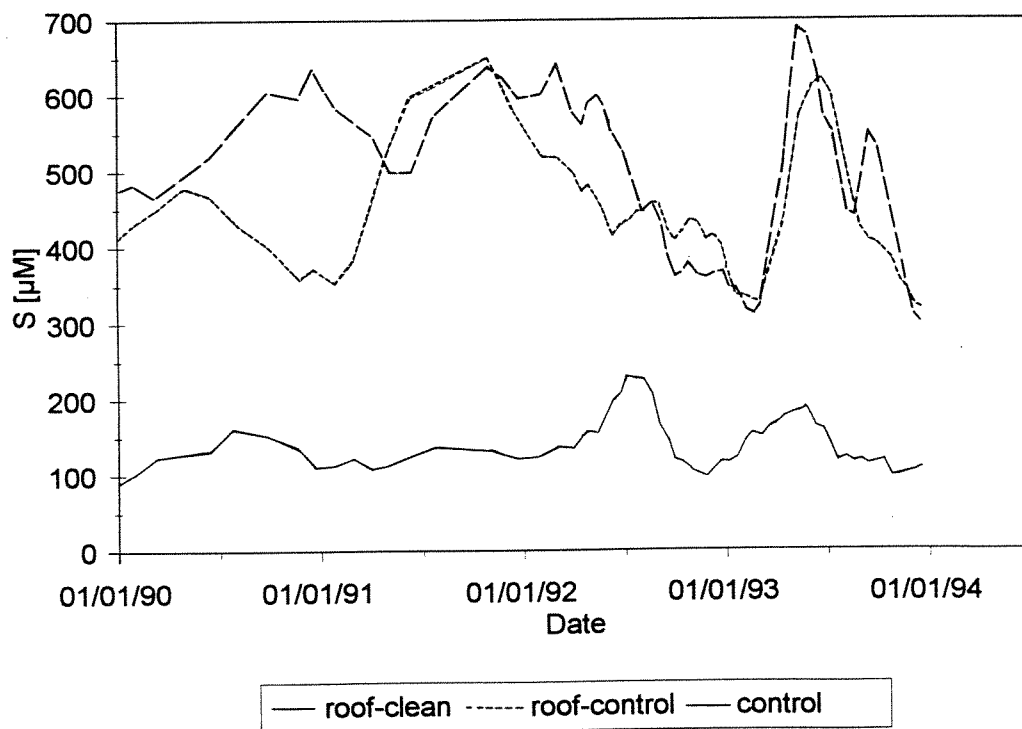


**Figure 2.** Ammonium concentrations at 10cm depth in the soil solution at Ysselsteyn.

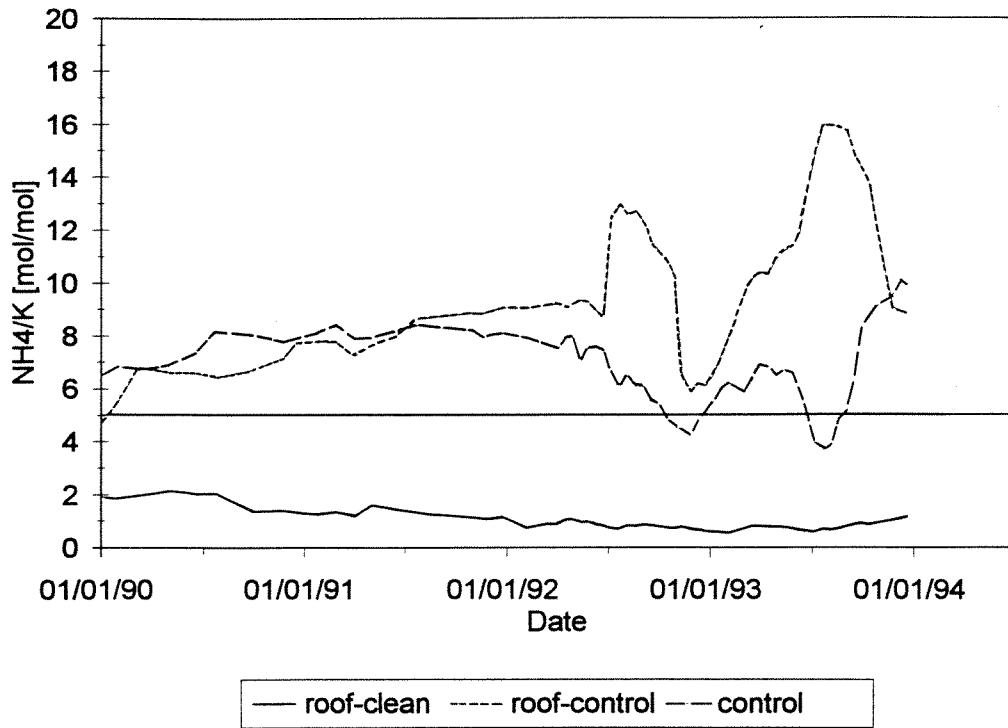




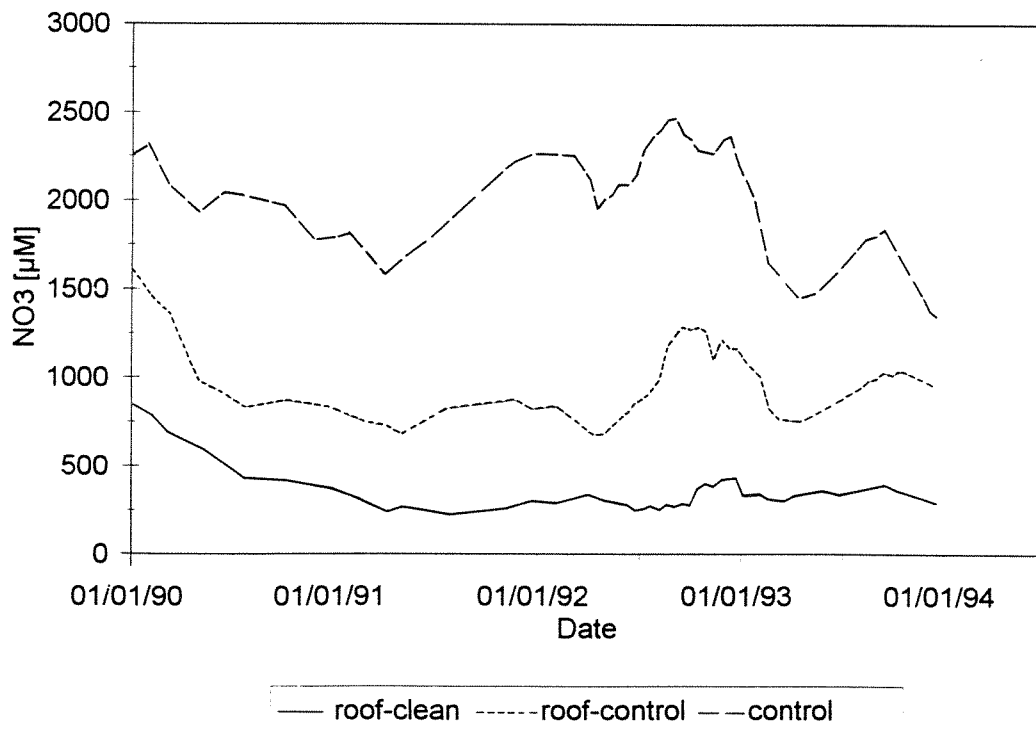
**Figure 3.** Nitrate concentrations at 10cm depth in the soil solution at Ysselsteyn.



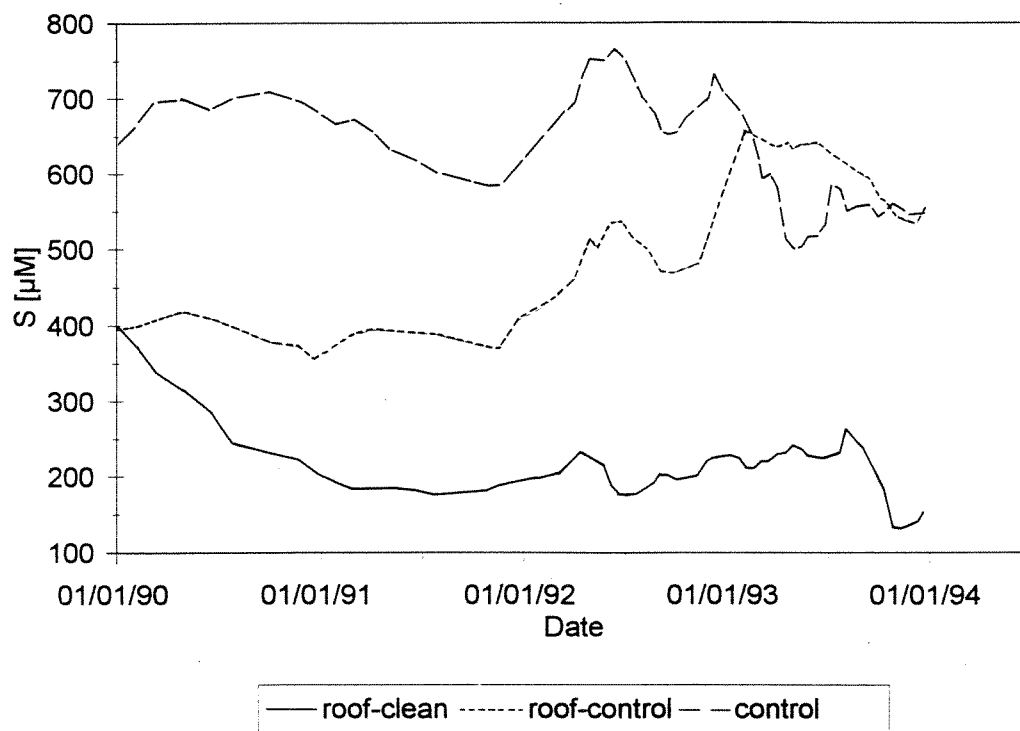
**Figure 4.** Total-S concentrations at 10cm depth in the soil solution at Ysselsteyn.



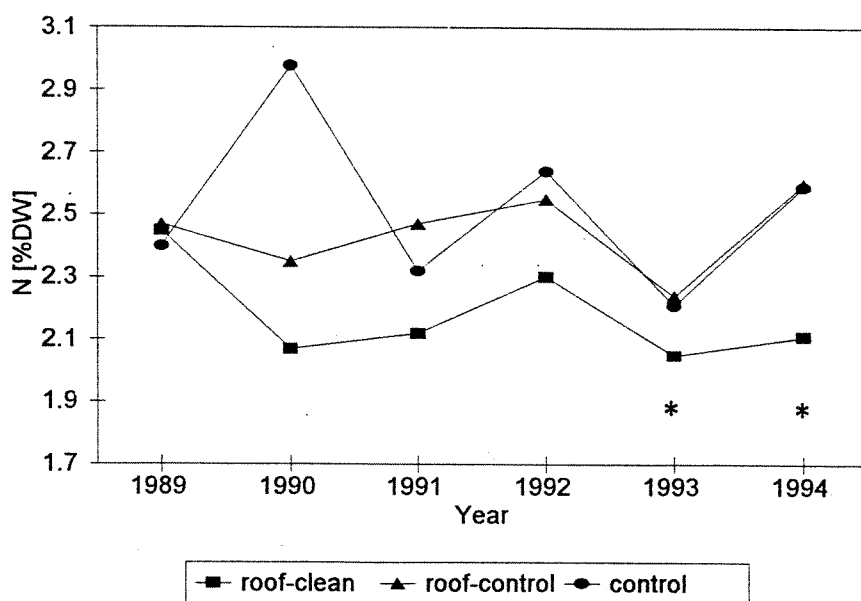
**Figure 5.** NH<sub>4</sub>/K ratio at 10cm depth in the soil solution at Ysselsteyn.



**Figure 6.** Nitrate concentrations at 90cm depth in the soil solution at Ysselsteyn.



**Figure 7.** Total-S concentrations at 90cm depth in the soil solution at Ysselsteyn.



**Figure 8.** Nitrogen concentration in the  $\frac{1}{2}$ -year-old needles at Ysselsteyn. \*: roof-clean plot is significantly different from both control plots at  $p \leq 0.05$ .

Nitrogen and sulphur leaving the ecosystem at 90cm have decreased significantly as a result of the treatment. Because of this, the leaching of the accompanying cations (Al, Mg, Ca and K) was significantly reduced (Boxman *et al.*, 1995). This means that the mineral balance in the roof-clean plots improved.

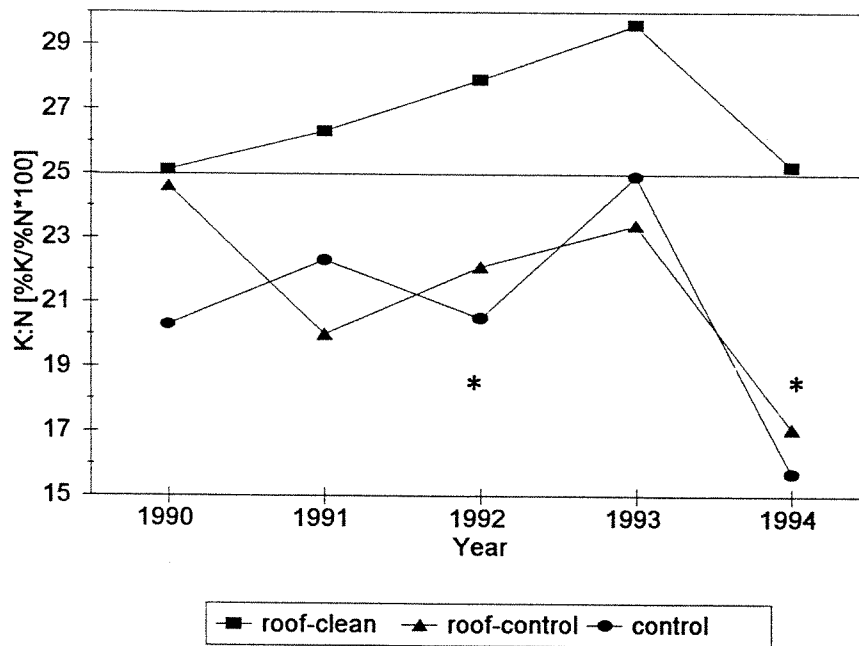
### **Nutrients in the needles**

Before the start of the manipulation experiment no significant differences were found in nutrient concentrations of the needles at both locations. At Ysselsteyn the needles had high nitrogen concentrations, whereas the other nutrient concentrations were very low. During the years of treatment the concentrations of nutrients varied considerably, which may be related to meteorological conditions (Boxman *et al.*, 1995 and references therein). The needles in the roof-clean plot responded to the treatment after a lag-time of approximately four years (Figure 8). The nitrogen concentration is still above 2%, which is considered as very high (optimal level is approximately 1.4-1.8%, Anonymous, 1990). The potassium and magnesium concentrations have increased significantly in the needles of the roof-clean plot as compared to both control plots (data not shown) and may be related to 1) a favourable  $\text{NH}_4^+/\text{NO}_3^-$  ratio in the soil solution, 2) a decreased leaching of base cations and 3) an increased root biomass (Boxman *et al.*, 1995). Consequently, the nutritional balance in the needles of the roof-clean plot has improved for potassium (Figure 9) and magnesium (data not shown) relative to nitrogen, the former even to a level above that is considered deficient ( $\text{K}:\text{N}>25$ , Anonymous, 1990).

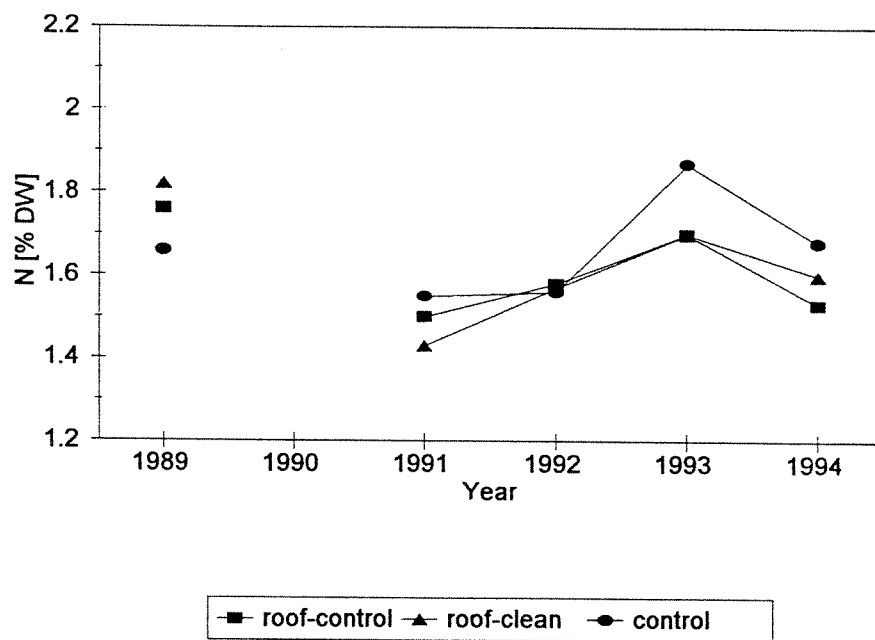
Although the ecosystem at Speuld is also nitrogen-saturated, the trees have normal nitrogen concentrations in their needles. In the control plot, however, nitrogen tends to increase (Figure 10). Since the trees are growing reasonably well, dilution effects in the needles may prevent nitrogen to become toxic. The nitrogen saturation effect is most pronounced in the older needles, which have higher nitrogen concentrations than the current needles (data not shown). Potassium, magnesium and calcium are sufficient in the needles, while phosphorus is somewhat low. No significant differences were found between the plots. As a result the nutritional balance of potassium (data not shown) and magnesium (Figure 11) relative to nitrogen have improved, although ratios are above the levels that are considered deficient (25 and 5, respectively; Anonymous, 1990).

Nitrogen taken up by the trees is incorporated into amino acids, and subsequently into proteins. The assimilation of ammonium is absolutely necessary as free ammonium is toxic because of its interference with many processes in the cell (Puritch and Barker, 1967; Wakiuchi *et al.*, 1970; Van der Eerden, 1982). If the rates of nitrogen uptake and subsequent amino acid synthesis exceed that of protein synthesis, free amino acids accumulate. Upon changes in nitrogen supply these amino acids show much greater relative changes in concentrations than the total nitrogen content. In coniferous trees arginine is most important in this respect, because of the low C/N ratio.

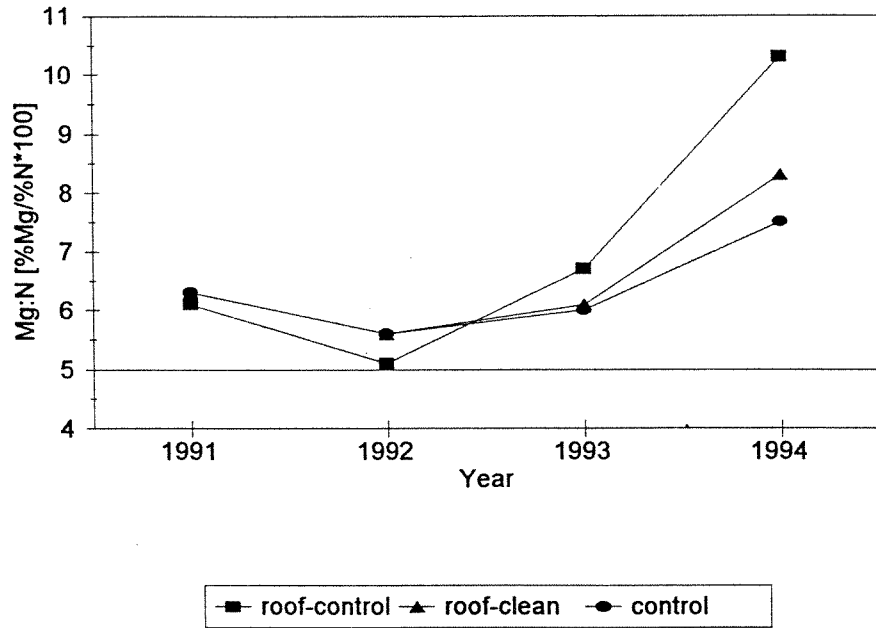
When the nitrogen concentrations in the needles increased, arginine seemed to accumulate at nitrogen concentrations above 1.5 to 1.6% (Figure 14).



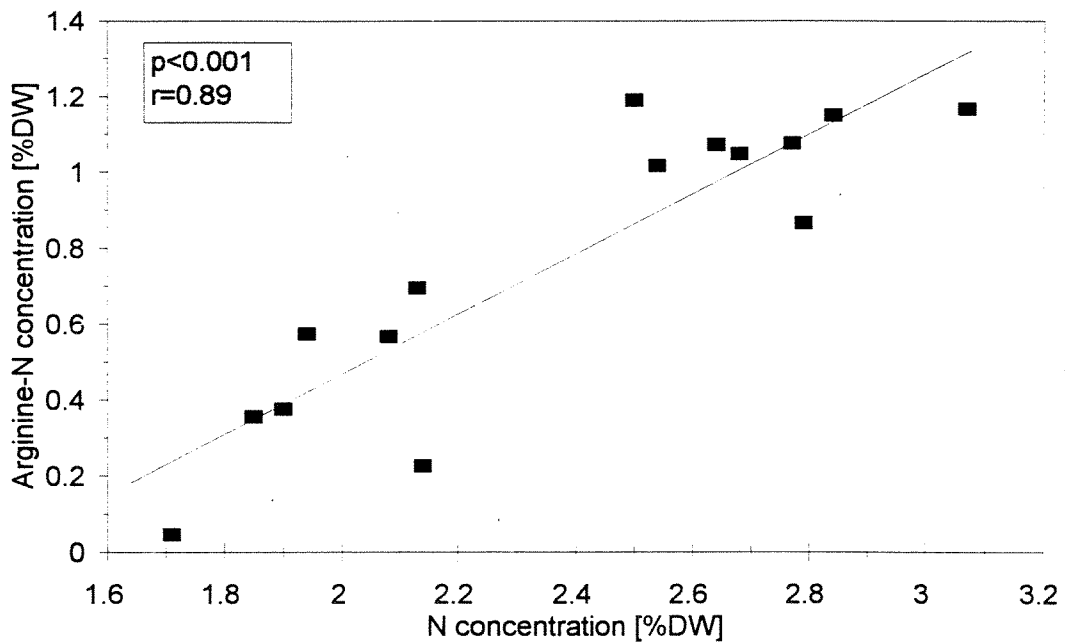
**Figure 9.** K:N balance in the 1/2-year-old needles at Ysselsteyn. \*: roof-clean plot is significantly different from both control plots at  $p \leq 0.05$ .



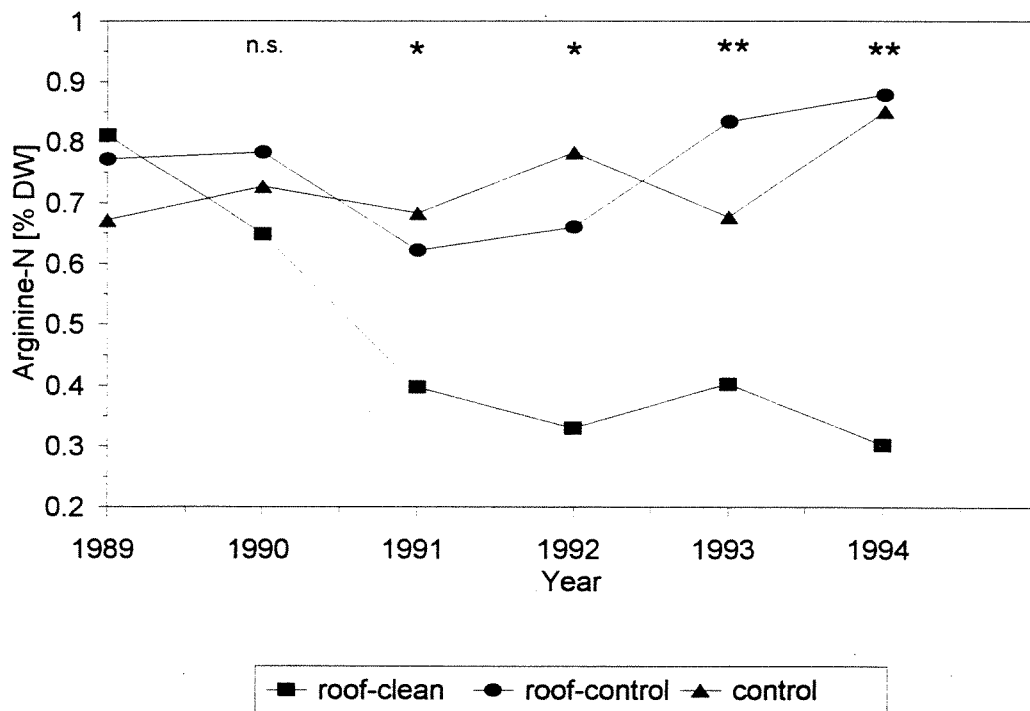
**Figure 10.** Nitrogen concentrations in the 1/2-year-old needles at Speuld.



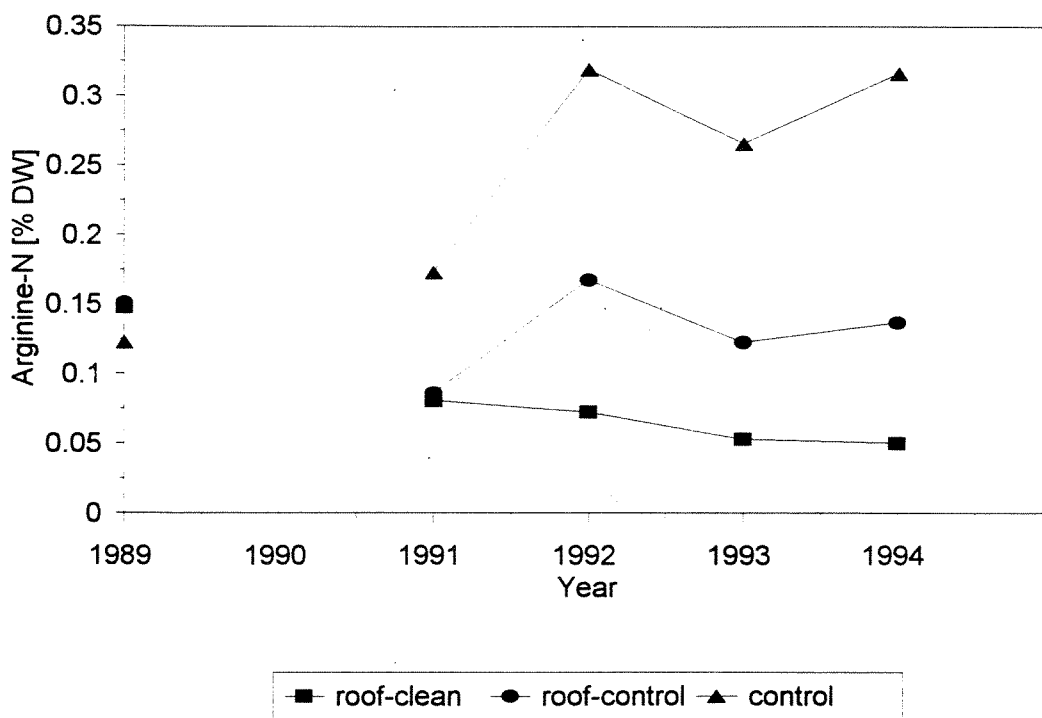
**Figure 11.** Mg:N balance in the ½-year-old needles at Speuld.



**Figure 12.** Correlation between the total-N and arginine-N concentration in the ½-year-old needles (collected January 1994) at Ysselsteyn.



**Figure 13.** Free arginine-N concentrations in the 1/2-year-old needles at Ysselsteyn. Roof-clean plot is significantly different from both control plots at \*:  $p \leq 0.05$ , \*\*:  $p \leq 0.01$ , n.s.: not significant.



**Figure 14.** Free arginine-N concentrations in the 1/2-year-old needles at Speuld.

Although total-N in the needles was still high, treatment significantly decreased the arginine concentration in the needles at both Ysselsteyn and Speuld. The response of arginine-N was fast: within one year changes could be observed. In this respect arginine can be regarded as a good indicator of detrimentally high ammonium deposition (Van Dijk and Roelofs, 1988; Ferm *et al.* (1990); Näsholm and Ericsson, 1990; Pietilä *et al.*, 1991).

Diameter growth of the dominant trees in the roof-clean plot improved ( $p=0.06$  for both roofed plots) and was inversely related to the arginine-N concentration in the needles (Figure 15). This is in agreement with the observation of Krauß *et al.* (1986) who found a clear reduction in growth in relation to inc

With respect to concentrations of arginine-N reported in the needles from pristine areas in northern Scandinavia ( $<0.06\%$ ), concentrations in the needles of Speuld were high (0.1-0.3%), but lower than at Ysselsteyn (0.65-0.9%). At the latter site this implies that almost 30% of total-N was stored as arginine-N.

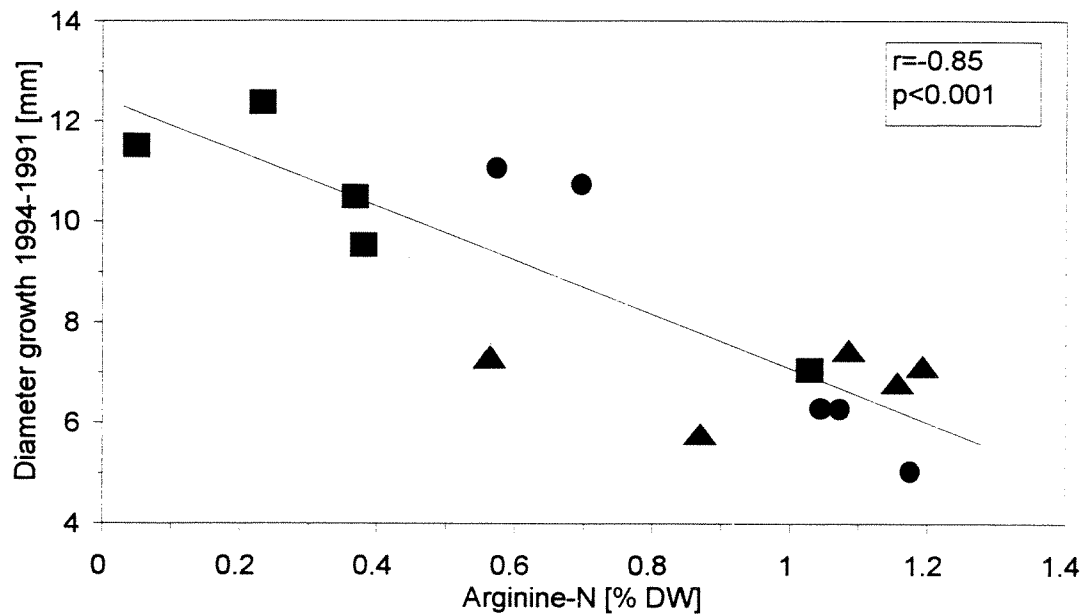
### **Fine-root growth**

A survey in 1992 revealed a significantly increased fine-root biomass and number of fine-root tips in the roof-clean plot at Ysselsteyn (Boxman *et al.*, 1995). Data from litter bag experiments of the Free University of Amsterdam confirmed these results. After one year more fine roots were grown into the litter bags, contained more root tips and had a higher degree of mycorrhizal infection (data not shown). These data suggest an enhanced uptake capacity of the roots, which is in accordance with the improved nutritional balance in the needles.

### **Ecosystem response**

Decreasing the input of nitrogen and sulphur strongly reduced the output of nitrate, sulphate, aluminium and base cations from both ecosystems, implying a tight input-output coupling. The N-cycle changed from open to more closed, indicating reversibility of nitrogen-saturation. However, the soil still contains a large amount of immobilized nitrogen and it is as yet still uncertain what will happen with this amount in the future. Both ecosystems are recovering from excess nitrogen availability, indicated by 1) a decreased leaching of base cations, 2) a more favourable  $\text{NH}_4^+/\text{NO}_3^-$  ratio in the soil solution, 3) increased root and 4) shoot growth, 5) a decrease in total-N and arginine-N in the needles and 6) an enhanced nutrient uptake and an improved nutritional balance in the needles.





**Figure 15.** Correlation between arginine-N concentration in the  $\frac{1}{2}$ -year-old-needles (collected in January 1994) and diameter growth of five dominant trees at Ysselsteyn. ■: roof-clean;  $\Delta$ : roof-control; ●: control.

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## 2. $^{15}\text{N}$ POOL DILUTION EXPERIMENTS

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### METHODS

#### $^{15}\text{N}$ pool dilution experiment with material from five NITREX sites

In April 1994, the organic layer of the control plot in the NITREX sites Gårdsjön, Klosterhede, Solling, Aber and Speuld was sampled. The  $^{15}\text{N}$  enrichment experiment carried out with this material (approximately the same method as described below) allowed the determination of the gross nitrogen (N) transformation rates along the N saturation gradient of the NITREX sites. A publication presenting this experiment is being prepared.

#### $^{15}\text{N}$ pool dilution experiment with material from the two Dutch NITREX sites

In May 1992, the organic layer of the low and high deposition plot in Speuld and Ysselsteyn were sampled. The material was incubated after addition of either  $^{15}\text{NH}_4^+$  or  $^{15}\text{NO}_3^-$ . After 0, 2, 5, 9 and 14 days of incubation, the atom%  $^{15}\text{N}$  in  $\text{NH}_4^+$  and  $\text{NO}_3^-$ , the total  $\text{NH}_4^+$  and  $\text{NO}_3^-$  concentration, the respiration rate and the microbial carbon (C) content were determined. A dynamic C and N transformation model was made to fit the experimental data. The model was based on the NIICCE model (Van Dam and Van Breemen, in press.) and contained three pools of C and organic N (labile organic matter, refractory organic matter and microbial biomass) and two pools of inorganic N ( $\text{NH}_4^+$  and  $\text{NO}_3^-$ ). By fitting the model on the  $^{15}\text{N}$  enrichment data, gross N transformations as well as microbial N and C parameters could be determined in these two N saturated sites at the two different deposition levels. The experiment has been reported in Tietema and Van Dam (in review).

#### $^{15}\text{N}$ tracer experiment in the two Dutch NITREX sites

During the period of May 1992 to May 1993,  $^{15}\text{N}$  was added to the throughfall applied on the low and high deposition plot in the two Dutch sites Speuld and Ysselsteyn. The fate of this  $^{15}\text{N}$  within the ecosystems was followed by sampling the pools and fluxes according to a common NITREX protocol. All pools and fluxes were sampled in both manipulated plots as well as in the control plot in the winters of 1992, 1993 and 1994, about 4 months before and 9 and 21 months after the start of the  $^{15}\text{N}$  application. Soil water at 90 cm depth was sampled fortnightly and bulked for three month periods from February 1992 until January 1994. The solid samples were analyzed for atom%  $^{15}\text{N}$  and total N; the liquid samples for atom%  $^{15}\text{NH}_4^+$  and  $^{15}\text{NO}_3^-$  and total  $\text{NH}_4^+$  and  $\text{NO}_3^-$  concentrations. Using the total N pool sizes, the distribution of the  $^{15}\text{N}$  within these pools could be calculated. The experiment will be reported in Koopmans *et al.* (in prep.).

### RESULTS

#### $^{15}\text{N}$ pool dilution experiment with material from five NITREX sites

Net N mineralization rates varied between 5.8 (Gårdsjön and Solling) and 8.9 (Klosterhede)  $\text{mg N kg}^{-1} \text{ day}^{-1}$  (Table 1). Only in Speuld and Solling could net production of  $\text{NO}_3^-$  be

measured. Gross  $\text{NH}_4^+$  mineralization and immobilization rates were highest in Aber (25.1 and 17.9, respectively) and in Speuld (22.9 and 16.6), leading to the fastest turnover of the  $\text{NH}_4^+$  pool at these sites. At none of the sites was significant immobilization of  $\text{NO}_3^-$  measured. The respiration rate was highest in Aber ( $388 \text{ mg C kg}^{-1} \text{ day}^{-1}$ ) and lowest in Klosterhede (123) and Gårdsjön (142) (Table 1).

<b>Table 1.</b> Net and gross N transformation and respiration rates in litter from several NITREX sites. All rates are in $\text{mg N (or C) kg}^{-1} \text{ day}^{-1}$ .						
process rates:	site:	Speuld	Solling	Aber	Klosterhede	Gårdsjön
net N mineralization		6.3	5.8	7.2	8.9	5.8
net nitrification		6.1	3.6	0	0	0
gross $\text{NH}_4^+$ mineralization		22.9	15.4	25.1	18.0	15.5
gross $\text{NH}_4^+$ immobilization		16.6	9.5	17.9	9.4	9.1
gross nitrification		6.1	3.6	?	0	0
gross $\text{NO}_3^-$ immobilization		0	0	?	0	0
respiration		306	200	388	123	142

### **$^{15}\text{N}$ pool dilution experiment with material from the two Dutch NITREX sites**

In general, the model could fit the experimental data within the range of observed means  $\pm$  S.D. Gross transformation rates were calculated with a variance of generally less than 10%. Gross  $\text{NH}_4^+$  transformation rates were slower at the Scots pine site than at the Douglas fir site (Table 2). At both sites reduced N-input resulted in increases in microbial biomass and in  $\text{NO}_3^-$  immobilization and autotrophic and heterotrophic nitrification rates. The  $\text{NH}_4^+$  immobilization rate per amount of microbial biomass decreased at reduced N deposition. The results indicated that gross nitrification at both sites was mainly (>75%) autotrophic nitrification.

**Table 2.** Modelling results for litter samples from the low and high deposition plots at Dutch NITREX sites. Simulated fluxes are means and standard deviations over the 14 days of the incubation experiment. MC = Microbial C; OM = Organic Matter.

	Ysselsteyn		Speuld	
	HIGH depo- sition	LOW depo- sition	HIGH deposition	LOW deposition
<b>Optimal parameter values</b>				
turnover time labile organic N (day)	10.9	86.6	3.6	46.7
turnover time refractory C (year)	3.24	2.61	2.91	3.84
turnover time NH <sub>4</sub> <sup>+</sup> (day)	13.4	8.1	6.7	7.4
NH <sub>4</sub> <sup>+</sup> -immobilization (mg N kg <sup>-1</sup> MC day <sup>-1</sup> )	17.4	14.0	14.9	7.5
NO <sub>3</sub> <sup>-</sup> -immobilization (mg N kg <sup>-1</sup> MC day <sup>-1</sup> )		0.54	0.04	0.16
heterotrophic nitrification (% of N min)	2.85	3.46	0.66	2.85
<b>Gross N fluxes (mg N kg<sup>-1</sup> OM day<sup>-1</sup>)</b>				
NH <sub>4</sub> <sup>+</sup> immobilization	16.8 ± 0.9	19.7 ± 1.7	33.1 ± 2.4	19.1 ± 1.5
NO <sub>3</sub> <sup>-</sup> immobilization	0.45 ± 0.18	0.75 ± 0.33	0.08 ± 0.02	0.41 ± 0.18
heterotrophic nitrification	0.60 ± 0.14	1.13 ± 0.45	0.33 ± 0.11	1.14 ± 0.24
autotrophic nitrification	3.00 ± 0.09	3.89 ± 0.20	2.53 ± 0.07	6.71 ± 0.19
NH <sub>4</sub> <sup>+</sup> mineralization	25.2 ± 1.2	31.4 ± 2.0	49.9 ± 2.9	38.9 ± 1.7
dissimilatory reduction of NO <sub>3</sub> <sup>-</sup> to NH <sub>4</sub> <sup>+</sup>	0.21 ± 0.06	0.28 ± 0.05	0.01 ± 0.00	0.11 ± 0.01

### <sup>15</sup>N tracer experiment in the two Dutch NITREX sites

In early 1993, nine months after the start of the <sup>15</sup>N application, 94 and 61% of the applied <sup>15</sup>N could be found in the investigated compartments and fluxes in the low and high deposition plot respectively at Speuld (Table 3). Almost 80% of the applied <sup>15</sup>N was retained in the organic-rich part of the soil in the low deposition plot compared to only 31% in the high deposition plot. In the latter plot 19% of the applied <sup>15</sup>N leached from the system during the first nine months, compared to only 2% in the low deposition plot. In the winter of 1994, nine months after the end of the <sup>15</sup>N application, the total amount of <sup>15</sup>N as a percentage of the <sup>15</sup>N applied found in these compartments was similar in both plots (71 and 86%). In the low deposition plot a relatively higher percentage of the <sup>15</sup>N applied was retained in the vegetation (31%) and in the soil (38%) compared to the high deposition plot (25 and 29%), whereas in the latter plot more <sup>15</sup>N was leached out of the system (33 compared to 2%). In the high deposition plot about 95% was leached as N-NO<sub>3</sub><sup>-</sup> (Table 3).

**Table 3.** Calculated  $^{15}\text{N}$  distribution for the Douglas fir site in Speuld. The numbers are percentages of the  $^{15}\text{N}$  applied as throughfall.

compartment / flux	low deposition plot		high deposition plot	
	retained 1993 (%)	retained 1994 (%)	retained 1993 (%)	retained 1994 (%)
<b>vegetation</b>				
needles (0.5 yr)	5.7	18.2	4.0	13.8
needles (1.5 yr)	5.7	10.4	5.8	9.1
twigs (0.5 yr)	0.5	0.9	0.4	0.8
twigs (2/3 yr)	0.8	1.4	0.5	0.9
<i>subtotal</i>	12.8	30.8	10.7	24.5
<b>soil</b>				
L horizon	22.9	5.6	13.5	2.9
F horizon	31.1	16.3	12.3	12.1
0-10 cm	25.3	16.0	5.5	13.8
<i>subtotal</i>	79.3	37.9	31.1	28.8
<b>leaching</b>				
NH <sub>4</sub> <sup>+</sup>	0.0	0.1	0.7	0.8
NO <sub>3</sub> <sup>-</sup>	1.6	2.0	18.3	32.3
<i>subtotal</i>	1.6	2.1	19.0	33.1
<b>total</b>	<b>93.7</b>	<b>70.8</b>	<b>61.0</b>	<b>86.4</b>

In Ysselsteyn, the same pattern was found (results not shown). The differences with Speuld were that in the Scots pine trees in Ysselsteyn a smaller proportion of the applied  $^{15}\text{N}$  was retained in the trees (2-12.5% in Ysselsteyn compared to 10-31% in Speuld). Also, in Ysselsteyn the differences in  $^{15}\text{N}$  retention between the high and low deposition plots were smaller in the vegetation and in leaching losses.

## DISCUSSION

The NITREX sites form a gradient of N saturation (Wright and Van Breemen, 1995). The Dutch site Speuld and the German site Solling are definitely N saturated with high ( $> 20 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ ) leaching losses of NO<sub>3</sub><sup>-</sup> comprising 50 - 60% of inputs (Tietema and Beier, 1995). In Aber, the Welsh site, NO<sub>3</sub><sup>-</sup> losses in the control site are moderately low (approximately  $8 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ ) (Emmett *et al.* 1995). However, this relatively low N output comprises about 50% of N input. Also, the extra amounts of N added to the system are for a large part leached out of the system, clearly indicating N saturation: the inability of the biota to retain the extra N. The two Scandinavian sites Klosterhede and Gårdsjön are N limited with very low NO<sub>3</sub><sup>-</sup> leaching.

The results show a general pattern of higher gross NH<sub>4</sub><sup>+</sup> transformation, higher respiration and higher net nitrification rates in N<sup>-</sup> saturated sites compared to N<sup>-</sup> limited sites. Exceptions to this general pattern were Solling with relatively low gross NH<sub>4</sub><sup>+</sup> transformation rates and

Aber with no net nitrification. The latter observation was not in agreement with the results of a year-round field incubation experiment which indicated net nitrification (Emmett *et al.* 1995). As conditions in lab incubations are generally more optimal for microbial transformations compared to field incubations, this discrepancy can only be explained by specific conditions in the material connected to the time of sampling. In Solling gross  $\text{NH}_4^+$  transformation rates were similar to those at N-limited sites, whereas the respiration rate was intermediate. In all sites gross  $\text{NO}_3^-$  immobilization rates were negligible. This finding was especially surprising in Klosterhede and Gårdsjön where microbial activity was expected to be strongly regulated by N and thus also  $\text{NO}_3^-$  availability.

The results from the  $^{15}\text{N}$  pool dilution experiment indicated differences between the Scots pine site in Ysselsteyn and the Douglas fir site in Speuld; gross  $\text{NH}_4^+$  transformation rates were much higher in the high deposition plot in Speuld than in Ysselsteyn. As Ysselsteyn is a highly N-saturated site with very high atmospheric N inputs, this seems to contradict the findings of the  $^{15}\text{N}$  pool dilution experiment with the five NITREX sites. In this latter experiment, gross  $\text{NH}_4^+$  transformation rates were higher in the N-saturated sites compared to the N-limited sites. At Ysselsteyn the excessive N-saturation has apparently led to conditions where the vitality and functioning of biota have been negatively influenced. In the vegetation this is illustrated by very high arginine levels in the needles, the occurrence of only two year classes of needles on the trees, and the extremely low fine-root biomass (Boxman *et al.* 1995). In the soil microbial community this is less obvious. Net N mineralization rates measured in a year-round field incubation study are even higher in Ysselsteyn than in Speuld (Koopmans *et al.* 1995a), but to our results indicate effects on gross  $\text{NH}_4^+$  transformation rates. The difference between vegetation and microbial community in a forest stand is the ability of the latter to adapt to changes in the environmental conditions as a result of the large diversity of the microbial community. From the microbial parameters determined with the  $^{15}\text{N}$  pool dilution experiment, it appears that bacteria may have a more prominent contribution at Ysselsteyn compared to Speuld. At Ysselsteyn as a result of decades of extremely high atmospheric N inputs, a shift to a community more dominated by bacteria has led to the lower gross  $\text{NH}_4^+$  transformation rates.

The  $^{15}\text{N}$  tracer experiment underlined the lower vitality of the Scots pine trees at Ysselsteyn relative to the Douglas fir trees at Speuld; the trees at Ysselsteyn retained only 2-4% of the applied  $^{15}\text{N}$  during the first growing season compared to the trees at Speuld 11-13%. The comparison between the low and the high deposition plot at both sites revealed a higher percentage of the  $^{15}\text{N}$  applied retained in the low deposition plot and a lower percentage leached out of the system. An exception is the vegetation at Ysselsteyn. Here a slightly higher percentage was retained by the trees in the high deposition plot. The results indicate that the decreased N deposition has indeed influenced the biota. The higher retention of deposited N indicates a change towards a more conservative strategy of the biota concerning N and thus a shift from N saturation towards N limitation.



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### 3. EFFECTS OF MANIPULATION OF NITROGEN DEPOSITION ON LITTER DECOMPOSITION AND SOIL BIOTA: A CROSS-SITE COMPARISON

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#### INTRODUCTION

Excessive nitrogen deposition affects the decomposition of organic material and nutrient flow in forest ecosystems (Fog, 1988). Soil organisms play an important role in the process of decomposition and contribute to the availability of nutrients (Swift *et al.*, 1979). High nitrogen input has detrimental effects on soil biota. When nitrogen is added to decomposing material, microbial activity is inhibited (Fog, 1988), and the abundance, diversity and contribution to mineralization by soil animals are negatively affected (Verhoef & Brussaard, 1990; Verhoef & Meintser, 1991; Boxman *et al.*, 1995). Microflora is a main food source for most of the soil fauna, and thus the negative effects on microflora caused by excessive nitrogen addition, may negatively affect soil fauna. As a result decomposition and mineralization rates may decrease.

This study is part of a research in which the effects of manipulation of nitrogen deposition on the soil biota (soil fauna and microflora) that affects decomposition is investigated in coniferous forests. In the present study results are presented on decomposition and microflora, conducted at the sites Ysselsteyn, the Netherlands (3 plots: roof, roof control, control), Klosterhede, Denmark (3 plots: roof, control, addition) and Gårdsjön, Sweden (3 plots: roof, control, addition) from May 1993 till May 1994. Site and treatment descriptions are given by Dise and Wright (1992). The 3 sites have different characteristics. From the Netherlands to Sweden ambient nitrogen deposition declines, precipitation increases and mean annual temperature declines. Furthermore, Ysselsteyn has a Scots pine (*Pinus sylvestris* L.) stand which is nitrogen saturated, while Klosterhede and Gårdsjön have Norway spruce (*Picea abies* L.) stands which are not saturated.

Litterbags (L, Aoo, mesh size 4 mm for pine and 2 mm for spruce) and fragmentation bags (F, Ao, mesh size 2 mm) were placed in the field as stratified litterbag sets. The litterbags in each plot were randomly distributed, side by side in rows, placed in an area of 1.5 x 2 m and with in a 10-cm strip of the original organic layer. The litterbag sets were randomly sequentially removed after 2, 4, 6, 8, 10 and 12 months. Each litterbag set had 12 replicates which were all analyzed for weight loss. Eight replicates were used for extraction of soil fauna (in the process of being analysed), while the other four were used for determining microbial abundance by means of fluorescence microscopy (FITC (fluorescein isothiocyanate) for fungi, DTAF (dichloro-triazin-aminofluorescein) for bacteria).

#### RESULTS

Over the 1-year period pine needle material of the fragmentation layer in all three plots at Ysselsteyn did not decompose (% dry weight loss, Fig. 1). Klosterhede and Gårdsjön

however, showed small weight losses. No effect of reduced nitrogen deposition (control -> roof) was found for decomposition in the F-layer at the 3 sites. For the L-layer, Scots pine needles decomposed faster than Norway spruce needles. Reduced nitrogen deposition did not affect decomposition of this layer at any of the 3 sites.

In Ysselsteyn, both fungi (Fig. 2) and bacteria (Fig. 3) are more abundant in the L-layer, while in Klosterhede and Gårdsjön fungi and bacteria are more abundant in the F-layer. The highest numbers are present in the winterperiod (November till March) and are the same in all plots in all sites for both layers. A reduction of nitrogen deposition results in a small decrease of the amount of fungi and bacteria in both layers in all 3 sites.

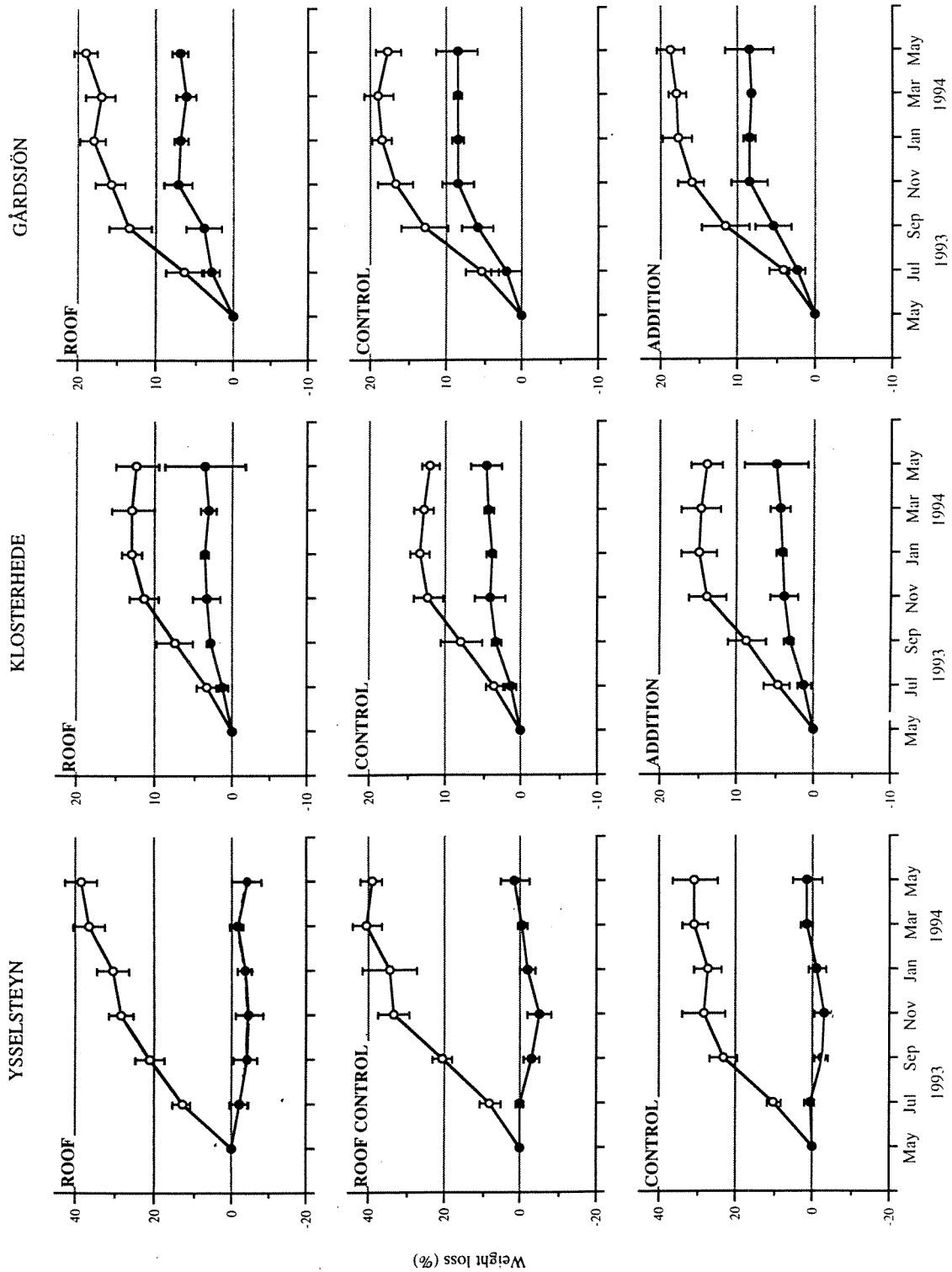
## CONCLUSIONS

Comparing the sites, the main contribution to the process of decomposition changes from the L- to the F-layer going towards the north. In the Netherlands the F-layer does not decompose at all, while in Sweden 33% of the organic material is decomposed in the F-layer. This coincides with an increase of microbial abundances in the F-layer from the Netherlands to Sweden. The much higher weight loss of the L-layer in Ysselsteyn relates to the different tree species. Scots pine needles decompose faster than Norway spruce needles, as spruce needles have a high content of lignin, as well as a resistant waxy surface. However, this does not explain the differences found for the F-layer. Although the process of decomposition is related to soil moisture and soil temperature, the differences found for the F-layer are probably not due to climatic influences. The Dutch site has more favourable climatic conditions for decomposition but shows no weight loss in the F-layer and a low amount of microflora. However, the differences in the F-layer could be explained by the amount of ambient nitrogen deposition, which in the Netherlands is 5 times higher than in Sweden. In literature reviews (Fog, 1988; Kuyper, 1989) added nitrogen is often beneficial to early stages (litter) of the decomposition process, but inhibits later stages (fragmentation), while microflora activity is reduced when nitrogen is added. However, for explaining the differences other factors, such as pH and soil fauna (abundances and composition), can not be ruled out.

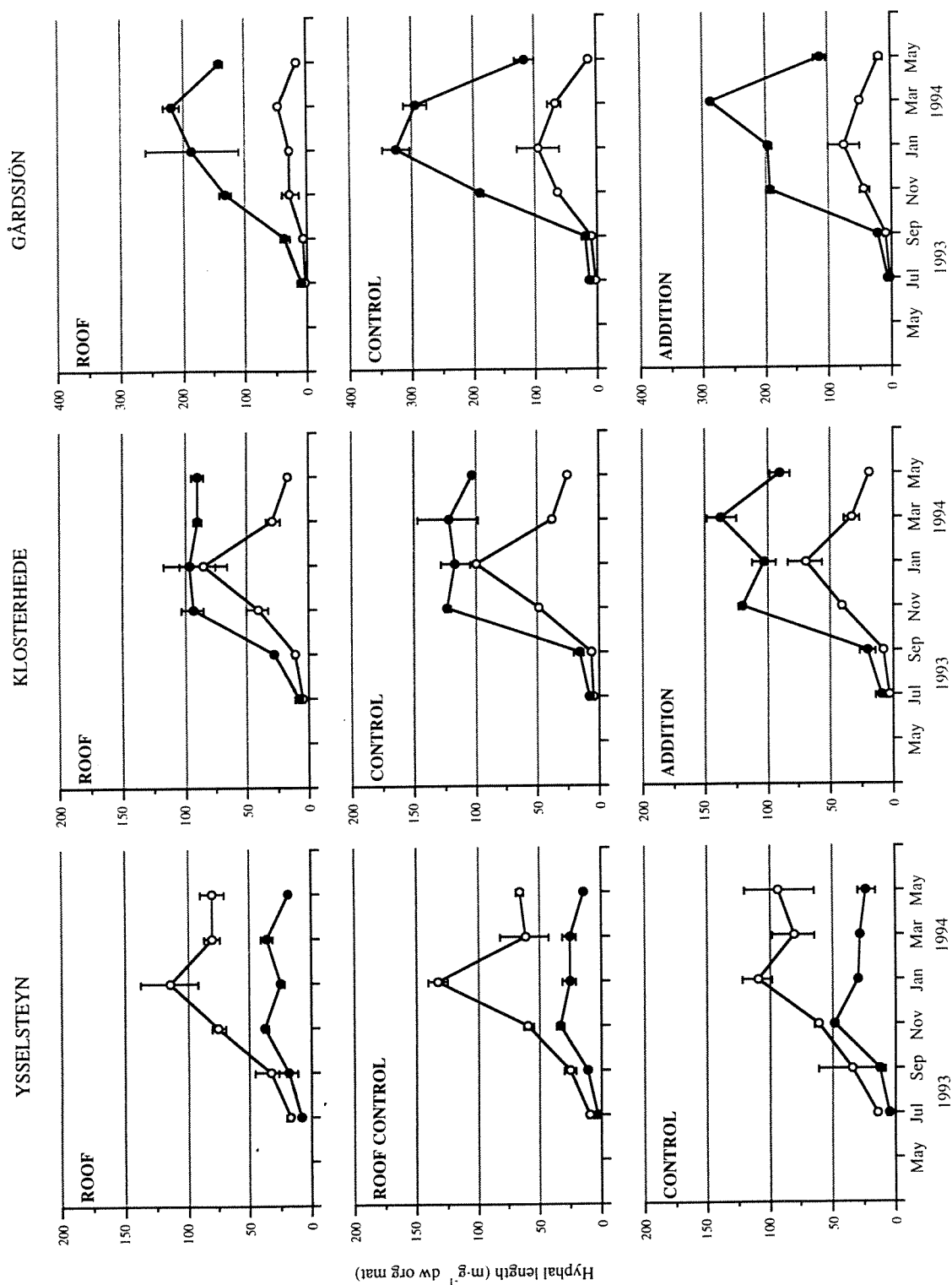
Concerning the reduction of nitrogen deposition, each site showed the same pattern. First, nitrogen reduction had no effect on the decomposition rate, neither in the L- nor in the F-layer. From the reviews cited above a decrease of the weight loss when nitrogen input is reduced might be predicted, although there are also studies in which nitrogen had no influence on decomposition rates. Secondly, decreased nitrogen deposition caused a reduction of the abundances of fungi and bacteria in both layers. The reduced numbers of microflora might be explained by a change in soil fauna. Diversity of soil fauna is known to increase when nitrogen deposition is reduced (Boxman *et al*, 1995). This diversity increase occurred in winter, at the time of the main differences in microflora. Changes in abundance and composition of soil fauna species may increase grazing pressure resulting in lower amounts of total microflora.

Addition of nitrogen (Klosterhede and Gårdsjön) did not change the decomposition rate, although enhanced decomposition was found after nitrogen addition in literature cited above. Nor did addition of nitrogen change the amount of microflora. Long-term differences in

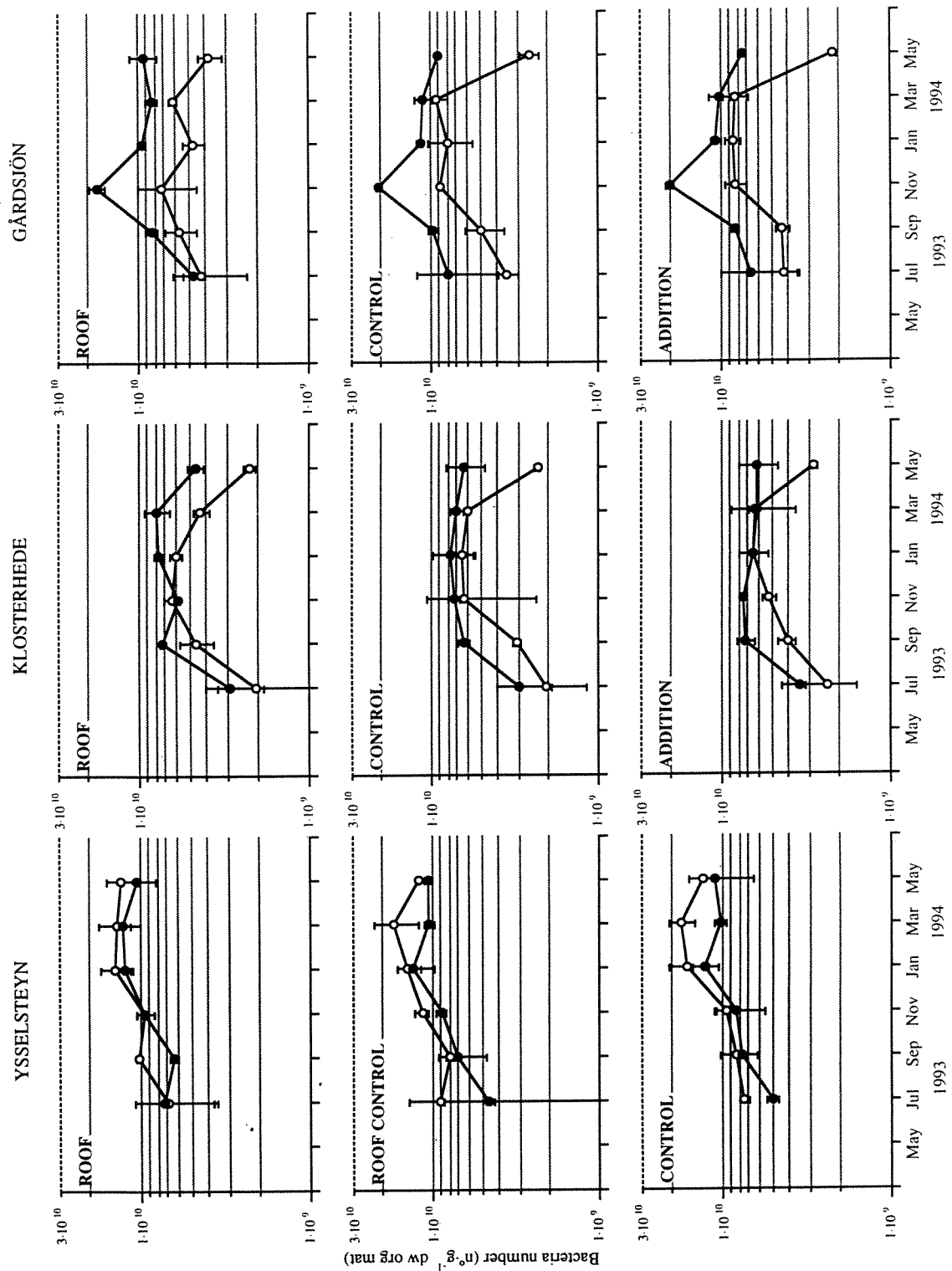
nitrogen deposition (between sites) have more pronounced effects on the ecosystem than short-term manipulation of nitrogen deposition (within sites).



**Figure 1.** Bi-monthly dry weight loss (5) of Scots pine (Ysselsteyn) and Norway spruce needles (Klosterhede and Gårdsjön) for the L (-o-) and F (-o-) layer during one year in three treatments differing in site-specific nitrogen deposition. Bars indicate standard deviations (n=12). Note the different scale of the y-axes.



**Figure 2.** Bi-monthly fungal hyphae length ( $\text{mg}^{-1}$  dry weight organic material) in the L (-o-) and F (-o-) layer of Scots pine (Ysselsteyn) and Norway spruce needles (Klosterheide and Gårdsjön) during one year in three treatments differing in site-specific nitrogen deposition. Bars indicate standard deviations ( $n=2$ ).



**Figure 3.** Bi-monthly numbers of bacteria ( $\text{n}^\circ\text{g}^{-1}$  dry weight organic material) in the L (-o-) and F (-o-) layer of Scots pine (Ysselsteyn) and Norway spruce needles (Klosterhehe and Gårdsjön) during one year in three treatments differing in site-specific nitrogen deposition. Bars indicate standard deviations ( $n=2$ ).

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#### 4. EFFECTS ON ENHANCED N DEPOSITION IN A SPRUCE FOREST AT KLOSTERHEDE, DENMARK, EXAMINED BY MODERATE $\text{NH}_4\text{NO}_3$ ADDITION

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#### MATERIALS AND METHODS

The fate and effects of increased N deposition were investigated by experimental manipulation in a mature Norway spruce plantation at Klosterhede, western Denmark. Ambient N deposition was  $23 \text{ kg N ha}^{-1}\text{yr}^{-1}$ . Addition of  $35 \text{ kg N ha}^{-1}\text{yr}^{-1}$  as  $\text{NH}_4\text{NO}_3$  to a  $500\text{-m}^2$  plot was carried out by handspraying monthly simulating a 2.5 increase of N deposition. The addition plot was compared to 3 parallel control plots. For soil solution sampling there were, however, 5 sets of samplers within the control plots.

The forest stand is second generation after heathland with 860 trees/ha and a basal area of  $29 \text{ m}^2$ . The soil type is a Typic Haplorthod (podzol). The soil is coarse sand with low base saturation. An organic layer of 7 cm with C/N ratio 33 has developed during the current rotation. Further experimental details are found in Gundersen and Rasmussen (1995). Here results from the pre-treatment period and the first 2.5 years of N addition are presented.

#### RESULTS AND DISCUSSION

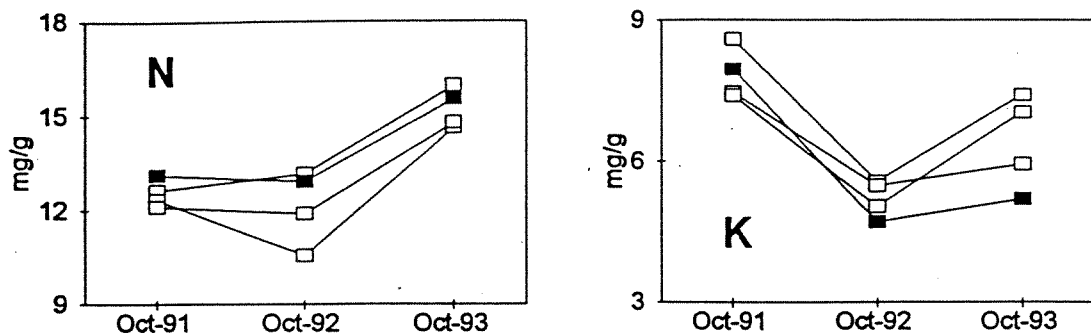
##### Tree response

The N treatment caused no detectable response on diameter growth, shoot length or needle weight during the first two growing seasons. But for both growing seasons tree growth was determined by very low water availability (drought) in the spring. Further there was no change observed in the N content of the needles (Figure 1a) although the foliage was considered N deficient. Only for K there was a significant decrease in the content of the needles compared to the controls (Figure 1b). The K/N ratio decreased from 0.6 before the treatment to 0.35 after treatment during two growing seasons. A K/N ratio below 0.3-0.4 may be considered as critical for the nutritional balance (Nihlgård, 1990). The decreased K content in the needles may result from an increased competition between  $\text{NH}_4^+$  and  $\text{K}^+$  in root uptake (Boxman *et al.*, 1991), since the soil water concentrations of  $\text{NH}_4^+$  after N addition were increased in the organic layer where most of the fine roots are present. In a complementary experiment in the Netherlands, where N deposition was decreased by a roof installation, an improvement of the K/N ratio and other nutrient ratios were found in the first years of treatment (Boxman *et al.*, 1995).

##### Decomposition and mineralization response

Needle litter decomposition was studied in litter bags in the N addition plot and in one of the control plots (2 plots x 5 sets x 15 replicates). The litter bags were incubated from the start of the treatment and retrieved after 3, 6, 9, 12, 24 months. Weight loss and N content were determined.





**Figure 1:** Content of a) nitrogen and b) potassium in current year needles (composite sample from 5 randomly selected trees) on the N addition plot (■) and 3 control plots (□).

The weight loss was not affected by the N treatment at any incubation time, but the N content of the remaining litter was increased on the N addition plot. The weight loss increased with i) increased distance to the nearest trunk, ii) increased moss cover, iii) and to some extent by a low position in old plough furrows. Therefore most of the variability of the decomposition was due to differences in moisture conditions. In a Sitka spruce forest Emmett *et al.* (1995) also found that decomposition did not respond to a similar increase of N deposition.

Mineralization was studied during 14 months (second year of treatment) by a sequential *in situ* incubation technique (e.g. Tietema *et al.*, 1990) in the N addition plot and in one control plot. Two intact soil cores (10 cm diameter) of the organic layer were sampled side by side in three replicates per plot. One soil core from each pair was taken directly to the laboratory for determination of the initial conditions, the other core was closed on top and left in the field for incubation in one month (3 months during winter). In the laboratory the cores were separated in L+F layer and H layer. From each layer 10 g fresh soil was used for determination of water content and 10 g (2 replicates) was extracted in 1 M KCl for determination of  $\text{NH}_4$  and  $\text{NO}_3$  concentrations. The accumulation of  $\text{NH}_4$  and  $\text{NO}_3$  in the incubated cores was taken as an estimate of net ammonification and net nitrification rates, respectively.

The results for the  $\text{NH}_4$  accumulation (Figure 2) showed no clear effect of the treatment. However, a possible treatment effect may be hidden by a within-plot variability and by a number of obscure results, which are currently being rechecked. The method is restricted by the large number of operations behind each data point. However, the calculated ammonification flux for a whole year increased from  $15 \text{ kg N ha}^{-1}\text{yr}^{-1}$  in the control plot to  $25 \text{ kg N ha}^{-1}\text{yr}^{-1}$  in the N addition plot. A drought in the spring 1993 inhibited the mineralization (Figure 2).

The soil was not accumulating  $\text{NO}_3$  (not nitrifying) except for a 'hot spot' in the N addition plot. This may be a result of the treatment or perhaps a type of hot spot was present in the control plot as well.

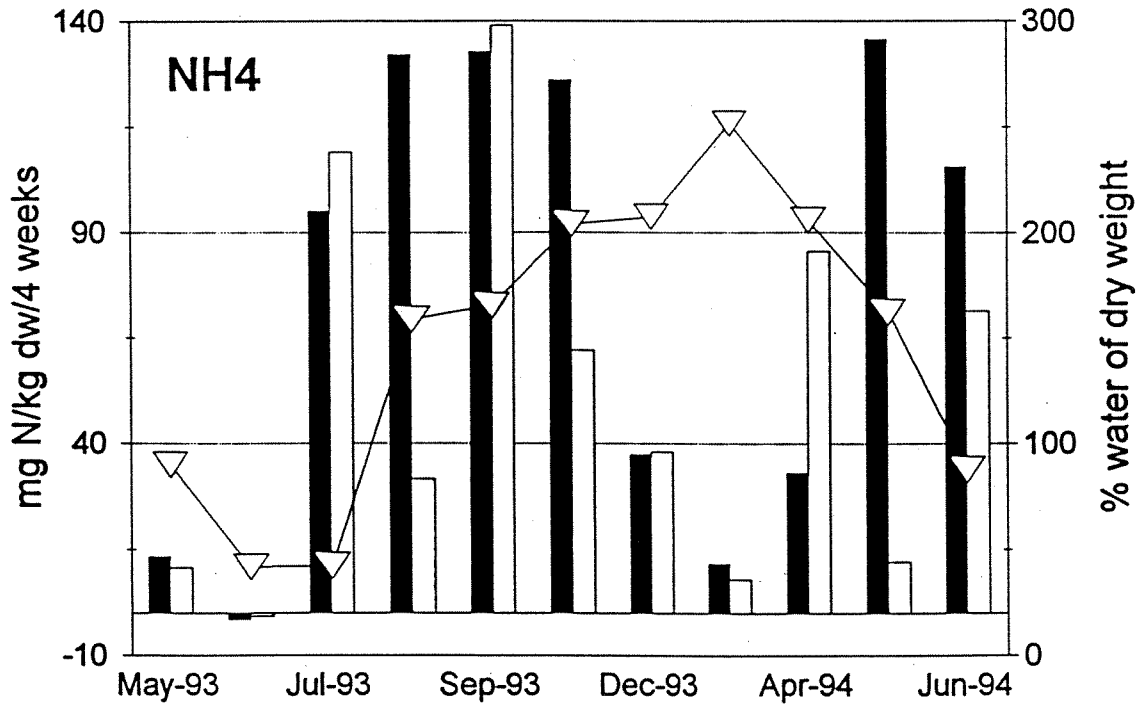


Figure 2: Seasonal pattern of the ammonium accumulation in the L+F layer (bars, left axis); mean of 3 replicates in the N addition plot (filled bar) and in one control plot (open bar). Soil moisture content is shown as a curve ( $\nabla$ , right axis).

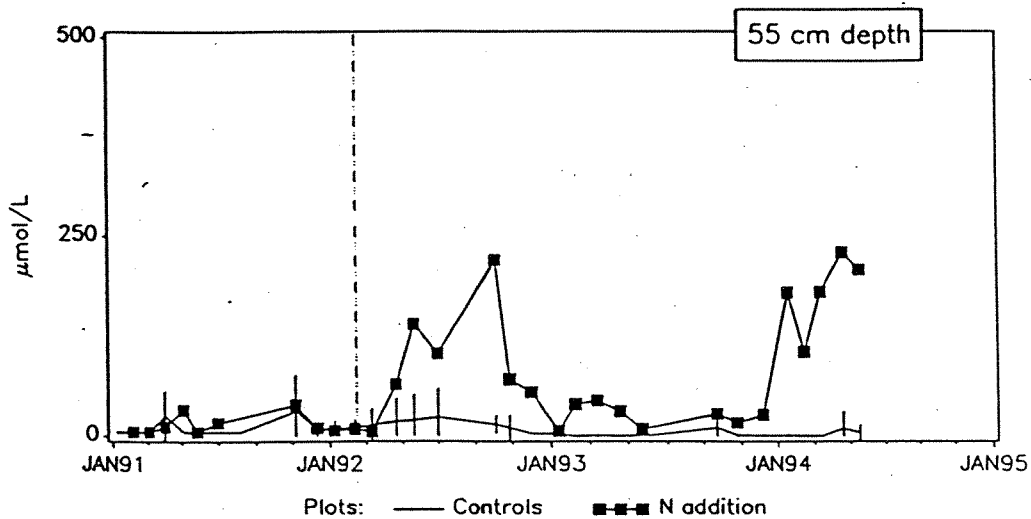


Figure 3: Nitrate concentration in soil water at 55 cm depth in the N addition plot (1 set of samplers;  $\blacksquare$ ) and in the control plots (5 sets of samplers; mean  $\pm$  std).

### Soil solution response

The soil solution chemistry responded promptly to the N application (Figure 3). Nitrate concentrations increased at all depths, and  $\text{NO}_3$  leaching increased from 0.3 to 2.3 kg N  $\text{ha}^{-1}\text{yr}^{-1}$ . Nitrate leached during winter and spring when the water transport was at the highest. Despite the increased  $\text{NO}_3$  leaching, 92% of the  $\text{NO}_3$  input was retained. The  $\text{NH}_4$  input was completely retained within the system, but soil water concentrations of  $\text{NH}_4$  increased below the organic layer and at 15 cm depth on the addition plot. An increase of exchangeable  $\text{NH}_4$  could account for 20 % of the added  $\text{NH}_4$  on the addition plot. No changes in concentrations of other major ions were detectable due to the N addition.

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## 5. EFFECTS OF ENHANCED NITROGEN DEPOSITION IN A SITKA SPRUCE STAND IN UPLAND WALES

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### INTRODUCTION

The Aber forest nitrogen critical load experiment was established to assess the effects of enhanced nitrogen deposition in both oxidised and reduced forms of nitrogen in a mature Sitka spruce stand in north Wales, UK. The site represents an intermediate point in the pollution gradient across which the NITREX series of nitrogen manipulation experiments have been established (Wright et al. 1995).

### METHODS

Nitrogen is applied weekly as a spray to the forest floor in combination with deionised water in a replicated block design experiment. Treatments are: sodium nitrate at 35 kgN ha<sup>-1</sup> yr<sup>-1</sup> (SN35), sodium nitrate at 75 kgN ha<sup>-1</sup> yr<sup>-1</sup> (SN75), ammonium nitrate 35 kgN ha<sup>-1</sup> yr<sup>-1</sup> (AN35), a no-spray control (C) and a water only or zero-N spray control (ZN). Soil water chemistry at three depths has been monitored and dissolved nitrogen leaching losses calculated using appropriate soilwater concentration data in combination with water fluxes quantified using a hydrological model for the site. The effect of nitrogen treatments on soil nitrogen transformations were determined using a combination of field and laboratory techniques. Litter decomposition rates were assessed using both a standard litter and litter collected from with the experimental plots. Effects on the growth and health of the trees were assessed using basal area growth increment techniques, foliar analysis and determination of fine root biomass. All methods are described in detail in Emmett et al. (1995a & b)

### RESULTS AND DISCUSSION

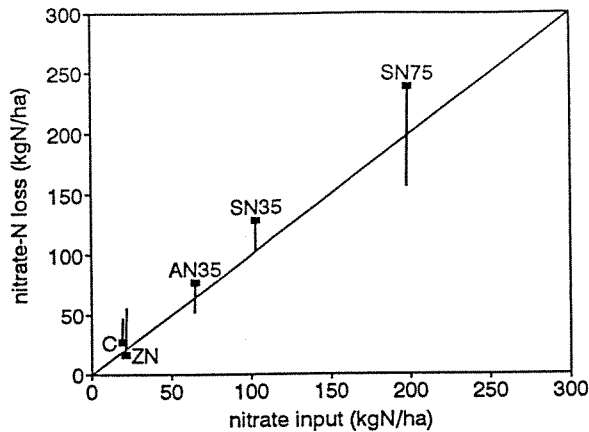
#### Soil water chemistry

Leaching losses of 100% of applied nitrate was observed after 2.5 years of nitrogen additions (Figure 1a) (Emmett et al. 1995a). In contrast, applied ammonium was retained in the ecosystem (Figure 1b). Immobilisation in the forest floor was observed in year 1 and in the lower mineral soil in year 2. Total dissolved-N leaching losses were therefore lower in the AN35 treatment relative to the SN35 treatment due to this ammonium retention (Figure 1c).

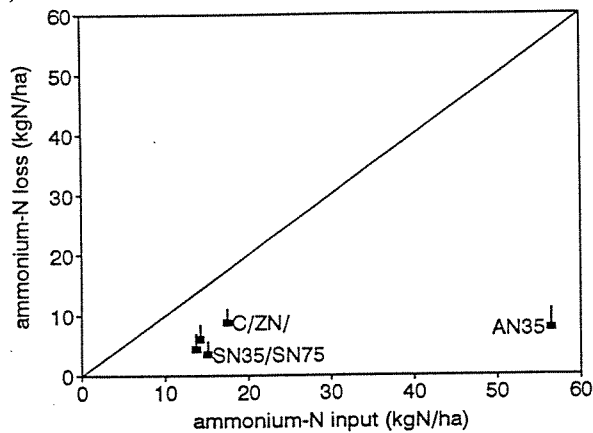
As the soil exchange complex is dominated by aluminium, the increase in salt loading and lack of nitrate retention in all treatments was associated with increased aluminium concentrations due to ion exchange between the incoming sodium or ammonium ions and

aluminium on the soil exchange complex (Emmett et al. 1995a). The fast response to nitrate addition at the Aber site contrasts with the low leaching losses observed in nitrogen addition experiments in more nitrogen-limited forest stands within the NITREX project (Wright and van Breemen 1995). These findings suggest that any increase in oxidised nitrogen deposition will result in increase nitrate and aluminium leaching at Aber, which can perhaps be best described as 'nitrate saturated'.

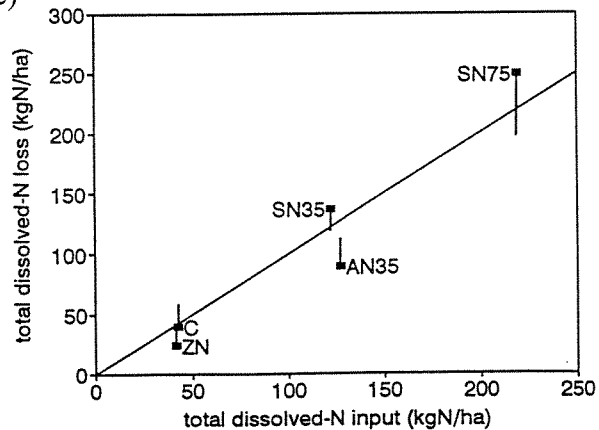
(a)



(b)



(c)



**Figure 1.** Input-output budgets in the control and treatment plots for (a) nitrate-N, (b) ammonium-N and (c) total dissolved-N for the period October 1990 to March 1993. Bars indicate standard errors.

### Soil nitrogen transformations and litter decomposition

No significant effect on nitrogen mineralisation or nitrification was observed in the different treatment plots after 2.5 years of nitrogen additions (Table 1) (Emmett et al. 1995b). There was also no effect on litter decomposition rates except in response to the water irrespective of nitrogen addition (Emmett et al. 1995b). There may be a delay in stimulation of soil nitrogen transformations when nitrogen is applied in small frequent doses in contrast to large single fertiliser doses. Effects on nitrogen transformations will continued to be monitored to determine any long-term effects.

**Table 1.** Annual rates of net N-mineralisation and nitrate accumulation in intact blocks of forest floor from the control and treatment plots. Given are means and s.e. of three replicate plots. No significant differences were observed between treatments.

Treatment	Net nitrate accumulation (kgN ha <sup>-1</sup> yr <sup>-1</sup> )	Net N-mineralisation (kgN ha <sup>-1</sup> yr <sup>-1</sup> )
C Control	7 ± 2	68 ± 17
ZN Water-only control	8 ± 5	73 ± 17
SN35 NaNO <sub>3</sub> 35kgN ha <sup>-1</sup> yr <sup>-1</sup>	12 ± 6	82 ± 4
SN 75 NaNO <sub>3</sub> 75kgN ha <sup>-1</sup> yr <sup>-1</sup>	2 ± 2	90 ± 4
AN 35 NH <sub>4</sub> NO <sub>3</sub> 35 kgN ha <sup>-1</sup> yr <sup>-1</sup>	6 ± 3	87 ± 10

### Tree health and growth

There has been no significant change in needle nutrient concentrations or annual growth increment. However, some effect of treatments on non-structural ephemeral components of the trees has been observed (Emmett et al. 1995b). There has been a reduction in standing crop root biomass (< 5mm) with high nitrate loadings and an increase in above-ground litterfall during the second winter of treatments. Continued monitoring is required to determine if these are early indications of changes in tree health and growth or reallocation of resources between above and below-ground components.

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## AKNOWLEDGEMENTS

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## 6. RESPONSE OF SOIL WATER CHEMISTRY TO EXPERIMENTAL "CLEAN RAIN" IN THE NITREX ROOF EXPERIMENT AT SOLLING, GERMANY

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### THE EXPERIMENT

At the Solling NITREX site in central Germany (51°31'N, 9°34'E, 500m a.s.l.) a manipulation study with three roof constructions of 300-m<sup>2</sup> each is run in a 60 year-old Norway spruce (*Picea abies* Karst.) stand. The roofs are underneath the canopy at ca. 3.5 m above the ground. They are covered with transparent polycarbonate. Water falling onto the roofs is collected in a central cabin, processed in different ways and redistributed to the roof plots by a sprinkling system.

The NITREX experiment comprises a "clean rain" treatment with simulated pre-industrial throughfall. Water is filtered, de-ionized, adjusted to clean rain concentrations and redistributed to the plot immediately.

The composition of the artificially prepared pre-industrial throughfall for the clean rain plot is shown in Table 1. Compared to the ambient control, reduction of sulfur input is ca. 85 % and reduction of total nitrogen (ammonium+nitrate) input 90 %.

**Table 1: Chemical composition of "clean rain" vs. ambient throughfall (control) [ $\mu\text{mol}/\text{l}$ ]**

	throughfall (1990/91)	"clean rain"	% reduction
pH	4	6	99.0
calcium	150	120	20.0
magnesium	54	50	7.5
potassium	108	80	25.0
sulfate-S	403	83	83.0
nitrate-N	213	43	80.0
ammonium-N	211	0	100.0

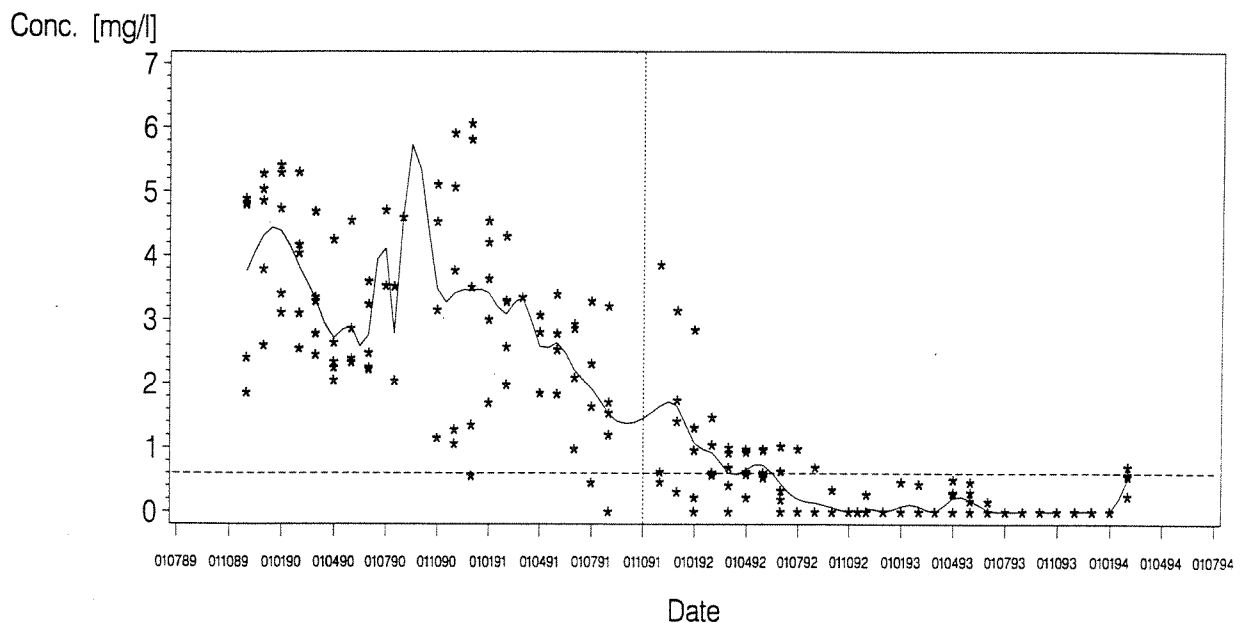


## RESULTS

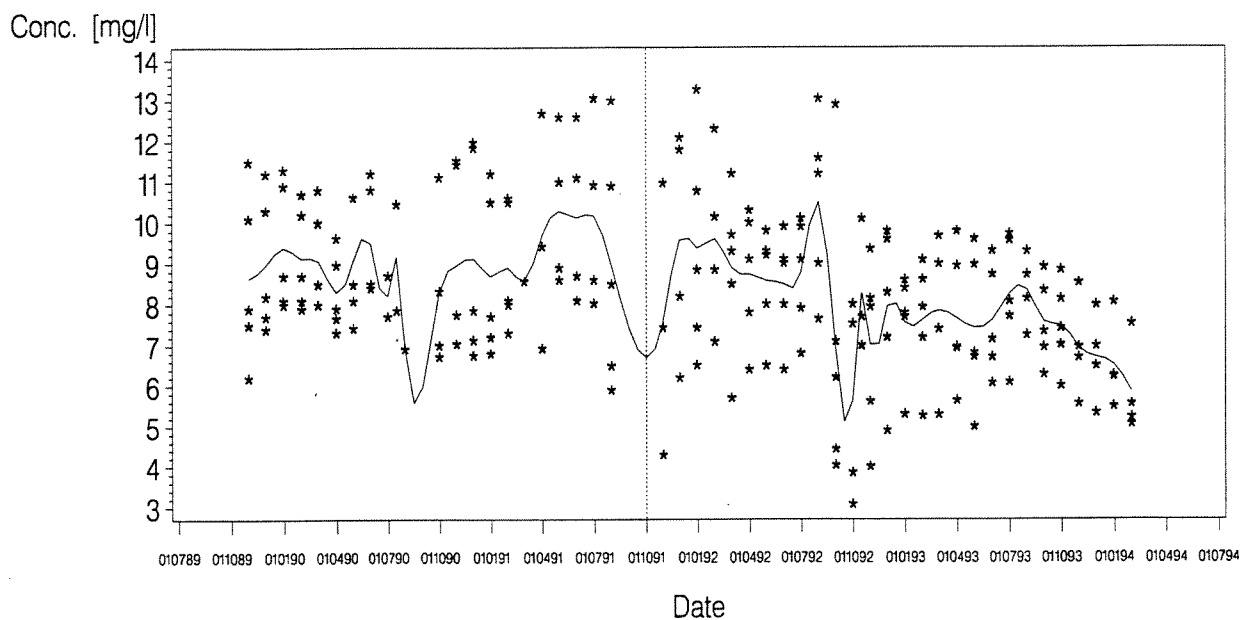
### Soil water chemistry

Soil water chemistry data now comprise about 2 yr. pre-manipulation and 2.5 yr. of manipulation treatment.

The response of soil water chemistry to reduced input fluxes of N and S was strong and fairly rapid. The decline of nitrate concentrations is more pronounced than that of sulfate. After only about 0.5 y of clean rain input the nitrate-N concentrations went *below* the input concentration level and have remained there since. This means that the leaching of nitrate to greater depths ceased. The sulfate-S concentration courses exhibit a less-rapidly decreasing trend. Aluminium (not shown) follows a very similar course, indicating that acid stress may gradually ameliorate at the clean rain site (Figures 1 and 2).



**Figure 1.** Concentrations of nitrate-N in soil solution at 40 cm depth in the clean rain plot. The horizontal reference lines indicate the concentration in the artificial "clean rain", whereas the vertical reference lines mark the date of roof closure and the beginning of the experimental manipulation.

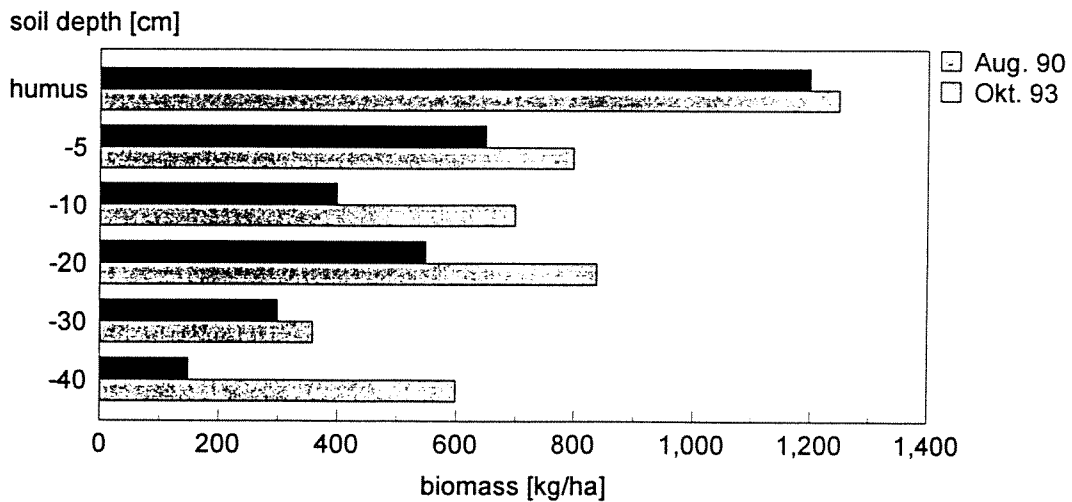


**Figure 2.** Concentrations of sulfate-S in soil solution at 40 cm depth in the clean rain plot.

Clear decrease in concentration can be recognized for nitrate and ammonium throughout the profile down to 100 cm soil depth, and for sulfate down to 70 cm depth. The ambient control plot (without roof) shows no apparent trends in soil water concentrations.

### **Root vitality**

The fine roots of the spruce trees reacted strongly to these changes in soil water chemistry. Living fine root biomass increased in the clean rain plot by about 40 % compared to pre-experimental conditions. This increase was strongest in the B-horizon, indicating that acid stress has ameliorated in the mineral soil (Figure 3).



**Figure 3.** Fine root biomass in soil of the clean root plot.

## CONCLUSION

There is a clear reflection of reduced input in soil solution concentrations, particularly for the N ions, but also for sulfate and Al down to ca. 70 cm depth.

Nitrogen ions virtually disappear from the soil water in the root zone (< 70 cm soil depth) after 6-12 months of clean rain application. If this trend continues, nitrogen leaching will cease within a relatively short period of time, and one criterion for "N saturation" will be effectively reversed.

The acidity parameters in soil solution apparently ameliorate more slowly, but already significantly in the upper soil. Regeneration of base saturation in the soil solid phase, however, will probably proceed slowly on the time scale of decades.

The results demonstrate that

- in the Solling spruce forests on acid soil atmospheric element input largely controls soil solution chemistry and that
- strict air pollution control measures would have a significant and quite rapid effect with respect to ameliorating nitrogen leaching, acid stress and forest health.

**7. NITREX project: ecosystem response to chronic additions of nitrogen to a spruce-forested catchment at Gårdsjön, Sweden.**

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## **INTRODUCTION**

We are conducting a whole-catchment experimental addition of nitrogen at Gårdsjön, SW Sweden, to investigate the risk and consequences of nitrogen saturation in coniferous forests typical of southern Scandinavia. The Gårdsjön experiment is part of the European NITREX project (Nitrogen Saturation Experiments). Beginning April 1991 we add in weekly portions about 35 kg NH<sub>4</sub>NO<sub>3</sub>-N ha<sup>-1</sup> yr<sup>-1</sup> in 5% extra water to the ambient 12 kg N ha<sup>-1</sup> yr<sup>-1</sup> in throughfall.

## **SITE DESCRIPTION**

The experimental site at Gårdsjön is located at 135-145 m elevation about 10 km from the Swedish west coast (58 04'N, 12 01'E), 50 km north of Gothenburg. The Gårdsjön region has a humid climate, with 1100 mm mean annual precipitation, 586 mm mean annual runoff, and mean annual temperature of 6.4°C. The area is characterised by an acid lake whose terrestrial catchment is dominated by shallow podzolic soils with inclusions of barren rock and peat

soils. The bedrock consists mainly of granites and granodiorites. The forest is mainly a naturally-regenerated mixture of 66-90 year old Norway spruce (*Picea abies* L. Karst) and some Scotch pine (*Pinus sylvestris* L.) (Olsson et al. 1985).

We simulate increased atmospheric deposition of nitrogen by weekly experimental additions of ammonium nitrate solution at a rate of about  $35 \text{ kg N ha}^{-1} \text{ yr}^{-1}$  to the G2 NITREX catchment.  $\text{NH}_4\text{NO}_3$  is dissolved in de-ionised water and applied by means of 270 ground-level sprinklers installed in a 5x5 m grid over the whole catchment. Additions are done weekly in amounts proportional to the volume of ambient throughfall which occurred the previous week. The additional water comprises about 5% of total throughfall volume. The average ammonium nitrate addition is in 1.4 mm of water and added during 30 minutes. This amount and intensity was chosen such that discharge is not affected on an event or an annual basis.

A separate manipulation experiment run in parallel is conducted at the adjacent catchment G1 ROOF (0.63 ha) (Hultberg et al. 1993). At G1 ROOF ambient inputs of N and S are removed by means of roof beneath the canopy and clean deionized water with the addition of natural sea salts spread underneath the roof by means of a sprinkling system. Water is sprinkled weekly beneath the roof.

A nearby 3.7-ha catchment (F1 CONTROL) with comparable soils and vegetation serves as a control for both G2 NITREX and G1 ROOF. Here inputs and outputs of major ions have been measured continuously since 1979. Details or methods are given by Moldan et al. (1995), Stuanes et. al. (1995), Clemensson-Lindell and Persson (1995), and Brandrud (1995).

The Gårdsjön area receives moderately high deposition of sulphate, nitrate and ammonium; 14-year mean (minimum and maximum) throughfall inputs at F1 CONTROL for 1979 - 1992 were  $25.0$  ( $18.9$  to  $31.6$ )  $\text{kg SO}_4\text{-S ha}^{-1} \text{ yr}^{-1}$ ,  $7.3$  ( $4.6$  to  $11.7$ )  $\text{kg NO}_3\text{-N ha}^{-1} \text{ yr}^{-1}$  and  $4.8$  ( $2.4$  to  $7.7$ )  $\text{kg NH}_4\text{-N ha}^{-1} \text{ yr}^{-1}$ , respectively.

## RESULTS

### Runoff

Nitrogen addition resulted in increased concentrations of nitrate in runoff at catchment G2 NITREX (Table 1, Figure 1).

During the second and third years of treatment. concentrations of nitrate were higher also during the growing season (Figure 1). The inorganic nitrogen (mostly as nitrate) lost during the first year of treatment was about  $0.6\% \pm 0.06\%$ , the second year was  $1.1\% \pm 0.1\%$  and the third year was  $5.2\% \pm 0.5\%$  of the total inorganic nitrogen input, respectively. Output of nitrate from the G2 NITREX catchment during pre-treatment years and similarly from the F1 CONTROL catchment during all years was  $<0.1\%$  of the nitrogen input (Moldan et al. 1995) (Table 1).

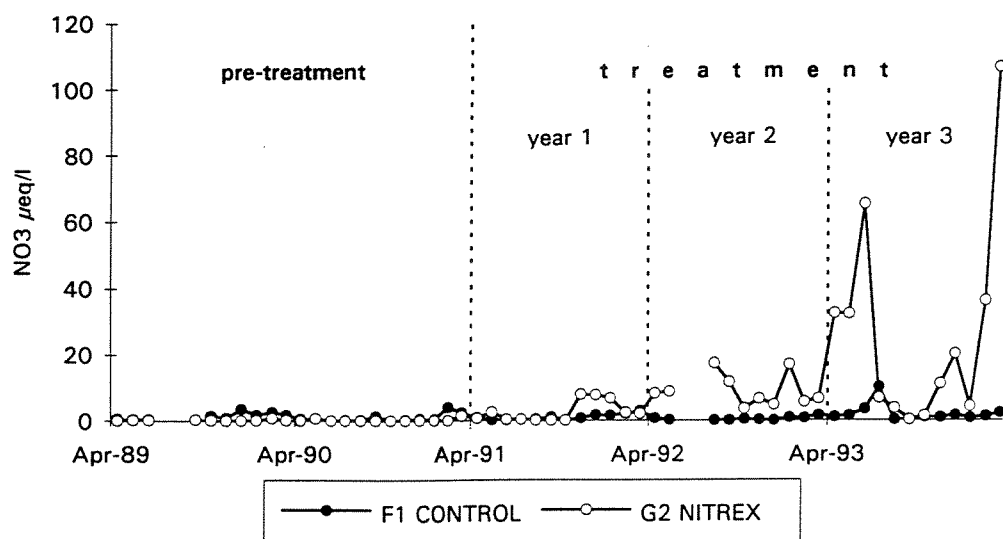
### Soil-solution

With few exceptions, the volume-weighted average nitrate concentrations in the soil solution increased from the pre-treatment year through the treatment years in G2 NITREX (Figure 2)

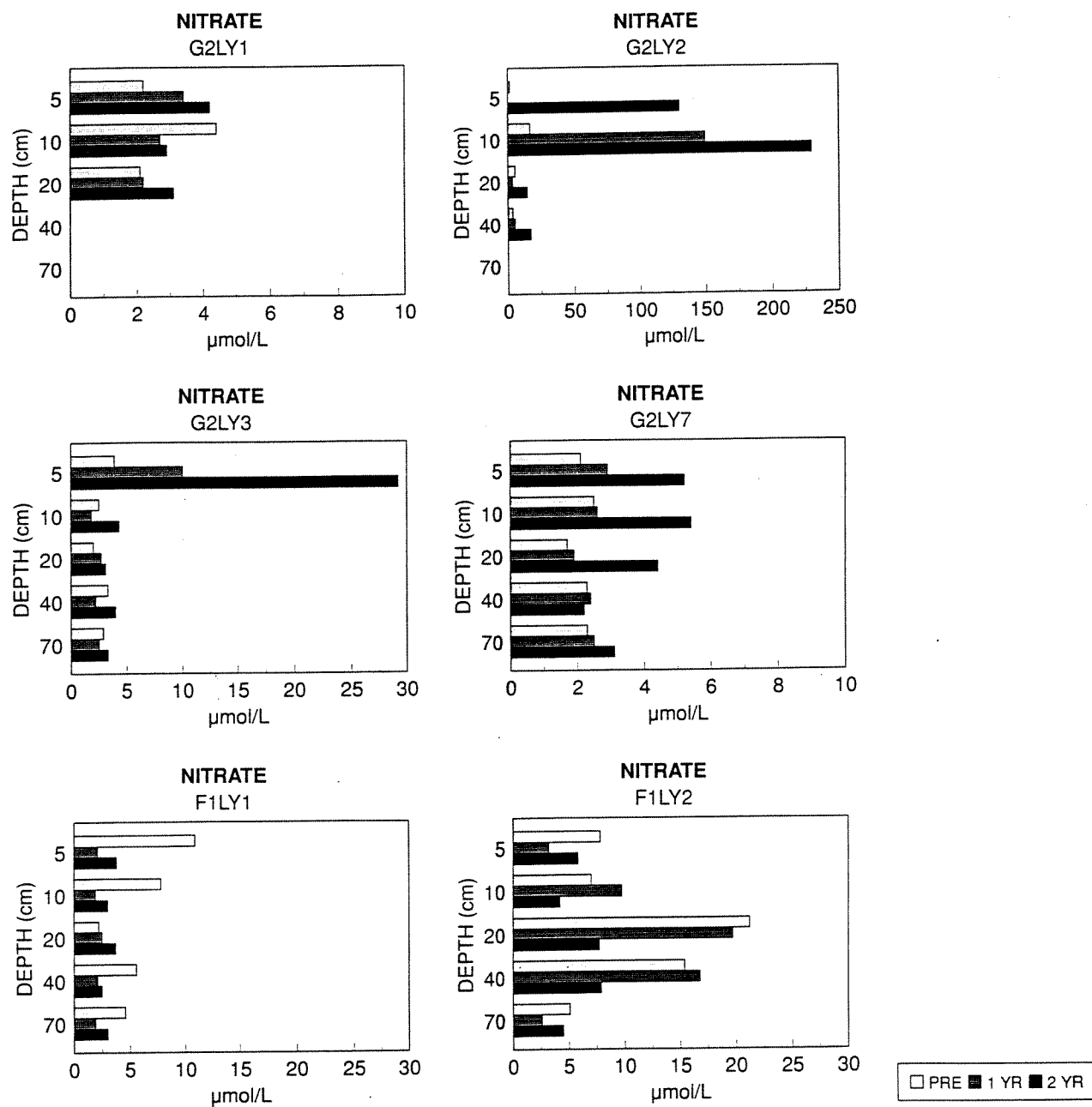
(Stuanes et al. 1995). No such time trend occurred at F1 CONTROL. On the contrary, in F1 CONTROL nitrate concentrations were generally highest in the pre-treatment year. The

**Table 1.** Fluxes of nitrogen species in bulk precipitation, throughfall (tf) and runoff during the 2-year pre-treatment period April 1989 - March 1991, and the 3 years of treatment April 1991 - March 1994 at catchments F1 CONTROL, G1 ROOF and G2 NITREX. Data from Hultberg et al. (1994) and Moldan et al. (1995) and Wright et al. (in press.). Units:  $\text{mmol m}^{-2} \text{ yr}^{-1}$  (1  $\text{mmol} = 1 \text{ meq}$ ;  $1 \text{ mmol m}^{-2} \text{ yr}^{-1} = 0.14 \text{ kg ha}^{-1} \text{ yr}^{-1}$ ).

	F1 CONTROL				G1 ROOF				G2 NITREX					
	NO3 in tf	out runoff	NH4 in tf	out runoff	NO3 in tf	out runoff	NH4 in tf	out runoff	NO3 in tf	in added	out runoff	NH4 in tf	in added	out runoff
pre-treatment														
Apr 89 - Mar 90	66	1	37	0	80	1	40	0	63	0	0	33	0	0
Apr 90 - Mar 91	51	0	26	0	46	0	22	0	42	0	0	21	0	0
treatment														
Apr 91 - Mar 92	56	1	32	1	0	0	0	1	54	115	2	36	115	1
Apr 92 - Mar 93	51	0	22	0	0	0	0	2	52	133	4	26	133	1
Apr 93 - Mar 94	36	1	17	2	8	0	0	1	40	146	17	30	146	2



**Figure 1.** Monthly mean volume-weighted nitrate concentrations in runoff from catchment G2 NITREX ( $\text{NH}_4\text{NO}_3$  addition) and F1 CONTROL (untreated control) at Gårdsjön during the 2 years prior to and 3 years of N addition.



**Figure 2.** Volume-weighted mean soil solution nitrate concentrations for the pre-treatment period and two treatment years. G2LY1 = treated, upper, dry; G2LY2 = treated, slope, dry; G2LY3 = treated, lower, wet; G2LY7 = treated, close to outlet, wet; F1LY1 = control, dry; F1LY2 = control, wet (from Stuanes et al. 1995).

response was most pronounced in the upper soil solutions. Nitrate concentrations at or below 20 cm soil depth were generally less than 5  $\mu\text{mol/L}$ .

Ammonium in solution followed a different trend: concentrations were generally higher in the pre-treatment year and the second treatment year compared to those from the first treatment year for both the treated and the control catchment (Figure 3). The addition of nitrogen thus caused a shift in the dissolved inorganic nitrogen pool towards a greater contribution from nitrate, especially in the upper part of the soil profile. Concentrations of organic nitrogen in the soil solution remained unaffected by treatment and were generally highest in the upper 10 cm of the soil in both catchments (Stuanes et al. 1995).

### **Soil nitrogen transformation processes**

Mineralization measurements from the first 2 years of treatment, 1991 and 1992, show no significant differences between the 3 catchments and no systematic change from 1991 to 1992. The fairly high C/N ratio of the soil, with a range between 35-52 in the organic layer suggests that immobilisation of added nitrogen is an important process during the seasons of microbial activity. The immobilisation of nitrate, however, appears to be reduced at site M2 in catchment G2 NITREX during the second year of treatment (Figure 4). This is reflected in increased levels of nitrate in the ion exchange resins under the soil cores. The same trend is found in the corresponding lysimeters. This is probably due to an increase in nitrification during the summer 1992 at this site (Figure 5) (Kjønaas in prep.). Additional years data are necessary to reveal trends over time.

### **Fine-roots**

There were large variations in the different root fractions in the core samples from all catchment areas between different years. Few significant changes were observed in diameter classes 1-2 and 2-5. The majority of changes in fine roots were observed in the humus layer (FH 0-5 and 5-10 cm, respectively) (Clemensson-Lindell and Persson 1995).

In the G2 NITREX catchment there was a tendency towards a decrease in the biomass of living fine roots in the FH layer in the moss-dominated plots (Figure 6). In contrast, at G1 ROOF catchment there was a significant increase in the amount of living fine roots in the FH layer. In F1 CONTROL there were no significant trends over time (Clemensson-Lindell and Persson 1995).

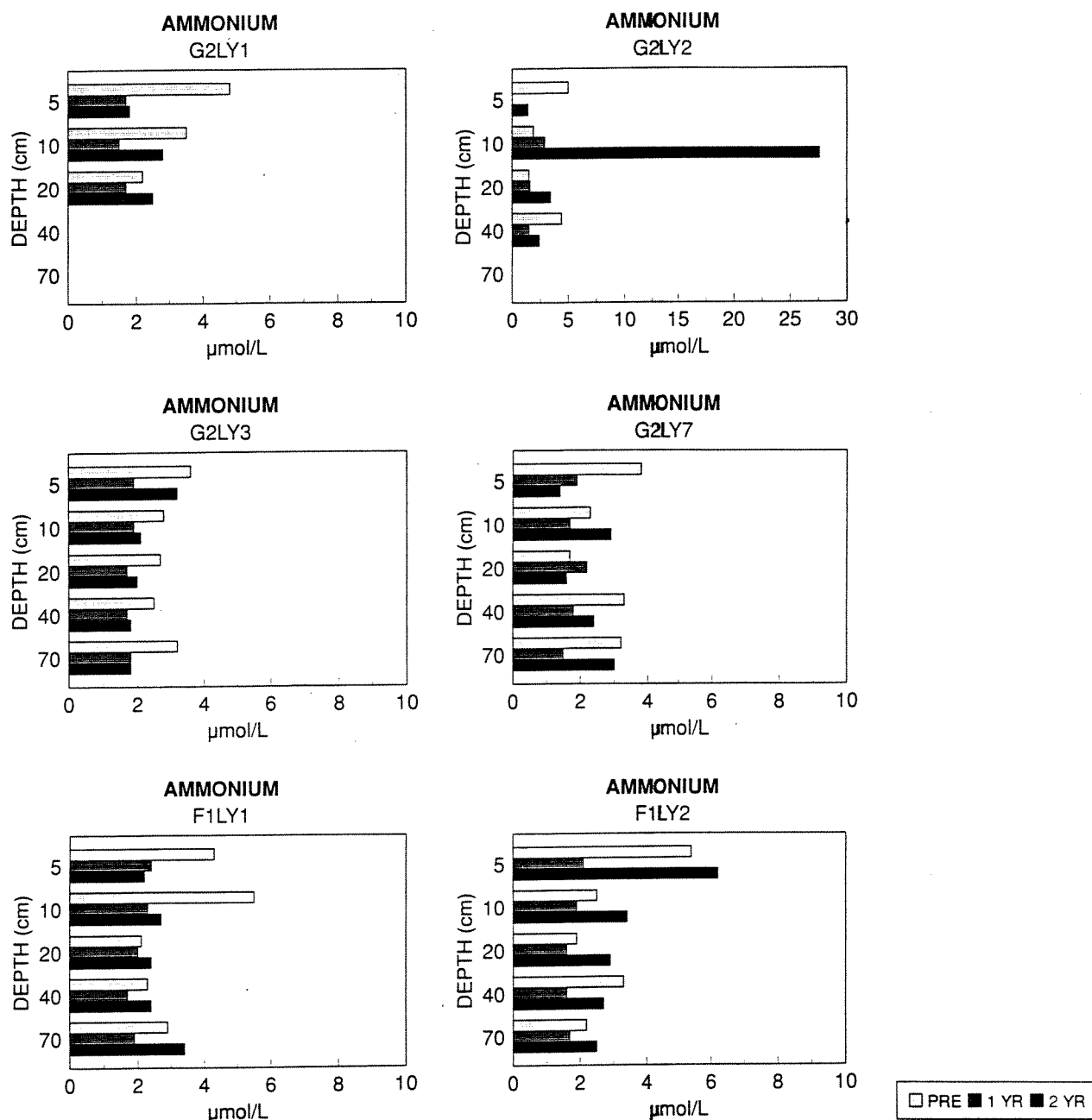
### **Mycorrhiza**

The species numbers of mycorrhiza decreased after N-treatment, from more than 60 species per year in 1990 and 1991 to less than 50 in 1992 and 1993. Results from 1993 at all 3 catchments indicate that species diversity of the G2 NITREX catchment was similar to that of the G1 ROOF catchment, but lower than in the F1 CONTROL catchment. The diversity of the F1 CONTROL catchment was more comparable to that of the G2 NITREX catchment before, and just after start of treatment.

In spite of the fairly low diversity, the fruit body production of the G1 ROOF catchment in 1993 was approximately twice as high as that of the other two catchments. The stress-tolerant

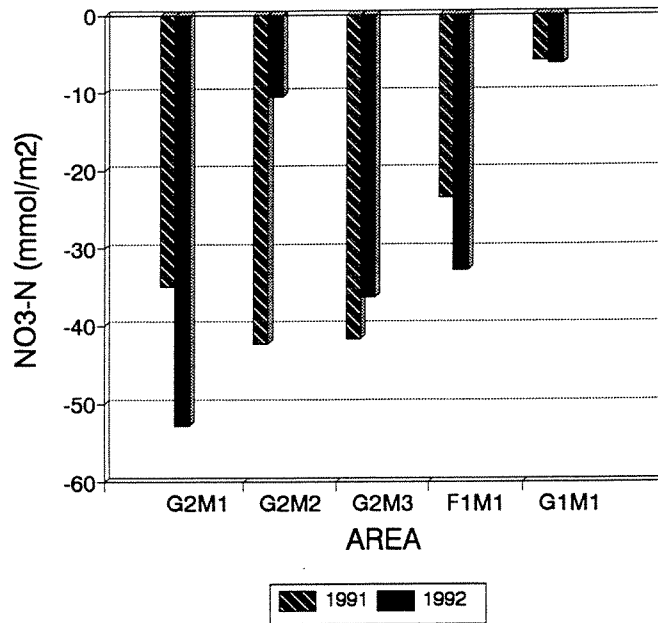


taxa *Lactarius* spp. (species such as *L. rufus*, *L. theiogalus*), *Cantharellus tubaeformis* and *Paxillus involutus* exhibited a comparatively low production at the F1 CONTROL catchment,



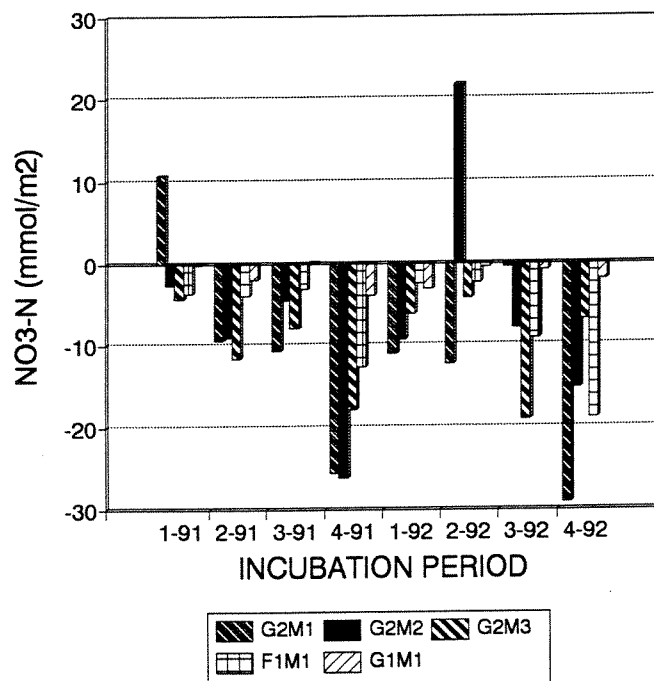
**Figure 3.** Volume weighted average soil solution ammonium concentrations for the pre-treatment period and two treatment years. G2LY1 = treated, upper, dry; G2LY2 = treated, slope, dry; G2LY3 = treated, lower, wet; G2LY7 = treated, close to outlet, wet; F1LY1 = control, dry; F1LY2 = control, wet (from Stuanes et al. 1995).

### NET MINERALIZATION Immobilization of NO<sub>3</sub>

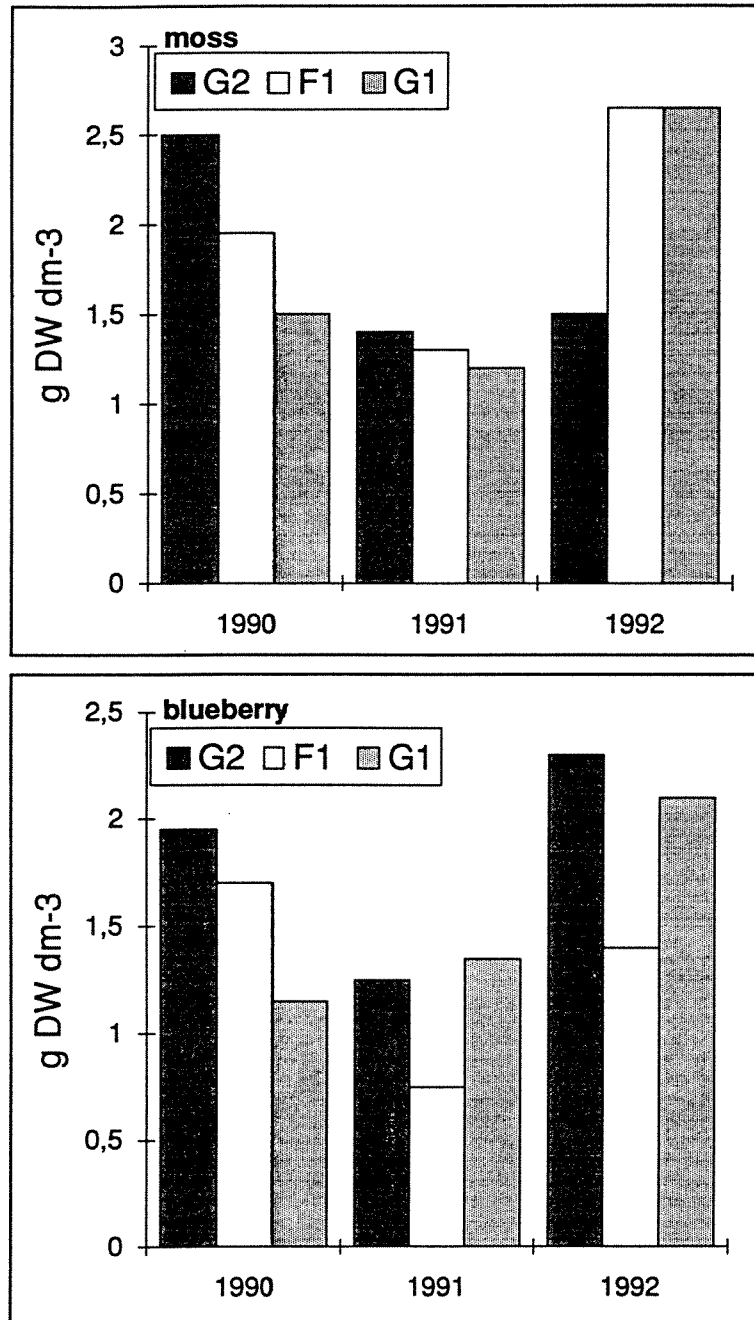


**Figure 4.** Immobilization of nitrate in soil at G2 NITREX, F1 CONTROL and G1 ROOF catchments in 1991 and 1992 as measured by soil core incubation technique (from Kjønås in prep.).

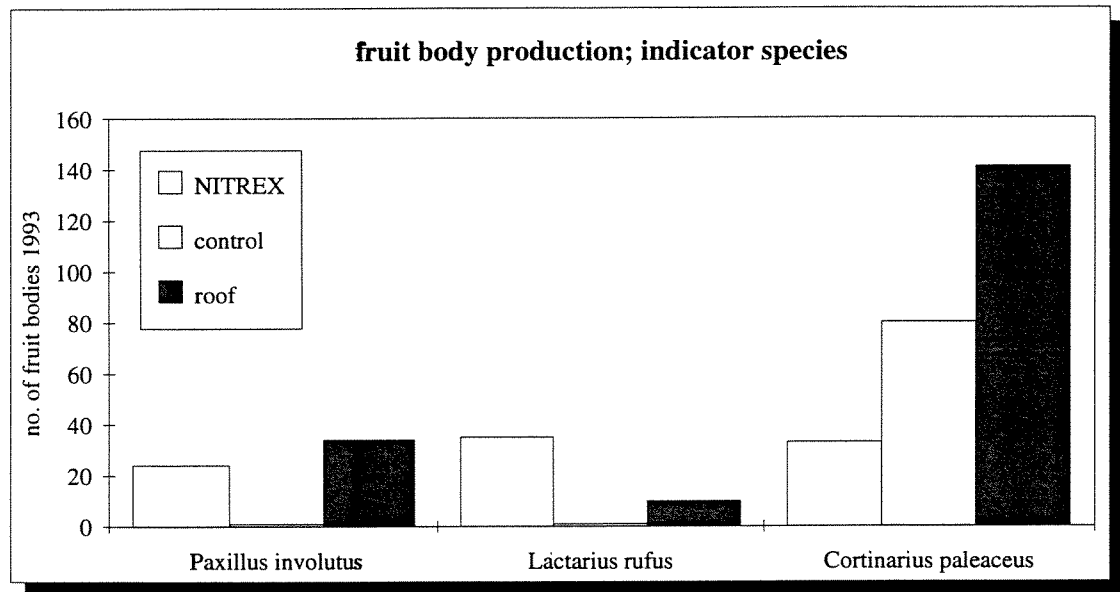
### NET NITRIFICATION



**Figure 5.** Net nitrification in soil at G2 NITREX, F1 CONTROL and G1 ROOF catchments in 1991 and 1992 as measured by soil core incubation technique (from Kjønås in prep.).



**Figure 6.** Living fine roots (classes 1 and 2) in soil core samples from the organic soil layer collected from catchments G2 NITREX, F1 CONTROL, and G1 ROOF in 1990 (pre-treatment), 1991 and 1992 (data from Clemensson-Lindell and Persson, 1995).



**Figure 7.** Fruitbody production of some selected indicator species of mycorrhizal fungi in the G2 NITREX catchment, the F1 CONTROL catchment and G1 ROOF catchment in 1993. Each catchment includes a 1300-m<sup>2</sup> transect (from Brandrud, 1995).

and a high production at the G1 ROOF and G2 NITREX catchments (Figure 7). The sensitive groups *Cortinarius* and *Russula* showed the highest production at the G1 ROOF and F1 CONTROL catchments and the lowest at the G2 NITREX catchment (Brandrud 1995).

## DISCUSSION

Together the results from G2 NITREX catchment indicate that the three years of nitrogen addition have resulted in changes across the forest ecosystem boundary as well as changes within the ecosystem. Most apparent and rapid is the increase in nitrate concentrations in runoff, whereas the changes in components and processes within the ecosystem are apparently slower and more difficult to detect.

The increased nitrate concentrations in runoff are related to the increased nitrate concentrations in soil solution in several of the lysimeters. Spatial differences in nitrogen demand by plant uptake and microbial immobilization and nitrogen supply through mineralization probably account for the large variation in nitrate concentration observed between the lysimeters at various points within the catchment. The soil nitrogen

transformation results suggest that the increased nitrate concentrations observed in soil solution (and hence runoff) are due to increased nitrification of ammonium to nitrate and reduced immobilization of nitrate in the soil. This is especially apparent in the uppermost soil layers where the major sources of ammonium are throughfall and the added ammonium nitrate.

Over the long term the nitrogen addition can be expected to affect productivity of trees and other vegetation, alter the relative abundance of annual and perennial lower vegetation, and change species composition. Thus far these types of changes are most apparent in the mycorrhizal fungus flora. The results after three years of treatment indicate that nitrogen sensitive species have become less abundant with lower fruit body production and nitrogen tolerant species have increased their fruit body production.

The fine-root data also point to changes due to the nitrogen addition. At G2 NITREX the amount of fine roots in the forest floor layer decreased while at G1 ROOF the amount increased. These changes are consistent with the hypothesis that nitrogen suppresses fine-root production.

As yet there are insufficient data available to assess the effects of nitrogen addition (and nitrogen removal at G1 ROOF) on the tree growth and vitality. The trees in both catchments are growing slowly and measurement of changes in growth parameters such as height and diameter require several years of data.

At Gårdsjön as well as most of the other NITREX sites (Wright et al.1995), the runoff responds immediately to changed nitrogen deposition, but the vegetation and soils lag behind by one or more years. Generally, biological responses are difficult to detect during the first stage of nitrogen saturation (Aber et al. 1989). The expected response of parameters such as nutrient content of foliage and growth of trees occur only several years after the onset of treatment. Apparently, this is the case for both increased as well as decreased (by roof) nitrogen deposition. The ecosystem as a whole shows a response to treatment (changed nitrate concentration in runoff), but only modest responses in individual components and internal processes are evident during the first two years of treatment.

## **ACKNOWLEDGEMENTS**

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## 8. Alptal, Switzerland: Activity Report 1994

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### INTRODUCTION

The Swiss project was imbedded into the European NITREX programme at the beginning of NITREX. The present report covers the period from 1 November 1993 to 31 December 1994.

The objectives of the project are:

- to measure key fluxes and pools of the nitrogen cycle in a subalpine forest ecosystem,
- to investigate changes due to an artificially increased nitrogen deposition,
- to assess the risk of a nitrogen saturation of the ecosystem and to define a critical load accordingly

### MATERIAL AND METHODS

#### Experimental plots

The experimental site of Alptal is situated south of Einsiedeln in the Swiss Prealps, at an altitude of 1200m and in a wet climate (2200mm of precipitation a year on average). Gleyic soils occur atop a flysch underground and are characterised by their very low water permeability. In the forested parts, they are covered by humus in the wet depressions and by a mor on the drier and more acidic mounds (Diserens, 1992).

Three experimental plots of about 1500 m<sup>2</sup> have been delimited by digging trenches. Because of the impermeable sub-soil, they can hydrologically be considered as catchments. Two are situated in a forest dominated by Norway spruce (*Picea abies*); the third is wetland. To measure the water runoff out of these catchments, weirs have been installed. Their measurements are stored along with meteorological parameters into a data acquisition system. At each weir, a water sampling device is activated proportionally to the discharge. Runoff, precipitation and throughfall are analysed weekly since February 1994. This allows the calculation of the input of nitrogen (nitrate + ammonium) and other elements as bulk deposition, as well as the output as leached solutes.

#### Soil

Both soil types are enriched in organic matter because of the poor oxygenation inhibiting the microbial decay. Soil nitrogen is therefore the major N pool of the ecosystem. To determine an appropriate sampling design, the spatial variability of the different soil-N pools was estimated by a pre-sampling. The main sampling was then carried out accordingly to perform a pre-treatment inventory of the soil nitrogen. Total, organic and inorganic nitrogen will be

determined from these samples. An additional experiment to characterise the fate of nitrogen in both soil types will start in September 1995. It will focus on the relations between hydrological processes and nitrogen leaching.

### **Vegetation**

Due to the relatively open tree canopy, the understory vegetation is well developed on the site. Its species composition has been mapped. To assess changes, permanent quadrates have been established on the plots; they are inventoried three times per vegetation period. Plant samples are taken for chemical analyses. Competition experiments are planned for 1995. Spruce needles (5 age classes) are harvested from the seventh whorl at the end of the year. Diameter growth is measured on trees equipped with micro-dendrometers. Photographs of tree crowns are taken to estimate growth in height and tree health.

### **Nitrogen treatment**

All the measurements so far have been done without manipulation of the nitrogen input. These background measurements are necessary because there is no replication in the experimental design. After one year, one of the plots will be treated with  $40 \text{ kgN ha}^{-1} \text{ yr}^{-1}$  by sprinkling an ammonium nitrate solution. The water for this solution is collected from natural precipitation falling on a plastic sheet. Since the treatment will result in a 10% increase in precipitation, the same amount of (unaltered) water will be applied to the control plot. All the installations to collect and store water, to add nitrogen and to sprinkle the solution were set up during the summer and fall 1994. The treatment starts 1 April 1995. It will include  $^{15}\text{N}$  in order to follow the fate of the added nitrogen (Kjønaas *et al.*, 1993).

## **RESULTS**

### **Soil**

The results from the pre-sampling showed that for a 4x4 m grid changes in exchangeable ammonium are expected after 2 years of treatment. Changes in the organic or total soil-N pools will not be measurable from chemical analyses. Determination of  $^{15}\text{N}$  will be necessary for this purpose.

### **Vegetation**

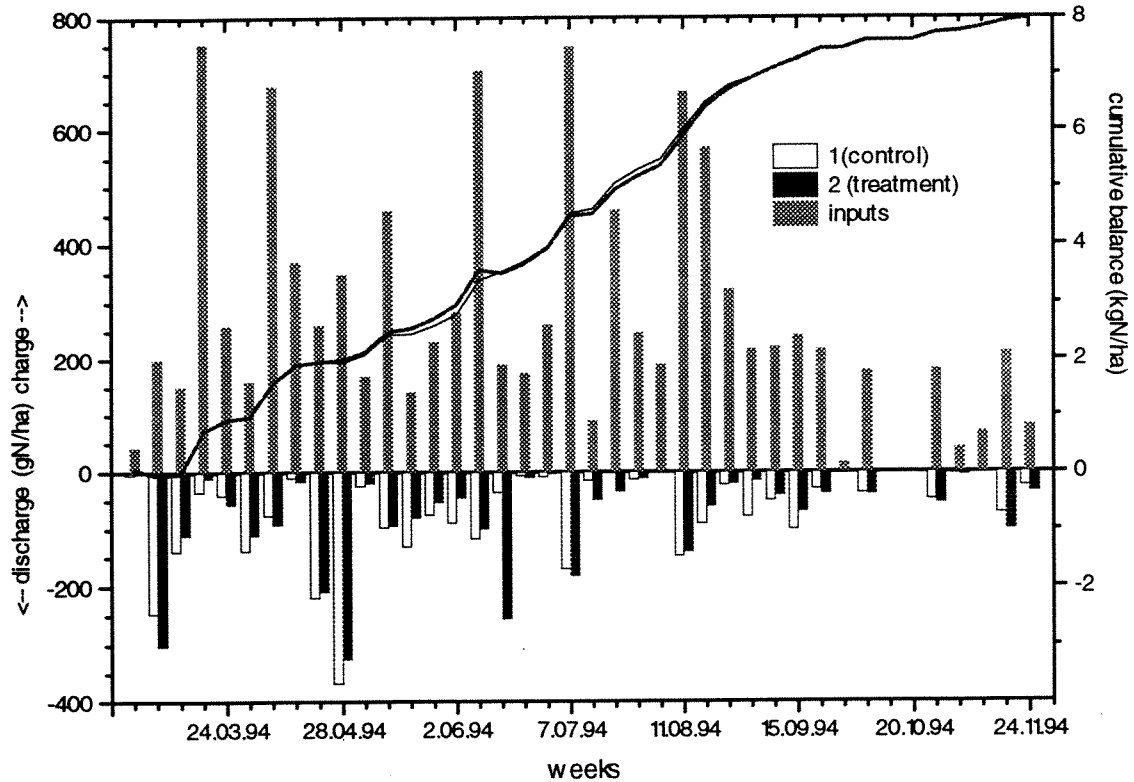
Three plant communities can be distinguished in the understory vegetation. One, dominated by *Vaccinium myrtillus* and *Vitis-idaea*, occupies the acidic and somewhat drier parts of the soil (mor type). *Caltha palustris* and *Petasites alba* dominate the wet depressions, except where more light reaches the ground. Then *Carex ferruginea* and *Poa trivialis* are dominant.

### **Nitrogen balance**

The spring of 1994 was characterised by an initial snowmelt in March, followed by new snow, and again melting at end of April. These melting events are reflected in two peaks of N (mainly nitrate) leaching (Figure 1). During the rest of the year, heavy rainfalls were associated with higher inputs as well as outputs generally large volumes of lower concentration. Measured concentrations are in the same range as reported by Hultberg *et al.* (1994) for the control catchment in Gårdsjön, (Sweden). The comparison of our plots (before any treatment) shows differences in the results of some weeks; on both plots, the overall N



budget indicates net retention of 8 kgN ha<sup>-1</sup> after 40 weeks of measurement (10.8 input minus 2.8 output).



**Figure 1.** Nitrogen balance of the experimental ecosystems: weekly inputs and outputs (left-hand scale, gN ha<sup>-1</sup>) as bulk deposition (positive bars), and leachates in runoff (negative bars) and cumulative differences (lines, right-hand scale, kgN ha<sup>-1</sup>).

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