ACTA UNIVERSITATIS AGRICULTURAE SUECIAE

AGRARIA 342

Nitrogen Turnover in Swedish Spruce Forest Ecosystems

Effects of nitrogen deposition

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Doctoral thesis
Swedish University of Agricultural Sciences
Uppsala 2002

Abstract

Andersson, P. 2002. Nitrogen turnover in Swedish spruce forest ecosystems - effects of nitrogen deposition. Acta Universitatis Agriculturae Sueciae, Agraria 342. Doctor's dissertation.
ISSN 1401-6249, ISBN 91-576-6194-4

Nitrate leaching from growing forests is not yet a widespread problem in Sweden, but nitrogen (N) deposition and high N retention in the soil during recent decades might have changed forest ecosystems towards N saturation. In this thesis net N mineralisation (measured with *in situ* incubations) was tested as an index of N status. It was shown that historic land use and N deposition, natural or simulated in fertilisation experiments, had caused great differences in this index (4-104 kg N $ha^{-1}yr^{-1}$) and that N leaching occurred at sites with high N status (net N mineralisation > 60 kg N $ha^{-1}yr^{-1}$). The net N mineralisation was well correlated to C/N ratio, which could also be used as a N status index. The amount of nitrate lost was not dependent on N status or current N deposition, but rather to the total N flux density (deposition + net mineralisation). The relatively fast change (30 years) from a highly N limited ecosystem to a system apparently approaching the first stage of N saturation upon moderate N fertilisation (35 kg N $ha^{-1}yr^{-1}$) motivates continued concern about N saturation in Sweden. Predicting future response to N deposition and forest management necessitates a thorough understanding of all important N fluxes, but the importance of organic N uptake in Swedish spruce forest ecosystems is still unclear. Budget calculations indicated that uptake of organic N is of great importance (80%) in low input areas. In high N-input plots the relative importance appeared lower (35%). However, soil solution samples from the forest floor contained low concentrations of both amino acids and inorganic N, and the lack of a clear dominance of amino acids might be explained by a tight circulation of amino acids, never releasing the amino acids into the soil solution fraction sampled with tension lysimeters.

Keywords: boreal, ecosystem recovery, fine root longevity, forest soil, mycorrhiza, *Picea abies,* PnET-CN.

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Appendix

Papers I-V

The present thesis is based on the following papers, which are referred to by their Roman numerals:

I. Andersson, P. Berggren, D. & Johnsson, L. 2001. 30 years of N fertilisation in a forest ecosystem – the fate of added N and effects on N fluxes. Water, air and soil pollution 130, 637-642.

II. Andersson, P. Berggren, D. & Nilsson, I. 2002. Indices for nitrogen status and nitrate leaching from Norway spruce (Picea abies (L.) Karst.) stands in Sweden. Forest ecology and management 157, 39-53.

III. Majdi, H. & Andersson, P. Root production, longevity and nitrogen turnover in response to nutrient availability in a boreal spruce forest. (Manuscript).

IV. Andersson, P. 2002. Amino acid concentration in sampled soil water – effects of sampling and storage using lysimeters with a small pore-size and sterile pre-evacuated sampling tubes. Communications in soil science and plant analysis (in press).

V. Andersson, P. and Berggren D. Organic and inorganic nitrogen in forest floor soil solution at low and high N input. (Manuscript).

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Introduction

Nitrogen and Swedish spruce forests

Too little or too much?

Nitrogen (N) in boreal forests has long been thought of as a potential problem, primarily as a problem of N deficiency and ecosystem deterioration due to N depletion through intensive harvest practices (Krapfenbauer and Buchleitner, 1981; Adams and Boyle, 1982). Although the soil N pool of boreal forests is normally large compared to vegetation pools and to annual vegetation need, this pool is not readily available to plants (Tamm, 1991). The organic matter, which is the main soil N pool, is only slowly decomposed (Swift *et al.*, 1979) and thus the release-rate of available N-forms is often limiting for growth (*cf.* Ågren and Bosatta, 1988). High emissions of N to the air through combustion (NO_x) and volatilisation processes (mainly NH_3 from agricultural practices) in the later part of the 20th Century and still ongoing (Berge *et al.*, 1999; Galloway, 2001), has now shifted the focus to the problem of N excess. In the 1980's it was noted that N input might soon relieve the forest ecosystems from N limitation (Ingestad *et al.*, 1981), and then that excess N input might cause both forest decline and acidification and eutrophication of surrounding aquatic ecosystems (Nihlgård, 1985).

Nitrogen saturation

One conceptual response to high N deposition (Aber *et al.*, 1989; Gundersen, 1991; Stoddard, 1994; and references therein) involves improved tree growth and a change in species composition as the N availability in the soil increases. Soil N pools increase as N is immobilised in the soil and as the N content in litter production increases. Greater amounts of organic matter with a lower C/N ratio lead to an increased net ammonification, and high availability of ammonium ions leads in its turn to an increased net nitrification. Pools and fluxes of N are thus increasing as long as the N input is higher than N losses through leaching and denitrification. As more N is circulated within the ecosystem, with a greater proportion being nitrate, the N losses also increase, and nitrification followed by nitrate leaching results in net soil acidification and nutrient imbalances as base cations are lost together with the nitrate. At a more advanced stage the nitrate is accompanied by aluminium instead of base cations in the acid run-off water. Eventually, forest decline might result from excessive N availability in several ways. An excess assimilation of inorganic N "stealing" carbon that otherwise could be used for growth and maintenance, an increased shoot/root ratio and related drought and wind problems, increased intensity and frequency of insect and pathogenic pests and nutrient deficiency and/or aluminium toxicity (although questioned lately, *cf.* de Wit, 2000) due to acidification by nitrification could all lead to impaired production.

Ågren and Bosatta (1988) defined N saturation as the stage when N losses equal N deposition and the system has no further capacity to retain N whereas Aber *et al.* (1989) used the same term for ecosystems in which availability of inorganic N exceeds the demand from microorganisms and plants and thus eventually leads to nitrate leaching with runoff water. The timing of N saturation, defined in either way, depends on N deposition rates as well as land use, both current and historical (Gundersen, 1991; Aber *et al.*, 1997). Many of the predicted effects have already been found in Europe and North America, including high nitrate leaching (Van Miegroet *et al.*, 1992; Dise and Wright, 1995; Gundersen *et al.*, 1998a; Bredemeier *et al.*, 1998) and impaired production (or rather increased production upon decreased N input) (Boxman *et al.*, 1998). Binkley and Högberg (1997) argued that there is no clear evidence of negative impact on forest growth due to N saturation in Sweden. However, in some specific forest stands the nitrate leaching is high (Nilsson *et al.*, 1998), locally even higher than the N deposition (Nohrstedt *et al.*, 1996; Tunby, Paper II). At least in the southwest of Sweden, N deposition is of such a magnitude, >15kg N $ha^{-1}yr^{-1}$ (Lövblad, 2000), that it exceeds the N removal with stem harvest and thus leads to N accumulation in accordance with what was described above, and obviously N saturation is also a potential problem in Sweden.

Monitoring changes towards nitrogen saturation

In order to monitor whether ecosystems change towards N saturation, different indices of N status have been proposed, *e.g.* foliage N concentration, soil N con-3centration, soil N pool, soil C/N ratio, net N mineralisation (Van Miegroet *et al.*, 1992; Gundersen *et al.*, 1998b). Aber *et al.* (1997) suggested that net N mineralisation relative to a site/species-specific maximum sustainable net N mineralisation would be a better index than net N mineralisation itself. This maximum sustainable net N mineralisation rate is theoretically reached in non-disturbed systems receiving N from the atmosphere when the system is truly saturated as discussed above and net tree growth is zero, mineralisation is equal to uptake, and deposition is equal to leaching. Although this situation is unlikely to occur in reality it may be simulated in computer models (Ingestad *et al.*, 1981; Aber *et al.*, 1997). Having such indices it would be possible to distinguish sites that are far from N saturation from sites close to N saturation, although neither of them currently show nitrate leaching, and a regional or national change in these indices would be an early warning of an approaching N saturation. In this respect one would also aim at finding a correlation between these indices and the beginnings of nitrate leaching. Gundersen *et al.* (1998a) thus found that high N leaching was likely at forest floor C/N-ratios below 30 and Aber *et al.* (1997) reported that a relative mineralisation (current net N mineralisation/ maximum sustainable net N mineralisation, see above) greater than 75% was correlated with a risk for nitrate leaching.

With this background in mind, there are several questions that await answers. Does the moderate N deposition rate in Sweden cause changes in the forest soil systems so that there is a risk for elevated N leakage now or in the future? If so, is it possible to monitor and model these changes so that one can predict the outcome of different N deposition scenarios or forest management practices?

Role of organic nitrogen

So far I have only discussed the role of inorganic N in forest ecosystems. However, forest ecosystems in areas with a natural (low) N deposition are rather dominated by organic N. Not only is the soil pool mainly organic, but also dissolved N in soil water and N lost in runoff are dominated by organic N forms (Hedin *et al.*, 1995). It has also long been hypothesised that uptake of organic N by mycorrhizae might be of great importance for N nutrition in boreal forests (Frank, 1894). Many experiments have supported this view (*cf.* Melin and Nilsson, 1953; Abuzinadah and Read, 1986; Finlay *et al.*, 1992; Schimel and Chapin, 1996; Näsholm *et al.*, 1998) and it is now widely accepted that many plants and mycorrhizal fungi have the ability to take up *e.g.* amino acids. Some ectomycorrhizal and ericoid mycorrhizal fungi also have the ability to mobilise amino acids from the soil organic material by producing and exuding proteolytic enzymes (Bajwa and Read, 1985; Abuzinadah *et al.*, 1986). It has been estimated that uptake of amino acids might account for up to 80% of the total N uptake based on soil solution concentrations and uptake kinetics in arctic ecosystems (Kielland, 1994). This is in contrast to most models of N turnover that are used to evaluate the problems concerning N deposition and N saturation, as these models usually only consider inorganic nitrogen species. Clearly the role of organic N in low deposition areas and in areas receiving high loads of N needs to be assessed if such models are to be used for evaluation of future risks of N saturation at different N deposition scenarios and forest management practices. In some model applications, uptake of organic N has been included (*cf.* Beier *et al.*, 2001). However there are no attempts in these applications to include release of amino acids through proteolysis, pools of amino acids or uptake of amino acids in intense competition with other microorganisms.

Objectives

- To assess the fate of added N in three long-term N-fertilisation experiments. Both long-term fate, measured as differences in soil and vegetation N pools between fertilised and non-fertilised plots 9-30 years after the start of the experiments, and short-term (15 months) fate of ^{15}N added to the soil surface.

- To assess how a hypothesised change in soil N pool and soil N concentration in the fertilised plots, as assessed above, affects net N mineralisation, and how net N mineralisation varies between sites in Sweden with different historic N deposition and land use.

- To evaluate the possibility of using net N mineralisation or soil C/N ratio as an index for N status and the risk for N leaching. Also, how these indices and the soil nitrogen flux density (N deposition + net N mineralisation) are related to the amount of N leaching from the soil.

- To clarify the importance of organic N uptake in low and high N input systems by two different approaches; (i) by budget calculations of total uptake, which includes the objective to assess N turnover connected with root litter production, and supply rate of inorganic N, and (ii) by assessing soil solution concentrations of inorganic N and amino acids.

Methodology

In this section I describe the general aspects of the methods used. For details the reader is referred to the method descriptions given in the specific paper of interest.

Study sites

I used two approaches to compare ecosystems with different N deposition. The first approach was to study ecosystems that differ because of their location in Sweden. There is a clear gradient in Sweden so that the N deposition is higher in the southwest (>15 kg N ha^{-1} yr^{-1}) and much lower in the north (2-3 kg N ha^{-1} yr^{-1}) (Lövblad, 2000). By studying seven sites located from the very south and west to the northern part of Sweden I covered a range of N deposition that is representative for Sweden as a whole (Table 1). This approach means that it is not only the current N deposition that differs between the sites, but also other climatic factors and site history. *Tunby*, *Klintaskogen* and *Skogaby* are all located in high deposition areas, but their previous land use differs markedly; from intensively grazed and burned heath-land at Skogaby to extensively managed deciduous forest at Tunby. At *Uddevalla 2* and *3,* the N deposition is somewhat lower and again the previous land use of the two sites differs. Whereas Uddevalla 2 was formerly used for agricultural purposes, Uddevalla 3 has a history of continuous spruce forestry. At *Stråsan* and *Flakaliden,* the N deposition is low and the sites have been continuously used for spruce forestry, including prescribed burning. The second approach I used was to isolate the effect of N deposition from other site differences by studying plots with artificial N deposition as compared with control plots at the same location. Therefore I also studied plots at Flakaliden, Skogaby and Stråsan to which N fertilisers had been added for 9-30 years (Table 1).

Net N mineralisation and indices for nitrogen status and nitrate leaching (Paper II) were studied at all sites, long-term fate of N was evaluated in the three fertilisation experiments (Paper I) and short-term fate (15 months) of N was studied at Stråsan (Paper I). Calculations of uptake of N and availability of inorganic N were made for Stråsan (Paper I) and Flakaliden, in the latter case including a detailed fine root dynamics study (Paper III). Finally a study on the presence of amino acids in the soil water was carried out at Flakaliden (Papers IV and V).

Long-term fate of added nitrogen (Paper I)

In the fertilisation experiment the amount of N in soil and vegetation in fertilised plots was compared with corresponding amounts in non-fertilised plots. The fate of added N (soil, vegetation or missing) could thus be assessed. The soil N pool was determined by sampling soil with cylinders (diameter 49 mm, see net N mineralisation below). Sample weight and total N concentration were used to calculate the

Table 1. *Descriptions of the sites included in the studies*

	Tunby	Klinta-skogen	Skogaby	Skogaby fertilised	Uddevalla 2	Uddevalla 3	Stråsan	Stråsan fertilised	Flakaliden	Flakaliden fertilised
Latitude	55°37'N	55°37'N	56°33'N	56°33'N	58°27'N	58°28'N	60°55'N	60°55'N	64°07'N	64°07'N
Longitude	14°06'E	13°27'E	13°13'E	13°13'E	11°46'E	11°44'E	16°01'E	16°01'E	19°27'E	19°27'E
Altitude (m asl)	125	105	95-115	95-115	120	110	360-410	360-410	310-320	310-320
Current N deposition (kg N ha^{-1}yr^{-1})	22[2]	22[2]	24[2]	24[2]	12[2]	12[2]	5[1]	5[1]	3[1]	3[1]
Current N addition (kg N ha^{-1}yr^{-1})				60				30		75
Mode of N application				weekly sprinkling				yearly solid		Daily sprinkling
Number of years				9				30		10
Total N addition (kg N ha^{-1})				780				1060		825
Leaching of inorganic N (kg N ha^{-1}yr^{-1})	25[5]	9[5]	<1[6]	26[6]	2[5]	<1[5]	<1[4]	<1[4]	<1[3]	<1[3]
Mean temperature °C	6.9[9]	7.9[10]	7.1	7.1	5.9[8]	5.9[8]	4.1[7]	4.1[7]	2.1	2.1
Precipitation mm yr^{-1}	774[9]	663[11]	1100	1100+	857[8]	857[8]	751[7]	751[7]	577	577+
Soil type (FAO)	Podsol	Podsol	Podsol	Podsol	Cambisol	Podsol	Podsol	Podsol	Podsol	Podsol
Stand age (yr)	47	40	33	33	32	55	41	41	35	35
Historical land use	beech forest	beech forest	calluna heath land	calluna heath land	agriculture	spruce forest	spruce forest	spruce forest	spruce forest	spruce Forest
Number of spruce generations	1	2	2	2	1	many	many	many	many	many
Number of replicates	1	1	3	3	1	1	2	2	4	4
Main source			Bergholm *et al.* (1995)	Bergholm *et al.* (1995	Nohrstedt (1992)	Nohrstedt (1992)	Tamm (1985)	Tamm (1985)	Linder (1995)	Linder (1995)

[1] http://www.smhi.se/sgn0102/n0205/atmosdep.htm: accessed 10-April-2002. [2] Calculated from measured wet deposition using dry deposition factors based on monitored wet deposition and calculated dry deposition (Lövblad *et al*., 1995). [3] Lars Lövdahl (1998, personal communication). [4] (Berggren *et al*., 1998). [5] Calculated from soil water concentrations of NO_3^- and NH_4^+ using Cl^- as a conservative ion. [6] (Bergholm and Nilsson, 1997). [7] Vintjärn Climatic Station (SMHI, unpublished data). [8] Svarteborg Climatic Station (SMHI, unpublished data). [9] Sankt Olof Climatic Station (SMHI, unpublished data). [10] Climatic stations at Lund and Sturup (SMHI, unpublished data). [11] Björnstorp Climatic Station (SMHI, unpublished data).

soil N pool, which thereafter was corrected for stoniness (Eriksson and Holmgren, 1996; Paper II). For Stråsan I calculated the above ground N pools from unpublished data (C.O. Tamm and H. Eriksson, pers comm.) of height, width and N concentrations using empirical biomass functions (Marklund, 1988). The roots were assumed to contain 15% of the total N pool (Lundmark, 1986). Ground vegetation biomass and its N content at Stråsan was assumed to be the same as in a similar experiment at Norrliden (C.O. Tamm (pers. comm.). For Flakaliden, the above ground tree pools were calculated in a similar way. (M. Strömgren, unpublished data.), and below ground pools were estimated from direct measurements of fine roots in soil cores (Paper III) and by assuming that the N content in coarse roots was 20% of the N content in stem and branches (Persson *et al.*, 2001). Both above ground (B. Olsson, pers. comm.) and below ground (Paper III) biomass and N concentration of the ground vegetation were measured at Flakaliden. The vegetation pool in Skogaby was estimated in a similar way (Persson *et al.*, 2001).

Short-term (15 months) fate of added nitrogen (Paper I)

$^{15}NH_4Cl$ (99% excess) was sprinkled onto the ground (0.1 g m^{-2}) in small subplots within a fertilised plot at Stråsan in June, 1995. The whole subplots were sampled 15 months later and divided into vegetation (above ground and below ground) and soil (L-, F- and H-layer). Adsorbed NH_4^+ was extracted with K_2SO_4, before the samples were hydrolysed (6M HCl, 100 °C, 24h). Hydrolysates were then fractionated into NH_4^+ (released during hydrolysis), amino sugars and amino acids, in which concentrations of ^{14}N and ^{15}N were determined.

Nitrogen fluxes (Papers I, II and III)

Nitrogen uptake by vegetation

Annual N uptake was estimated for Stråsan and Flakaliden as the sum of N accumulation in vegetation and N in litter production. The accumulation was calculated from annual increases in vegetation N pools (see above).

Above ground litterfall was measured 1981-1988 (Stråsan; C.O. Tamm unpublished data) and 1987-present (Flakaliden; M. Strömgren unpublished data) with litterfall collectors placed on the ground. In fertilised plots at Stråsan, the maximum size of the tree canopy was already reached in 1982 (Axelsson, 1985) and the litterfall in 1996 was assumed to be the same as the average litterfall during 1981-1988. In control plots the maximum canopy size was not yet reached and the litterfall increased during 1981-1988. The litterfall in 1996 was extrapolated from these earlier data. N content in the litterfall was thereafter calculated from measured N concentrations. Litterfall from field layer vegetation and mosses was estimated at 40% (B. Olsson, pers comm.) and 18% (Rüling and Tyler, 1970) of the standing biomass (see above).

The below ground litter formation at Stråsan was estimated to be 2.3 times the above ground litterfall (Nilsson and Wiklund, 1994; Persson and Wiren, 1995) whereas at Flakaliden root litter formation was calculated from standing fine root biomass and fine root longevity (Paper III). The fine root dynamics were studied using minirhizotrons (Johnson *et al.*, 2001) and the longevity of fine roots was estimated with the median longevity based on root length (the time by which 50% of any new root length produced had died) as calculated by a Kaplan–Meier survival analysis. The fine root production (g $m^{-2}yr^{-1}$) was calculated as the ratio between standing biomass (g m^{-2}) and longevity (yr). Assuming steady state concerning fine root biomass, fine root litter formation equals fine root production.

Net nitrogen mineralisation

Net nitrogen mineralisation was measured with *in situ* incubations of soil cores (Raison *et al.*, 1987). In each plot, 8-9 pairs of steel cylinders were pushed into the soil. One cylinder of each pair was sampled immediately and the other was capped with a plastic lid and left in the field for 6 weeks before sampling and insertion of new pairs of cylinders for a new incubation period. During the winter season the cylinders were left for 21-34 weeks. The measurements continued for one year. Sub-samples from each plot, soil horizon and sampling event were bulked into one sample. Net N mineralisation was then calculated as the difference in inorganic N (extracted by 2 M KCL) between the field-incubated soil samples and the non-incubated samples.

Nitrogen losses with percolating soil water

The losses of nitrogen in runoff water were estimated from soil solution concentration and water transport. Soil water was sampled with tension lysimeters under the root zone at 50 cm depth. Runoff was estimated by using the SOIL model (Jansson and Halldin, 1979; Jansson, 1998) for Flakaliden (L. Lövdahl pers comm.) and Skogaby (Bergholm and Nilsson, 1997). At the other sites, runoff was estimated from the input-output balance of chloride ions, which were assumed to behave in a conservative way in the soil. Data concerning nitrate losses from Tunby, Klintaskogen and Uddevalla were provided by O. Westling (pers. comm.).

Modelling of potential net nitrogen mineralisation (Papers I, II)

I used the computer-based model PnET-CN (Aber *et al.*, 1997) in order to estimate the potential net N mineralisation. PnET-CN is a generalised lumped parameter model that simulates water, carbon and nitrogen dynamics in tree stands with a monthly time resolution. Driving climatic variables include latitude, monthly means of daily maximum and minimum air temperatures, global radiation, precipitation and N deposition. The model was parameterised for North American sites using values from Aber *et al.* (1995a, 1995b, 1996, 1997). Where appropriate, available data for Swedish conditions were used (Paper II). The maximum sustainable net N mineralisation was estimated as the simulated net N mineralisation of an undisturbed forest at steady state. As long as the N deposition is higher than the losses, N will accumulate in the system and net mineralisation will increase from year to year. At last steady state is reached in the model when there is no net tree growth and N mineralisation is equal to N uptake and N leaching is equal to N deposition. For Stråsan, the dynamic performance of the model was tested by comparing simulated values of tree growth and net N mineralisation with measured values between 1967 and 1996. This necessitated a longer simulation period including simulated whole tree harvest and burning of the clear-cut areas every hundred years since the 16th Century. Burning of the site was implemented as a reduction in the soil C and N pools of 20%.

Organic and inorganic nitrogen forms in forest floor soil solution (Papers IV, V)

Soil water from the forest floor at Flakaliden was sampled with Rhizon SMS tension lysimeters (Rhizosphere research products, Wageningen, the Netherlands). These lysimeters are small (2.5 mm in diameter *100 mm long) and made from a porous polymer with a mean pore size of 0.1 µm (Meijboom and van Noordwijk, 1992). The small size makes them easy to insert into either the F- or H-layer of an organic soil horizon and minimises the soil disturbance. The small pore size theoretically gives a sterilising effect through filtration. In combination with pre-evacuated sterile sample tubes, this makes it possible to analyse free amino acid concentrations in water samples even some time after the start of sampling.

Before the main study, the sterilising effect of the lysimeters was confirmed in the laboratory (Paper IV). In the main study at Flakaliden (Paper V), soil solution concentrations of inorganic nitrogen, total dissolved nitrogen and amino acid nitrogen were investigated by installing 8 lysimeters in each plot; 4 in the F-layer and 4 in the H-layer. Samples were taken during 1 night (≈14h) every third week during the growing season. Before analysis, the samples were pooled into one F-layer and one H-layer sample per plot and sampling event.

Chemical analyses

Total C and N in soil and plant material (Papers I, II, III)

Total C and N concentrations were determined on dried and milled samples using an elemental analyser (LECO CNS 932, USA). In samples from the ^{15}N addition experiment, ^{15}N was analysed using mass spectrometry (Thermoquest Delta plus - continuos flow, USA).

Ammonium and nitrate (Papers I, II, IV, V)

Ammonium and nitrate in both soil extracts and soil water were measured using flow injection techniques (Tecator Fiastar, Sweden). Nitrate was reduced to nitrite on a cadmium column, and the concentration was measured as the colour intensity of an azo dye formed after reaction with two reagents (Tecator, 1992b). Ammonium was measured as a colour change of an indicator caused by ammonia diffusion from the sample into the indicator after alkalisation of the sample with NaOH (Tecator, 1992a).

Amino acids (Papers IV, V)

Amino acids were separated and quantified by reverse phase high performance liquid chromatography (HPLC) using a method developed by and commercially available from Waters under the name AccQ•Tag™ (Waters, 1993). This is a pre-column derivatisation method yielding stable derivatives that are detected with a fluorescence detector at the sub pico-mole level. Alpha butyric acid (ABA) (Sigma-Aldrich, Germany) was used as an internal standard. Hydrolysable amino acids in the soil water were analysed in the same manner after vapour phase acid hydrolysis in 150°C for 1 h (Tarr, 1986).

TON/TOC (Paper V)

Total organic nitrogen was calculated as the difference between total dissolved nitrogen (TDN) and inorganic nitrogen. TDN was measured using a total nitrogen analyser which oxidises N to NO that is detected (Mitsubishi TN-05, Japan). TOC was measured using a carbon analyser (Shimadzu TOC-5000A, Japan) which detects CO_2 produced by combustion of the sample (total C) and CO_2 evolved upon acidification and sparging of the sample (inorganic C), where TOC = total C-inorganic C.

Results and Discussion

Fate of added N

The long-term fate of added N was evaluated in the fertilisation experiments where there was a well-defined extra addition of N to the ecosystem. By comparing fertilised plots with control plots, there is no need to know the pool sizes from the beginning of the experiment or to measure the total atmospheric N deposition, assuming that there is no difference between plots in these respects. In this case "long term" means 9-30 years and "fate" includes the possibilities that N could be found in the soil or in the vegetation or that it could be missing and thus assumed to be lost from the system. The measurements of the soil pools indicate that rather large amounts of N could be recovered in the soil, although there were great differences between the different sites (Table 2). In the highly N limited experiment Stråsan, about 75% of the added N was recovered in the soil in N1 and N2 plots 30 years after the start of the experiment. This increase in soil N was almost entirely located in the organic soil layer. Higher N addition (N3) did not lead to a further increase in the soil N pool. At Flakaliden, with a similarly low N deposition, the increase in the organic layer was only 20% of the added N, and in the mineral soil, fertilised plots had even lower amounts compared to non-fertilised, although the difference was non-significant. The difference between Flakaliden and Stråsan might be due to the different N addition methods. At Flakaliden, N was added throughout the growing season together with other nutrients in optimal proportions with the aim of maximising tree uptake and growth, whereas at Stråsan N was added as a solid salt once a year.

Table 2. *Amounts of added N and N pools (kg N ha^{-1}) in soil and vegetation in 1996 (Stråsan) or 1997 (Flakaliden, Skogaby)*

	Stråsan				Skogaby		Flakaliden	
	NO	N1	N2	N3	I	IF	I	IL
Total N addition	-	1060	1760	2820	-	780	-	825
Soil								
L	30	120	160	170	80	200	20	30
F+H	540	1260	1730	1440	850	930	330	500
E	100	120	100	90	680	770	290	310
$B_{0\text{-}5cm}$					330	390	280	260
$B_{5\text{-}20cm}$					990	1090	410	310
Soil total	670	1490	1990	1710	2930	3380	1330	1420
Soil increase	-	820	1320	1040	-	450	-	90[a]
Vegetation	370	670	640	610	570	910	280	660
Vegetation increase	-	300	270	240	-	340	-	380
Total	1040	2160	2630	2320	3500	4290	1610	2080
Total increase	-	1120	1590	1280	-	790	-	470
Missing	-	-60	170	1540	-	-10	-	360

[a] There was an increase (+180 kg N ha^{-1}) in the organic layer but a decrease (-90 kg N ha^{-1}) in the mineral soil of the fertilised plots.

At Flakaliden, a greater amount was taken up in vegetation compared to what was found in the soil, while the opposite was true for Stråsan. The average annual accumulation in vegetation was also higher for Flakaliden (38 kg N ha^{-1}yr^{-1}) than Stråsan (10 kg N ha^{-1}yr^{-1}), although this could be explained by differences in stand development. The fertilised plots at Stråsan reached maximum canopy size in the 1980s (Axelsson, 1985) and have since then only accumulated N in relatively N-poor stems, branches and coarse roots, whereas the IL plots at Flakaliden were still increasing their canopy size at the time of this study. The annual N accumulation was thus mainly due to an increased amount of N in needles (Strömgren, 2001). The annual N accumulation in vegetation at Stråsan might have been as great as that at Flakaliden before crown-closure. The smaller average annual increase in the N pool in the soil organic layers at Flakaliden (18 kg N ha^{-1}yr^{-1}) compared to Stråsan (27 kg N ha^{-1}yr^{-1}) might have the same explanation, namely that the IL plots had not yet reached crown closure or maximum canopy size and accordingly the litter production was still low. If the accumulation of N in the organic horizons was mainly due to increased amounts of N in litter formation at Stråsan, the lower increase in litter formation at Flakaliden would lead to the observed lower N accumulation in organic horizons.

As no or only minor losses of nitrate were recorded from IL plots at Flakaliden and N1 plots at Stråsan, one would expect to find an extra amount of N corresponding to the total fertilisation-N added. This is the case at Stråsan where the extra amount N found in the system as a whole well matches the total N additions. At Flakaliden, the extra amounts in vegetation and soil together only account for less than 65% of the added N. If the pool estimates are true, there must have been high N losses from IL plots at Flakaliden (36 kg N ha^{-1}yr^{-1}). Johnson (1992) pointed out that N added continuously in low doses (atmospheric deposition or a sprinkling system like at Flakaliden) rather than in a few high doses, as is typical in fertilisation experiments, is retained in the system to a lesser extent, and also that the soil retention in such systems is more or less non-existent. According to this assumption, one would expect some nitrate leaching from the site. In contrast to this, Nilsson *et al.* (1998) found soil retention in most Swedish ecosystems, where they compared stem accumulation with deposition and leaching. The fertilisation scheme used at Flakaliden, where nutrients were applied continuously only during the most active growing season, would also minimise leaching losses. Denitrification is another possible loss of N. In well-drained soils this process is restricted to micro-sites with low redox potential, and often limited by the availability of nitrate (Henrich and Haselwandter, 1997). Thus a daily supply of nitrate together with irrigation might provide best possible conditions for denitrification in a well-drained acid forest soil. Very high denitrification rates have been observed in fertilisation experiments in a spruce stand growing on a partly waterlogged soil (Mohn *et al.*, 2000); up to 13 mg $m^{-2}d^{-1}$, which could be compared with the average fertilisation rate at Flakaliden which is 110 mg $m^{-2}d^{-1}$ during the growing season. The rate in drained soils is however usually much lower, although in some cases reaching up to 7 kg N $ha^{-1}yr^{-1}$ (Brumme and Beese, 1992; Mogge *et al.*, 1998; Papen and Butterbach-Bahl, 1999) and would thus not explain the missing N at Flakaliden. Denitrification measurements at Flakaliden would however be of great interest.

At Skogaby, the atmospheric deposition of N is much higher. The control plots are receiving about two thirds of what the moderately fertilised N1 plots at Stråsan receive, and the fertilised plots at Skogaby receive much more N than any treatment at Stråsan or Flakaliden (Table 1). Sixty percent of the added N was recovered in the Skogaby soil, an increase found in both organic soil layers and in the mineral soil. The increased amount of N in the mineral soil is an indication of downward transport of N in the soil with the soil solution and/or an increased root litter formation. High nitrate leaching has been detected from fertilised plots, which confirms that N is transported in the soil in fairly high amounts. Adding the difference in vegetation pools between IF and I plots as estimated by Persson *et al.* (2001) to the soil pool difference, the retention in Skogaby IF plots apparently exceeds 100%, despite the fact that great nitrate losses have been observed (Bergholm and Nilsson, 1997).

The extra N input at Skogaby could possibly be higher than the actual additions through the sprinkling system. As the fertilisation has led to an increased tree growth, the atmospheric dry deposition of N, which is important at Skogaby, might have increased. The throughfall actually contained about 5 kg N ha^{-1} yr^{-1} more in plots treated with ammonium sulphate (which like IF plots showed increased tree growth) compared to control plots (Bergholm and Berggren, 2001). However, this increase is probably due to a lesser uptake of N being intercepted in the crown rather than to an increased dry deposition of N, as indicated by the fact that there was no difference in sulphate amount in throughfall between control plots and NS plots.

The apparently poor match between addition and retention + observed losses that was found at Flakaliden, could be due to both incorrect pool estimates and real differences between plots even before the N additions. The soil pool estimates excluded soil layers below the 10-20 cm layer in the B-horizon, and any changes at these depths were not recorded in this investigation. As only low concentrations of both DON and inorganic N have been found in the soil solution in the mineral soil, the transport of dissolved N to deeper soil layers is probably small. The standard error of each plot-wise estimate of the total soil N pool calculated from the eight within-plot replicates (see soil sampling in connection with net N mineralisation measurements) was 30-70 kg N ha^{-1}. The main reason is more likely to be differences between plots even before the start of the experiment. The variation in the soil pool estimates of the different plots within the same treatment (and within blocks) is great. Despite the high variance of the mineral soil N pool estimates, the

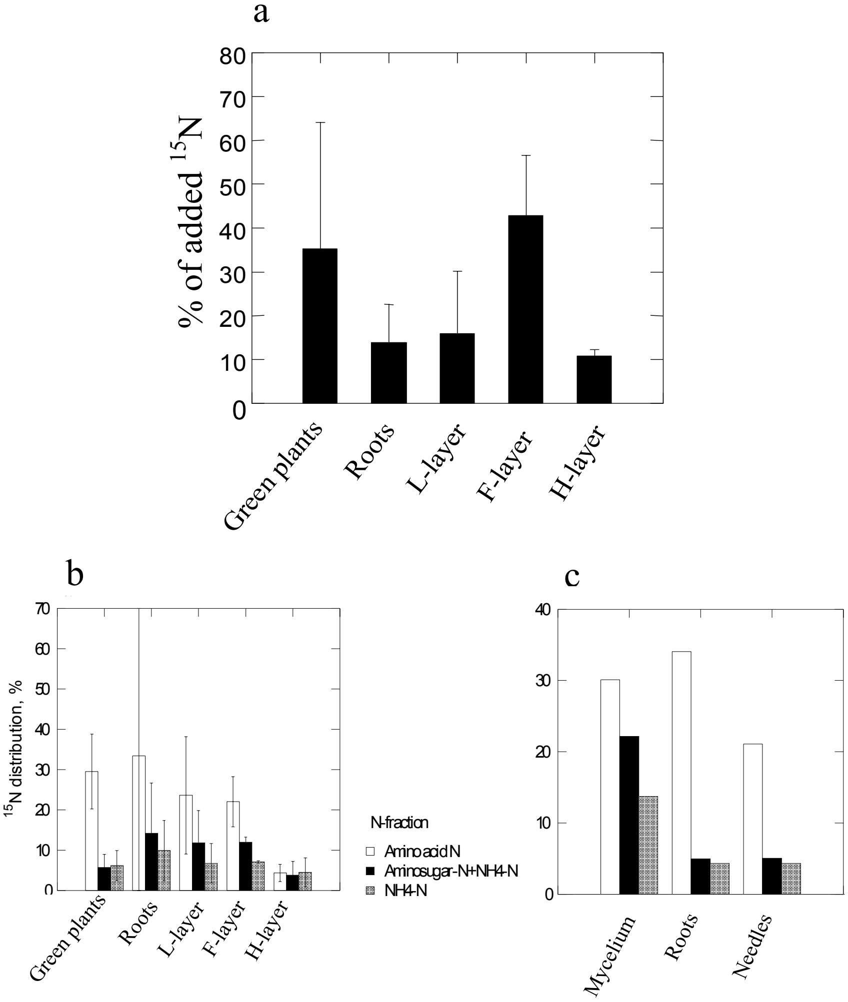

Figure 1. a; Recovery of ^{15}N (% of added) in vegetation and soil fractions 15 months after addition of $^{15}NH_4Cl$ (0.1 g N m^{-2}) on the forest floor. b-c; Distribution of ^{15}N in hydrolysed soil and vegetation fractions (% of total ^{15}N in the hydrolysate), b; samples from the field experiment in (a), and c; samples from a laboratory experiment where fungi and spruce plants were grown separately with $^{15}NH_4^+$ as nitrogen source. Before acid hydrolysation (6 M HCl) the samples were washed with 0.25 M K_2SO_4.

measured *lower* N pools in mineral soil in fertilised plots indicate that the retention of fertiliser-N in the mineral soil is of minor importance. If the non-significant difference between mineral soil N pools is disregarded and only organic layer retention is considered, the missing N in Table 2 becomes 270 kg N ha^{-1}.

The short-term (15 months) fate of added ^{15}N at Stråsan indicated a strong N immobilisation in the uppermost part of the soil and in vegetation. Fifty-five per cent of the added ^{15}N was found in the L- and F-layers, whereas only small amounts were found in the H-layer (Fig. 1a). Most of the ^{15}N in the soil was recovered in organic form and only small amounts were extracted as NH_4^+ with K_2SO_4. Later experiments in our laboratory with sterile and non-sterile soil material indicate that the immobilisation was biological rather than chemical (L. Johnsson, pers. comm.). Chemical immobilisation of ammonium was very low at prevailing soil pH (pH 4), whereas in soil where the pH was adjusted to pH 7, the chemical immobilisation was higher. The proportions of ammonium, amino acids and amino sugars in the hydrolysates of the L- F- and root-fraction resembled the proportions found in hydrolysates of fungal mycelium, indicating that fungal immobilisation of added N was an important immobilisation process (Fig. 1b-c).

Nitrogen status of Swedish spruce forest ecosystems

The sites investigated in this thesis had very different N status in terms of net N mineralisation or C/N ratio (Table 3). At the two northern sites with a low N deposition and with a land use history of several generations of spruce forestry including prescribed burning, the net N mineralisation rate was 4-5 kg N ha^{-1} yr^{-1} and the C/N ratio in the F+H layer was 34-39. In the southernmost part of Sweden, net N mineralisation was 104 kg N ha^{-1} yr^{-1} and the C/N ratio 24 at a site with high N deposition (Tunby). This site had a first generation stand of Norway spruce after European beech. The question is whether it is the deposition or the land use history that is most important for the current N status. Comparing the three sites in the high deposition area (Skogaby, Klintaskogen and Tunby), there are great differences despite the similar N deposition. Skogaby's land use history (Malmström, 1951) consists of hundreds of years of intensive grazing in combination with repeated burning of the site every 4-6 years in order to control the heather (*Calluna vulgaris* (L.) Hull.). This land use would have depleted the soil of N before the site was reforested at the beginning of the 20th Century. Indeed the N status is much lower than at Tunby with a net N mineralisation rate of 37 kg N ha^{-1} yr^{-1} and a C/N ratio of 29 in the F+H layer. Klintaskogen is intermediate with a second-generation spruce forest after beech. A similar importance of the land use history can be seen At Uddevalla where the former agricultural field had a much higher net N mineralisation rate (86 kg N ha^{-1} yr^{-1}) compared to the site with continuous forestry (35 kg N ha^{-1} yr^{-1}), although this difference might rather be seen as an effect of historic N additions resulting from an agricultural land use including fertilisation.

The effect of N deposition could be evaluated by means of the fertilisation experiments. In all three experiments the net N mineralisation rate had increased significantly in the fertilised compared to the non-fertilised plots. At Stråsan it had increased tenfold to 50 kg N ha^{-1} yr^{-1}, at Flakaliden it had increased 4.5 times to 18

Table 3. *Annual net N mineralisation, nitrification, modelled maximum net N mineralisation and soil C/N ratio at seven Swedish spruce forest sites, including three sites with N-fertilised plots. The data are based on field measurements during one year*

Flux	Tunby	Klinta-skogen	Skogaby	Skogaby fertilised	Udde-valla 2	Udde-valla 3	Stråsan	Stråsan fertilised	Flakaliden	Flakaliden fertilised
Net mineralisation (kg N $ha^{-1}yr^{-1}$)										
L	18.2	10.6	5.5	19.8	9.2	3.8	0.3	6.7	0.0	2.8
F+H/A_h	45.3	31.4	21.8	34.2	37.5	22.5	4.1	40.4	2.1	10.1
E	9.5	8.9	7.8	9.6		5.7	0.6	1.8	1.2	3.3
B	31	13.9	1.7	6.3	39	3.2	0.3	0.9	0.7	1.5
Total	104	65	37	70	86	35	5.2	50	4.1	18
Net nitrification (kg N $ha^{-1}yr^{-1}$)										
L	1.3	1.2	0.0	3.9	3.3	0.0	0.0	0.8	0.0	0.0
F+H/A_h	3.4	7.8	0.0	5.1	12.1	0.0	0.1	0.1	0.0	-0.1
E	1.9	3.4	0.3	0.5		0.0	0.0	-0.3	0.0	-0.1
B	21.1	10.5	0.2	4	23.3	1.6			0.4	0.7
Total	28	23	0.4	14	35	1.6	0.1	0.6	0.4	0.5
Maximum mineralisation[b] (kg N $ha^{-1}yr^{-1}$)	68	69	67	67	70	70	56	56	60	60
Relative mineralisation	1.53	0.94	0.55	1.04	1.22	0.50	0.09	0.89	0.07	0.3
C/N ratio (g g^{-1})										
L	27	28	33	27	34	40	41	28	42	32
F+H /A_h	24	25	29	27	18	33	34	27	39	32
E	23	21	25	24		29	25	23	27	24
$B_{0\text{-}5cm}$	22	20	24	23	14	25			26	24

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[a] B-horizon mineralisation estimated from the ratio between mineralisation in B and E horizons in the other podzols in this study.

[b] As simulated with the PnET-CN model.

kg N ha^{-1} yr^{1} and at Skogaby it had doubled to 70 kg N ha^{-1} yr^{-1} (Table 3). C/N ratios had also changed considerably due to the fertilisation (Table 3) and the two indices (C/N ratio and net N mineralisation per unit weight of C) were well correlated in the F+H layer (Fig. 2) regardless of land use history and mode of N addition (atmospheric deposition or fertilisation on the ground). In fact the C/N ratio of the F+H layer was also fairly well correlated to the total mineralisation rate (kg N ha^{-1} yr^{-1}) including mineral soil (r^2=0.78, p<0.001). Scott and Binkley (1997) found a non-linear relationship between lignin/N-ratio in the litter and the mineralisation rate, but the partly similar relationship (C/N ratio in the litter and total net N mineralisation rate) in our data set was weaker than for the F+H layer. For use as an N status index, I propose that the C/N ratio of the F+H layer rather than that of the litter should be used. The larger and older pool of C and N in the F+H layer would have a longer memory of historic land use and N deposition, which is important for the current N status as discussed above. The relative net N mineralisation, *i.e.* the ratio between the current measured net N mineralisation and the maximum sustainable net N mineralisation at the site (as modelled by PnET-CN), ranked the sites with respect to N status in exactly the same order as did the net N mineralisation rate itself. This was due to the fact that the same tree species was growing at all sites, resulting in similar maximum sustainable net N mineralisation rates at all sites (Table 3). However, this approach indicates that the net N mineralisation of 50 kg N ha^{-1} yr^{-1} at Stråsan would mean the same high N status as the 65 kg N mineralised per year at Klintaskogen, as the net N mineralisation at Stråsan is already close to the maximum possible level according to the PnET-CN model (Table 3).

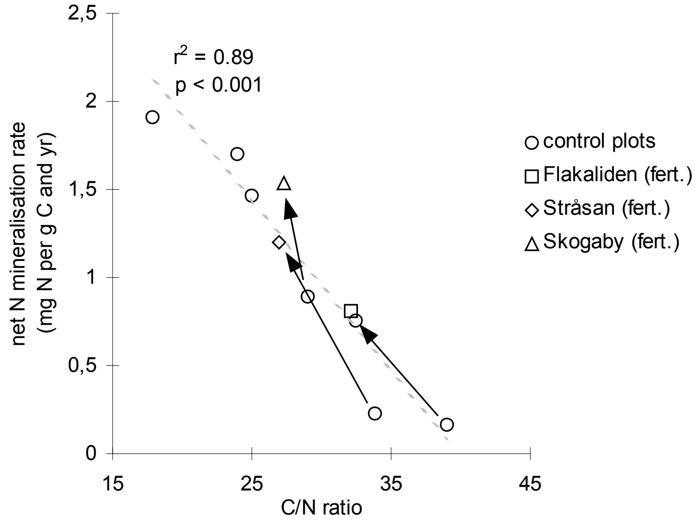

Figure 2. Net N mineralisation rate vs. C/N ratio in the F+H layer. The arrows show how net N mineralisation and C/N ratio in the F+H layers have changed in response to N fertilisation at three of the sites. p and r^2 values refer to a linear regression analysis (dotted line).

In the discussion above, only steady state conditions as simulated by the PnET-CN model were considered, but this raises the question of how the model performed dynamically before steady state was reached. The tree growth and net N mineralisation rate in control plots were simulated in a satisfactory way although the initial growth was somewhat high. However, the response to the rapidly increasing N additions at Stråsan was too slow (Fig. 3a). While measurements showed no N leaching and an almost immediate response to the N additions, the

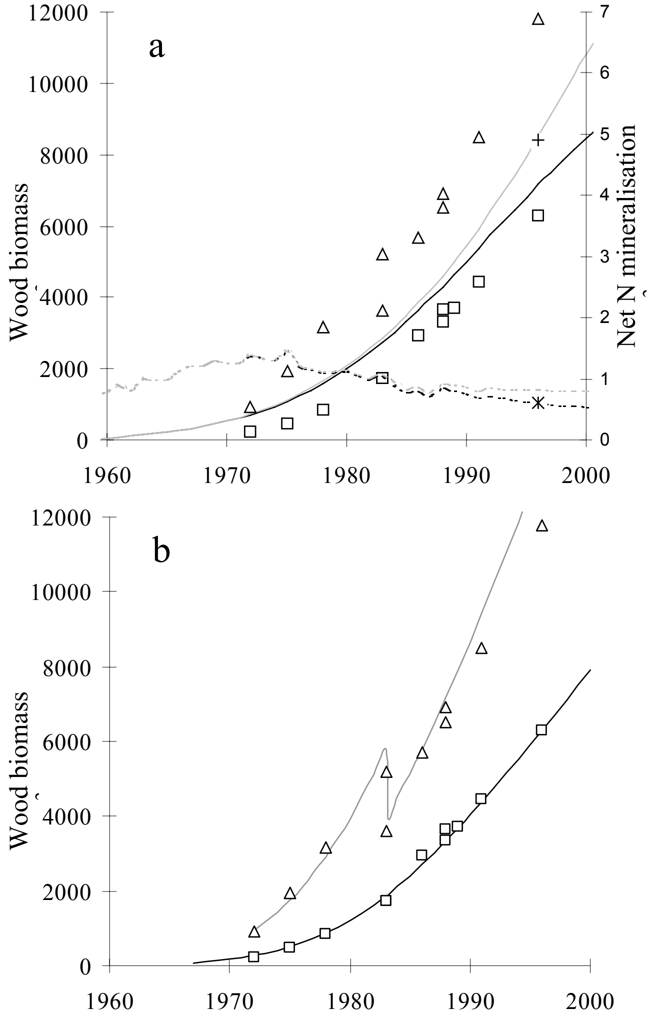

Figure 3. a; Measured wood biomass in control plots (□) and N1 plots (Δ), modelled (PnET-CN) wood biomass (solid line) in control plots (black line) and N1 plots (grey line), measured net N mineralisation (1996) in control plots (✶) and N1 plots (+), and modelled net N mineralisation (dotted line) in control plots (black line) and N1 plots (grey line). b; Legend as above, wood biomass was modelled with PnET-V2 and foliage N concentration was fixed at values measured in the early period of the experiment and N1 biomass in 1972 was set to measured value. The sudden decease in wood biomass in 1983 was due to thinning.

modelled demand for N in the small plants was low and leaching substantial, and hence there was no growth difference between treatments. As plants increased in biomass, yearly growth and N demand, the extra N additions kept foliage N concentration higher in fertilised plots than in control plots, resulting in a difference in growth rate. The model predicted a temporary decrease in foliage N concentrations, implying N limitation also in fertilised plots, which was not confirmed in the field, and accordingly the growth rate kept lagging behind compared to field measurements. However, using the model PnET-V2 (Aber *et al.*, 1995b), in which foliage N concentration is given as a model input according to measured values, modelled growth matched measured growth fairly well (Fig. 3b). The dynamic problem was thus located in the soil- and N availability part of the model. An important factor concerning N retention in the system in this application was the lack of understorey vegetation that would have circulated N and kept it in the system during the first years after plantation. The use of a single homogeneous soil pool does not seem to hold when dramatic and sudden changes are introduced into the ecosystem. However, the ability to simulate "stable" control plots and the tree-part performance of the PnET-V2 model in fertilised plots still makes it relevant to apply steady state conditions as simulated by PnET-CN, despite the poor dynamic model performance at the Stråsan site.

Nitrogen leaching related to nitrogen status and nitrogen deposition

As presented above, it is evident that very great changes take place in the forest soil when large amounts of N are added over many years. Not only is the soil N pool increasing as a result of increasing amounts of organic matter with higher N concentration, but the turnover rate of this pool is also increasing. The question then is wheteher the N status, depending on the historic N input and land use, correlates with N leaching from these sites. The answer is both yes and no. As seen in Fig. 4a, all sites having a net N mineralisation greater than 60 kg N ha^{-1} yr^{-1} leach nitrate, and no sites with a net N mineralisation less than 60 kg N ha^{-1} yr^{-1} leach nitrate. However, there was no correlation between the amount of nitrate leached and the net N mineralisation above 60 kg N ha^{-1}yr^{-1}. The use of relative net N mineralisation did not change these findings at all. Again this was mainly because only sites with the same tree species within a narrow climatic gradient showed enhanced nitrate leaching. These sites had almost the same modelled maximum sustainable net N mineralisation rate. The strength of the relative mineralisation approach is rather to make it possible to compare different tree species, both coniferous and deciduous, growing under different climatic conditions (Aber *et al.*, 1997), and the value of such a use would still be very interesting to test further.

The C/N ratio also divided the sites between leaching (C/N≤ 27 in the F+H layer) and non-leaching (C/N≥27), although there was an overlap at C/N=27 (Fig. 4b) which is in line with the findings of Gundersen *et al.* (1998a). It was the fertilised plots at Stråsan that showed no nitrate leaching despite a C/N ratio of 27. The same plots had the highest net N mineralisation of the non-leaching sites, all suggesting that these plots are now close to nitrate leaching. The relatively fast change (30 years) in this initially N deficient site to a site close to N saturation caused by moderate fertilisation (35 kg N $ha^{-1}yr^{-1}$) shows that the risk for regional N saturation in Sweden should be taken seriously.

As expected and as has been shown before (Johnson, 1992; Dise and Wright, 1995; Nilsson *et al.*, 1998), there was no good correlation between current N deposition and nitrate leaching (Fig. 4c). Leaching occurred at sites with as low N input rate as 12 kg N ha^{-1} yr^{-1}, whereas 78 kg N ha^{-1} yr^{1} was received at another site without resulting in nitrate leaching. Summarising Fig. 4a-c, it appears that nitrate leaching does not occur in systems with low availability of inorganic N in terms of either low N status or low N deposition. At sites with high N status or high N deposition, the nitrate losses are varying. It then falls naturally to combine N status with current N deposition to test if the two interact, *i.e.* as N status increases, N leaching occurs at lower N deposition rates. The most straight forward way of combining N deposition and N status is simply to add N deposition and net N mineralisation, as they are given in the same unit. The resulting soil flux density of mineral nitrogen (SFDN) represents available mineral N. Figure 4d shows that SFDN is much better correlated to nitrate leaching than either N status or N deposition alone. In our investigation there was a threshold value around 90 kg N $ha^{-1}yr^{-1}$ so that leaching of nitrate only occurred at higher SFDN values, and this leaching was correlated to SFDN (r^2=0.74, p=0.06, n=5). All sites within our investigation were mid-age spruce stands, and other species and stand-ages might show other threshold values. Interestingly, the sites within the European NITREX project (Gundersen *et al.*, 1998b) also fit into this relationship reasonably well (Paper II).

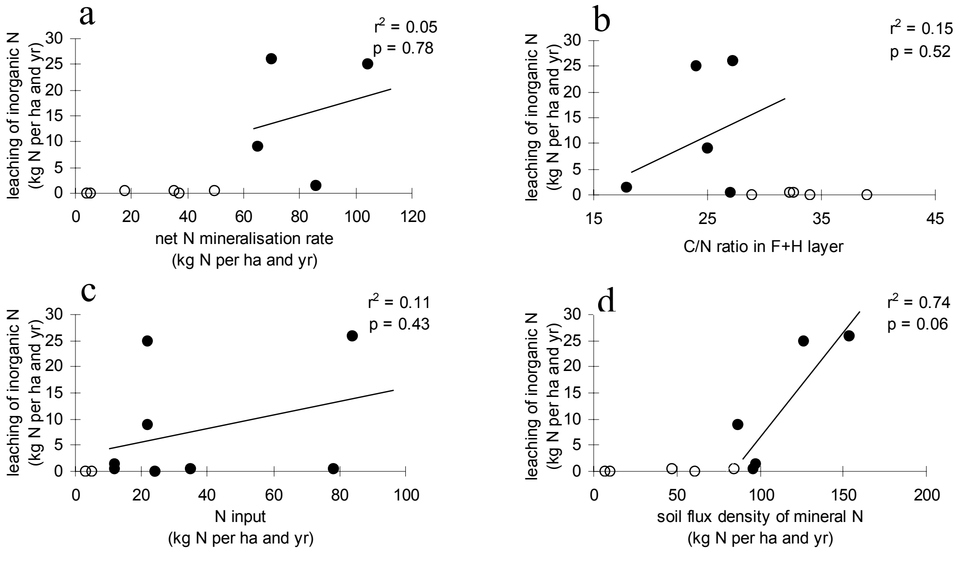

Figure 4. Leaching of inorganic N vs. (a) net N mineralisation rate, (b) C/N ratio in the F+H layer, (c) N input (deposition + fertilisation) and (d) soil flux density of mineral N (deposition + fertilisation + mineralisation). The linear regression models are based on data points beyond the threshold value at which leaching starts to occur (filled symbols).

In other investigations the use of both N mineralisation rate and N input rate either improved the fit of a linear regression model explaining NO_3^- leaching (r^2 = 0.64) compared with using N mineralisation or N input alone (r^2 = 0.44 and r^2 =0.11 respectively) (Van Miegroet *et al.*, 1992) or did not improve such a regression (Gundersen, 1995). However, in the cited studies, mineralisation rates and the soil flux density of mineral N were not based on both total net N mineralisation rates and total N deposition rates. Van Miegroet *et al.* (1992) used the N mineralisation rate in the upper mineral soil (0-10 cm) and the total input of N in a multiple linear regression analysis, whereas Gundersen (1995) used N flux in throughfall and N flux in throughfall + litterfall as substitutes for the N input and soil flux density of mineral N respectively. Matzner and Grosholz (1997) and Gundersen *et al.* (1998) combined N deposition with another index of N status, the C/N ratio in the F+H layer, and could thereby improve the fit (r^2 = 0.74 and r^2 = 0.69 respectively) of a regression model explaining N leaching compared with using only the C/N ratio (r^2 = 0.51 and r^2 = 0.60 respectively) or N deposition (r^2 = 0.25 and r^2 = 0.48 respectively).

Thus summarising observed effects of N deposition to some Swedish spruce stands, it is evident that, depending on site history, the retention of N varies considerably. At sites with low N status, the retention is high, leading to an increased N status. Leaching of N occurs when nitrate is present in the percolating soil solution. This happens when the supply of inorganic N temporarily or chronically exceeds the demand of N, so that nitrate from deposition or nitrification is not immediately immobilised by microorganisms or taken up by trees. As the supply of mineral N depends on both N deposition and internal net N mineralisation, leaching is better correlated to the sum of these two fluxes than to either of them alone. Monitoring of national N status, and changes in this status, could be done by net N mineralisation rate measurement or, which might be more realistic, by measurements of C/N ratio in the F+H layer, which is strongly correlated to the net N mineralisation rate. However, a wish to evaluate the future risk of nitrate leaching with different deposition scenarios or different forest management practices necessitates the use of models. In view of the increasing knowledge of the possible importance of internal ecosystem fluxes of organic N, these models might also have to include these fluxes. The remainder of this thesis addresses the importance of organic N in Swedish spruce forest ecosystems.

Estimating organic nitrogen uptake by the budget method

The calculations of N uptake for Stråsan show that the availability of mineral N far from covered the need (Table 4). In control plots the net mineralisation and deposition of N (11 kg N $ha^{-1}yr^{-1}$) was not enough to sustain even the accumulation in tree biomass (18 kg N $ha^{-1}yr^{-1}$). The deficit of mineral N became even higher when litterfall was included in the analysis, and only 25% of the apparent need was then covered by mineral N. In fertilised plots, the accumulation of N in vegetation was actually lower than in control plots as the stands had already reached their maximum canopy size, so that accumulation of N only took place in the relatively

Table 4. *Fluxes of N in Stråsan and Flakaliden (kg N ha^{-1}yr^{-1})*

	Stråsan				Flakaliden	
	N0	N1	N2	N3	I	IL
(1) N input (deposition + fertilisation)	6	36	6	6	3	78
(2) Net N mineralisation	5.2	50	40	47	4.1	18
(3) Net N accumulation in trees	18	16	14	10	16	79
(4) N content in litter production	30	122	115	115	40	54
(above+below ground litter production)	(9+21)	(37+85)	(35+80)	(35+80)	(15+25)	(14+40)
(5) N leaching	<1	1	<1	<1	<1	<1
(6) Total uptake (3)+(4)	48	138	129	125	56	133
(7) Available inorganic N (1)+(2)	11	86	46	53	7	96
(8) Organic uptake (6)-(7)	37	52	83	72	49	37
(9) Soil balance (1)-(3)-(5)	-12	+19	-8	-4	-13	-1

N-poor wood of stem, branches and coarse roots. However, the need for N was still high, as part of the N-rich needles need to be replaced each year. The result is that 35% of the need was covered by net N mineralisation and deposition in the currently non-fertilised plots (N2 and N3) and that 60% was covered in the currently fertilised N1 plots. By estimating uptake of organic N in this way, it appears that the uptake is not decreased due to the increased availability of inorganic N in fertilised plots as compared to non-fertilised plots. A preference for inorganic N is often assumed (Beier *et al.*, 2001; Persson *et al.*, 2001), but is not supported in laboratory experiments (Padgett and Leonard, 1993; Jones and Darrah, 1994; Causin and Barneix, 1994).

The weakest point in the estimation of N uptake for Stråsan would be the root litter formation. Roots were not investigated at the site and the value of 2.3 as the ratio between root litter and above ground litter formation was taken from the rather different site Skogaby. It is not known whether this factor is the same for Stråsan, and indeed whether it is the same in all treatments. There are many other estimates of this factor, claiming that the root litter formation is anything from much smaller to many times greater than the above ground litterfall in different ecosystems. There is, however, a tendency for higher factors in later studies. In a more recent study at Skogaby, it appears that the ratio is not the same in plots receiving high loads of ammonium sulphate as in control plots. Whereas the ratio in control plots was comparable to the older figures used in this thesis, the ratio in NS plots was only 1.2. In contrast, the study at Flakaliden showed a higher ratio in the N fertilised plots; 1.7 in I and 2.9 in IL (Table 4). In N1 at Stråsan, the mineral N would cover the total need of N if the ratio between above and below ground litter production was set to 1.

The detailed root production study at Flakaliden increased the reliability of the N budget at that site in this respect. The biomass sampling showed no difference in fine root biomass in the mineral soil between I and IL plots (Paper III). In the organic soil layer, the biomass was about twice as high in the IL plots. There was also a significant difference in the longevity of fine roots between the treatments. In IL plots, the fine roots lived on average for a shorter period than in I plots (0.9 and 1.1 years respectively). Both a higher biomass and a shorter lifespan result in a higher production of fine roots (and litter production) in IL plots when production (g $m^{-2}yr^{-1}$) is calculated as the ratio between average biomass (g m^{-2}) and longevity (yr). The resulting estimated N uptake for Flakaliden exceeds the net N mineralisation in both non-fertilised plots and in fertilised plots. Again the organic uptake seems to be as high in the fertilised plots as in non-fertilised plots.

Persson *et al.* (2001) made a similar N budget for Skogaby where they found that the organic N uptake in control plots was 17 kg N $ha^{-1}yr^{-1}$, whereas they concluded that no organic N was taken up in plots that had received 100 kg N $ha^{-1}yr^{-1}$ as ammonium sulphate for 10 years. In that study the net N mineralisation rate was calculated from known relationships between net N mineralisation in sieved soil in the laboratory and soil moisture and temperature. This method gave higher estimates of the net N mineralisation than those reported in this thesis, which is one explanation for the lower apparent need for organic N in Skogaby. Persson *et al.* (2001) concluded that the root litter production is the most uncertain part of the budget, which also seems to be relevant for the Stråsan and Flakaliden sites.

There is an obvious problem regarding studies of root dynamics; the roots cannot be observed in their natural environment without being disturbed. One approach is to let the roots first grow in their natural environment and then observe them in a destructive manner. The problem associated with this approach is of course that one can never observe the same roots again, and it is impossible to know the dynamics from a number of observations of the average standing biomass alone. The minirhizotrone approach I used is in a way the opposite. The environment is first disturbed by inserting observation tubes, from which the roots can be observed in a non-destructive manner. In this way individual roots can be followed from emergence to death, and one also has a check from observation to observation on which roots are newly formed and which roots existed at the time of the previous observation. The main drawback is that any effect of the disturbance is included in the observations. Taken together, I believe that the use of minirhizotrons is the best available method to clarify root dynamics.

Organic and inorganic nitrogen in the forest floor soil solution

Organic N dominated the soil solution in both fertilised (IL) and non-fertilised (I) plots at Flakaliden. In I plots, DON constituted more than 95% of total dissolved N on average, and never less than 80%. This is a commonly reported proportion in ecosystems with a low natural N input whereas the opposite is often true in areas with high N deposition (Hedin *et al.*, 1995). However, despite the high N input of inorganic N to the IL plots, the sampled soil solution from fertilised plots was also dominated by DON. On average 75% (F-layer) and 90% (H-layer) of total dissolved N was in the form of DON. Note that this refers to the average of the DON proportion of each sample. In a few samples, the nitrate concentration was very high, also leading to high yearly average nitrate concentrations as shown in Table 5. Concerning N nutrition, much of this DON is thought not to be directly available to plants or to microorganisms, but rather only a small part, of which free amino

Table 5. *Concentration (mg N l^{-1}) of dissolved organic N (free amino acids, hydrolysable amino acids, total organic N), dissolved inorganic N (ammonium, nitrate) and dissolved organic C. Yearly average (standard errror) calculated from all individual samples (n=9-22). In samples with concentrations below the detection limit, half the detection limit was used rather than zero. IL = irrigation and liquid fertilisation, I = irrigation*

	IL		I	
	F-layer	H-layer	F-layer	H-layer
Amino acid-N	0.003 (0.001)	0.007 (0.005)	0.015 (0.007)	0.007 (0.003)
NH_4^+-N	0.145 (0.067)	0.031 (0.010)	0.023 (0.008)	0.025 (0.006)
NO_3^--N	2.54 (1.33)	0.391 (0.273)	0.038 (0.028)	0.015 (0.004)
DON	2.03 (0.38)	2.03 (0.30)	1.50 (0.24)	1.60 (0.54)
Hydrolysable amino acids	1.43 (0.34)	0.64 (0.09)	0.76 (0.17)	0.40 (0.10)
DOC	101 (12)	121 (19)	105 (14)	88 (15)
Sampled volume (ml)	7 (2)	11 (2)	3 (1)	10 (1)

acids have been identified as a potentially important N source. These directly accessible free amino acids constituted less than 1% of DON in soil water from the organic soil layers at Flakaliden.

The concentrations of free amino acids were thus generally very low, and in fact only half of the samples contained detectable concentrations of any amino acid at all (the detection limit for individual amino acids was around 0.2 pmol). The most common amino acids were glycine (identified in 37% of all analysed samples), glutamine/histidine (22%), aspargine/serine (21%) and aspartic acid (15%). There was a difference between IL plots and I plots in that free amino acids were even less commonly found in fertilised plots (in 38% vs. 68% of all samples). This difference indicates that amino acids are either released into the soil solution at a slower rate or taken up at a faster rate in IL plots compared to I plots. A possible explanation for the slower release of amino acids is that the production of proteolytic enzymes was reduced by the increased availability of ammonium, an effect that has previously been reported from laboratory experiments (Leake and Read, 1991). Another explanation could be that microorganisms specialised in mobilising and capturing organic N with the use of exoenzymes might be out-competed by mineral N species (Read *et al.*, 1989). Indeed the N fertilisation at Flakaliden has been shown to alter the mycorrhizal community structure (Fransson *et al.*, 2000), but whether there has been any functional change caused by this community change is yet unclear. An increased uptake of amino acids is also possible. The fertilisation has led to an increased net primary production, an increased above and below ground biomass and to an increased demand for N. An increased size of the extramatrical mycelium of the ectomycorrhizae could lead to an increased efficiency of organic N uptake. Increased N availability has previously been reported to either increase or decrease the size of the extramatrical mycelium depending on nutritional status (Ekblad *et al.*, 1995; Wallenda and Kottke, 1998). In laboratory experiments, an optimum N availability has often been found, although many field experiments have shown a decreased amount of extramatrical mycelium as an effect of N additions (Wallenda and Kottke, 1998). At Flakaliden, where an optimal nutrient status is achieved by balanced fertilisation, it is as yet unclear whether the mycelium has increased or decreased, and a "priming effect" of the fertilisation resulting in higher organic N uptake could not be ruled out. A similar tendency for lower amino acid concentrations in water extracts from the more fertile end of a boreal productivity gradient compared to the less fertile end was found by Nordin *et al.* (2001).

As expected, the concentrations of ammonium and nitrate were higher in fertilised compared to non-fertilised plots (Table 5). Concerning nitrate, very high concentrations were found in both soil layers (F and H) of the fertilised plots during the period of fertilisation (14 June-22 August), whereas the difference between treatments was not so obvious outside this period (Paper V), indicating that the high nitrate concentrations originate directly from the fertilisation rather than from an enhanced net nitrification. This is in line with the *in situ* incubation investigation (Paper II) where no enhanced net nitrification was found. Although ammonium concentrations in the samples from the F layer of the fertilised plots were considerably higher than in any other samples during the period of fertilisation, the differences were quite small at other times despite a much higher net N mineralisation. A probable explanation for this is that the mineralisation is most intensive during the same period as the fertilisation is carried out, and the absolute difference at other times could be rather small. Interestingly, the ammonium concentration in the soil water sampled from the H layer of the fertilised plots appears to be little affected by the fertilisation. There is no peak in the concentration during fertilisation and there is no big difference between the H layers of the

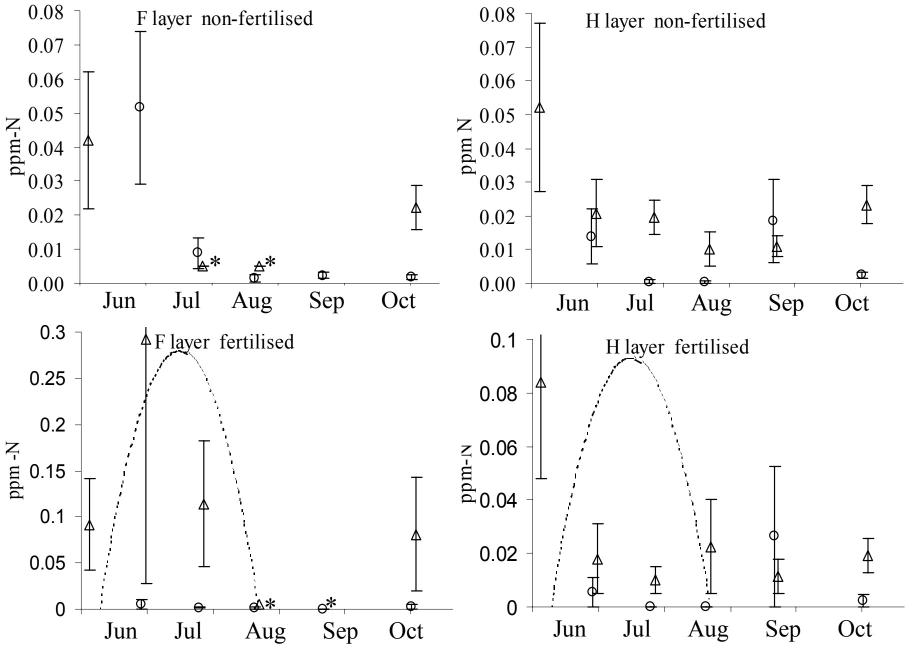

Figure 5. Ammonium (△) and amino acid (○) concentration in soil water from F and H layer of non fertilised (I) and fertilised (IL) plots in Flakaliden. Error bar indicates standard error. Samples below the detection limit are shown as half the detection limit and denoted with*. The dotted curve shows the fertilisation intensity during 1999 with a top value of 0.15 g N m^{-2}d^{-1} in mid July.

two treatments (Fig. 5, Table 5). The additions of $^{15}NH_4^+$ at Stråsan (Paper I) indicated the importance of the F layer with its dense mat of mycelium, resulting in only very small amounts of ^{15}N being recovered in the H layer. However, even if ammonium does enter the H-layer with the infiltrating water, it would primarily be in the macropores with a slow diffusion into smaller pores. The lysimeters, on the other hand, sample water even from these smaller pores and a difference in the macropore water composition would partly be masked by the composition of the water from smaller pores This fraction of the soil solution is in turn to a greater degree controlled by the solid phase, as the residence time is higher (Zabowski and Ugolini, 1990).

The budget calculations for Flakaliden indicated that organic N is of dominating importance in the non-fertilised plots, and as similar uptake kinetics for ammonium and several amino acids have been reported (Kielland, 1994; Schimel and Chapin, 1996; Wallenda and Read, 1999), one should expect the soil solution to be dominated by amino acids. However, looking at Fig. 5 this is not evident. In the H layer, ammonium is the dominating ion on most occasions, especially during the most biologically active period. In the F-layer, on the other hand, there are no reliable measurements of the ammonium concentrations as there is one missing value and two occasions below the detection limit. There is no doubt that the ammonium concentrations are very low, but so are amino acid concentrations. It is possible that exoenzyme exudation, hydrolysis and uptake mainly take place in an even tighter context, never letting the amino acid out in the soil solution, at least not into the fraction sampled with rhizon tension lysimeters. I believe this explanation to be the most probable for why the expected dominance of amino acids could not be confirmed in the non-fertilised plots.

Acid hydrolysis of the soil water resulted in a release of amino acid N that on average corresponded to 40% of DON. The variance was high, partly depending on the many different analytic steps involved in the assessment of this proportion (TDN, NH_4^+, NO_3^-, free amino acids, total amino acids after hydrolysis), and no significant effects were found of either treatment or soil depth. A possible explanation for the relatively big difference between soil depths, 55% in the F layer and 30% in the H-layer ($p=0.07$), is that DOM produced from a more decomposed material in the H layer, the effect of microbial exploitation for energy and nutrients, would contain less attractive amino acids that are released upon further acid hydrolysis in the laboratory. Michalzik and Matzner (1999) did not find such a difference in an N saturated forest in Germany. They found that 60-65% of the DON from organic soil layers was released as amino acids upon an alkaline hydrolysis. However, that study was based on zero-tension lysimeters, and as discussed above, the water thus sampled from macro-pores might be quite different from a zero-tension lysimeter solution.

Ecosystem recovery or nitrogen depletion?

Comparing ecosystem N input to N accumulation in the vegetation, it was seen that in control plots at Stråsan and Flakaliden, the vegetation increased its N pool at the expense of the soil N pool (Table 4). In N1 (Stråsan) the fertilisation means that more N is added to the system than is accumulated in vegetation, and the soil pool increases together with an increasing N mineralisation from this N pool as discussed above. When the additions are stopped (N2 and N3), the input becomes smaller than the accumulation in vegetation again, leading to a slowly decreasing soil N pool and net N mineralisation. The recovery in terms of N status might thus be a slow process, depending on trees gradually mining the soil for N and subsequent tree harvest. Gundersen *et al.* (1998b) found such slow changes in N status in the NITREX sites and Strengbom (2002) found that vegetation recovery was not complete 50 years after the last N addition in a fertilisation experiment. In contrast, nitrate leaching seems to cease almost instantaneously upon reduced N input. This could be seen at Stråsan where the leaching from N2 and N3 was low, as well as in the NITREX sites Ysselsteyn and Speuld where the leaching decreased rapidly when the high N deposition in the area was excluded from some plots by a roof construction (Gundersen *et al.*, 1998b). This is in line with the finding discussed above that nitrate leaching is dependent on both N deposition and N status. A high N status alone would not be enough to cause nitrate leaching.

Interestingly the accumulation in fertilised IL-plots at Flakaliden between 1995 and 2000 (Strömgren, 2001) equalled the input from deposition and fertilisation, and accordingly one should not expect the soil pool to have increased during this period. The net N mineralisation on the other hand might still increase as more fresh and N-rich litter is returned to the soil each year by an increased litterfall from the growing crowns. Until the stand reaches its maximum crown size, the soil might actually get depleted of N despite the high N input.

In the non-fertilised plots of Stråsan and Flakaliden, where N accumulation in trees exceeds the input from deposition, continuous harvesting would not be sustainable as N would become scarcer from generation to generation. If whole tree harvesting for energy production is employed, the problem would be even more accentuated (Rolff and Ågren, 1999). Although this might be in line with ecosystem recovery in areas with a former high N deposition, it is a delicate question to define when ecosystem recovery turns into ecosystem deterioration. One may also raise the question of whether or not forestry in low N deposition areas should include N fertilisation in order to protect "natural" ecosystem functioning and productivity, as well as species composition to an acceptable degree.

Conclusions

- The retention of added N in vegetation was similar at all sites (240-380 kg N ha^{-1}), as maximum canopy size and needle N concentration was approached in fertilised plots. Results indicated that a major part of the added N was taken up by the trees before it was recycled to the soil, *i.e.* the soil retention was to a great extent a result of increased formation of litter with a high N concentration. At Stråsan, where canopy closure was reached 15 years before the time of this study, the N pool in the forest floor had increased greatly, whereas in Flakaliden, where the litterfall still was low even in fertilised plots, the soil N increase was low.

- Current N status of Swedish spruce ecosystems is dependent on site history concerning both N deposition and land use. National or regional changes in N status could be evaluated by monitoring net N mineralisation or C/N ratio of the humus-layer.

- Nitrate leaching from healthy Swedish spruce forest stands is at risk when net N mineralisation is greater than 60 kg N $ha^{-1}yr^{-1}$ or C/N ratio is lower than 30. The actual nitrate leaching rate from the stands at risk is also dependent on current N deposition.

- Measured values of inorganic N availability do not meet estimated N uptake in vegetation, indicating the importance of organic N uptake in boreal forest ecosystems. In non-fertilised plots at Stråsan and Flakaliden (Northern Sweden) the uptake was estimated to be approx. 50 kg N $ha^{-1}yr^{-1}$, and the mineral N supply only covered approx. 20% of this demand. In fertilised plots at the same sites, the uptake was estimated to be approx. 135 kg N $ha^{-1}yr^{-1}$ whereof the mineral N supply covered approx. 65%. Although the relative importance of organic N thus varied, the absolute organic N uptake was similar in fertilised and non-fertilised plots (37-52 kg N $ha^{-1}yr^{-1}$).

- Amino acid concentrations in the soil water of the forest floor were very low. This indicates that amino acids were held within a tight circulation and not released into the soil water fraction sampled with tension lysimeters.

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Acknowledgements

In chronological order, I thank Birger and Lise-Lotte for setting me into this world and, together with Jens, bringing me up to who I am.

More direct contributions to this thesis come from all teachers who taught me all this and that it has been necessary to know. Especially important was Mr Dunn in Maryborough High school (VIC) who taught me to write in a somewhat structured way. Directly responsible for making me a soil scientist is Ingrid, who found me in the entrance one day not knowing what to do as I just had been refused from the limnology course in Uppsala (I thought 3rd reserve was safe). By offering me a late place in her course and then work of all sorts, she kept me in the Department until a position as a graduate student kindly financed by SLU made it possible to become a full time student again. At this stage Dan and Ingvar entered the scene with good ideas and great support when forming my PhD project and finding assistant supervisors in Lars, Hooshang and Henrik. During the following years, the work went on in the laboratory and in the field with supervision mainly from Dan, but with all sorts of support from the staff in the Department and at the experimental forests Jädraås, Flakaliden and Skogaby. Although the list for these years could be very long, I especially want to thank Anne, Bengt-Olov, Gunilla and Ragnar who have been exceptionally service-minded and immediately solved any problem I have ever presented to them. I also want to thank Hillevi Eriksson, Lars Lövdahl, Bengt Olsson, Monika Strömgren, Carl Olof Tamm, and Olle Westling who have made unpublished results available to me, and Professor John Aber who helped us with the PnET-CN model. Coming closer to the end of the project and the need to produce publishable papers, Lars, Hooshang and Henrik, together with Dan, have constituted the supporting group needed for the completion of this thesis, and Anna, Ingvar and Lisbet have had valuable comments on earlier versions of the manuscript.

Undoubtedly, non-work support from friends within and outside the Department has been just as important. Naturally, Linnea has meant most in this respect, not least the past months when she has taken care of most things that have needed attention at home.