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Chemical and Biological Effects of Artificially Increased Nitrogen Deposition to the Ground in a Swedish Beech Forest

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During 1985–90, effects of N deposition to a beech forest floor in south Sweden were studied by supplying 12 and 36 kg N ha⁻¹, as NH₄NO₃, on 25 occasions, i.e. 60 and 180 kg ha⁻¹ yr⁻¹, corresponding to ca. 3 and 9 times the ambient deposition rate. Treatments raised the output of NO₃⁻ and several base and metal cations from the soil. There was some increase in the total N content of the leaves and a considerable increase in the contents of free amino acids, whereas phenols decreased. Leaf concentrations of Ca, P and K were lower than in controls. Throughfall chemistry was generally little influenced by the treatments. There was no measurable change in the wood production. Fruitbody production of ectomycorrhizal fungi almost ceased, whereas that of major decomposer species increased considerably. The biomass of most field layer species was reduced to some extent in the treated plots and no new vascular plant species appeared during this five-year period as a result of the treatments. *Key words:* *Fagus sylvatica*, nitrogen, forest, soil, soil solution chemistry, nutrient, deposition, flux, biological effects, wood production, plants, macrofungi.

INTRODUCTION

The deposition of NH₄⁺ and NO₃⁻ over central and northern Europe is a question of great environmental concern. There are indications that the availability of mineral N no longer limits the primary productivity of many forest sites in this area. This surplus of sufficiency, characterizing the state of N saturation, may bring about structural and functional changes of natural ecosystems.

Forest dominated by beech (*Fagus sylvatica* L.) plays an important role in the landscape of southwestern Scandinavia. Changes in structural properties and vitality of the beech have been observed in Sweden during the last 10–15 years (Nihlgård, 1985). Retrospective work has documented floristical changes in the beech forest floor (Falkengren-Grerup, 1986; Falkengren-Grerup & Tyler, 1991) and comparative studies have indicated diversity changes in the macrofungal flora of Scanian deciduous forests (Rühling & Tyler, 1990).

Increased supply of mineral N to soils may alter the mineralization rate of soil organic matter. Nitrogen application is often known to increase the C mineralization rate, e.g. of compost, but reduced rates have been reported in fertilization experiments in coniferous forest soils (Nohrstedt *et al.*, 1989). Increased N deposition to the forest floor might influence the fruitbody production and the diversity of litter decomposer and mycorrhizal macrofungi (Hora, 1972; Ohenoja, 1978).

The primary production of forests usually increases as a result of N application. However, a stage of 'N saturation' might have been attained in some sites or areas in central Europe and, possibly, south Sweden. This implies that tree production is no longer primarily limited by the N supply. What 'N saturation' actually will imply for the normal functioning of the forest ecosystem is not well known, though misfunction of the ectomycorrhiza (Richards, 1965), leading to mineral nutrient deficiencies, might be a consequence, as well as leaching losses of NO_3^- to the subsoil and surface water (Grennfelt & Hultberg, 1986).

Nutrient concentrations in beech leaves exposed to different amounts of pollutants or supply of N, may vary within rather wide ranges (Bergmann, 1988; Le Tacon & Toutain, 1973; Wöhler, 1988). In a study of leaf chemistry of beech in Scania (Balsberg Pålsson, 1989) the mean N concentrations proved to be higher than usually reported in this species. Nitrogen in excess may change the carbohydrate metabolism (Givan, 1979; Haynes, 1986), considerably increasing soluble form concentrations of N, e.g., some free amino acids (Zedler *et al.*, 1986; Van Dijk & Roelofs, 1988; Näsholm & Ericsson, 1990), whereas the content of phenolics may decrease (Tuomi *et al.*, 1990). These metabolic changes might increase the susceptibility of the plant to diseases and parasites.

The vegetation of the forest floor may change as a result of increased N supply. The forest vegetation is made up of species representing a wide variety of N demands (Ellenberg, 1979). A gradual change in the proportions of the currently occurring species might take place as a result of a higher N deposition or otherwise increased supply of mineral N (Tyler, 1987). A rapid colonization of 'new' species from the seed bank is another possibility. In open land, species diversity is often lowered by N application, as some grasses seem to be efficient competitors at a high N supply (Thurston *et al.*, 1976; Heil & Diemont, 1983). Nitrogen effects on species diversity of forests are less apparent and probably sometimes even positive.

During 1985–90, a study on the effects of artificially increased N deposition to a beech forest floor was carried out by the Soil and Forest Ecology Groups at the University of Lund. The objectives of this coordinated work were to characterize structural, functional, and chemical changes caused by an increase in the deposition of NH_4^+ and NO_3^- , corresponding to about 3 and 9 times the current deposition rate. To simulate this, NH_4NO_3 was supplied at the rate of 60 and 180 kg N ha⁻¹ yr⁻¹, i.e. 12 and 36 kg N ha⁻¹ on 25 occasions during this 5-year period. Observations were made on changes in (a) throughfall and litterfall chemistry and amounts, (b) soil and soil solution chemistry, (c) amounts of soil organic C and N, (d) leaf chemistry and nutrient dynamics in the canopy, (e) tree growth and performance, (f) fruitbody production of macrofungi, and (g) vegetation of the forest floor.

MATERIALS AND METHODS

Site description and climate

The experimental site is situated within a large beech forest, Maglehems Ora (55°45' N, 14°07' E) in eastern Scania, ca. 4 km from the Baltic Sea. Elevation above sea-level is 76–78 m, i.e. well above the highest coastline since the last glaciation. The bedrock is a finegrained gneiss, which is covered by a deep delta deposit of glacialfluvial sand, only slightly mixed with moraine. The texture is mainly fine sand (dominating fractions 0.06–0.6 mm) with a low clay (2–4%) and a low boulder content. The study area is level, very well drained, the soil profile a dystric cambisol, transitional to a cambic arenosol, with less distinct horizons. The reaction is acidic, pH-KCl being 3.5–3.9 in the upper horizons (Table 1). The humus type is a weakly aggregated sandy mull with little or no transitional characters to moder.

The area has been covered mainly by deciduous forest for at least three centuries and by

Table 1. *pH-KCl ($\bar{X}$) of the soil at the start (1985) and at the end (1990) of the experiment*

Horizon	Control		60 kg N ha ⁻¹ yr ⁻¹		180 kg N ha ⁻¹ yr ⁻¹	
	1985	1990	1985	1990	1985	1990
A1	3.49	3.62	3.60	3.61	3.55	3.58
A2	3.64	3.70	3.61	3.57	3.57	3.59
B1	3.86	3.78	3.84	3.77	3.76	3.73
B2	3.89	3.82	3.84	3.78	3.84	3.76

beech for at least two centuries, according to local maps. The present beech stand is even-aged, 120 yr, established after an ample beech mast production in the late 1860's. The canopy is closed and no thinning of the stand has occurred during the last decade. The number of trees (only beech occurs) is ca. 210 ha⁻¹, mean height being 32 m and the total basal area of stems at breast height ca. 30 m² ha⁻¹. The shrub layer is rather sparse and the forest floor vegetation not completely closed, dominated by *Oxalis acetosella*, *Anemone nemorosa*, and *Dentaria bulbifera*, with some *Maianthemum bifolium*, *Viola riviniana* and *V. reichenbachiana*, *Rubus idaeus*, and a few other species.

The climate of the site may be characterized as temperate suboceanic and subhumid. The mean annual precipitation is ca. 600 mm, rather evenly distributed throughout the year, though somewhat lower during the spring months. Mean temperature of the warmest month (July) is +17°C and of the coldest month (February) ca. -1°C. The years, in particular the winters, of 1985-87 were colder than average, whereas there were positive temperature anomalies in 1989-90, e.g. +5.5°C in February, 1990. Annual precipitation figures were close to (1986-87) or above (1985, 1988) normal, except in 1989, when only 427 mm was measured at Vitemölla, the most adjacent meteorological station, ca. 7 km away. The total precipitation sum for 1985-90 was normal. Cumulative temperature and precipitation curves, calculated from national Swedish meteorological statistics for the most adjacent stations, are illustrated (Fig. 1).

Design of the experiment

The experimental area covers ca. 1.3 ha. One block of nine 25 × 25 m plots, with 5 m buffer strips between the plots, was laid out in 1985. Controls and two levels of N treatment were randomized in triplicates. Finely granulated commercial NH₄NO₃ (27.7% N), containing 4% Ca and 2% Mg as carbonates to counteract acidification effects on the soil, was distributed by hand on 25 occasions, once per month during May to September, between August 1985 and September 1990. Two treatment levels were used, 12 and 36 kg N per ha and occasion (60 and 180 kg N ha⁻¹ yr⁻¹).

Methods of sampling and analysis

Precipitation, throughfall, soil solution and litterfall. Open-field precipitation (bulk deposition) was collected in four shaded samplers (funnels, diam. 20 cm, equipped with sieves, connected to bottles, all in polyethylene PE) placed 2 m above ground in a clear-cut area 0.7 km from the site. In each plot, throughfall was collected in five shaded samplers (funnels as above) placed ca. 50 cm above ground and randomly distributed in the central 10 × 10 m to avoid border effects. In these funnels litterfall was also collected. Bulk deposition and throughfall volumes were determined in the field. Bulk deposition liquids were treated as single samples, whereas the five throughfall samples were pooled before analysis.

Soil solution was collected using ceramic cup lysimeters (P 80; tension -0.8 bar). Four

Fig. 1. Cumulative precipitation and mean monthly temperature sums, °C, for the period 1985–90, calculated as deviation from normal values, at the SMHI station Vitemölla, 7 km south of the experimental site (precipitation) and four other SMHI stations in Scania (temperature).

lysimeters were installed at 30 cm soil depth in a regular pattern in the central 10 × 10 m of each plot. Collecting bottles (Duran glass) were placed in a container with cover, below ground to keep samples dark and cool. The lysimeters were allowed to equilibrate with the soil solution for about 2 months prior to the first sampling.

Bulk deposition, throughfall, litterfall and soil solution were sampled continuously during eighteen periods from May 1989 to November 1990. Period duration was 12–39 d during the growing season, otherwise 40–46 d.

The aqueous samples were frozen at -20°C in the laboratory. After thawing, throughfall samples were filtered (filtering paper OOR), whereas bulk precipitation and soil leachates were not. Subsamples for determination of NH_4^+ , NO_3^- , SO_4^{2-} , Cl^- and dissolved organic carbon (DOC) were analyzed without further treatment. Aliquots (700–1 000 ml) for metal analysis were transferred to a 1 l Erlenmeyer flask which was sealed with a hood of filtering paper and evaporated at 105°C . The residue was treated with 10 ml conc. HNO_3 to destruct organic matter and convert the metals into a chemically uniform and soluble state. The volume was reduced by evaporation to ca. 2 ml and diluted to 25 ml prior to analysis.

The litterfall samples, stored at -20°C , were dried at ca. 40°C , separated into leaf litter and other litter and weighed. Samples from November 1989 to November 1990 were pooled. Leaf litter subsamples were digested in conc. HNO_3 , evaporated, filtered and diluted.

For the determination of inorganic and organic N compounds, carbohydrates, phenols and DOC, throughfall water was collected both monthly and at four rain events (May–October) in both 1989 and 1990. Ten gauges were placed at random in the inner 20×20 m area of each plot. Every gauge consisted of a PE bottle and a funnel fitted with a filter. For event sampling the bottles were also equipped with an inner plastic bag and the funnels with a glass wool stopper which were replaced at every sampling event. Between the collection events the funnels were covered.

The following analytical methods were used. pH was determined electrometrically in untreated aqueous samples. Ammonium was determined by flow injection analysis, NO_3^- , SO_4^{2-} , Cl^- and F^- by ion chromatography, Rb (at 2000 mg K l^{-1} as KCl in solutions) by flame emission, Cd by AAS, P, Al, Ca, Cu, Fe, K, Mg, Mn, Na and Zn by ICP-EMS. DOC in aqueous samples was analyzed by IR technique. Litter C was determined by IR technique following combustion in a C determinator, and N by Kjeldahl analysis.

Total sugars and phenols were determined colorimetrically by the anthrone method and the Folin–Denis method, respectively (Hansen & Møller, 1975; Swain & Hillis, 1959).

Soil organic carbon and nitrogen

The amounts of C and N were measured in the humus horizon (sampling depth 0–10 cm) at the end of the experiment in November 1990. Twelve cylinder samples, volume 395 cm^3 , were taken according to a regular pattern in every 25×25 m plot, thus totally 36 samples per treatment. They were sifted (6 mm) and dried at 105°C to constant weight. Carbon content was determined using the C determinator, and organic N + NH_4^+ N (considered as organic N due to the very low share of NH_4^+) by a semi-micro Kjeldahl procedure. The amounts were calculated as g m^{-2} of the 0–10 cm layer.

Plant material from beech

For the analysis of N and organic compounds beech leaves were sampled in August 1989. Leaves were taken from trees in the centre of each plot, 10–12 m above ground, and immediately frozen on dry ice. Analysis was carried out on the freeze-dried and finely ground leaves. Nitrogen was determined by a semi-micro Kjeldahl method, NH_4^+ and NO_3^- colorimetrically from aqueous ethanolic extracts. Soluble carbohydrates, phenols and protein were estimated according to Hansen & Møller (1975), Swain & Hillis (1959) and Bradford (1976), respectively. High-performance liquid chromatography and fluorescence detection were used for the free amino acid analysis, as described by Einarsson et al. (1983) and Näsholm et al. (1987).

For sampling of crowns of mature beech trees, a 30 m sky-lift was utilized. Trees chosen for the study had to be within the working range of the lift standing in the buffer areas surrounding the plots, though border trees were avoided. Six trees from each treatment were sampled in order to study the nutrient dynamics within the crown. One branch was cut in each of the four cardinal points of the compass, in the upper, middle and lower level of the crown, and from the centre of the crown. Sampling took place in August 1989, when the leaves were fully developed but still retained a high photosynthetic capacity. In studying the nutrient dynamics over the summer months, six trees in each plot were chosen. In one of the high-N plots only three trees were reached by the lift. In June, July and August

1990 one lateral branch was cut from the same main branch in the upper part of the crown. Results from 1989 had shown that the exact position of the main branch was of no importance. Twenty leaves from each sample were chosen at random and dried at 40°C for 24 h. Nutrient concentrations were determined after sample digestion with HNO₃ using ICP-EMS.

Forest floor vegetation and fruitbody production of macrofungi

Nine permanent subplots of 2 × 2 m were distributed in a regular grid in each plot. The vegetation was described annually in 1986–90 according to 5%-cover classes. In 1990, shoot densities and sizes of five frequent herbs were measured. A trenching experiment was also performed just outside the main study area in order to estimate the influence of root interactions between trees and the vegetation of the forest floor with and without N supplied in excess. For the experiment 4 × 5 plots (2 × 2 m) were used, either trenched or fertilized, or both. The N application corresponded to 750 kg N ha⁻¹, distributed on five events in 1989. The vegetation of these plots was described in the same way as above.

The treated and control plots of the main experiment were searched for macrofungal fruitbodies three times in 1985 and 1986, five times in 1988, and six times in 1989, chiefly in September–October, the period of main fruitbody production. The fungi were removed from the site only when necessary for species identification.

Wood increment in beech

The circumference at breast height (1.3 m above the ground) was measured annually in 1987–91 in all trees of the plots, using a previously fixed position on the trunk. The cross-sectional area of each trunk at this height was calculated. From the total number of trees in each plot, all essentially even-aged, the basal area per plot was estimated. Relative basal area increment was calculated as a percentage of basal area measured in 1987. The stem diameter in 1990 varied from 30 to 56 cm, with a mean of 41.8 cm. Five of the trees were lost during the study period and were excluded in the calculations.

Statistical treatment

Throughout, samplings were replicated, the number of replicates given in connection with the results of the various studies. Significance of the differences between means was tested using analysis of variance and Tukey test (Wilkinson, 1989), or student's t-test.

RESULTS

Deposition, throughfall and litterfall chemistry

Bulk deposition pH was 4.37 as a mean for the 17-month sampling time (Table 2). The throughfall pH varied between 4.2 and 4.6 during the defoliated, and between 5.8 and 7.0 during the foliated periods. None of the elements differed significantly in mean throughfall concentrations between treatments and control. On passage through the canopy, concentrations increased of DOC, NH₄⁺, NO₃⁻, SO₄²⁻, P, Cl, Ca, Mg, Na, Rb and especially K and Mn, whereas the concentrations of Al and Zn decreased compared to precipitation water.

During the growing season the mean concentrations of NH₄⁺ in throughfall water were lower at event sampling than when sampled continuously for one month, whereas organic C concentrations were 2–3 times higher. The leaching of organic compounds was related to season and pH of the water. Generally, concentrations were several times higher at the

beginning of the season than later on. Only small differences between the treatments in the concentrations of organic compounds were noted at some rain events. Based on seasonal averages, the influence of the treatments seemed insignificant and not statistically ascertained (Table 3).

In the one-year period November 1989–November 1990, the leaf litter had a significantly lower C:N ratio in the high-N plots than in the controls (Table 4). Both treatments had higher S and lower P concentrations than in controls, whereas none of the other elements analyzed differed significantly between control and treatments.

Soil solution chemistry

The treatments had an acidifying influence on the soil solution, mean pH being 4.5 in the controls, 4.0 in the low-N plots and 3.8 in the high-N plots (Table 2). The pH-KCl of the soil, however, was almost similar in controls and treatments in 1985 and 1990 (Table 1).

Concentrations of NH_4^+ in soil solutions from the treated plots were about five times higher than in the controls, both in the low-N and in the high-N treatments (Table 2). The NO_3^- level was much more influenced by the treatments, being nine and fifteen times higher than in the control. Metal ion concentrations were often 3–10 times those of the control plots. There were also higher DOC concentrations in the soil solutions from the treated plots.

Nitrogen fluxes through the ecosystem

Nitrogen fluxes in bulk deposition and throughfall were calculated for each sampling occasion separately in multiplying water fluxes by N concentrations. The N fluxes with litterfall were calculated by multiplying the concentrations in the leaf-litter fraction by the dry weight of the pooled one-year total litterfall. The soil solution N fluxes were estimated using measured lysimetric soil solution concentrations and calculated soil water fluxes, assuming the flux of Cl in throughfall to be equal to the flux of Cl in the soil solution of the control plots. Element fluxes for the one year period (October 1989 to October 1990) were calculated as the sum of the corresponding sampling occasions. A tentative soil budget is given in Table 5.

In beech forests, stemflow is an important pathway to the soil for many elements. In our study we have no measurements of the fluxes through the soil in the vicinity of stems, which certainly differ from areas at greater distance from stems. In this well-drained sandy soil lateral movements of water is almost negligible and the stemflow area is, therefore, quite limited. Our calculated fluxes are not valid for the closest one meter of the stems but should be valid for the main part of the beech forest, where lysimeters were installed.

The N input with bulk deposition was $24 \text{ kg ha}^{-1} \text{ yr}^{-1}$. The fluxes differed slightly between bulk deposition and throughfall, indicating some dry deposition of N to be concealed by leaf uptake. The treatments did not obviously influence the N fluxes with the throughfall. Of N reaching the soil as throughfall, 72% was leached from the rooting zone in the control plots. The NO_3^- leaching through soil was >50% greater than the amount deposited with throughfall but only one tenth of the deposited NH_4^+ was leached through the soil, which indicates nitrification to be important also in this acid soil. Both NH_4^+ and NO_3^- had a negative soil budget in the control plots. In the low N plots N added was almost quantitatively nitrified and leached from the rooting zone as NO_3^- . In the high-N plots, the NO_3^- leaching was greatly enhanced.

Table 2. Mean pH and element concentrations in bulk deposition, throughfall and soil solution

Eighteen sampling occasions (May 1989–November 1990). mg l^{-1} . DOC = dissolved organic carbon. Means lacking common letters differ significantly ($p < 0.05$) between treatments (Tukey test)

	pH	DOC	NH ₄ -N	NO ₃ -N	SO ₄ -S	P	Cl
Bulk deposition	4.37	–	1.25	1.04	1.69	0.066	4.16
Throughfall							
Control	6.08 a	11.50 a	2.52 a	1.85 a	2.92 a	0.716 a	9.00 a
Low N	6.16 a	11.00 a	2.63 a	1.86 a	2.82 a	0.472 a	9.17 a
High N	6.11 a	11.90 a	2.63 a	2.18 a	3.01 a	0.538 a	9.96 a
Soil solution							
Control	4.49 a	12.00 c	0.92 b	4.10 c	6.39 a-		23.00 a
Low N	4.04 b	17.80 b	4.77 a	35.10 b	5.82 a-		23.40 a
High N	3.80 c	23.90 a	4.86 a	60.50 a	6.13 a-		27.10 a

	Cd	Cu	K	Mg	Mn	Na	Rb
Bulk deposition	0.00015	0.00500	0.53	0.18	0.01	1.92	0.018
Throughfall							
Control	0.00012 a	0.00400 a	4.67 a	0.87 a	0.36 a	2.87 a	0.042 a
Low N	0.00011 a	0.00400 a	4.05 a	0.80 a	0.27 a	2.60 a	0.038 a
High N	0.00011 a	0.00400 a	4.56 a	0.92 a	0.36 a	3.00 a	0.043 a
Soil solution							
Control	0.00100 c	0.00069 a	0.54 b	1.48 c	0.48 c	6.53 b	0.002 c
Low N	0.00400 b	0.00081 a	3.36 a	4.81 b	4.52 b	7.82 a	0.018 b
High N	0.00800 a	0.00087 a	3.87 a	8.74 a	10.31 a	8.44 a	0.030 a

Nitrogen and organic compounds in beech leaves

After four years of treatment, total N in beech leaves had increased by 14%, but there was no difference between the two treatment levels (Table 6). Ammonium was not found in detectable amounts, whereas some NO₃⁻ accumulated in leaves of the treated plots. The contents of free amino acids in the leaves also increased significantly, whereas soluble proteins decreased slightly. No changes due to N application were obtained in the carbohydrate content, but the phenol concentrations of the leaves were 30% lower in the treated plots than in the controls. The results are presented more in detail in Balsberg Pålsson (1992).

Table 3. pH and concentrations (mg l^{-1}) of soluble N compounds, sugars, phenols and organic carbon in throughfall water at event samplings

Means (and ranges) for the growing seasons of 1989 and 1990

	Control		Low N		High N	
pH	6.5	(5.1–7.1)	6.5	(5.3–7.1)	6.5	(4.6–7.2)
NH ₄ ⁺ – N	1.8	(0.21–3.0)	1.8	(0.32–2.9)	1.8	(0.20–3.3)
NO ₃ ⁻ – N	1.8	(0.87–3.8)	1.7	(0.39–3.0)	1.6	(0.81–3.2)
Org. N	2.2	(1.3–3.4)	2.4	(1.3–4.2)	2.3	(0.76–4.0)
Tot. N	5.7	(3.1–9.1)	5.9	(2.9–9.7)	5.7	(2.8–9.9)
Tot. sugars	6.1	(1.4–23)	5.5	(1.4–23)	5.5	(1.0–24)
Tot. phenols	15	(5.2–30)	14	(5.2–33)	14	(4.3–35)
Org. C	30	(15–78)	31	(16–63)	29	(13–64)

Al	Ca	Zn
0.06	0.51	0.024
0.04 a	2.36 a	0.015 a
0.04 a	2.17 a	0.014 a
0.04 a	2.37 a	0.016 a
1.39 c	7.79 c	0.083 c
7.18 b	23.28 b	0.300 b
19.20 a	31.80 a	0.456 a

Nutrient dynamics in the canopy

The N treatments decreased leaf concentrations of P, K and Ca, whereas concentrations of Mg, Cu and Fe were not affected. Leaf area, or dry weight per leaf unit area were not influenced by the treatments. In K and Ca, treatment effects were most pronounced in the upper part of the crowns. Nutrient concentrations of leaves in August usually differed significantly between different levels of the canopy (Table 7), the P, K, Mg, Cu, and Fe concentrations being higher in the lower part of the crown in the control plots. There were indications that treatments equalized the concentration differences between the crown levels, only the K concentrations being significantly different within the crown at high N application.

With the exception of N, nutrient concentrations in leaves varied according to season (Table 8). Concentrations of most elements were lower in July than in June or August in the controls. However, Mg and Cu were highest in June, Mn and Ca in August. Nitrogen treatment equalized the differences between months in Mg, Cu and K. The P concentration of leaves from control plots was lowest in July. The low N plots showed no seasonal variability in P over the summer months, whereas there were indications ($p < 0.10$) of

Table 4. Mean element concentrations, $\mu\text{g g}^{-1}$, in beech leaf litter, pooled from November 1989 to November 1990

Means lacking common letters differ significantly ($p < 0.05$) between treatments (Tukey test, $n = 3$)

	C%	N%	C/N	S	P	Al	Ca	Cd
Control	50.4 a	1.16 b	43.5 a	1 160 b	1 200 a	85.9 a	10 900 a	0.12 a
Low N	50.3 a	1.26 ab	40.0 ab	1 210 a	1 010 b	86.3 a	11 000 a	0.13 a
High N	51.0 a	1.36 a	37.6 b	1 200 a	1 010 b	85.8 a	10 200 a	0.11 a
	Cu	Fe	K	Mg	Mn	Na	Rb	Zn
Control	4.73 a	105 a	3 080 a	1 090 a	2 090 a	368 a	12.0 a	31.6 a
Low N	4.12 a	105 a	3 190 a	1 200 a	1 880 a	369 a	12.3 a	31.0 a
High N	4.22 a	104 a	3 060 a	1 180 a	1 790 a	341 a	12.6 a	28.7 a

Table 5. Fluxes and calculated soil budget of inorganic N ($\text{g m}^{-2} \text{yr}^{-1}$) for the beech forest soil during the period October 1989–October 1990

Soil-water fluxes estimated assuming CI input equal to CI output. Nitrogen in litter-fall and biomass increment (uptake in above-ground biomass) considered as $\text{NH}_4\text{-N}$. Biomass increment (tree stem and twigs) was calculated using data from two mature beech stands in North-Central Scania (Bergkvist, Folkeson and Olsson, unpublished)

	$\text{NH}_4\text{-N}$	$\text{NO}_3\text{-N}$	Tot-N
Bulk deposition (BD)	1.39	1.03	2.42
Throughfall (TF)			
Control	1.04	0.88	1.92
Low N	1.09	0.82	1.91
High N	1.01	0.88	1.89
Litterfall (LF)			
Control	1.37	–	1.37
Low N	1.16	–	1.16
High N	1.58	–	1.58
Fertilizer addition (FA)			
Control	0.00	0.00	0.00
Low N	3.31	3.29	6.60
High N	9.94	9.86	19.80
Biomass increment (BI)	1.50	–	1.50
Soil solution (SS)			
Control	0.24	1.19	1.43
Low N	0.77	9.62	10.39
High N	1.04	16.74	17.78
Soil budget (BD + FA – BI – SS)			
Control	–0.35	–0.16	–0.51
Low N	2.43	–5.30	–2.87
High N	8.79	–5.85	2.94

decreasing P concentrations during the course of the summer at high-N treatment. The total N concentrations of the crown leaves were higher in the treated plots compared to controls at all three sampling occasions; at low-N ca. 10% and at high-N ca. 24% higher than in the controls.

Soil organic carbon and nitrogen

No difference ($p > 0.05$) between treatments and control was detected in amounts of C present in the 0–10 cm layer of the soil at the end of the experiment (Table 9). Amounts of

Table 6. Influence of nitrogen additions on the concentrations of 'total nitrogen', NO_3^- and soluble organic compounds in the beech leaves

The figures in the table are the percentage of the control. Significance levels: * $p \leq 0.05$, ** $p \leq 0.01$ (Student's t-test)

	Low N	High N
Nitrogen	114**	114**
Nitrate	150**	167**
Protein	86	92
Free amino acids	169*	191*
Total sugars	94	96
Starch	101	102
Phenols	72*	69*

Table 7. *Chemical composition of beech leaves in different parts of the crown as influenced by nitrogen treatments, in August 1989, and calculated as % of control concentrations*

A = low N treatment, B = high N treatment. Differences between crown levels and treatments were tested using two-way analysis of variance. *p*-values are given for the independent variables, n.s. = not significant (*n* = 24)

Treatment	P		K		Mg		Cu		Fe		Ca	
	A	B	A	B	A	B	A	B	A	B	A	B
Upper crown	90	90	77	73	110	116	76	115	110	128	86	77
Middle crown	87	85	73	81	109	111	82	106	107	105	97	85
Lower crown	73	70	82	79	106	113	82	82	108	96	86	83
<i>p</i> values:												
Treatment		0.001		0.001		n.s.		n.s.		n.s.		0.001
Crown position		0.001		0.001		0.003		0.012		0.009		n.s.
Treatment × Crown position		n.s.		n.s.		n.s.		n.s.		n.s.		n.s.

Table 8. *Differences between summer months (1990) in the chemical composition of beech leaves*

Concentrations calculated as % of concentrations in June. Only upper crown leaves of control plots are considered. Differences between months and treatments were tested using two-way analysis of variance. *p*-values are given for the independent variables, n.s. = not significant (*n* = 18)

	N	P	K	Mg	Cu	Fe	Al	Mn	Ca
July % June	90	79	85	77	81	85	70	130	109
August % June	95	86	108	69	81	109	139	160	124
<i>p</i> -values:									
Treatment	0.001	n.s.	0.002	0.001	0.001	0.015	n.s.	n.s.	0.039
Time (month)	n.s.	0.001	0.017	0.004	0.028	0.001	0.001	0.001	0.001

Table 9. *Amounts of carbon and nitrogen in the humus layer (depth 0–10 cm) at the end of the experiment (Nov. 1990)*

Each mean ($\bar{X} \pm 95\%$ confidence limit) is based on 36 samples, volume 395 cm³; the < 6 mm fraction used for analysis; basis of calculation g m⁻². a, b and c show *p* > 0.05, *p* < 0.05 and *p* < 0.01, respectively, for difference from control

	Control	60 kg N ha ⁻¹ yr ⁻¹	180 kg N ha ⁻¹ yr ⁻¹
Carbon	2076 ± 107	1997 ^a ± 104	2006 ^a ± 125
Nitrogen	132 ± 6.8	122 ^b ± 6.5	112 ^c ± 7.4
C : N	15.7	16.2	17.8

N, however, differed significantly, particularly between the high-N treatment and the control (*p* < 0.001). Notable is that the amounts of N were lower, and that the C:N ratio consequently higher, in the treatments than in the control. A net loss of organic N from the humus component seems to have been the consequence of the increased supply of mineral N. This result, which has been carefully controlled, was unexpected and would probably demand more elaborate studies to be explainable.

Effects of wood increment

Total basal area per plot in 1987 varied between 23.0 and 30.7 m² ha⁻¹ with no consistent difference between control and treatments, though with a tendency of low values for two of the high-N plots. Stem growth during the period 1987–91, estimated by absolute basal area increment, did not deviate significantly between control and treatments (Table 10). The relative basal area increment differed but little, except for one high value in a low-N plot.

As a consequence, no consistent changes in stem increment as a result of the treatments could be measured during the study period.

Effects on species of the field layer

The number of plant species in the studied stand was relatively high compared to many beech forests in Sweden: 15 herbs, 4 grasses, 4 woody species and 1 fern were observed. However, ca. 50% of the ground was not covered by vegetation, possibly an effect of the closed tree canopy. The cover varied between the years, mainly according to amount of precipitation, which was above average during the earlier part of the 5-year period and later on below average (Fig. 1). Changes in cover were related to the first observation year and corrected for the difference between the years in the cover of the controls. Total cover decreased slightly in the treated plots. Out of the eight most common species, there was a decrease in cover of all but two (Table 11).

Shoot density, length or weight was measured. Changes were mostly in agreement with the cover changes. *Maianthemum bifolium*, *Oxalis acetosella* and *Anemone nemorosa* decreased to some extent with N supply, whereas a larger biomass of *Dentaria bulbifera* was measured in the treated plots (Table 12). The density of *Fagus* seedlings seemed to increase with N supply, which is in agreement with N fertilization experiments. This was not due to a greater fruiting in the treated plots, but occurred also in the treated small plots (4 m²) of the one-year trenching experiment. The high N application (750 kg ha⁻¹ in one year) of the trenching experiment changed the biomass of the studied species in a way similar to, though

Table 10. Basal area (m² ha⁻¹) of beech stems in late autumn 1987–1991, mean annual increment and relative mean annual increment of the basal area during this period

Plot no.	Basal area, m ² ha ⁻¹					Mean annual increment	Relative mean annual increment, %
	1987	1988	1989	1990	1991		
Control 1	30.72	31.20	32.48	34.08	34.24	0.88	2.86
Control 2	28.80	29.76	30.72	31.36	32.00	0.80	2.78
Control 3	24.00	24.64	25.12	26.08	26.40	0.60	2.50
Mean	27.84	28.53	29.44	30.51	30.88	0.76	2.73
SD	2.83	2.82	3.14	3.32	3.30	0.12	0.16
Low-N1	28.32	30.40	32.16	32.64	33.12	1.20	4.24
Low-N2	26.56	27.20	28.32	28.96	29.60	0.76	2.86
Low-N3	29.92	30.40	31.41	32.32	33.01	0.77	2.58
Mean	28.27	29.33	30.63	31.31	31.91	0.91	3.22
SD	1.37	1.51	1.66	1.66	1.63	0.20	0.72
High-N1	24.00	24.32	25.44	26.24	26.72	0.68	2.83
High-N2	28.80	29.60	30.40	31.36	31.84	0.76	2.64
High-N3	23.04	23.20	24.00	25.12	25.44	0.60	2.60
Mean	25.28	25.71	26.61	27.57	28.00	0.68	2.69
SD	2.52	2.79	2.74	2.72	2.77	0.07	0.10

Table 11. Mean change (%) in cover between 1987 and 1990 as related to 1986 in low-N and high-N plots

Species with a cover less than 2% are included in 'other species'

Species	Cover 1986 Control	Mean change (%)	
		Low-N	High-N
Increasing			
<i>Dentaria bulbifera</i>	5.2	37	32
<i>Rubus idaeus</i>	8.1	24	25
Decreasing			
<i>Anemone nemorosa</i>	5.0	-14	-34
<i>Fagus sylvatica</i> < 20 cm	2.6	-32	-29
<i>Maianthemum bifolium</i>	10.9	-22	-42
<i>Oxalis acetosella</i>	37.0	-12	-13
<i>Poa nemoralis</i>	4.3	-21	-45
<i>Viola riviniana/reichenbachiana</i>	5.9	-28	-59
Other species	13.5	-7	-10
Total cover	50.2	-7	-22

Table 12. Mean weight (mg), length (mm) or number of individuals (n) of the most common species of the forest floor, in relation to nitrogen application in the main and the trenching experiments

In the trenching experiment, nitrogen effects (N-, N+) and trenching effects (T-, T+) are tested separately. Means lacking common letters differ significantly ($p < 0.05$) between treatments (Tukey test)

	Main experiment			Trenching experiment			
	Control	low-N	high-N	N-	N+	T-	T+
<i>Maianthemum bifolium</i> (mg)	45 ^a	32 ^b	37 ^{ab}	36 ^a	34 ^b	34 ^b	39 ^a
<i>Oxalis acetosella</i> (mg)	7.3 ^a	7.1 ^{ab}	6.6 ^b	6.6 ^a	6.5 ^a	5.9 ^b	7.1 ^a
<i>Viola riviniana/reichenbachiana</i> (mg)	82 ^b	98 ^a	117 ^a	85 ^a	66 ^a	65 ^b	95 ^a
<i>Dentaria bulbifera</i> (mg)	118 ^c	241 ^a	207 ^b	—	—	—	—
<i>Anemone nemorosa</i> (mm)	115 ^a	117 ^a	90 ^b	8.3 ^a	7.1 ^b	7.8 ^a	7.7 ^a
<i>Fagus sylvatica</i> seedlings (n)	1.7 ^a	2.0 ^a	3.7 ^a	1.8 ^a	5.7 ^a	1.9 ^b	6.1 ^a

somewhat less than in the main experiment. *Maianthemum* and *Oxalis* decreased in the treated plots of both experiments. Both species are frequent on acidified soils (Falkengren-Grerup, 1990) and should be able to tolerate the low pH of the soil water (cf. Table 2). The cover of *Viola* spp. diminished by more than 30% and their number of shoots, though not their weights, was greatly reduced by the treatments.

The two species of the spring aspect, *Anemone nemorosa* and *Dentaria bulbifera*, reacted differently. The biomass of *Anemone* decreased, that of *Dentaria* increased by the treatments. These measurements were made before the first application of the year and soil analyses in the trenching experiment indicated that little NO_3^- remained in the rhizosphere in the spring following the heavy application of the preceding season. The excluded competition from tree roots resulted in a larger biomass in most species considered, indicating that other factor(s) than N limited the production (Table 12).

Effects on fruitbody production of macrofungi

Out of the 134 species of macrofungi which produced fruitbodies during the course of the experiment, 49 belonged to mycorrhiza genera, 25 were mainly wood decomposers, and 60 were decomposers of leaf-litter and soil organic matter. A drastic effect of the treatments was the disappearance of fruitbodies of mycorrhiza species, evident even at the low-N treatment. On the contrary, fruitbody production of several litter and humus fungi, e.g., species of *Clitocybe*, *Mycena*, etc., increased considerably, whereas the wood and bark decomposers did not react in an obvious way. More details are given in Rühling & Tyler (1991).

DISCUSSION

Nitrogen input may acidify forest soils, either due to increased primary production and uptake of base cations, or due to leaching losses of NO_3^- together with base cations. Inorganic N does not usually accumulate in forest soils. The NO_3^- is only weakly adsorbed and amounts of NH_4^+ on exchange sites are usually low, as any NH_4^+ not transformed to proteins in biological tissue is usually nitrified, except possibly in the most acid mor podzols. The balance between N supply and biological demand for N generally controls NO_3^- leaching and the effects of N deposition on soil acidification (Tamm, 1991).

Nutrient application often results in raised element concentrations in throughfall soon after the treatment (Parker, 1990). This was not the case in our experiment with many repeated applications, in spite of the increase in soil-solution concentrations of most elements analyzed. The main part of the N naturally deposited and artificially added to the forest was clearly lost to deep soil layers. Even in the control plots, leaching amounted to much more than half the input with throughfall. Dry deposition was not estimated separately, however, and the total input was therefore probably underestimated.

Despite the location of this forest away from large pollution sources, N input with bulk deposition was slightly above the range, 10–20 kg N ha⁻¹ yr⁻¹, suggested to constitute the 'critical' N load for south Sweden (Nilsson & Grennfelt, 1988). It has been shown for a number of coniferous forests in northern and central Europe that NO_3^- outputs are <1 kg ha⁻¹ yr⁻¹ where inputs are <10 kg ha⁻¹ yr⁻¹ (Grennfelt & Hultberg, 1986). Above this level, NO_3^- leaching increases drastically, and at an input of 23 kg ha⁻¹ yr⁻¹ the leaching loss of NO_3^- is almost 20 kg ha⁻¹ yr⁻¹. When the 'critical' load is exceeded, vegetation is no longer capable of transforming additional N into more biomass. Apart from one plot of the low-N treatment there was no indication that this beech forest reacted on the additional N supply by increased stem growth.

An insignificant root uptake of added NO_3^- and nitrification of excess NH_4^+ are the most probable explanations of the high NO_3^- concentrations in the soil solution of the treated plots. It is obvious that nitrification played an important role in soil N transformation also in the controls, as demonstrated by the rather high NO_3^- concentrations. Nitrification does occur also in very acidic soils, and the C : N ratio found in this soil was well below the upper limit, ca. 25, at which nitrification may occur (Kriebitzsch, 1978). The C : N ratio in litterfall found here, ca. 40, is also at the threshold under which ecosystems have been suggested to possess a potential for NO_3^- leaching (Gundersen & Rasmussen, 1990).

The net effect of increased leaching of NO_3^- is a H^+ contribution to the soil (Reuss & Johnson, 1986) and increased solubilization of Al-containing minerals, e.g., gibbsite (Thomas & Hargrove, 1984). The solubility of Al, Ca, Cd, K, Mg, Mn, Na, Rb and Zn is very susceptible to changes in soil acidity, as demonstrated by experimental acidification of lysimeter soils (Bergkvist, 1986). A minor pH decrease within the measured pH range of the

soils will raise the soil-solution concentration of these metals considerably (Bergkvist, 1987; Tyler *et al.*, 1987).

Compared to inorganic components, canopy leaching of organic constituents has received little attention. Changes in the leaf concentrations of organic compounds, the vitality of the trees and possibly a greater susceptibility to parasite attack (Nihlgård, 1985), due to N in excess, may alter the leaching rates. Although the N treatments significantly changed the leaf concentrations of NO_3^- , free amino acids and soluble phenols, this was not reflected in the canopy throughfall. Leaching rates of organic compounds were more related to pH, as previously observed in tree seedlings exposed to simulated acidic fog (Scherbatskoy & Klein, 1983; Mengel *et al.* 1990).

The N treatments increased the N content of the leaves to some extent, though certainly not in proportion to the increase in mineral N measured in the soil solution. Excess N is reported to result in an accumulation of NO_3^- in plant tissues (Mills & Jones, 1979; Smirnov & Stewart, 1985). Also asparagine and glutamine accumulated in the leaves from the treated plots (Balsberg Pålsson, 1992), whereas no change in the carbohydrate concentrations was detected. The observed changes in the leaf phenolics may explain the increased susceptibility to insects and pathogens in trees exposed to excess N, as observed by Roelofs *et al.* (1985) and Flückiger *et al.* (1989).

As to the differences in mineral contents between leaves from various parts of the beech canopy, most elements decreased with increasing level above ground in untreated plots. Nitrogen treatment partly equalized this difference in some elements. This higher uniformity most likely had different explanations. In elements showing decreasing concentrations with the treatments (P, K and Ca), the greatest changes took place in the lower parts of the canopy, whereas the changes in Al and Fe, elements which increased with the treatments, were greatest in the top position. Differences in concentrations of elements between the summer months in the control plots were most likely caused by leaf growth dynamics, as there is a rapid leaf biomass increase in Swedish beech from June to July, but not later in the season (Staaf & Stjernquist, 1986). However, differences between months disappeared for N, P, K, Mg and Cu with the N treatments. Beech growing on nutrient-rich soils were reported to differ considerably over the summer season with respect to mineral nutrient concentrations. However, a minimum leaf content in July was also measured for K by Le Tacon & Toutain (1973) and Wöhler (1988).

The almost complete failure of ectomycorrhizal macrofungi to produce fruitbodies following 3–4 years of the low-N treatment is a matter of concern. Mycorrhizal infection rate was reduced at high concentrations of mineral N (Richards, 1965) and NO_3^- may sometimes inhibit mycorrhizal infection (Theodorou & Bowen, 1969). NPK fertilization of beech forest reduced mycorrhizal frequency (Blaise & Garbaye, 1983) and 50 kg N ha^{-1} , as $(\text{NH}_4)_2\text{SO}_4$ decreased the fruitbody production of the most common mycorrhiza species in British pine plantations (Hora, 1972). Adverse effects from regular N fertilization on macrofungal fruitbody production are known from Scandinavia (Ohenoja, 1978; Wästerlund, 1982), though partly conflicting results have also been reported (Kardell & Eriksson, 1987).

Mechanisms behind the N effects on macrofungi are still obscure. It seems, however, likely that the high N deposition rates in central and northern Europe gradually change the species composition of the macrofungal flora and possibly have adverse effects on the functioning of the tree ectomycorrhiza.

The productivity of vascular plants in the beech forest floor did not seem to be limited by scarcity of N. Instead most species decreased their cover of the ground to some extent as a result of the treatment. The trenching experiment rather indicated that other nutrients, or water (but not light) were the limiting factors, since four out of five species increased in

cover, when competition from the tree roots was eliminated. The seemingly favourable effect of N on the development of *Dentaria bulbifera* might be related to the observation that this species lacks mycorrhiza (Mayr & Godoy, 1989) and is therefore possibly less sensitive to elevated soil N levels. Of the more frequent plants of the study site, *Dentaria* is known to be the most demanding with respect to conditions generally favourable to plant growth. Such species usually prefer a high ratio of NO_3^- to NH_4^+ in the solution (Bogner, 1968), as found in the solution of the treated plots.

CONCLUSIONS

The N treatments increased the NH_4^+ and, particularly, the NO_3^- concentrations of the soil solution, as well as the output of NO_3^- and several base and metal cations to the lower soil horizons. Most of the NH_4^+ was removed from the soil by other means than NH_4^+ leaching and evidence of the rapid nitrification was obtained. The observation that the amount of organic N in the humus layer decreased as a result of the treatments is not readily explained and would deserve closer examination. It seems that the mineralization of N was stimulated by an increased supply of mineral N without changing the amount of soil C.

The treatments raised the total N contents of beech leaves to some extent, including NO_3^- but not NH_4^+ . The concentrations of free amino acids in the leaves increased considerably, whereas phenol concentrations were depressed. Concentrations of P and K were reduced, those of Fe and Al higher than in controls. This pattern is fairly consistent with earlier findings of mineral uptake in forest plants and mechanisms involved would comprise both increased availability due to soil acidification and high NO_3^- concentrations and antagonism with mineral N ions in plant uptake. However, there was no increase in the leaching of N from the canopy and throughfall chemistry was generally little influenced by the treatments. The amounts of organic compounds in throughfall were much more influenced by rainfall pH, increasing at episodes with a high acidity.

There was no measurable change of the wood production estimated as increase in cross-sectional area of the beech stems, as a result of the N treatments. As a 5-year period might have been too short for negative effects to appear, it cannot be excluded that such effects would have appeared during an even more extended application period. The biomass of most field-layer species was reduced in the treated plots and the fruitbody production of ectomycorrhizal fungi ceased after 3–4 years of N application. Instead, there was an increase in the fruitbody production of the main decomposer macrofungal taxa.

The overall conclusion must be that no positive effects on the primary productivity were obtained by the N treatments and that this forest ecosystem was primarily not limited by nitrogen.

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