

**Soil acidification and imbalanced nutrient  
availability in Scots pine forest soils in the  
Netherlands**

**causes, extent and control**

Ontvangen

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**Soil acidification  
and  
imbalanced nutrient availability  
in Scots pine forest soils  
in the Netherlands**

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**causes, extent and control**

Proefschrift  
ter verkrijging van de graad van doctor  
in de landbouw- en milieuwetenschappen  
op gezag van de rector magnificus,  
dr. C.M. Karssen,  
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The research reported in this thesis was conducted at the Department of Soil Science and Plant Nutrition, Wageningen Agricultural University, P.O. Box 8005, 6700 EC Wageningen, The Netherlands.

## Stellingen

1. Zuuruitscheiding door boomwortels onder invloed van een verhoogde ammonium-beschikbaarheid is in Nederland een belangrijke bron van bodemverzuring.  
*Dit proefschrift*
2. Het is niet correct om te spreken van 'ammoniumpreferentie', wanneer een plant ammonium wel sneller opneemt dan nitraat, maar schade ondervindt van dit ammonium wanneer het de overheersende stikstofbron in het voedingsmedium is.  
*Dit proefschrift.*  
*C. Kamminga-Van Wijk & H.B.A. Prins, 1993. Plant and Soil 151: 91-96.*  
*A.J. Gijsman, 1990. Nitrogen Nutrition and Rhizosphere pH of Douglas-fir. Proefschrift, Rijksuniversiteit Groningen.*
3. Toedienen van mineralen aan N-verrijkte ecosystemen kan een belangrijke rol spelen in het herstellen van voedselarme milieus.  
*Dit proefschrift.*  
*J.M. Klap & P. Schmidt, 1993. Nederlands Bosbouw tijdschrift 65: 15-21.*
4. Wie kan constateren dat na bekalking het ontzuringfront in de strooisellaag voortschrijdt met een snelheid van ca. 1 cm per jaar, dient zich af te vragen waar de bodemfauna gebleven is.  
*K. Kreutzer et al., 1991. Ökosystemforschung Högwald, Forstwissenschaftliche Forschungen, Heft 39, p. 186-204.*
5. Een bezwaar tegen schattingen van nitraatuitspoeling in veldincubaties met open en afgedekte buizen is dat in beide typen buizen verschillende omstandigheden heersen.  
*W. de Boer et al., 1993. Biology and Fertility of Soils 15: 120-126.*
6. Iedere multiple comparison procedure die 'significante' hoogteverschillen detecteert op een biljartlaken, zoals Duncan's Multiple Range Test, zegt in feite niets, en het gebruik ervan is niets anders dan wishful ranking.

7. De Samenwerkende Electriciteits-produktiebedrijven kunnen onze CO<sub>2</sub> emissie nooit wegmoffelen achter een op zichzelf prijzenswaardig initiatief als het herinplanten van bossen die al deels afgestorven zijn en dus reeds een geschiedenis van eigen CO<sub>2</sub> emissie achter de rug hebben. Zo kan de stichting FACE beter haar naam veranderen in: Forests Absorbing Carbondioxide After Dieback-induced Emission.  
*J. Eekelboom, 1993. Nederlands Bosbouwtijdschrift 65: 34-37.*
8. Milieuonderzoek wordt niet zozeer ingegeven door zorg voor het milieu, als wel door zorg voor het in stand houden van een onverantwoorde levenswijze.
9. De grootste paradox van onze tijd is dat weinigen zich een moment gunnen om over de eeuwigheid na te denken.
10. Multiraciale samenlevingen lopen niet stuk op culturele verschillen tussen bevolkingsgroepen, maar op intolerantie en hebzucht, en deze zullen door een strakker vreemdelingenbeleid niet verdwijnen, maar juist gevoed worden, en zich dan met hernieuwde kracht richten op een volgend slachtoffer.
11. Wie gelooft in de historische authenticiteit van Jezus, maar niet in zijn claim dat Hij de enige weg tot God is, blijft altijd geconfronteerd met het feit dat zijn lijdelijk ondergaan van een executie als misdadiger dan wel een erg zwakke en zinloze afgang was voor een 'groot mens'.

Stellingen behorend bij het proefschrift *Soil Acidification and Imbalanced Nutrient Availability in Scots Pine Forest Soils in the Netherlands – Causes, Extent and Control*.  
Gert Arnold, Wageningen, 15 september 1993.

## Voorwoord

Dit proefschrift vormt de afsluiting van een onderzoek dat ik heb uitgevoerd aan de Vakgroep Bodemkunde en Plantenvoeding van de Landbouwwuniversiteit in Wageningen. Zoals al uit de titel blijkt, bestond het onderzoek uit twee onderdelen. Het deel dat zich richtte op de gebalanceerde voeding van grove den speelde zich voornamelijk af in een bemestingsproef in de gemeentebossen van Harderwijk. Nu zou het natuurlijk weinig zin hebben om aan het begin van een vierjarige periode van onderzoek een veldproef met volwassen bomen in te zetten, want daarvoor reageren bomen veel te langzaam. De mogelijkheid om toch een dergelijk onderzoek te doen heb ik dan ook voornamelijk te danken aan het feit dat de bemestingsproef in Harderwijk reeds in 1985 werd gestart. En dit is weer voornamelijk te danken aan de visie en inzet van Ir. A. Hekstra, die, om een lang verhaal kort te maken, kennishiaten niet langer wilde vullen met praten, maar met gedegen onderzoek. Gedreven pionier als hij is, wist hij in korte tijd de nodige contacten te leggen met deskundigen en geldschietters, zodat het niet alleen bij een goed idee bleef. Gedurende de periode dat het Nederlands Meststoffen Instituut een deel van de financiering op zich nam, tot 1990, was A. Hekstra de drijvende kracht achter al het werk dat de veldproef met zich meebracht. Pas tegen het eind van die periode raakte ik erbij betrokken. Het heeft me altijd een beetje in verlegenheid gebracht om als aanvankelijke buitenstaander zomaar in een lopend onderzoek 'in te breken' om vervolgens alle gegevens voor een eigen promotie te gebruiken. Ik dank dan ook de toenmalige begeleidingscommissie dat zij mij deze brutaliteit niet alleen toegestaan heeft, maar me er zelfs in aanmoedigde. Het gaat daarbij met name om A. Hekstra, K. Dilz, A. van Diest en J. van den Burg.

Een onvoorstelbare hoeveelheid veldwerk was dus al verricht voor ik zelfs maar iets wist van deze proef. In de loop der jaren, ook nadat ik mee ging doen, groeide het aantal betrokken mensen tot een klein legertje, dat voornamelijk werd gerecruteerd uit stagiaires van de Christelijke Agrarische Hogeschool in Dronten. Het is dan ook onmogelijk om alle namen van deze zandhazen te noemen, maar zonder hen was het onderzoek absoluut onmogelijk geweest. Daarnaast heeft de afdeling Plantsoenen van de gemeente Harderwijk o.l.v. L. de Vries een groot aandeel gehad in veldwerkzaamheden en het broodnodige onderhoud van het proefveld.

Tijdens mijn werk kwam ik erachter dat de vakgroep zich gelukkig kan prijzen met een groep toegewijde en kundige analisten. Als een onderzoeksinstelling een lichaam is, dan vormen de analisten de zintuigen, en het wetenschappelijk personeel voornamelijk de mond. Ik vraag me wel eens af of de huidige waardering voor deze functies wel een juiste weerspiegeling geeft van hun belang. Wie zijn neus schendt....

Eric Heij leek nooit moe te worden van mijn continuous-flow van monsters. Hij vormde een menselijke oase te midden van een toenemend gerobotiseerde omgeving. Arie 'professor' van den Berg wist het altijd klaar te spelen dat geestdodend routinewerk toch gezellig werd. Bij Wim Menkveld kon ik altijd terecht voor de meest uiteenlopende karweitjes. Simon Maasland heeft dagen achtereen PVC-pijpjes staan zagen en schuren, tot hij ze 's-nachts nog zag. Ik weet niet hoe vaak Winnie van Vark en Frans Möller me steeds opnieuw allerlei analytische handigheidjes hebben uitgelegd, omdat ik ze weer vergeten was. Waardering had ik ook voor de kwartiertjes in de koffiekamer. Ergens voelde je: hier worden de werkelijk belangrijke zaken besproken. En al verdienden sommige details misschien nog Eckstra

uitwerking, op de een of andere manier was je na de bespreking weer grondig van de grote lijnen ver-Wittich-t.

Waardevol waren de kritieken en suggesties die ik tijdens werkbesprekingen kreeg. Bert Janssen heeft volgens mij een roeping als microchirurg misgelopen. Ik heb enorm veel baat gehad bij de aanwezigheid van Pieter de Visser, eerst als buurman en later als kamergenoot. Hij was altijd bereid om van gedachten te wisselen over allerlei aspecten van het bosonderzoek, of om me te voorzien van gegevens die voor mijn onderzoek relevant waren. Rien van Beusichem heeft de laatste paar jaar van mijn werkperiode en tijdens de afronding van dit proefschrift voortdurend een vinger aan de pols gehouden, en is in die tijd publiek verantwoordelijk geweest voor mijn werk. Deze inzet is uiterlijk nooit zo zichtbaar geworden, maar een ernstige kink in de kabel zou hem als eerste ge(k)raakt hebben. A. van Diest, tenslotte, heeft op allerlei terreinen bijgedragen aan mijn onderzoek, van het creëren van mijn AIO-plaats tot de uitwerking van dit slotdokument, van het bespreken van experimenten tot het uitleenen van zijn privé-auto voor talloze tochtjes naar Harderwijk.

Zo staan dus vele mensen aan de basis van het voor u liggende werk. Er mag maar één naam op gezet worden. Het lijkt haast een willekeurige keuze...



*Aan mijn ouders,  
die hier ongeveer zes keer zo lang aan gewerkt hebben als ik.*

Omdat er geen trouw, geen liefde  
en geen kennis Gods is in het land  
Daarom treurt het land  
en al wat erin woont verkwijnt

Hosea 4: 1, 3

**Arnold, G., 1993. Soil acidification and imbalanced nutrient availability in Scots pine forest soils in the Netherlands — causes, extent and control. (Bodemverzuring en onevenwichtig nutriëntenaanbod in bodems van grove-dennenbossen in Nederland — oorzaken, omvang en beheersing.)**

*Ph. D. thesis, Wageningen Agricultural University, Wageningen, Netherlands. 139 pp.*

Deposition in the Netherlands contains high amounts of nitrogen, predominantly ammonium originating from livestock production. When ammonium is deposited in forests, soil acidification may occur as a result of increased nitrification and/or through an increased cation-over-anion root uptake, causing an increased proton excretion into the rhizosphere. In the present research the contribution of these processes to soil acidification in Scots pine forest soils is assessed. In a pot experiment the ammonium:nitrate uptake ratio of young Scots pine trees was found to be 7 times that in the soil solution; an acidifying nutrient uptake pattern occurred when the contribution of ammonium to total nitrogen uptake was larger than 30%. It was estimated that under field conditions soil acidification due to nitrogen uptake of Scots pine amounted to approx.  $2.2 \text{ kmol ha}^{-1} \text{ y}^{-1}$ . In an in-situ incubation experiment, nitrogen mineralization in the forest floor and the topsoil was found to amount to 13 kg ammonium and 26 kg nitrate  $\text{ha}^{-1} \text{ y}^{-1}$ , causing soil acidification of approx.  $1 \text{ kmol H}^+ \text{ ha}^{-1} \text{ y}^{-1}$ . Liming increased nitrogen mineralization, but not the proportion of mineralized nitrogen that was nitrified (65%).

Further, increased nitrogen depositions may induce an imbalanced tree nutrition and nutrient deficiencies. In a field experiment it was investigated whether these effects and soil acidification can be corrected by forest fertilization and liming. Several years after application, lime had positively influenced pH and extractable Al to a 50-cm depth. Possible beneficial and adverse effects of liming are discussed. P and K additions readily increased foliar P and K and, after a few years, stem volume growth. However, it can be questioned whether in a situation of high growth rates ( $16 \text{ m}^3 \text{ stem wood increment ha}^{-1} \text{ y}^{-1}$ ) increased growth indicates an increased tree vitality.

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# 1

## General introduction

## **A diseased atmosphere**

The atmosphere has long been seen as a boundless reservoir freely to be filled with waste products. For ages there was little or no awareness of the fact that this seeming indestructibility was caused by a constant recycling of most gaseous waste products and particles in a range of chemical and physical processes. Of course, locally discomforts due to various emissions into the atmosphere were felt, and sometimes prevented. For instance, in the 12th century, burning smoke-producing pit-coal instead of charcoal was allegedly liable to the death penalty. However, on a global scale problems started to arise, when mankind persisted in burdening the atmosphere with emissions so immense that the existence of recycling processes became visible in their uncomfortably swelling outputs, or when compounds were introduced that could not conventionally, or not at all, be recycled. Today we are faced with disturbances in several strata of the atmosphere, the most alarming of which may turn out to be that in the fragile ozone layer.

In the lower parts of the atmosphere recycling of pollutants became visible in accelerated wet and dry depositions of various compounds. As an example, in the 19th and 20th century, large areas in Europe and the United States were stained with soot produced by coal- or lignite burning. In 1952 some 4000 people died of lung- and heart diseases when the city of London was stricken with a phenomenon since known as "smog".

## **Diseased forests**

But not only man was affected. In 1883, in the Harz mountains in Germany forest damage was observed as a consequence of exposure to industrial fumes (Von Schröder and Reuss, 1883). Nevertheless, it was not until the 1960s that environmental health gradually gained public concern. Since the 1970s, when in Germany a 'new type' of forest decline was observed (Prinz et al., 1987), the terms 'acid rain' and 'forest die-back' have been heard more and more. The reason why these terms are often mentioned in one breath, lies in the long lifetime of trees. Because of this longevity, effects of air pollution, if present, can accumulate, and will become visible while other short-lived organisms may remain unaffected.

The occurrence of forest decline in many industrialized countries is now indisputable. However, there has been much controversy concerning underlying mechanisms, and until today many causes of forest decline are yet to be elucidated. In the Federal Republic of Germany several site-specific types of forest damage were recognized, which helped to disentangle distinctive causes. It lies beyond the scope of this introduction to enter into more details. The reader is referred to an annotation by Blank et al. (1988) and to the subsequent comments by Hauhs and Ulrich (1989).

## Forest decline due to increased nitrogen deposition

Nihlgård (1985) reviewed several hypotheses for explaining forest decline, and advanced a theory that ammonium played a major role. This certainly was not the first time (although claimed as such by the author) that ammonium was associated with forest damage. For some decades it had been observed in several western European countries that a strongly intensified livestock farming raised  $\text{NH}_3$  emissions and, consequently, depositions of  $\text{NH}_x$  (i.e.  $\text{NH}_3$  and  $\text{NH}_4^+$ ). In the Netherlands excessively high  $\text{NH}_x$  depositions have developed, increasing from ca. 500 mol (7 kg N)  $\text{ha}^{-1} \text{y}^{-1}$  in the 1950s to ca. 1500 mol (21 kg N)  $\text{ha}^{-1} \text{y}^{-1}$  in the 1980s (Arts, 1988). These average values can be exceeded near emission sources (Kaupenjohann et al., 1989; Berendse, 1988). In 1982, Van Breemen et al. described how these depositions could give rise to acidification of forest soils. In forests, concentrations of  $\text{NH}_4^+$  (and  $\text{SO}_4^{2-}$ ) in water passing tree canopies are usually found to be 4 to 6 times higher than those in rainwater, because of the interception of  $\text{NH}_4\text{-SO}_4$  aerosols by tree canopies or a mutually enhanced deposition of  $\text{NH}_3$  and  $\text{SO}_2$  on leaf surfaces (Van Breemen et al., 1982; Velthorst and Van Breemen, 1989). Especially in coniferous forests, with this 'dry deposition' on needles proceeding during the full year, annual throughfall depositions can be very high. Local amounts of throughfall deposition under Douglas fir corresponding with 160 kg N  $\text{ha}^{-1} \text{year}^{-1}$  were reported by Ivens et al. (1988). Deposition of  $\text{NH}_4^+$  can cause soil acidification through several mechanisms (see below).

Besides  $\text{NH}_x$  emissions, mainly due to agricultural sources,  $\text{NO}_x$  ( $\text{NO}$ ,  $\text{NO}_2$  and  $\text{NO}_3^-$ ) emissions occur. On a global scale the main sources of  $\text{NO}_x$  are natural processes, like lightning and denitrification, but in industrialized regions the combustion of fossil fuels is the main source (Söderlund, 1980).

The amounts of  $\text{NH}_x$  and  $\text{NO}_x$  in deposition are variable because they depend on weather conditions that greatly influence transport and deposition rates. They are usually not simply related to the proximity of sources. Nevertheless, throughfall deposition in the Netherlands usually contains more ammonium than nitrate. Of both inorganic nitrogen species, ammonium may be the most harmful to forests (see below).

## Specific effects of increased nitrogen depositions

### *Plant-nutritional disorders - soil acidification*

The nitrogen cycle consists of several processes, nearly all of which are associated with  $\text{H}^+$  consumption or production. Fig. 1 in Chapter 2 (page 21) shows a simplified diagram. Deposited nitrogen will be incorporated into this cycle, which may have several effects, either direct ones, or indirectly through biological activities.

Possible direct effects of  $\text{NH}_x$  deposition:

- [1] In case of  $\text{NH}_3$  deposition: a pH increase by the formation of  $\text{NH}_4^+$ , which consumes  $\text{H}^+$ .
- [2] Displacement of other cations from the exchange complex by adsorption of  $\text{NH}_4^+$ , possibly followed by:
- [3] Increased leaching of these cations.

Possible indirect effects of  $\text{NH}_x$  deposition through biological activities:

- [4] An increased nitrogen assimilation by macro- and microflora, possibly leading to relative deficiencies of other nutrients. When the additional nitrogen uptake mainly consists of  $\text{NH}_4^+$ , the flora is likely to develop an excess cation-over-anion uptake pattern. This will induce plant roots to excrete  $\text{H}^+$  into the rhizosphere in order to maintain internal charge balance. The result will be:
- [5] Soil acidification due to an increased root proton production.
- [6] Strong soil acidification due to an enhanced nitrification process, more so when followed by:
- [7] Increased  $\text{NO}_3^-$  leaching (see below).

Possible direct effects of  $\text{NO}_x$  deposition:

- [8] Increased  $\text{NO}_3^-$  leaching.
- [9] When deposited as  $\text{HNO}_3$ : a pH decrease, affecting microbial and chemical processes (e.g. weathering of minerals).

Possible indirect effects of  $\text{NO}_3$  deposition through biological activities:

- [10] Acid consumption by the macro- and microflora when  $\text{NO}_3^-$  is assimilated (the reverse of process [5]).

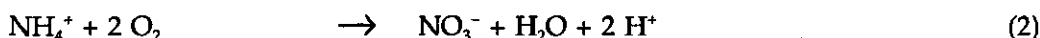
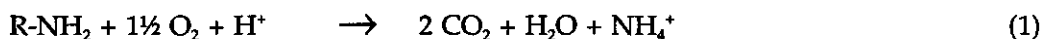
The importance of nitrification [6] in acid forest soils has long been a matter of controversy. Evidence of nitrifying microorganisms existing in strongly acid forest soils was given at least 35 years ago (Corke, 1958), but at low pH values their nitrification rate was considered negligible. However, increased nitrate concentrations observed in forest soils subject to high nitrogen immisions (Van Breemen and Van Dijk, 1988) suggested the occurrence of nitrification in acid soils under conditions of high ammonium availability. Stams and Marnette (1990) found considerable nitrification rates developing in soil columns filled with acid oak-birch leaf litter, but could not characterize the involved mechanism. De Boer et al. (1989) found evidence of chemolithotrophic nitrification in acid heathland soils. Although the mechanism of the nitrification reaction in acid soils (autotrophic or heterotrophic) can not always be

determined, its occurrence is now beyond doubt. The question remains how large the contribution of the nitrification process is relative to that of other acidifying soil processes (see below).

Processes [3] and [4] may result in nutrient deficiencies, absolute, or relative to N. The latter is referred to as an imbalanced nutrition. In most terrestrial ecosystems of temperate and boreal regions, nitrogen usually is the element limiting plant growth. In such regions vegetations are adapted to preserving nitrogen and, thus, may consist of plant species having insufficient internal protection mechanisms against excessive nitrogen availability. As a consequence, pertaining to trees, an effect of the increased nitrogen depositions mentioned above may be that growth is stimulated even when other elements become limiting. In addition, an excessive uptake of ammonium may cause several deleterious effects within the plant (see page 37).

#### *Interference with the nitrogen mineralization/uptake balance*

Nutrient uptake is counterbalanced by mineralization, i.e. the formation of inorganic components during microbial decomposition of organic matter. In the case of nitrogen, mineralization is defined as the formation of inorganic nitrogen ( $\text{NH}_4^+$  and  $\text{NO}_3^-$ ) from organically bound nitrogen, i.e. ammonification, possibly followed by nitrification:



From Eqs. (1) and (2) it is apparent that ammonification is acid-consuming, while nitrification produces acidity. Nitrogen mineralization is neutral with respect to the  $\text{H}^+$  budget when 50% of the formed ammonium is nitrified. Lower or higher percentages result in  $\text{H}^+$  consumption or production, respectively. To know which of these two possibilities prevails, only the net mineralization rate is to be known, i.e. the result of gross mineralization (Eqs. 1 and 2) and reimmobilization of inorganic nitrogen by microorganisms.

In most natural ecosystems in steady state inorganic nitrogen is efficiently absorbed by the flora and little is lost through leaching or volatilization. In such a situation, any  $\text{H}^+$  production or consumption due to mineralization is compensated for by the flora's nutrient uptake pattern (c.f. processes [5] and [10]). For instance, when nitrification is important, the vegetation will adapt to a relatively high availability of nitrate, and exhibit an acid-consuming uptake pattern in which total anion uptake exceeds total cation uptake. However, there may be circumstances in which decomposition of organic matter is inhibited, e.g. low temperatures or anaerobic conditions, or does not fully compensate the accumulation of organic matter, e.g. in aggrading forests. In such situations soil acidification may occur as a natural process.



A profoundly different situation may arise when deposition becomes an important nitrogen source. When predominantly ammonium is deposited, nitrogen accumulation in the ecosystem's biomass will give rise to soil acidification. Direct uptake of 1 mol ammonium produces 1 mol  $H^+$ . When 1 mol ammonium is nitrified, 2 mol  $H^+$  is produced (Eq. 2), while subsequent uptake of 1 mol nitrate consumes only 1 mol  $H^+$ . However, when the capacity of the ecosystem to assimilate deposited nitrogen is limited, nitrification is likely to induce nitrate leaching. In that case, the neutralization of its  $H^+$  production by nitrate uptake is further reduced, and the result is a strong soil acidification.

### *Nitrogen saturation*

Forest stands with closed canopies have little potential to increase standing biomass. Therefore, in such cases an increased nitrogen accumulation in biomass can only be achieved by removing biomass to create space for new growth, or through increased nitrogen concentrations in living and dead biomass.

The former option implies frequent interventions, e.g. in the form of cutting. Nutrients other than nitrogen are necessarily removed as well, which for proper regrowth may require nutrient additions. Furthermore, removal of mineral cations may promote soil acidification and will necessitate frequent liming.

The viability of the latter option depends on the nitrogen status of the ecosystem as a whole, or: on the extent to which the ecosystem is saturated with nitrogen. The term nitrogen saturation is rather popular in this context, but it is difficult to describe in an operational manner. Several definitions were posed (discussed by Tietema, 1992). However, it should be kept in mind that an ecosystem that at present is not nitrogen saturated, i.e. is characterized by a nitrogen availability smaller than, or equal to its nitrogen demand, can lose this property when circumstances change, e.g. by disturbances or by climatic or vegetational changes. Ecosystems that are presently allowed to accumulate nitrogen may in the future turn into unwanted nitrogen sources.

### **Forest nutritional status and fertilization**

Nutrient deficiencies due to processes [3] or [4] (see above) may play a role in causing a decreased forest vitality. These deficiencies may be visible in deficiency symptoms (Hüttel, 1986; Taylor, 1991), or appear from a determination of the forest's nutritional status. The latter may be carried out both when deficiency symptoms are visible or invisible. However, the methodology of determining the nutritional status of a forest is a very undefined and controversial subject. Both soil and plant analysis have been used for this purpose. Their possibilities and constraints will be discussed below (partly based on J. van den Burg, personal communication). Meanwhile, it should be kept in mind that when nutrient deficiencies are observed, an addition of the lacking

nutrients may not always be effective. As explained on page 40, there may be situations in which forests can not utilize added nutrients.

### *Soil analysis*

Soil analytical methods used were first adopted from agricultural research. However, the applicability of such methods for assessing the nutrient status of forests is restricted by a number of factors:

First, the lifetime of a forest typically spans several decades. The moment of soil sampling, therefore, is rather arbitrary, and it is not always clear which soil parameter optimally reflects the availability of a nutrient pool in relation to the time-span over which it is exploited. This dilemma is illustrated e.g. by the growing diversity in methods for the analysis of phosphorus availability. A number of extraction methods have been developed. Dighton and Harrison (1990, 1983) used bio-assays, and more recently determinations of labile organic P concentrations and phosphatase activity proved useful as P availability indices in Mountain Ash forests (Polglase et al., 1992). Further, due to the long lifetime of a forest stand, research in this area is forced to proceed at a low pace. Therefore, it takes more time to obtain conclusive results than in agricultural research.

Second, nutrient pools that would be unavailable for plant roots, may be accessible to mycorrhizae. However, the role of mycorrhizae in making nutrients available to higher plants is hard to evaluate in soil analytical procedures.

Third, forest soils are notoriously variable. It is often questionable if the number of samples taken to assess a soil's nutrient status is adequate to represent the area under investigation.

Fourth, forest tree species have a greater and more variable rooting depth than have agricultural crops, which makes it hard to choose the sampling depth that most correctly represents the soil body to be tapped by tree roots. Also, the rooting intensity is lower than that of agricultural crops and often an abundant undercover vegetation comprises a large part of total root biomass. Thus, a bulk soil sample may not adequately represent the soil compartments exploited by tree roots.

For these reasons, literature on forest nutrition shows a wide diversity in adopted sampling times and depths, analytical methods and in interpretations of results, thus making mutual comparisons difficult. So far, only regional standards (often preliminary) were developed (e.g. Anon. 1990). Due to such a lack of standards, in this thesis 0.01 M  $\text{CaCl}_2$  solution was used as an extractant for the annual evaluation of the availability of nutrients in the forest floor and mineral soil. This extractant is commonly used in agricultural soil testing and occasionally in forest soil research (McCray and Sumner, 1990). The choice of  $\text{CaCl}_2$  was also motivated by the availability in our laboratory of a large number of routine-analysis methods for nutrients extracted with this 0.01 M  $\text{CaCl}_2$  solution.

## *Plant analysis*

Plant analysis is a more direct way of assessing a forest's nutritional status. However, since a plant's nutrient concentration is a synthesis of a range of concurrent processes, taking place both in the plant and in the soil, plant analysis is less powerful in diagnosing specific causes of possible nutritional disorders than is soil analysis.

Plant analysis begins with choosing the plant part to be sampled, which choice depends on the objective of the analysis. For the diagnosis of nutrient deficiencies limiting plant performance mostly foliage is sampled. This is not only because of convenience, but mainly because of processes in the leaf being pivotal for the functioning of the whole plant. Foliar analysis methods and interpretations in forest research are reasonably well standardized (see also page 76), although also these standards usually have no more than a regional scope.

## **Aim and contents of this thesis**

This thesis discusses two lines of examination, pertaining to increased  $\text{NH}_x$  depositions in the Netherlands. The first one deals with the question whether or not trees can be considered important sources of soil acidification by exhibiting an excess cation-over-anion uptake pattern, possibly enhanced by an increased ammonium availability. In Chapter 2 the background of nitrogen acquisition by plants is presented in more detail and a preliminary investigation to estimate ammonium:nitrate uptake ratios is described. The method tested in Chapter 2 is applied in an extended form in Chapter 3, which describes the ammonium:nitrate uptake ratio of Scots pine for various ammonium:nitrate availability ratios. Scots pine was chosen as experimental tree species, because it is, and will remain, the most important forest tree species in the Netherlands, covering over 40% of the forested area (Hinssen, 1991). In many other European countries Scots pine is a major tree species as well. Based upon ammonium:nitrate uptake ratios and the uptake of major nutrients other than nitrogen, estimates are made of soil acidification due to Scots pine growth. The question whether or not these estimates are high in comparison with other possible sources, or may be balanced by proton sinks related with nitrogen transformations, led to the research described in Chapter 7. In this chapter, nitrogen mineralization in the forest floor and the upper horizons of the mineral soil is quantified *in situ*, using undisturbed soil cores equipped with layers of ion exchange resin. From these measurements the amount of soil acidification due to net nitrogen mineralization could be calculated. The effects of liming on net mineralization and soil acidification were also investigated.

The second series of investigations dealt with the possibility of induced nutrient deficiencies and soil acidification due to an increased  $\text{NH}_x$  deposition (processes [3] and [4], and [5] and [6], respectively, mentioned above). The underlying question is

whether forest fertilization and liming can serve to ameliorate such negative effects, and possibly stimulate the utilization of deposited nitrogen. In 1985 in Harderwijk, the Netherlands, experimental forest fertilization and liming were started in a 25-y-old Scots pine plantation. This stand was a homogeneous forest of good provenance on a virtually level drift sand plane. The stand was located in an area with intensive duck breeding farms. Initial foliar analysis suggested that the forest's nutritional status was insufficient with respect to P, Ca and Mg, whereas foliar N concentrations were high (Van Diest, 1989). However, at that time only German guidelines on how to interpret foliar nutrient concentrations were available. Dutch standards, proposed since (Anon., 1990), would have judged the nutritional status slightly less pessimistic (see page 77). Although nutrient deficiencies initially appeared to exist, it was as yet unclear whether they had developed under the influence of increased  $\text{NH}_x$  depositions, or had been caused by other factors like natural depletion of soil pools, drought, or impaired root functioning. Therefore, the results could not be expected to give an undisputed answer to the basic questions mentioned above. Nevertheless, the experiment would provide more insight in the response of Scots pine to applications of various nutrients and lime, which may help to develop more accurate fertilization recommendations.

From 1986 until 1989, in the field experiment soil and needle analyses were carried out. Tree height and diameter measurements were conducted in the same period, and in 1991. In [Chapter 4](#) soil analysis results are discussed. [Chapter 5](#) deals with element concentrations in the soil solution, and [Chapter 6](#) presents the results of foliar analysis and stand measurements and discusses growth responses in the light of soil and needle analyses.

Finally, in [Chapter 8](#) the various parts of knowledge concerning N cycling and the related  $\text{H}^+$  budget obtained in the previous chapters are assembled and discussed, and the applicability of forest fertilization and liming for ameliorating nutrient deficiencies and acidification is evaluated.

## **Nitrogen supply, tree growth and soil acidification**

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**Key words:** *Larix kaempferi*,  $\text{NH}_4\text{:NO}_3$  uptake ratio, cation-anion uptake balance, tree nutrition,  $^{15}\text{N}$  isotope, soil acidification

### **Abstract**

Also by trees nitrogen is absorbed in quantities larger than those of any of the mineral nutrients. Nitrogen can be taken up by trees as  $\text{NH}_4$  and as  $\text{NO}_3$ , but the relative ease with which they are absorbed does not necessarily reflect the degree of preference shown by tree species for either of the N forms.

In an experiment with larch trees grown on mineral soil in 60-liter containers and supplied with  $^{15}\text{N}$ -enriched  $\text{NH}_4$  or  $\text{NO}_3$ , it could be shown that  $\text{NH}_4$  is absorbed more readily than  $\text{NO}_3$ . With information available on the  $\text{NH}_4\text{:NO}_3$  uptake ratio, a cation-anion uptake balance sheet could be constructed for larch trees, showing a large excess of cations absorbed over anions. The resulting acidifying effect on the soil must be viewed as one of the major causes of acidification of forest soils in the Netherlands. This process, however, is but one in a cyclic sequence of N transformations in forest ecosystems, each one having its specific influence on soil pH. A model of these N transformations is presented and discussed.

### **Introduction**

#### *Nitrogen in plants*

Among plant nutrients, nitrogen takes first place in quantity present per unit weight of phytomass. In the form of agricultural produce, the average nitrogen concentration in phytomass was estimated at 1.5% on a dry-weight basis, followed by potassium at 1% (Stout, 1961). For forest ecosystems, the dominance of N, relative to other nutrients, is less pronounced, but still present. An estimated quantity of 1001 kg N per ha was reported to be present in the phytomass of an average, terrestrial vegetation, still heavily dominated by woody material (Deevey, 1970). Here, calcium takes second place with 754 kg per ha, followed by K, Mg and P, in that order. These figures may serve to show that also in forest ecosystems a steady supply of nitrogen is essential to a balanced nutrition and to a high degree of vitality of trees.

More so than mineral nutrients, nitrogen inside trees is present predominantly in leaves and needles. Little of it is translocated to the wood. In experiments with use made of  $^{15}\text{N}$ -labelled N fertilizers (Nambiar and Bowen, 1986), it was observed that in *Pinus radiata* 45 months after application of the fertilizer, 72% of the fertilizer-N originally accumulated in needles was still present in, mainly younger, needles. Retranslocation to stem and branches accounted for only 8% and 2%, respectively, of

fertilizer-N absorbed. Such data make it clear that nitrogen is involved mainly in metabolic processes and has no important functions in storage tissues, but also that coniferous trees have an ability to retain sizeable portions of N, once absorbed, in the needles over some years.

### *Nitrogen supply*

On a global basis, many forests are found on deeply weathered soils, unable to provide mineral nutrients in amounts needed for vigorous tree growth. In such ecosystems, nitrogen is often present in relatively ample quantities, due to two sizeable modes of influx, viz. nitrogen in wet and dry deposition, and nitrogen biologically fixed from the atmosphere. It could be shown (Poels, 1987) that in a rainforest in Surinam (South America), 80 km from the Atlantic Ocean, in an area without traffic, industry or intensive livestock production, the annual influx of N in wet deposition was  $10.5 \text{ kg ha}^{-1}$ . It could further be measured that the annual net accumulation of N in the profile was  $18.3 \text{ kg ha}^{-1}$  versus 7.0, - 2.1, 10.9 and  $0.6 \text{ kg ha}^{-1}$  for Ca, Mg, K and P, respectively. It may therefore be concluded that in tropical rainforests nitrogen is generally not the nutrient serving as minimum factor in the nutrition of trees. On the other hand, when in temperate and boreal regions forests on relatively young soils of glacial origin are not exposed to much atmospheric pollution, tree growth may be hampered primarily by lack of nitrogen (Hüttl, 1986; Nõmmik and Larsson, 1989).

Natural influxes of N into forest ecosystems are smaller in temperate than in tropical regions. In Europe, *Alnus* is the only indigenous forest tree species serving as host plant to  $\text{N}_2$  fixing organisms, whereas in tropical forests many leguminous tree species are available to guarantee a steady influx of N into these ecosystems. Furthermore, due to a higher frequency of electrical discharges during thunderstorms, formation of nitrogen oxides is higher in tropical than in temperate atmospheres. Little is known about the origin of  $\text{NH}_4$  naturally occurring in wet and dry deposition. It is, however, likely that high evaporation rates of tropical ocean water are responsible for high rates of  $\text{NH}_3$  volatilization from this alkaline medium, thus leading to relatively much  $\text{NH}_4$  in the wet and dry deposition of tropical regions.

In Europe, natural sources for nitrogen immissions into forest ecosystems have become overshadowed by man-made ones. Internal combustion engines of automobiles, emitting nitrogen oxides, have become the source primarily responsible for additional influxes of nitrogenous compounds into forests. In certain regions particularly inside the Netherlands,  $\text{NH}_3$  emission from intensive livestock production units contributes heavily to N immissions into forests.

This situation of a combined immission of  $\text{NH}_4$  and  $\text{NO}_3$  has primarily generated interest in environmental problems, including soil acidification. In addition, attention is being paid to the extent to which forests can accumulate the deposited N, and to the response of trees to variations in the ratio of available  $\text{NH}_4$  and  $\text{NO}_3$ .

*NH<sub>4</sub>:NO<sub>3</sub> uptake ratios*

With respect to this last issue, it is known that most plants possess an ability to absorb both NH<sub>4</sub> and NO<sub>3</sub>. However, plant species lacking the enzyme nitrate reductase (NR) can only metabolize NH<sub>4</sub>. Since NH<sub>4</sub> tends to predominate in acid soils due to a low activity of nitrifying bacteria, plant species without NR seemingly show a preference for acid soils. In reality, however, they do not prefer, but only tolerate such soils in which they find available N mainly in the NH<sub>4</sub> form on which they depend exclusively for their N nutrition.

Theoretically, all plants should prefer NH<sub>4</sub>, since the amount of energy to be invested in the production of proteins is less when NH<sub>4</sub> than when NO<sub>3</sub> is the N source. In practice, however, it is often observed that plants give preference to NO<sub>3</sub> as N source, as evidenced by a higher growth rate. The reasons for this preference are not always clear. It is known that the uptake of the divalent cations Ca and Mg is more inhibited by NH<sub>4</sub> than is that of the monovalent cation K. Since for dicotyledonous plants the Ca- and Mg demands are higher than for monocotyledonous ones it might be justified to consider this as the fact underlying the NO<sub>3</sub> preference shown by the former. Furthermore, dicotyledonous plants have relatively high carboxylate contents. Since carboxylate synthesis is metabolically associated with nitrate reduction (Dijkshoorn, 1962), it is to be expected that plants requiring high carboxylate levels prefer NO<sub>3</sub> as N source.

In case of an equal supply of NH<sub>4</sub> and NO<sub>3</sub>, plants that are known to prefer NO<sub>3</sub> still absorb NH<sub>4</sub> more rapidly (Van Diest, 1976). The physiological background of this anomalous behavior is largely unknown. The deleterious influences of the phenomenon on plant growth are observed most strikingly in nutrient-solution experiments. Under such conditions, the dominating NH<sub>4</sub> uptake is responsible for an extrusion of protons by the roots leading to strong acidification of the nutrient solution. The plant may then suffer more from high acidity of the growth medium than from NH<sub>4</sub> uptake.

Under more natural conditions it has frequently been observed that plants absorb NO<sub>3</sub> more easily from acid soils and NH<sub>4</sub> more easily from alkaline soils. It has been suggested (Rao and Rains, 1976) that a reduction in NO<sub>3</sub> uptake at high pH levels may be due to a competitive effect of OH ions on NO<sub>3</sub> uptake mechanisms. Likewise, the uptake of NH<sub>4</sub> from acid soils may be inhibited by a predominance of protons blocking NH<sub>4</sub> uptake mechanisms. For practical forest tree nutrition, a consequence of these findings would be that with progressing forest soil acidification the NH<sub>4</sub>:NO<sub>3</sub> uptake ratio would be shifted in favor of NO<sub>3</sub> uptake. It was found (Van den Driessche, 1987) that Douglas fir seedlings grown on sand made most growth on NO<sub>3</sub> under acid (pH4) conditions, and most growth on NH<sub>4</sub> under neutral (pH7) conditions. An additional factor involved is the uptake of iron. It has frequently been shown that NO<sub>3</sub> uptake suppresses iron availability. The pH increasing effect exerted by NO<sub>3</sub> uptake on the rhizosphere might be responsible for a lowering of the iron



availability. Douglas fir seedlings receiving  $\text{NO}_3\text{-N}$  were found to respond to the addition of Fe chelate, which was not the case for seedlings receiving  $\text{NH}_4\text{-N}$  (Van den Driessche, 1987).

In an early study on the responses of tree species to variation in N nutrition (Evers, 1962) it was observed that poplar trees prefer  $\text{NO}_3$ , and that Norway spruce grows best on a combination of  $\text{NH}_4$  and  $\text{NO}_3$  in an acid medium, and on  $\text{NH}_4$  in an alkaline medium. The negative response to  $\text{NH}_4$  under acid conditions was ascribed to  $\text{NH}_4$ -induced Ca deficiency.

As mentioned before, the N form preferred by trees is not always the N form most easily absorbed. In nutrient-culture experiments (Keltjens and Van Loenen, 1989) it was experienced that from an  $\text{NH}_4$   $\text{NO}_3$ -containing nutrient solution all five tree species included in the experiment absorbed  $\text{NH}_4$  preferentially, with greater preference shown by the broad-leaved species oak and birch than by the coniferous species larch, Scots pine and Douglas fir.

#### *$\text{NH}_4\text{:NO}_3$ availability ratios*

A study of the N uptake characteristics of trees under natural conditions is faced with several difficulties not encountered in experiments involving nutrient solutions or sand cultures. In nutrient culture experiments the ratio in which  $\text{NH}_4$  and  $\text{NO}_3$  are absorbed can simply be determined by monitoring the rate of disappearance of the two ionic species from the solution. Also in sand cultures, complicating factors such as N mineralization-immobilization turnover and  $\text{NH}_4$  adsorption on colloidal material can be avoided.

However, when N uptake ratio studies are conducted with soil as growth medium, account must be taken of the fact that potentially available and readily available quantities of  $\text{NO}_3$  are virtually identical, whereas potentially available  $\text{NH}_4$  may be a quantity many times larger than the quantity of readily available  $\text{NH}_4$ , the former being  $\text{NH}_4$  in exchangeable positions and the latter  $\text{NH}_4$  in the soil solution. Quantification of the two modes of N nutrition can in such cases be achieved only through the use of isotopic N.

Knowledge is then needed of the sizes of the two pools of plant-available N. With this information at hand, quantities of  $\text{NH}_4$ - and  $\text{NO}_3$  salts can be added to the soil in a ratio similar to the existing  $\text{NH}_4\text{:NO}_3$  ratio in the soil, with each time one of the N sources being enriched with  $^{15}\text{N}$ . As long as the added quantities of both N forms constitute only minor portions of the total available quantities of  $\text{NH}_4$  and  $\text{NO}_3$ , the addition will not bring about disturbing shifts in the pools of  $\text{NH}_4$  and  $\text{NO}_3$  in the soil.

The trees whose  $\text{NH}_4\text{:NO}_3$  uptake ratio is under examination can now be expected to absorb portions of the added  $\text{NH}_4$  and  $\text{NO}_3$  in the same ratio as in which residual  $\text{NH}_4$  and  $\text{NO}_3$  will be absorbed. With use made of the isotope-dilution technique, determinations of total N absorbed by the trees and of  $^{15}\text{N}$  absorbed will now permit

estimates to be obtained of the ratio in which N is absorbed as  $\text{NH}_4$  and  $\text{NO}_3$ .

It must be remarked here that the above described technique is beset with a number of complications. First, partial nitrification of added and residual  $\text{NH}_4$  during the experimental period may affect the  $\text{NH}_4:\text{NO}_3$  ratio originally met in the soil and used as a guideline for the ratio in which  $\text{NH}_4$  and  $\text{NO}_3$  were added to the soil. In case nitrification occurs after addition of  $^{15}\text{N}$ -labelled  $\text{NH}_4$ , some of the  $^{15}\text{N}$  present in the plant parts to be analyzed will be mistaken as having been absorbed as  $\text{NH}_4$ , whereas in reality the N was absorbed as  $\text{NO}_3$ . The result will be an overestimation of the quantity of N absorbed as  $\text{NH}_4$ . Conversely, an underestimation of  $\text{NO}_3$  absorption will result when due to nitrification after addition of  $^{15}\text{N}$ -labelled  $\text{NO}_3$ , the pool of available  $\text{NO}_3$  is increased. A means of avoiding these complications is the use of a nitrification inhibitor.

Second, microbial activity may lead to a partial immobilization of added inorganic N. In such cases, preferential use of  $\text{NH}_4$  by microorganisms may result in relatively more  $\text{NH}_4$ - than  $\text{NO}_3$  immobilization, and thus can give rise to an underestimation of the proportion of N absorbed by trees in the form of  $\text{NH}_4$ . Such an immobilization of inorganic N by microorganisms can be followed by a remineralization. If  $\text{NO}_3$  were the originally immobilized N form, remineralization would primarily result in  $\text{NH}_4$  formation. Uptake of this N by trees would lead to an overestimation of the relative contribution of  $\text{NH}_4$  and to an underestimation of that of  $\text{NO}_3$  to the nutrition of the trees. However, as microbes show a strong preference for  $\text{NH}_4$  as N source,  $\text{NO}_3$  immobilization is likely to be of little significance.

The extent of such complications is a function of the rate of turnover of N, which in turn can be affected by the amount of N added. When the quantities of added  $^{15}\text{N}$ -labelled  $\text{NH}_4$  and  $\text{NO}_3$  are small, relative to the existing pools of residual  $\text{NH}_4$  and  $\text{NO}_3$ , the additions will not bring about serious disturbances in existing quantities and ratios of  $\text{NH}_4$  and  $\text{NO}_3$  in the soil and, thus, will not serve as important incentives to additional microbial activities.

Another way of circumventing complications associated with such microbially induced N transformations would be the use of a broad-spectrum pesticide wiping out all microbial activities in the soil. A disadvantage of such an intervention is the elimination of mycorrhizae as well. It has been shown (Bledsoe and Zasoski, 1983) that mycorrhizae promote the uptake of  $\text{NH}_4$  by Douglas fir from a medium containing both  $\text{NH}_4$  and  $\text{NO}_3$ .

### *Experimental*

In the experiment discussed below three-year old larch trees, grown in a sandy mineral soil, received limited quantities of  $^{15}\text{N}$ -enriched  $\text{NH}_4$  and  $\text{NO}_3$  in a ratio similar to the one encountered in atmospheric deposition in Dutch forests. The labeling of the fertilizer sources will allow estimates to be obtained of ratios of  $\text{NH}_4$  and  $\text{NO}_3$  absorbed by the trees.

With information at hand on the relative quantities of  $\text{NH}_4$  and  $\text{NO}_3$ , a further chemical analysis of the needles enables one to construct a cation-anion uptake balance sheet for the larch trees examined. With the aid of this information it can be calculated whether or not through their nutrient absorption pattern trees make a contribution to the acidification of forest soils.

Any such contribution, if verified, must be viewed in a larger perspective of nutrient cycling through a forest ecosystem. The extent to which and the velocity with which organic N in the shed needles will be subject to ammonification and, subsequently, to nitrification, will eventually contribute as well to the overall pH changes to be expected in a soil under forest subjected to influxes of ammonium and nitrate through dry and wet deposition. In the present chapter, main emphasis will be placed on the contribution of trees, through their uptake pattern, to pH changes in the soil.

## Materials and methods

The sandy soil used in the trial was collected from a forested area adjacent to the site of a forest fertilization experiment, with Scots pine as experimental tree species, conducted in the central part of the Netherlands (Van Diest, 1989). After the LFH horizons had been removed, the soil was excavated to a depth of 30 cm and thoroughly mixed. The pH value measured in 0.01 M  $\text{CaCl}_2$  was 4.0. Total N amounted to 420 mg N  $\text{kg}^{-1}$ , and the concentrations of  $\text{NH}_4$ ,  $\text{NO}_3$  and dissolved organic N in 0.01 M  $\text{CaCl}_2$  solution were 3.5, 0.7 and 2.3 mg N  $\text{kg}^{-1}$ , respectively.

Eight 60-liter round, plastic containers (upper diameter 58 cm, lower diameter 48 cm, height 30 cm) were each filled with 63.7 kg dry soil. Per container, the following quantities of fertilizer had previously been added to the soil: 17.1 g  $\text{K}_2\text{SO}_4$ , 10.6  $\text{MgSO}_4 \cdot \text{H}_2\text{O}$ , 19.3 g  $\text{Ca}(\text{H}_2\text{PO}_4)_2$ , 79 g  $\text{CaSO}_4 \cdot \text{H}_2\text{O}$ , and 2 g  $\text{CuSO}_4$ . These applications were equivalent to those of the PKCa Mg treatment of the abovementioned Scots pine fertilization experiment, with the exception that in the containers Ca was applied as gypsum and not as limestone.

In March 1989, three-year old larch (*Larix kaempferi* Lamb.) trees were collected from a one-year old plantation. The adhering soil was washed from the roots and per container one tree was planted. The root systems were allowed to recover for a period of 7 weeks before the actual experiment started.

The larch tree trial reported on in this chapter is a portion of a larger experiment in which the  $\text{NH}_4:\text{NO}_3$  uptake ratio of Scots pine will be investigated. The larch tree was chosen as preliminary experimental species for the following reasons. First, as a coniferous tree the larch tree bears a greater resemblance to Scots pine than do broad-leaved, deciduous tree species. Second, a very large percentage of absorbed nutrients is each year stored in new larch needles, which will all again be shed at the end of the growing season. This characteristic enables recovery of a large portion of the absorbed nutrients in the needles, and thus ensures that relatively small quantities of

$^{15}\text{N}$ -labelled N sources are sufficient to allow reliable recovery percentages of added N to be obtained.

Before the actual  $^{15}\text{N}$  experiment started, 1g N (3:1  $\text{NH}_4:\text{NO}_3$ ) was applied per container to prevent the trees from developing an extreme N demand possibly leading to the absorption of all available N, irrespective of N species. The 3:1  $\text{NH}_4:\text{NO}_3$  ratio is similar to the ratio in which  $\text{NH}_4$  and  $\text{NO}_3$  in wet and dry deposition enter the soil of the Scots pine experimental site, as measured in an adjacent Scots pine stand.

The N to be applied was dissolved in 1-L volumes of deionized water, together with a first application of N-serve (140 ml Dow N-serve 24, 100x diluted) serving as nitrification inhibitor. To ensure uniform distribution of the N to be applied, the solution was injected in 30-40 dosages. Needle analysis performed two weeks after N addition showed that the N-nutritional status of the trees was slightly better than that of corresponding trees in the original plantation (N percentages of 2.4 and 2.0, respectively). When the trees thus had shown normal N-uptake characteristic, the application of labelled N was started.

Two applications of labelled N were made, on June 6 and June 23. Each time, per container 1-L volumes of solution containing 0.375 g N as  $(\text{NH}_4)_2\text{SO}_4$ , 0.125 g N as  $\text{KNO}_3$ , and N-serve were injected. Four containers received labelled  $\text{NH}_4$  and four containers labelled  $\text{NO}_3$  (both 10%  $^{15}\text{N}$ ). N-serve was injected biweekly until August 28.

The containers were kept inside a greenhouse to prevent atmospheric nitrogen and moisture depositions. The greenhouse was ventilated well to avoid temperatures far exceeding the natural ones.

The moisture content of the soil in the containers was originally adjusted to 18% on a dry-weight basis, and readjusted to that level after weekly weighings, through addition of deionized water. The volumes of intermediate water dosages were calculated from estimates of daily losses due to evapotranspiration. The soil moisture contents were not allowed to drop below 10%.

In mid-September all predominantly green needles were harvested, dried at  $70^\circ\text{C}$ , weighed and ground. Subsamples were digested in a  $\text{H}_2\text{SO}_4$ , salicylic acid, selenium,  $\text{H}_2\text{O}_2$  mixture (Walinga et al., 1989). In the digest, N and P were determined colorimetrically, K, Ca and Na by atomic emission, and Mg by atomic absorption spectrometry. Cl was determined in water extracts (1:50) with the use of a chlorocounter. For lack of a reliable method for S determination, the organic S content of the needles was estimated at 6.5% of the total-N content (Van den Burg, 1979), and the inorganic S content was assumed to be equal to the organic S content.

Analyses of  $^{15}\text{N}$  abundance in needle samples were made with the use of a mass spectrometer.  $^{15}\text{N}$ - and total-N analyses of needles of trees having received  $^{15}\text{NH}_4$  allowed estimates to be obtained of the sizes of fractions of total N absorbed as fertilizer  $\text{NH}_4$ . Likewise, the sizes of the fractions of total N absorbed as fertilizer  $\text{NO}_3$  could be estimated through  $^{15}\text{N}$ - and total-N analyses of needles of trees having received  $^{15}\text{NO}_3$ . When the  $\text{NH}_4:\text{NO}_3$  ratio of uptake from fertilizer sources is

considered to be representative of that ratio of uptake from soil sources, the latter ratio can be calculated.

With knowledge of the ratios of  $\text{NH}_4$  and  $\text{NO}_3$  uptake, cation-anion uptake balance sheets could be constructed. From the results of these balance-sheet calculations estimates of net  $\text{H}^+$  extrusion by roots could be obtained. It should be taken into account that these  $\text{H}^+$  extrusion estimates were based on chemical composition values and estimated weights of needle biomass only. Consequently, the reported values should slightly underestimate the real values.

## Results and discussion

### *Nutrient uptake pattern of the larch tree*

As mentioned before, four trees grew on soil to which  $^{15}\text{N}$ -enriched  $\text{NH}_4$  and  $^{14}\text{NO}_3$  had been added, and four on soil to which  $^{15}\text{N}$ -enriched  $\text{NO}_3$  and  $^{14}\text{NH}_4$  had been added. In Table 1, information is supplied on the quantities of  $\text{NH}_4$ -N and  $\text{NO}_3$ -N absorbed by the trees from the fertilizer sources and from the soil sources, as estimated from the quantities of  $^{15}\text{N}$  encountered in the needles with the aid of mass-spectrometer analysis.

Table 1. N in larch needles, as derived from  $\text{NH}_4$ - and  $\text{NO}_3$  sources ( $\text{mmol N kg}^{-1}$  dry needles).

measured as being absorbed from fertilizer source (means of 4 trees)		calculated as being absorbed from soil source (means of 8 trees)	
as $\text{NH}_4$	as $\text{NO}_3$	as $\text{NH}_4$	as $\text{NO}_3$
247 $\pm$ 37	71 $\pm$ 13	987	284

The average  $\text{NH}_4$ -N: $\text{NO}_3$ -N uptake ratio was found to be 3.5, which value is higher than the one reported earlier (Keltjens and Van Loenen, 1989) for larch, being 2.8, as obtained in the situation of a 1:1  $\text{NH}_4$ : $\text{NO}_3$  availability ratio established in a nutrient solution. In the present situation the approximate initial ratio of potentially available  $\text{NH}_4$  and  $\text{NO}_3$  in the soil in the container was 5:1. Under field conditions it was found with the use of porous ceramic cups that for this soil such a 5:1 ratio corresponds with a 1:3 ratio for concentrations of  $\text{NH}_4$  and  $\text{NO}_3$  in the soil moisture.

Two phenomena may have influenced the numerical value of the  $\text{NH}_4$ : $\text{NO}_3$  uptake ratio. First, during the period of N uptake following the application of  $^{15}\text{N}$ -enriched N sources, the use of a nitrification inhibitor may have caused a gradual depletion of the  $\text{NO}_3$  supply, whereas the formation of new  $\text{NH}_4$  in the ammonification process was not affected. This development could have led to a situation in which during the uptake period every  $^{15}\text{NH}_4$  absorbed represented a stable or possibly even growing quantity of soil  $\text{NH}_4$  absorbed, the latter leading to an underestimation of the soil  $\text{NH}_4$  quantity absorbed and thus of the  $\text{NH}_4$ : $\text{NO}_3$  uptake ratio.

Table 2. Cation-anion uptake balance sheets for *Larix kaempferi* trees, as estimated from the quantities of nutrients measured in the needles.

<sup>15</sup> N-enriched N source and no. of tree	Concentrations (mmol kg <sup>-1</sup> dry needles)											
	NH <sub>4</sub>	K	Ca	Mg	Na	ΣC <sub>a</sub>	NO <sub>3</sub>	P	S	Cl	ΣA <sub>a</sub>	Σ(C <sub>a</sub> -A <sub>a</sub> )
NH <sub>4</sub> 1	1418	408	87	98	9	2205	363	39	101	76	680	1525
NH <sub>4</sub> 2	1347	482	88	98	8	2210	389	34	98	73	692	1517
NH <sub>4</sub> 3	980	562	100	116	8	1983	364	27	76	81	624	1359
NH <sub>4</sub> 4	1376	443	95	108	8	2234	363	38	99	71	669	1564
NO <sub>3</sub> 1	1201	463	145	137	8	2236	255	35	83	68	523	1713
NO <sub>3</sub> 2	1308	515	145	122	8	2365	401	38	97	50	684	1681
NO <sub>3</sub> 3	1107	372	100	106	17	1908	318	30	81	52	561	1347
NO <sub>3</sub> 4	1139	504	125	127	0	2147	388	38	87	71	670	1477

ΣC<sub>a</sub> and ΣA<sub>a</sub>: summations of cations and anions absorbed (mmol<sub>c</sub> kg<sup>-1</sup>)

Second, an absence of formation of new soil-NO<sub>3</sub> could have resulted in a gradual depletion of available NO<sub>3</sub> and thus in a widening of the ratio of available NH<sub>4</sub> and NO<sub>3</sub>. Such a development, if occurring, might have favored the uptake of NH<sub>4</sub> and might have led to an overestimation of the real NH<sub>4</sub>:NO<sub>3</sub> uptake ratio under natural conditions. This hazard of NO<sub>3</sub> depletion was reduced by postponing the use of the nitrification inhibitor until the moment of the first application of NH<sub>4</sub> and NO<sub>3</sub>, and by applying a quantity of unlabelled NO<sub>3</sub> prior to the application of labelled NO<sub>3</sub>. Analyses performed on soil samples at the end of the experimental period showed that the NH<sub>4</sub>:NO<sub>3</sub> ratio in soil extracts had fallen from the initial value of 4.9 to a value of 6.2.

With estimates available of the quantities of N absorbed as NH<sub>4</sub> and NO<sub>3</sub> per unit dry needle weight, a cation-anion uptake balance sheet could be constructed, as presented in Table 2.

It can be observed that the uptake of cations far exceeds that of anions, and that this cation uptake excess is due mainly to a preponderance of NH<sub>4</sub> uptake. When it is assumed that the uptake of every equivalent of cations and anions is accompanied by an extrusion of an equivalent of protons and hydroxyls, respectively (Van Diest, 1989), it can be deduced that the uptake pattern of the larch tree exerts an acidifying effect on the rhizosphere of the tree and, more indirectly on the soil as a whole.

The average excess of cation-over-anion uptake per kg of dry needles, as calculated from the values in the last column of Table 2 amounts to 1522 me. For a conversion of this value into an estimate of the quantity of acidity produced per ha of larch, an estimate is needed of the quantity of needles produced annually per ha. When such a quantity is estimated at 2000 kg dry needles, the acidity produced annually per ha will be 2000 × 1522 mmol<sub>c</sub> or 3.04 kmoles of H<sup>+</sup>.

### Hydrogen budget

For the construction of an  $H^+$  production-consumption balance sheet this value of 3.04 kmoles  $H$  must be compared with a number of other values representing transformations of N in a forest ecosystem, as shown in Fig. 1. In the following, these transformations and their effects on soil pH will be discussed. In some instances, no quantitative information is as yet available to allow calculations to be made of quantities of  $H^+$  consumed or produced. As mentioned before, the larch trial dealt with in this chapter constitutes a part of a larger experiment in which more quantitative information on the various N transformations will be collected.

The above discussed transformations in which soil- $NH_4$  and soil- $NO_3$  are absorbed by the trees and converted into organic N in phytomass are listed in Fig. 1 as process d and f, respectively. The first one is acid-producing (+1), the second acid-consuming (-1). It must be emphasized again that the influence of nutrient uptake by trees on soil pH is not determined by N transformations

only, but by uptake of mineral cations and anions as well. It can, however, be calculated from the data of Table 2 that, on an equivalence basis,  $NH_4$  uptake represented 57% of total cation uptake and  $NO_3$  uptake 56% of total anion uptake, which illustrates the dominating influence of the nutrient N on the cation-anion uptake balance of forest trees in the Netherlands.

It can further be calculated from the data of Table 2 that for an annual needle production of 2 tons, larch trees absorb from the soil a quantity of 44.6 kg N, of which 34.6 kg are taken up as  $NH_4$  and 10.0 kg as  $NO_3$ . Annual N depositions per ha measured in the throughfall of a forested site near the Scots pine fertilization experiment were 31 kg  $NH_4$ -N (the sum of processes a and b in Fig. 1) and 9 kg  $NO_3$ -N (process c). In addition 28 kg  $SO_2$ -S are deposited per ha (P.H.B. de Visser, unpublished). The potentially acidifying effects of these  $NO_3$  and  $SO_2$  depositions are equivalent to 0.64 and 1.75 kmoles  $H^+$ , respectively. For an evaluation of the actual

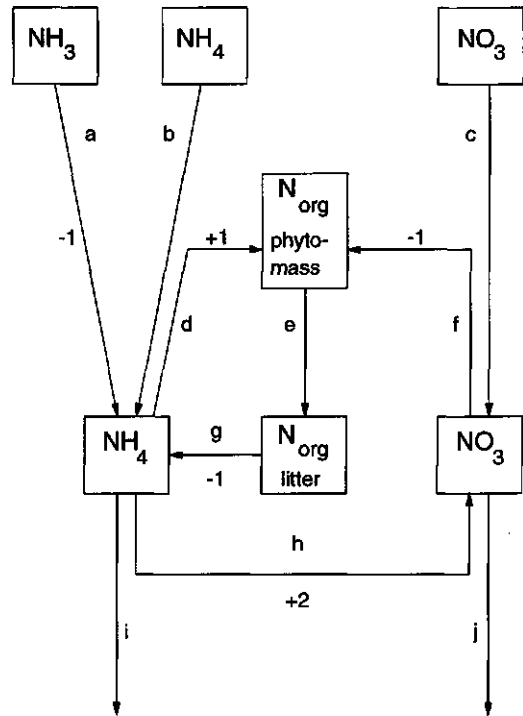


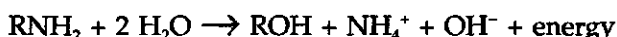
Fig. 1. A qualitative presentation of processes of N transformation in a forest ecosystem affecting soil pH. The various processes, which are discussed in the text, are listed with the letters a-j. The moles of  $H^+$  consumed (-) and produced (+) per mole of N transformed are also listed in the figure.

acidifying effects, the annual absorption of these anions by the trees should be taken into consideration. In the present case of the larch trees the quantity of  $\text{NO}_3$  annually absorbed approximately equals the quantity deposited, but annual S absorption by the trees only represents 20% of S deposited. As in both cases the absorption of these nutrients takes place in the form of anions, the absorption processes will have pH-raising effects on the soil (for  $\text{NO}_3$ , process f in Fig. 1).

Whether or not such effects will be lasting ones depends on the fate of the N- and S compounds after needle fall. The extent to which the originally pH-raising effects of the uptake of  $\text{NO}_3$  and  $\text{SO}_4$  will be maintained, corroborated or nullified after needle fall depends on the degree of mineralization of the organic N- and S compounds present in the shed needles. For N, the effect on soil pH will be determined by the magnitudes of the processes g and h in Fig. 1.

The situation pertaining to  $\text{NH}_4$  is still more complicated. The quantity listed as annually deposited (31 kg  $\text{NH}_4\text{-N}$ ) per ha approximates the estimated quantity annually absorbed by larch trees (34.5 kg  $\text{NH}_4\text{-N}$ ). An additional quantity of  $\text{NH}_4$  will annually appear in the soil as a result of the ammonification process.

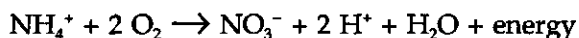
The pH-raising effect of this process is illustrated in the following reaction:



Newly deposited  $\text{NH}_4$  sources reach the soil partially as  $\text{NH}_3$  in the form of dry deposition (process a) and partially as  $\text{NH}_4^+$  in the form of wet deposition (process b). In moist soil, the deposited  $\text{NH}_3$  will soon attract a proton (-1, Fig. 1).

Consequently, newly mineralized  $\text{NH}_4$  (process g) and newly deposited  $\text{NH}_3$ , subsequently converted to  $\text{NH}_4^+$  (process a) will exert pH-raising influences on soil before such a pH rise may be partially reversed by tree roots absorbing the  $\text{NH}_4$  and extruding a proton for every  $\text{NH}_4^+$  absorbed (process d).

In addition to this acidifying  $\text{NH}_4$ -absorption process, a second process involving  $\text{NH}_4$  and having an acidifying effect is nitrification (process h). This process results in the release of two protons for every  $\text{NH}_4$  nitrified:



The classical view of nitrification being dependent on soil pH levels higher than 4.5 has been challenged by researchers claiming activities of heterotrophic (Ishaque and Cornfield, 1976) and of chemolithotrophic (De Boer et al., 1988) nitrifiers in acid soils. Although it remains to be shown unequivocally that such processes, being qualitatively feasible, are of any quantitative importance under field conditions, a model approach should take account of the possibility that nitrification may occur in acid forest soils and thus may contribute to forest soil acidification (process h).

Any  $\text{NO}_3$  produced in a nitrification process is likely to add to the quantity of  $\text{NO}_3$



not absorbed by the trees. The  $\text{NO}_3$ -retention capacity of sandy soils is slight, so that  $\text{NO}_3$  is subject to leaching during periods of precipitation exceeding evapotranspiration (process j). In this leaching process  $\text{NO}_3$  moves together with a cation. In very acid soils Al may be the primary accompanying cation. In less acid soils Ca and Mg may serve as such thus depriving these soils of highly valuable mineral nutrients. Depending on the circumstances,  $\text{NH}_4$  might also be an important cation accompanying  $\text{NO}_3$  in the leaching process. To which extent  $\text{NH}_4$  and  $\text{NO}_3$  will leach together well depend on the magnitudes of a number of processes in Fig. 1, such as the balance of  $\text{NH}_4$  precipitation (processes a and b) and  $\text{NH}_4$  uptake (process d), and the balance of litter-N ammonification (g) and nitrification (h).

It must, however, be emphasized that more cationic nutrients are likely to be lost in a leaching process together with  $\text{SO}_4$  ions, formed from  $\text{SO}_2$  deposition, than with  $\text{NO}_3$  ions. Of the 28 kg S entering Dutch soils annually per ha through deposition, only a small fraction (in the present experiment estimated at 6 kg) is absorbed by trees, thus leaving much room for  $\text{SO}_4$  to accompany nutrient cations in a leaching process.

#### *Nitrogen nutrition of trees*

The primary objective of the above discussed experiment was to evaluate the importance of the nitrogen uptake process by trees in the overall nitrogen budget for a forest ecosystem in the Netherlands. It could be shown that a substantial portion of the annual N influx can be absorbed by the trees, mainly as  $\text{NH}_4$ . This preference for  $\text{NH}_4$ , as demonstrated with larch trees, appears to make an important contribution to overall acidification of Dutch forest ecosystems. It remains to be substantiated, however, that soil acidification per se is a major cause of forest decline. There are numerous reports (e.g. Keltjens and Van Loenen, 1989; Ryan et al. (1986a, b)) suggesting that trees can be remarkably insensitive to high soil acidity and to the ensuing high solubility of soil Al.

As was mentioned earlier, the preference trees seem to have for  $\text{NH}_4$ -N declines with increasing soil acidity. In the form of an increased uptake of  $\text{NO}_3$  exerting a pH-increasing effect on soil, trees thus appear to be able to retard an ongoing soil acidification process. A complicating factor is, however, that with increasing soil acidification ammonification (process g in Fig. 1) is less obstructed than is nitrification (process h), which would result in a raised  $\text{NH}_4$ : $\text{NO}_3$  availability ratio. Under such circumstances  $\text{NO}_3$  deposition (process c) would become the primary source of  $\text{NO}_3$  for trees. A reduced nitrification process and an increased aptitude for  $\text{NO}_3$  uptake by the trees would then together contribute to a reduction in  $\text{NO}_3$  leaching. The two major advantages of such a development would be a reduction in contamination of deep groundwater with  $\text{NO}_3$ , and a reduction in quantity of mineral cationic nutrients lost due to leaching along with  $\text{NO}_3$ . These advantages of ongoing soil acidification may, however, not outweigh certain disadvantages such as toxicity of Al and/or Mn

and P fixation.

When forest soils have become extremely acid, liming may be practiced. In case dolomitic limestone is used, the primary advantages are a reduction in Al solubility and increased availabilities of Ca and Mg. Gradually developing secondary effects will influence soil N transformations and N uptake characteristics. Due to a rise in pH, both ammonification and nitrification will increase, but with a higher soil pH level the  $\text{NH}_4\text{:NO}_3$  uptake ratio will shift towards relatively more  $\text{NH}_4$  absorbed. Increased  $\text{NO}_3$  production and reduced  $\text{NO}_3$  uptake could promote leaching of  $\text{NO}_3$  and accompanying cationic nutrients, unless liming stimulated tree growth enough to substantially raise N demand and N uptake by trees.

Presently in the Netherlands major efforts are made to reduce the emission of  $\text{NH}_3$  associated with the production, storage and application of liquid manure. If such efforts would lead to a more rapid reduction in  $\text{NH}_x$ - than in  $\text{NO}_x$  immissions into forests, the  $\text{NH}_4\text{:NO}_3$  uptake ratio would shift toward relatively more  $\text{NO}_3$  absorbed by trees. The resulting pH-raising effect would likely be partially offset by an induced rise in preference for  $\text{NH}_4$  uptake by the trees, but any such pH-lowering effect may be limited due to a reduced availability of  $\text{NH}_4$ . A more complete uptake of  $\text{NH}_4$ , becoming available due to immission and ammonification, would lower the quantity of  $\text{NH}_4$  available for nitrification. Consequently the strong acidification, caused by nitrification, and the ensuing leaching of  $\text{NO}_3$  and accompanying mineral cations would all be alleviated. It can be concluded, therefore, that measures taken to reduce  $\text{NH}_3$  emissions in the Netherlands would be effective in reducing both soil acidification and loss of mineral nutrients. An exception to this statement would have to be made for cases in which a major portion of the immission consists of  $\text{NH}_3$  reaching the soil as such and consuming a proton for a conversion to  $\text{NH}_4$  (process a in Fig. 1).

The objective of the present chapter has been to illustrate the importance of processes of N uptake by trees and their contribution to overall pH changes in a Dutch forest ecosystem subjected to influxes of  $\text{NH}_x$  and  $\text{NO}_x$ . It was shown that larch trees display a strong preference for  $\text{NH}_4$  as N source, that such a preference in itself can exert a strongly acidifying effect, but that the effect can be modified in several ways by N transformations in the ecosystem taking place before N uptake by the trees and after needle fall.

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**Soil acidification as caused by the nitrogen uptake pattern of  
Scots pine (*Pinus sylvestris*)**

**Key words:** cation-anion uptake balance,  $^{15}\text{N}$  labelling,  $\text{NH}_4/\text{NO}_3$  uptake ratio, nitrogen source, *Pinus sylvestris*, soil acidification, soil solution

### Abstract

Three-year-old Scots pine (*Pinus sylvestris*) trees were grown on a sandy forest soil in pots, with the objective to determine their  $\text{NH}_4/\text{NO}_3$  uptake ratio and proton efflux. N was supplied in three  $\text{NH}_4\text{-N}/\text{NO}_3\text{-N}$  ratios, 3:1, 1:1 and 1:3, either as  $^{15}\text{NH}_4 + ^{14}\text{NO}_3$  or as  $^{14}\text{NH}_4 + ^{15}\text{NO}_3$ . Total N and  $^{15}\text{N}$  acquisition of different plant parts were measured. Averaged over the whole tree, the  $\text{NH}_4/\text{NO}_3$  uptake ratios throughout the growing season were found to be 4.2, 2.5, and 1.5 for the three application ratios, respectively. The excess cation-over-anion uptake value ( $C_a - A_a$ ) appeared to be linearly related to the natural logarithm of the  $\text{NH}_4/\text{NO}_3$  uptake ratio. Further, this uptake ratio was related to the  $\text{NH}_4/\text{NO}_3$  ratio of the soil solution. From these relationships it was estimated that Scots pine exhibits an acidifying uptake pattern as long as the contribution of nitrate to the N nutrition is lower than 70%. Under field circumstances root uptake may cause soil acidification in the topsoil, containing the largest part of the root system, and soil alkalization in deeper soil layers.

### Introduction

In the Netherlands relatively large quantities of  $\text{NH}_x$  ( $\text{NH}_4^+$  and  $\text{NH}_3$ ), emitted by intensive livestock farms, are subsequently deposited. In two ways  $\text{NH}_x$  may cause soil acidification, i.e. by nitrification (e.g. Van Breemen et al., 1987; De Boer, 1989) and by an  $\text{NH}_4$ -induced excess cation-over-anion uptake by roots, accompanied by net proton extrusion. The latter only occurs if ammonium is taken up to such an extent that anion uptake insufficiently balances the influx of positive charges. As most plants absorb more cations than anions, other than ammonium and nitrate (Mengel and Kirkby, 1987), soil acidification may occur even if ammonium uptake does not exceed nitrate uptake. Thus, Raven (1986) states that herbaceous plants of similar composition exhibit an intracellular  $\text{H}^+$  production of 1.22 and an  $\text{H}^+$  consumption of 0.78 mole per mole N consumed in case of an exclusive ammonium or nitrate nutrition, respectively. This implies that  $\text{H}^+$  production occurs if ammonium and nitrate are taken up in a ratio higher than 0.78:1.22, i.e. when the nitrate contribution to total N nutrition is smaller than 61%. Accordingly, Gijsman (1990a) found that Douglas fir acidifies its rhizosphere when the contribution of nitrate to N uptake falls below 65%.

Thus, if a plant would take up ammonium and nitrate indiscriminately, the rhizosphere is more likely to be acidified than alkalized. However, plants usually show a preference for either one of the N species. If ammonium is preferred the rhizosphere will be acidified. In this chapter ammonium (or nitrate) preference is

defined as uptake of  $\text{NH}_4$  and  $\text{NO}_3$  at an  $\text{NH}_4/\text{NO}_3$  uptake ratio higher (or lower) than the same ratio in the soil solution. The pertinent question in the present investigation is to what extent tree species prefer ammonium or nitrate and which consequences such a preference may have for acid- or base production in the rhizosphere.

It has frequently been shown that seedlings of a number of forest tree species grow better when nitrogen is supplied in the ammonium form or as ammonium plus nitrate (Adams and Attiwill, 1982; Nelson and Selby, 1974). Douglas fir may be an exception (Bigg and Daniel, 1978; Van den Driessche and Dangerfield, 1975). However, better growth with either one of the N forms can at best serve as an indication, but not as evidence of ammonium or nitrate preference as defined above. For acquiring such evidence the uptake of ammonium and nitrate should be measured separately. This is easily accomplished in nutrient solution experiments. For example, Keltjens and Van Loenen (1989) grew five tree species, including Scots pine and Douglas fir, on nutrient solutions containing  $\text{NH}_4\text{NO}_3$ . All species showed an  $\text{NH}_4/\text{NO}_3$  uptake ratio  $> 1$ . However, the uptake ratio of ammonium and nitrate from soils can only be unravelled when use is made of labelled N sources. With this technique Gijsman (1991) showed that Douglas fir exhibited an  $\text{NH}_4/\text{NO}_3$  uptake ratio higher than the  $\text{NH}_4/\text{NO}_3$  ratio in the soil solution. In an earlier pot experiment (Chapter 2) the  $\text{NH}_4/\text{NO}_3$  uptake ratio of larch (*Larix kaempferi* Lamb.) was investigated. This experiment served as a preliminary study to test a method of diversified  $^{15}\text{N}$  application. It appeared that larch absorbed 3 times as much  $\text{NH}_4\text{-N}$  as  $\text{NO}_3\text{-N}$ , while the soil solution's  $\text{NH}_4/\text{NO}_3$  ratio was 0.33 in the same soil under field circumstances. The  $\text{NH}_4\text{-N}/\text{NO}_3\text{-N}$  ratio of the soil solution in the pots was not determined. In soil extracts this ratio was  $> 3$ , so ammonium preference could not be proven beyond doubt. Extract concentrations primarily reflect the sizes of the pools of potentially available nutrients, whereas the pools of nutrients in the soil solution are more directly available. Especially for  $\text{NH}_4$  the former may be large as compared to the latter.

In the present experiment, therefore, emphasis was laid more on soil solution than on extract concentrations of N species as a factor determining the N uptake pattern. The experiment was designed to investigate whether and, if so, to what extent soil-grown Scots pine (*Pinus sylvestris*), as most common forest tree species in the Netherlands, exhibits ammonium preference.  $^{15}\text{N}$ -labelled nitrogen was applied in three different  $\text{NH}_4/\text{NO}_3$  ratios, thus supplying an opportunity to examine to what extent the uptake ratio is influenced by the application ratio. In this chapter "application ratio" and "uptake ratio" refer to the  $\text{NH}_4\text{-N}/\text{NO}_3\text{-N}$  ratio of the N addition and N uptake, respectively.

## Materials and methods

### *Experimental lay-out*

In January 1990 sandy forest soil was collected from an area adjacent to a fertilizer experiment described earlier (Van Diest, 1989), also with Scots pine as experimental species. After the LFH horizon had been removed, the soil was excavated to a depth of 30 cm and thoroughly mixed. The pH value measured in a 1:10 (w:v) 0.01 M  $\text{CaCl}_2$  extract of fresh soil was 4.0. The soil concentrations of 0.01 M  $\text{CaCl}_2$ -extractable  $\text{NH}_4$  and  $\text{NO}_3$  were 2.7 and 3.2 mg N  $\text{kg}^{-1}$ , respectively. Total N and C amounted to 0.42 and 11.3 g  $\text{kg}^{-1}$ , respectively. The CEC (silver thiourea) was 13.7 mmol $_c$   $\text{kg}^{-1}$ .

Twenty-four round, plastic containers (upper diameter 58 cm, bottom diameter 48 cm, height 30 cm) with perforated bottoms were first filled with a 5-cm layer of coarse sand. This layer was confined by two sheets of root cloth. Next, the pots were each filled with an amount of the moist forest soil equivalent to 60.2 kg oven-dry (105°C) soil. Per pot, the following amounts of fertilizer were mixed through the soil: 4.5 g  $\text{K}_2\text{SO}_4$ , 11.4 g  $\text{MgSO}_4 \cdot \text{H}_2\text{O}$ , 9.0 g  $\text{Ca}(\text{H}_2\text{PO}_4)_2$ , 21.5 g  $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$  and 1 g  $\text{CuSO}_4$ . On a surface basis, the quantities of P, K, Ca and Mg equalled those applied in the PKCaMg treatment of the above-mentioned fertilizer experiment. The pots were placed under a translucent shelter to prevent wet deposition of atmospheric nitrogen. Underneath the pots plastic sheets were laid out with raised edges to allow watering from below. This method of watering was chosen, as the more accurate method of weighing was too elaborate and tensiometers did not function properly (most likely due to a poor contact between the cups and the sandy soil). Tap water was applied weekly. Saturation of the bottom part of the soil was prevented by the layer of coarse sand. A separate experiment showed that capillary rise of water in a 30-cm column of the potted soil resulted in an average moisture content of 18%. Once every three or four weeks the topsoil was wetted to prevent formation of a salt crust.

In March 1990, per pot one three-year-old Scots pine tree was planted with adhering soil. The trees quickly sprouted. Early in July, before the actual  $^{15}\text{N}$  experiment started, unlabelled N was applied to prevent an extreme N demand which, if occurring, would likely result in the uptake of all subsequently added N, irrespective of N species. Three groups of 8 pots received unlabelled N (1 g N per pot) with  $(\text{NH}_4)_2\text{SO}_4$ -N /  $\text{KNO}_3$ -N ratios of 3:1, 1:1 and 1:3, respectively. The 3:1 ratio had been used before (Chapter 2). The smaller ratios were chosen to examine the influence of changing application ratio on the uptake ratio. The application was carried out by homogeneously injecting 1 L of water containing the required amounts of N and nitrification inhibitor (Dow N-serve 24, about 10 mL per L soil solution).

On July 25 and August 6, to each pot 0.5-g quantities of labelled N were applied having the above mentioned ratios and compositions, now with 1 mL N-serve per L soil solution. Within each group of 8 pots 4 pots received labelled ammonium and unlabelled nitrate and 4 pots received the reverse (labelled ammonium and nitrate

contained 10%  $^{15}\text{N}$ ). The total amount of N applied per pot thus added up to 2 g. The uptake ratio per plant part was calculated by dividing the  $^{15}\text{N}$  percentages of the  $^{15}\text{NH}_4/^{14}\text{NO}_3$ -fed trees by those of their  $^{15}\text{NO}_3/^{14}\text{NH}_4$  counterparts. Dry weights per plant part were used to calculate the uptake ratios of whole trees. These ratios, together with the uptake of other cations and anions were used in calculating the  $\text{H}^+$  production per tree during the season. Details of these calculations will be given later.

#### *Soil solution sampling*

As it was intended to relate the uptake ratio to the ratio of available  $\text{NH}_4/\text{NO}_3$ , soil solution sampling was carried out. For this purpose in each pot a ceramic tension cup (Soil Moisture Equipment Corporation) was inserted at a depth of 15 cm. The soil solution was collected by connecting the cups to vacuumed bottles (ca. 20 kPa). Sampling took place after N had been injected. Unplanted soil at 18% moisture content was used to analyze the solution of the soil before N addition. In the solution samples pH,  $\text{NH}_4^+$  and  $\text{NO}_3^-$  were measured as described below.

#### *Harvest and measurements*

On October 9 the trees were harvested. The current year's needles and wood, the older needles, the older wood and the roots were collected separately. The tissues were dried in a forced-draft oven at  $70^\circ\text{C}$  and ground. Soil- and root sampling took place in two subsequent days. All trees had developed a root system throughout the entire soil volume. The roots were collected by sieving the soil through a 10-mm sieve and subsequently washing the roots on a 2-mm sieve. The piece of stem above the root collar was cut off and added to the older wood. Undoubtedly some losses of root material occurred, but probably a good estimate of the root mass increment was still obtained as the trees had been planted with incomplete root systems as well.

From the sieved soil a sample was taken which was again sieved through a 2-mm sieve. Of this fresh sample 3.00 g was extracted with 30 mL 0.01 M  $\text{CaCl}_2$  solution (Houba et al., 1990), to compare the final amounts of extractable  $\text{NH}_4$  and  $\text{NO}_3$  with the initial amounts. In the extracts pH,  $\text{NH}_4^+$  and  $\text{NO}_3^-$  were measured, the latter two colorimetrically in an automated continuous-flow system, i.e.  $\text{NH}_4^+$  by indophenol blue colouring, and  $\text{NO}_3^-$  after reduction to nitrite, followed by conversion to a red-coloured diazo compound.

The plant samples were digested in an  $\text{H}_2\text{SO}_4$ , salicylic acid,  $\text{H}_2\text{O}_2$  medium, catalyzed by selenium. In the digests N and P were measured colorimetrically, K, Ca and Na by atomic emission and Mg by atomic absorption spectrometry. Cl was measured in water extracts (1:50 w:v) using a coulometer. The organic S content of the needles was estimated as 6.5% (on a weight basis) of the total N content (Van den Burg, 1979), i.e. an S/N equivalence ratio of 0.057, and inorganic S was assumed to equal organic S. P is assumed to have been absorbed as  $\text{H}_2\text{PO}_4^-$ .

Uptake rates of nutrients were calculated by subtracting the initial amounts from the final amounts. The initial amounts were determined by analyzing pooled samples of 10 trees from the planting stock. Any increase in biomass of the older needles and wood could not be observed (i.e. growth of new biomass of the stem, previous years' needles and branches after planting). Part of the older needles may have been shed, whereas any mass increment of the older branches and the stem was obscured by variability. Therefore, these parts were not included in the uptake calculations.

The  $^{15}\text{N}$  percentages of the tissues were measured by an on-line Dumas-combustion gas-isotope ratio mass spectrometer (SIRA II, VG Instruments) and corrected for natural  $^{15}\text{N}$ . For statistical analysis SAS software (SAS Institute Inc., 1988) was used. Soil solution concentrations were log-transformed prior to statistical analysis, to obtain normally distributed data. Separation of means was carried out by the Ryan-Einot-Gabriel-Welsch multiple F test ( $\alpha = 0.05$ ) as supplied by the SAS GLM procedure.

## Results

### Soil data

The soil solution data show wide variations in time with respect to  $\text{NH}_4$  and  $\text{NO}_3$  concentrations (Table 1). However, after log transformation only few significant differences between the three sampling dates occurred (not indicated in the table). At the 1:1 treatment  $\log(\text{NH}_4)$  at 21/9 was significantly lower than the previous values. At the 1:3 treatment both  $\log(\text{NH}_4)$  and  $\log(\text{NO}_3)$  decreased consistently with time. With respect to pH in the soil solution, only the 1:3 treatment shows significant differences, viz. an increase with time. Per sampling date, the nitrate concentrations in the soil solution were not significantly (and not consistently) changed by variation in the application ratio, but the ammonium concentrations were (note that statistical testing was carried out with log-transformed data, while untransformed data are shown). The pH values of the soil solution did not show significant differences among treatments within one sampling date.

Table 1. pH and ammonium and nitrate concentrations in soil solution samples ( $\text{mmol m}^{-3}$ ) and soil extracts ( $\text{mg kg}^{-1}$ ) after injection of N in three  $\text{NH}_4\text{-N}/\text{NO}_3\text{-N}$  ratios (Ratio). Arithmetic means ( $n=8$ ) of untransformed data.

	Ratio	Date			
		31/8	7/9	21/9	9/10
		Soil solution		Extract	
pH	3:1	4.19	4.31	4.28	4.24
	1:1	4.23	4.38	4.39	4.26
	1:3	4.26	4.52	4.52	4.26
$\text{NH}_4\text{-N}$	3:1	757a <sup>*</sup>	243a	520a	8.9a
	1:1	172ab	132b	105ab	6.4a
	1:3	287b	86b	69b	3.2b
$\text{NO}_3\text{-N}$	3:1	1229	357	1001	10.4a
	1:1	1056	564	626	17.7b
	1:3	2565	629	868	21.8b

<sup>\*</sup>Significant differences in one column (if occurring) are denoted by a different letter (Ryan-Einot-Gabriel-Welsch multiple F test at  $\alpha = 0.05$ ; soil solution data were log-transformed prior to testing)



Table 2. Nutrient concentrations (mmol kg<sup>-1</sup>) in different parts of 4-y-old Scots pine grown in pots on a sandy forest soil amended with 2 g of nitrogen in three NH<sub>4</sub>-N/NO<sub>3</sub>-N ratios (Ratio).

Element	Ratio	Plant part				
		Nc <sup>a</sup>	Np	Wc	Wp	R
N	3:1	1207	848	541	347	597
	1:1	1161	789	577	343	614
	1:3	1214	829	557	344	641
P	3:1	53	33	36	20	34
	1:1	54	32	34	20	37
	1:3	57	32	35	20	39
Na	3:1	18	28	7	7	28
	1:1	21	33	8	8	24
	1:3	22	28	9	7	23
K	3:1	210	132	117	71	109
	1:1	214	123	121	78	126
	1:3	237	137	123	77	141
Ca	3:1	87	149	78	74	67
	1:1	81	144	79	75	64
	1:3	84	152	73	74	68
Mg	3:1	47	47	37	29	29
	1:1	43	43	33	29	28
	1:3	47	43	37	29	31
Cl	3:1	39	34	13	9	26
	1:1	32	32	21	11	27
	1:3	39	40	18	13	35

<sup>a</sup>N = needles, W = wood, R = root, c = current year's, p = previous years'.

Dry weight (g)

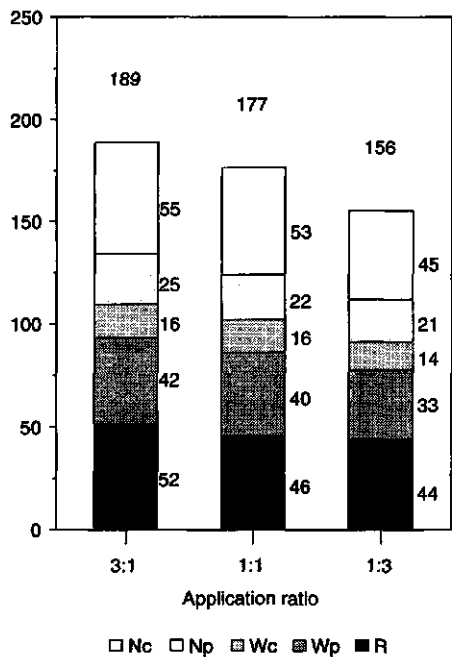


Fig. 1. Dry weights (g per plant) of various components of 4-y-old Scots pine trees after one growing season on a sandy forest soil in pots amended with 2 g of N in 3 NH<sub>4</sub>/NO<sub>3</sub> application ratios. Dry weights per component are listed beside the columns; total dry weights above the columns; N = needles, W = wood, c = current year's, p = previous years', R = roots.

In the soil extracts nitrate and ammonium were significantly altered by variation in the application ratio, but not pH (Table 1). A comparison of the original and final amounts of the two N species shows that the amounts of extractable NH<sub>4</sub> and especially NO<sub>3</sub> increased during the growing season, as a consequence of the N applications. At the 1:3 treatment, where 1.5 g NO<sub>3</sub>-N had been added, extractable NO<sub>3</sub>-N amounted to about 1.3 g per pot at the end of the experiment.

Balance calculations including soil and plant data showed that during the growing season about 0.4 g N per pot was mineralized.

#### Plant data

The amounts of biomass harvested seem to decrease with decreasing application ratio

Table 3. Acquisition of applied labelled N (mmol per plant) in different plant parts of Scots pine for three different  $\text{NH}_4/\text{NO}_3$  ratios of N application (Ratio).

Ratio	Labelled N source	Plant part					Whole plant	Percentage of applied labelled N
		Nc <sup>a</sup>	Np	Wc	Wp	R		
3:1	$\text{NO}_3$	2.9	0.2	0.3	0.4	0.8	5	26%
3:1	$\text{NH}_4$	15.6	1.4	1.9	2.0	4.4	25	47%
1:1	$\text{NO}_3$	5.5	0.6	0.8	0.8	2.0	10	27%
1:1	$\text{NH}_4$	9.8	0.7	1.3	1.4	2.9	16	45%
1:3	$\text{NO}_3$	6.6	0.7	0.8	0.9	2.5	11	21%
1:3	$\text{NH}_4$	6.2	0.5	0.8	0.8	1.8	10	56%

<sup>a</sup>for an explanation see Table 2

(Fig. 1), but the differences are not significant, neither is the difference in total biomass harvested per treatment.

The tree nutrient concentrations were not significantly influenced by variations in the application ratio (Table 2), except for P in the current needles and in the roots, showing an increase with an increasing proportion of nitrate in the N application.

The acquisition of labelled N was highest when supplied as  $\text{NH}_4$  (Table 3). Most of the applied N was recovered in the current needles and only little in the wood and in older needles.

The uptake ratios as calculated from the  $^{15}\text{N}$  data were always 2 to 3 times higher than the application ratio (Table 4). This qualitatively indicates that Scots pine shows ammonium preference.

### *Quantifying the acidification*

For an assessment of the acidifying effect of this ammonium preference a quantitative approach is required. In a previous article (Chapter 2) it was assumed that the N uptake from soil sources and from applied nitrogen occurred in identical  $\text{NH}_4/\text{NO}_3$  ratios throughout the growing season. The validity of this assumption is however in doubt: in the present experiment it appeared that the uptake ratio was governed largely by the application ratio, while it may be assumed that before the application of N all trees showed approximately the same, unknown, uptake ratio. Thus, two periods must be distinguished: one period with identical soil solution  $\text{NH}_4/\text{NO}_3$  ratios in all pots, and a second period, starting with the first (unlabelled) N application, in which the soil solution ratios and the uptake ratios were affected by variations in the application ratio.

Table 4.  $^{15}\text{NH}_4/^{15}\text{NO}_3$  uptake ratios in different plant parts of Scots pine, measured for three different  $\text{NH}_4/\text{NO}_3$  application ratios (Ratio).

Ratio	Plant part				
	Nc <sup>a</sup>	Np	Wc	Wp	R
3:1	5.4	6.1	6.7	5.5	5.2
1:1	1.8	1.2	1.7	1.8	1.3
1:3	0.9	0.7	1.0	0.8	0.8

<sup>a</sup>for an explanation see Table 2

Table 5. Regression of  $\text{NH}_4/\text{NO}_3$  uptake ratio (UR) of Scots pine trees on the  $\text{NH}_4/\text{NO}_3$  ratio of the soil solution (SSR):  $\text{Ln}(\text{UR}) = a + b \cdot \text{Ln}(\text{SSR})$ .

	Plant part				
	Nc <sup>a</sup>	Np	Wc	Wp	R
a	1.8	1.9	2.0	1.9	1.7
b	0.9	1.2	1.0	1.0	1.0
r <sup>2</sup>	0.96	0.90	0.91	0.97	0.92

<sup>a</sup>for an explanation see Table 2

The uptake ratio in the first period could be estimated from the data obtained in the second period as follows. There appeared to be a linear relationship between the natural logarithms of the measured uptake ratios and the average  $\text{NH}_4/\text{NO}_3$  ratio in the soil solution as measured after the applications of labelled N (Table 5). Logarithms were used to linearize the relationship and to stabilize the variances. The average  $\text{NH}_4/\text{NO}_3$  ratios in the soil solution over the three sampling dates after the N additions (Table 1) were 0.76, 0.33 and 0.12 for the 3:1, the 1:1 and the 1:3 treatments, respectively. In the first period this ratio was 0.5 (measured in unplanted soil). Using the relationships in Table 5, the uptake ratio in the first period before N addition could be interpolated for each plant part. As the slopes of the regression lines in Table 5 are near 1 and the intercepts near 2, the uptake ratio is always ca. 7 times higher than the  $\text{NH}_4/\text{NO}_3$  ratio of the soil solution (Table 5:  $\text{Ln}(\text{UR}) \approx 2 + \text{Ln}(\text{SSR})$ , so  $\text{UR} \approx e^2 \cdot \text{SSR}$ ).

Further, it was assumed that the average N uptake rates for both periods (before and after the first N application, respectively) were equal. With this assumption made, the total amount of N taken up by the plants could be partitioned in fractions taken up before and after the first addition of N. Finally, with the estimated and measured uptake ratios for both periods the amounts of ammonium and nitrate taken up could be calculated. The results of these calculations are presented in Table 6. Only the data of the current needles and wood and of the roots are presented as the older needles and wood did not show any perceptible dry mass increment. Clearly, ammonium uptake always exceeds nitrate uptake, even though nitrate is the dominating N species in the soil solution (Table 1). Averaged over the whole tree, the  $\text{NH}_4/\text{NO}_3$  uptake ratios throughout the growing season were 4.2, 2.5, and 1.5 for the three application ratios, respectively.

#### *Cation-anion uptake balance*

Knowing the uptake of  $\text{NH}_4$  and  $\text{NO}_3$  and of the other major nutrients, a cation-anion uptake balance sheet can be constructed. The weighted averages of the nutrient concentrations per plant part are used to calculate the cation-anion uptake balance of

the whole plant (Table 7). It can be seen that there is a larger uptake of positive than of negative charges in all treatments, implying an extrusion of  $H^+$  into the rhizosphere. This  $H^+$  production increases with increasing  $NH_4/NO_3$  application ratio, which, however, did not result in significant changes in the pH of the soil solution (Table 1). Thus, depending on the composition of the soil solution the trees produced 0.40 to 0.71 moles  $H^+$  per kg dry mass increment. The relationship between the uptake ratio and the  $(C_a - A_a)$  value is illustrated in Fig. 2. The amount of  $H^+$  produced is linearly related to the natural logarithm of the uptake ratio ( $r^2 = 0.95$ ,  $P = 0.0001$ ).

Table 6. Quantities of N taken up as  $NH_4$  or as  $NO_3$  ( $mmol\ N\ kg^{-1}\ DW$ ) and stored in different parts of Scots pine trees during one season, for three  $NH_4$ -N/ $NO_3$ -N application ratios (Ratio).

N source	Ratio	Plant part		
		Nc <sup>a</sup>	Wc	R
$NH_4$	3:1	970	448	283
	1:1	833	421	286
	1:3	739	342	211
$NO_3$	3:1	237	93	79
	1:1	329	155	125
	1:3	474	215	156

<sup>a</sup>for an explanation see Table 2

Table 7. Quantities of nutrients absorbed by Scots pine trees in one growing season and the resulting nutrient uptake balance sheets.  $C_a$  =  $mmol_c$  cations absorbed;  $A_a$  =  $mmol_c$  anions absorbed.

Ratio	Nutrient uptake ( $mmol\ kg^{-1}$ )									Balance ( $mmol_c\ kg^{-1}$ )		
	$NH_4$	$NO_3$	P	Na	K	Ca	Mg	S	Cl	$C_a$	$A_a$	$C_a - A_a$
3:1	641	160	39	19	148	72	36	45	27	1026	315	711
1:1	581	234	41	19	157	69	33	46	25	962	390	572
1:3	483	317	42	19	169	69	36	45	31	880	482	399

## Discussion

There is no independent way to check if nitrification was effectively inhibited, as neither soil solution nor extract  $^{15}N$  percentages were measured. If labelled ammonium is nitrified, also labelled nitrate will contribute to the plant's  $^{15}N$  content, which is assumed to have been derived from ammonium only. If unlabelled ammonium is nitrified in the presence of a labelled nitrate pool, this pool will be diluted with unlabelled nitrate, so the amount of  $^{15}N$  in the plant would represent a larger amount of absorbed nitrate than the calculated amount. Thus, in both situations unaccounted nitrification would cause an over-estimation of the amount of ammonium taken up.

However, the soil solution data (Table 1) do not indicate that nitrification occurred. Nitrification under circumstances of preferred  $NH_4$  uptake would probably have resulted in  $NO_3$  accumulation and, hence, increasing  $NO_3$  concentrations. Moreover, the ammonium concentrations in the extracts reflected the amounts in which it was

applied. Nitrification would have reduced or even reversed these differences between treatments.

Nitrification was suppressed, but ammonification could proceed constantly, thus causing dilution of the labelled ammonium pool. As a consequence, the amount of ammonium taken up (and thus the uptake ratio) may have been underestimated.

The assumption of a linear N uptake rate probably obscured any differences in uptake ratios and ( $C_a - A_a$ ) values between the three treatments. It is likely that the trees' growth showed an exponential trend so that a disproportionately large part of the biomass increment occurred in the second half of the season, where differences in uptake ratios existed. Accounting for exponential growth would have created greater differences between uptake ratios and ( $C_a - A_a$ ) values over the whole growing season than shown in Table 7. However, the regression line of Fig. 2 would not necessarily have had a significantly different slope and intercept, it would have merely covered a broader range.

By extrapolating the fitted line of Fig. 2 to the x-axis it can be estimated at which uptake ratio the root system's  $H^+$  production would be zero. In that case the contribution of nitrate to total N nutrition would be 70% (Fig. 2,  $\text{Ln}(\text{uptake ratio}) = -0.84$ ). Normally, extrapolating a fitted curve far beyond the range of experimentation is not recommendable. Closer to and beyond the origin, for instance, the linear relationship may disappear as uptake of other anions and cations may be influenced by the proportion of nitrate-N taken up (Mengel and Kirkby, 1987). Here, extrapolating to the left seems justified, as the estimated value of 70% corresponds well with results reported by Gijsman (1990a). He found a neutral uptake pattern for Douglas fir if the contribution of nitrate to the total N uptake was 65%. However, the relationship between the soil solution  $NH_4/NO_3$  ratio and the uptake ratio may differ among tree species.

Another way of estimating the  $NO_3$  percentage with zero  $H^+$  efflux is to calculate the  $(C-A)/N_{org}$  ratio according to Troelstra (1983). Here C-A refers to the difference in accumulated equivalence amounts of cations and inorganic anions (i.e.  $C_a - A_a$  as mentioned here, excluding absorbed N and S incorporated in organic substances). The proton efflux can be written as (Troelstra, 1983, with an  $S_{org}/N_{org}$  equivalence ratio of 0.057):

$$H^+ \text{ efflux} = (C-A) + 0.943 * N_{org} - 2 * (N_{org} \text{ from } NO_3).$$

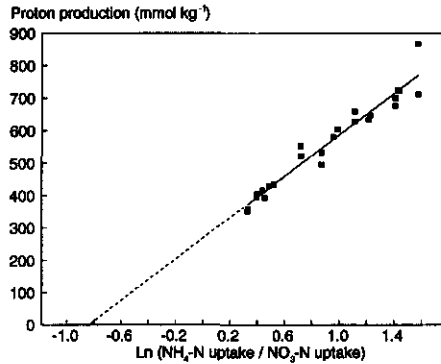


Fig. 2.  $H^+$  production of 3-y-old Scots pine trees for one season's growth on a sandy forest soil in pots with a range of  $NH_4/NO_3$  uptake ratios.

Thus, per unit  $N_{org}$  assimilated, originating from  $(1-x)$   $NH_4$  and  $x$   $NO_3$  the  $H^+$  efflux equals  $(C-A)/N + 0.943 - 2x$ . If the  $H^+$  efflux of each plant is calculated this way and fitted on  $x$ , also a linear relationship is obtained ( $r^2 = 0.94$ ). Here, extrapolation shows a zero  $H^+$  efflux if  $x = 0.66$  (i.e. 66% of N nutrition derived from  $NO_3$ ).

The throughfall deposition in the field has an  $NH_4/NO_3$  ratio of 3:1 (Chapter 2). However, this would not justify a conclusion that the calculated acidification of the 3:1 treatment may serve as an estimate of possible acidification caused by tree uptake in the field. In the field a large part of the immitted ammonium may be adsorbed onto exchange sites in the litter layer or be subjected to nitrification (Stams et al., 1990, 1991). The litter leachate will therefore usually have an  $NH_4/NO_3$  ratio lower than that in the throughfall. Moreover, ammonium is retained in the organic matter of the topsoil. Another difference between the pot experiment and the field is that in the former only the top 30 cm of the profile was used and that the soil was mixed. This probably caused a higher ammonium availability than under field circumstances. Also a higher rooting intensity in the pots as compared to the field will have enhanced ammonium availability, as ammonium is relatively immobile. To estimate acidification occurring in the field the relationship shown in Fig. 2, together with the correlation between the uptake ratio and the  $NH_4/NO_3$  ratio of the soil solution (Table 5) can be used. In the field at 30-cm depth an ammonium concentration of about  $40 \text{ mmol m}^{-3}$  is measured in the soil solution, while nitrate averages  $400 \text{ mmol m}^{-3}$  (data not shown). Usually the  $NH_4$  concentration decreases quickly with depth, whereas the  $NO_3$  concentration is more constant (e.g. Tiktak et al., 1988), causing the  $NH_4/NO_3$  ratio to decrease with depth. If the soil solution  $NH_4/NO_3$  ratio at 30 cm is taken (i.e. 0.1) it follows that an uptake ratio of 0.6 may be expected (Table 5). As Fig. 2 indicates, this would mean that the trees exhibit a slightly acidifying uptake pattern at that depth. Thus, above 30-cm depth rhizosphere acidification and well below 30-cm depth rhizosphere alkalization might occur. Although Scots pine roots generally do reach a far greater depth than 30 cm (Lehnardt and Brechtel, 1980), the highest rooting density usually occurs in the topsoil. De Visser (1990) found that the top 30 cm of a sandy forest soil planted with Scots pine contained 71% and 73% of total root length and root biomass, respectively, in 90 cm mineral soil. Furthermore, the litter layer may contain a considerable amount of fine roots (Persson, 1988), obviously exposed to high  $NH_4$  concentrations. A high  $NH_4$  concentration in the soil solution may even entirely suppress  $NO_3$  uptake, irrespective of the  $NO_3$  concentration (Marschner et al., 1991). This may occur in the litter layer or under circumstances favouring mineralization. It can therefore be concluded that besides the uptake pattern, the rooting pattern is an additional factor to be considered when soil acidification is assessed. Further research should reveal which portions of the active root system are exposed to which  $NH_4$  and  $NO_3$  concentrations.

The  $NH_4/NO_3$  uptake ratio depends not only on the soil solution's  $NH_4/NO_3$  ratio. It has been found to be temperature-dependent as well (Clarkson and Warner, 1979; Marschner et al., 1991). A dependence on pH has also been clearly established (e.g.

Evers, 1963). Indirectly it may also depend on soil moisture content. If the soil solution evaporates, the concentrations of  $\text{NH}_4$  and  $\text{NO}_3$  increase. The  $\text{NH}_4$  concentration may thereby reach a level at which  $\text{NO}_3$  uptake is inhibited (Marschner et al., 1991), even though the  $\text{NH}_4$ -concentration is buffered. However, under dry circumstances  $\text{NH}_4$  transport is retarded by adsorption of  $\text{NH}_4$  onto the solid phase (Gijsman, 1991), and the  $\text{NH}_4/\text{NO}_3$  ratio in the rhizosphere soil solution will drop by depletion of  $\text{NH}_4$ . Gijsman (1991) stated that under relatively dry soil conditions the plant could not express its ammonium preference. It may be better to state that ammonium preference still occurs (i.e. a higher uptake ratio than the rhizosphere soil solution  $\text{NH}_4/\text{NO}_3$  ratio), but that it not necessarily results in a higher ammonium than nitrate uptake.

The term "ammonium preference" may not be correct in a mechanistic sense. Plants might not actually prefer ammonium as a major N source, but may rather suppress predominant nitrate nutrition in cases where nitrate is more readily available than ammonium. Mengel and Kirkby (1987) state that supplying ammonium may be energy-conserving, as the required reduction of nitrate to  $\text{NH}_3$  takes energy. However, if ammonium is available in larger quantities than nitrate, a self-protecting mechanism against ammonium surplus may be initiated, as a preponderant ammonium uptake may give rise to depletion of the plant's carboxylate pool, to internal acidification or to toxic internal levels of  $\text{NH}_4^+$  (Gijsman, 1990b; Hageman, 1984).

It has been suggested that nitrate inhibits the translocation of P from roots to shoots (Van den Driessche and Dangerfield, 1975), whereas ammonium has been found to stimulate P uptake in a pot study with Douglas fir (Bledsoe and Zasoski, 1983) and in a short-term uptake study with Scots pine (Boxman and Roelofs, 1988). These observations are not confirmed by the data presented here. No correlations were found between P uptake and  $\text{NH}_4$  or  $\text{NO}_3$  uptake ( $\text{mmol kg}^{-1}$ ) in any tree part or for the tree as a whole.

## Conclusions

Since the pH values of most agricultural soils in the Netherlands are high enough to allow nitrification to take place,  $\text{NH}_x$  immission into these soils is bound to result in soil acidification. The situation is different in forest soils. The low pH values of these soils usually reduce autotrophic nitrification, causing lower nitrification rates than in agricultural soils (e.g. Vonk et al., 1988). The results of the present research indicate that as a consequence of preferred  $\text{NH}_4$  uptake the nutrient uptake pattern of Scots pine induces  $\text{H}^+$  extrusion. Therefore, unlike in agricultural soils, in forest soils the excess cation-over-anion uptake may be a major source of acidification.

This acidification will partly be reversed by mineralization. If the needle and litter layer mass are at a steady state, no net accumulation of living and shed needles occurs, and the acidification produced by needle growth is completely neutralized by

mineralization. In that case, forest growth, accompanied by timber harvesting, produces minimum acidification, to be estimated by the  $H^+$  production of the annual wood increment. In the previously mentioned field experiment with Scots pine (Van Diest, 1989), an annual wood increment of about  $15 \text{ m}^3 \text{ ha}^{-1}$  is found. This equals ca.  $7000 \text{ kg wood DM ha}^{-1} \text{ yr}^{-1}$ . The acidification produced by this wood increment would be 4.2, 3.5 and  $2.5 \text{ kmol H}^+ \text{ ha}^{-1} \text{ yr}^{-1}$  for the three  $\text{NH}_4/\text{NO}_3$  application ratios, respectively, as calculated from the  $C_a - A_a$  value found for wood in the pot experiment (not separately mentioned).

When besides the  $7000 \text{ kg wood increment ha}^{-1}$  an annual growth of  $2000 \text{ kg needle DM ha}^{-1}$  would occur without mineralization, the acidity generated would be 6.2, 5.0 and  $3.6 \text{ kmol H}^+ \text{ ha}^{-1} \text{ yr}^{-1}$  for the three application ratios, respectively. These amounts may serve as estimates of maximum possible acidification, assuming that no significant accumulation of root litter occurs. They may be reached e.g. in young plantations, when there is relatively little return of above-ground biomass to the soil.

Even if mineralization does occur, however, it not necessarily alleviates soil acidification, as it mainly takes place in the litter layer, while proton production occurs in deeper soil horizons ("spatial de-coupling of ammonification and nitrogen uptake", Ulrich, 1986).

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**Response of a Scots pine (*Pinus sylvestris*) stand to application of phosphorus, potassium, magnesium and lime. I. Soil data**

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**Keywords:** fertilizer recommendations, forest fertilization, liming, nitrogen, *Pinus sylvestris*

## Abstract

In 1985-1988, a 25-y-old Scots pine forest was limed and fertilized with P, K and Mg in a 2<sup>4</sup> factorial design and in an experiment with 5 liming levels, in order to alleviate potential nutrient deficiencies as a result of high N depositions (ca. 40 kg ha<sup>-1</sup> y<sup>-1</sup>). In this chapter, the first of three, the effects of fertilization and liming on nutrients in the forest floor and mineral soil are discussed to serve as a basis for the explanation of foliar nutrient concentrations and tree performance. Of all treatments, liming showed the widest array of effects, especially in the forest floor. It raised the pH in the forest floor and after 4 y that in 0-50 cm mineral soil, and it lowered extractable Al in the forest floor and in 0-50 cm mineral soil in 1989. These effects, except for that on forest floor pH, were stronger with increasing lime dosages. Liming showed transient effects on the amounts of soil inorganic N (N<sub>i</sub>). Until 1989, N<sub>i</sub> in the forest floor was lowered and that in the mineral soil was raised by liming. This could be attributed to the formation of NO<sub>3</sub>, which is more mobile than NH<sub>4</sub>. Plots limed with 3 Mg (tonnes) lime ha<sup>-1</sup> appeared to have higher N<sub>i</sub> concentrations in the whole sampled profile than those limed with > 3 Mg ha<sup>-1</sup> or the unlimed plots, the reason of which is unclear. Liming removed extractable K and Mg from the forest floor probably by exchange against Ca. The residence time of added P and possibly Mg in the forest floor was increased by liming, probably by a reduced solubility of the added fertilizers. Added K was poorly retained in the forest floor and probably quickly leached to soil layers deeper than 50 cm.

## Introduction

Forest fertilization was first applied and scientifically examined in the 19th century (Baule and Fricker, 1967), as was liming (Trümper, 1936). Both were common practice in the Netherlands, especially in reforestation programmes of heathlands around the beginning of this century. Around 1950, foresters lost interest in it, until the 1980s when it was suggested as a tool to revitalise declined forests (Van den Burg, 1989, 1991). However, in each specific case of forest decline it should be carefully evaluated whether forest fertilization is appropriate. In Germany, a number of "damage types" could be distinguished, which demonstrated that forest decline can not always be attributed to one distinct cause, but that locally different complexes of causes were involved. It was shown that in some areas decline corresponded with a nutrient deficiency, although the mechanisms inducing the deficiency were not always clear (Blank et al., 1988). Although the causes of forest decline are very diverse and specu-

lative (Foster, 1989), there are cases where nutrient deficiencies are definitely involved and amelioration is to be expected after application of those nutrients (e.g. Hüttl, 1990; Zöttl et al., 1989). However, even when a nutrient deficiency is involved, it should be examined whether or not the trees are able to utilize an increased supply of nutrients. When also drought and/or root damage are involved the effect of fertilization may be nullified.

In areas with intensive livestock breeding, large quantities of  $\text{NH}_x$  ( $\text{NH}_4^+$  and  $\text{NH}_3$ ) are emitted. This occurs in several areas in the Netherlands, but also e.g. in Germany (Kaupenjohann et al., 1989; Uebel, 1991). Forest canopies are exposed to dry deposition of  $\text{NH}_x$ , causing higher ammonium concentrations in throughfall than in bulk precipitation (e.g. McLeod et al., 1990; Velthorst and Van Breemen, 1989). Thus, on forest soils in the Netherlands an average of 40 kg N ( $\text{NH}_x$  and  $\text{NO}_x$ )  $\text{ha}^{-1} \text{year}^{-1}$  is deposited in throughfall precipitation, but quantities locally exceeding 160 kg N  $\text{ha}^{-1} \text{year}^{-1}$  have been reported (Ivens et al., 1988). Bredemeier (1988) reported ammonium-enriched throughfall in an area in Germany with intensive livestock farming.

As forests in the Netherlands are mainly planted on sandy soils low in mineral nutrients (Van den Burg, 1991) N deposition may result in an overabundant N availability causing induced deficiencies of other nutrients. This effect may be aggravated when on the exchange complex  $\text{NH}_4^+$  replaces other cations, that are subsequently leached with  $\text{SO}_4^{2-}$  or  $\text{NO}_3^-$  as counterions. Weathering of silicates, even when stimulated by a lower pH, proceeds too slowly to replenish such losses of cations (Van Grinsven et al., 1989). An initial effect of excessive N may be an induced growth push, possibly causing internal nutrient dilution, and thus aggravating the relative deficiency of other nutrients. Several workers have reported an increased tree growth during the past two decades, possibly due to an increased atmospheric N inputs, (Rehfuess et al.; 1991, Sauter, 1991). Research in De Peel, the Netherlands, showed forest decline as a consequence of excessive N deposition (Van den Burg et al., 1987).

In the present research a mature Scots pine plantation was amended with P, K, Mg and lime, in order to alleviate potential nutrient deficiencies. Scots pine is the major forest tree species in the Netherlands and in large parts of Scandinavia, Germany, Poland and Russia. Especially in Sweden many fertilization experiments were carried out with Scots pine (e.g. Tamm and Hallbäcken, 1988). Recently, forest fertilization recommendations were presented in the Netherlands (Anon., 1990). The present research partly evaluates these guidelines.

## Materials and methods

### *Pine forest fertilization experiment*

The experimental area is situated near Harderwijk, the Netherlands (52°20'N; 5°35'E).

The soil is a coarse sandy fluvioglacial deposit covered by a fine drift sand layer of varying depth (10–40 cm) with a weak podzolic soil formation and a forest floor that is distinct from the underlying mineral soil. The water table is at ca. 9 m. The site was planted with Scots pine (*Pinus sylvestris* L.) in 1960.

In 1985 the stand was thinned (Chapter 6) after which the research started. Initial soil data are presented in Table 1. P, K, Mg and lime were applied in a 2<sup>4</sup> factorial complete randomized block design (CRBD, 3 replicates). These treatments are denoted as P, K, Mg and Ca, respectively.

In a separate experiment the effect of different lime rates was investigated. Five amounts of lime (0, 3, 6, 9 and 18 Mg ha<sup>-1</sup>, denoted as 0tL, 3tL, etc.) were applied, also as a CRBD with 3 replicates, accompanied by applications of P, K and Mg as in the factorial experiment. The PKMg treatment of the factorial experiment served as the 0tL treatment. The PKMg additions in the liming experiment served to bring out the effects of liming, since other nutrients would not be limiting. The applied fertilizers, rates and application dates of the factorial experiment and the 3tL level of the liming experiment are presented in Table 2. In autumn 1985, all lime treatments received 3 Mg lime ha<sup>-1</sup>. Three, 6 and 9 Mg ha<sup>-1</sup> were applied to the 6tL, 9tL and 18tL treatments, respectively, in autumn 1986. Finally, 6 Mg ha<sup>-1</sup> was applied to the 18tL treatment in spring 1987. The 1986 lime applications were carried out after soil sampling, so in 1986 the liming experiment actually had only one lime level (3tL).

The individual plot size was 22 × 25 m. Within these plots, 5-m-wide boundary zones were observed from which no soil and litter samples were taken.

### Sampling and analyses

From 1986 to 1989, in autumn forest floor and mineral soil samples were collected. The forest floor was sampled by quickly forcing a 5-cm bulk density core into the soil and removing the mineral soil collected in the core. At the same point the 0–25-cm and 25–50-cm mineral soil layers were sampled with a gouge. Per plot 20 samples were collected following a grid, and pooled. Until 1989 no treatment had perceptibly influenced the forest floor mass (data not shown).

The forest floor samples were dried at 70°C in a forced-draft oven and ground; soil samples were air-dried and sieved through a 2-mm sieve. To obtain a measure of the

Table 1. Initial element concentrations in the forest floor, and in the 0–25 and 25–50 cm mineral soil segments.

Element	Soil layer		
	Forest floor (g kg <sup>-1</sup> )	0–25 cm (mg kg <sup>-1</sup> )	25–50 cm (mg kg <sup>-1</sup> )
EON <sup>a</sup>	-	4.6	2.3
NO <sub>3</sub>	-	0.0	0.0
NH <sub>4</sub>	-	4.0	1.1
K	-	5.8	2.1
Mg	-	1.7	0.6
pH	-	4.24	4.56
N-total	15.7	454	263
P-total	0.62	102	142
K-total	0.80	632	728
Ca-total	1.20	69	74
Mg-total	0.33	29	38
Zn-total	-	8	10

<sup>a</sup>Extractable organic nitrogen.

Table 2. Amounts and compositions of fertilizers and lime applied in the factorial experiment and at the 3tL level of the liming experiment.

Nutrient	Amount (kg ha <sup>-1</sup> )	Fertilizer	Date of first application
P	25	Rock phosphate	autumn 1985
	25	Triple superphosphate	autumn 1985
K	100 <sup>a</sup>	Potassium sulphate	spring 1986
Mg	100 <sup>a</sup>	Kieserite	spring 1986
Ca	1200	Calcium carbonate <sup>b</sup> (powder, 3000 kg)	autumn 1985

<sup>a</sup>K and Mg applications were split, with additional applications made in the springs of 1987 and 1988, to a total of 100 kg ha<sup>-1</sup> each.

<sup>b</sup>Contained 3.6% MgCO<sub>3</sub> (1% Mg).

total contents of all major nutrients were assessed. Portions of 0.400-g (litter: 0.300-g) were digested in an H<sub>2</sub>SO<sub>4</sub>, salicylic acid, H<sub>2</sub>O<sub>2</sub> medium, catalyzed by selenium. This digestion was devised for plant samples; the soil particles were not completely dissolved by the reagents, so for soil the term 'total analysis' cannot be applied. However, probably all N and large fractions of P were digested, because these elements are only or mainly found in organic matter.

In the extracts NH<sub>4</sub>, NO<sub>3</sub>, P, K, Mg and total extracted N were measured in an automated continuous-flow system: the N species colorimetrically, i.e. NH<sub>4</sub> by indophenol blue colouring; total dissolved N after digesting the extract with K<sub>2</sub>S<sub>2</sub>O<sub>8</sub>, and oxidizing all native and produced NH<sub>4</sub> to NO<sub>3</sub>; NO<sub>3</sub> after reduction to nitrite, followed by conversion to a red-coloured diazo compound; P colorimetrically after formation of a blue phosphomolybdenum complex. Extractable organic N was calculated as total extracted N - (NO<sub>3</sub>+NH<sub>4</sub>). The pH(CaCl<sub>2</sub>) was measured with a glass electrode. P in the soil extracts was below the detection limit. In the digests N (as NH<sub>4</sub>) and P were measured as mentioned. In the extracts and the digests K and Ca were measured by atomic emission and Mg by atomic absorption spectrometry. In the 1989 extracts Al was measured colorimetrically.

### Data analysis

For statistical tests SAS software (SAS Institute Inc., 1989) was used. The significance levels were differently chosen for the two experiments. In a 2<sup>4</sup> factorial design a maximum of 15 effects are tested simultaneously. Thus, the probability of obtaining at least one *P* value <  $\alpha$  due to coincidence is conservatively estimated as 1-(1- $\alpha$ )<sup>15</sup>. At  $\alpha$  = 0.01 this probability ( $\leq 0.14$ ) was considered acceptable. At more liberal *P* values too many effects would be mistaken as significant.

In the liming experiment effects with *P* < 5% were considered significant. For the 1986 data all liming levels were equal. For the data acquired in 1987-1989 curve-fitting

available fractions of nutrients 3.00 g dry material was extracted with 30 mL 0.01 M CaCl<sub>2</sub> solution (Houba et al., 1990). This extraction procedure is of common use in agriculture in the Netherlands, but less common in forestry. In forestry in the Netherlands it is common to assess P<sub>total</sub> (Anon., 1990). However, to obtain additional information on the fate of each applied compound the

was carried out, as is appropriate for describing responses to quantitative factors (Mize and Schultz, 1985). Polynomial and exponential models were tested. Polynomial models with significant linear and/or quadratic and insignificant lack-of-fit contrasts were selected. Exponential curve-fitting was carried out with Genstat's Fitcurve directive (Rothamsted Experimental Station, 1990). Exponential models were favoured above polynomial models if the former had higher  $R^2$  values. Thus, polynomial models with cubic contrasts were always rejected. For exponential models only the rate parameter,  $r$ , is reported. Here,  $r$  expresses the measure to which the effectiveness of further increasing lime applications declines. Note, however, the large interval between 9 and 18 Mg lime  $\text{ha}^{-1}$ , that adds uncertainty to the correct shape of fitted curves.

Three- or 4-year averages were tested by repeated measures analysis of variance (RMAOV) (Moser et al., 1990). Significant treatment effects according to RMAOV are only listed if the effects in separate years were unidirectional.

## Results and discussion

The tables 3-11 present relevant soil analysis results of the factorial and the liming experiments. When significant main effects occurred, the data of the factorial experiment are grouped accordingly. Thus, for instance, "-P" denotes the average of

Table 3. Concentrations of  $\text{CaCl}_2$ -extractable elements in the forest floor of the factorial experiment ( $\text{mg kg}^{-1}$  DM).

Treat-ment	Year			
	1986	1987	1988	1989
<b>NH<sub>4</sub></b>				
Control	237	189	174	193
-Ca	205	190	202	184 ooo
+Ca	141 ***	124 ***	107 ***	114 ***
<b>NO<sub>3</sub></b>				
Control	4	6	29	81
-Ca	4	8	42	36 ooo
+Ca	34 ***	27 ***	51	103 ***
<b>P</b>				
Control	-	129	78	75
-P	-	102	98	87 ooo
+P	-	141 ***	117 **	107 ***
-Ca	-	133	107	89
+Ca	-	110 *	108	105 **
<b>K</b>				
Control	-	340	209	303
-K	-	253	166	317 ooo
+K	-	301 *	213 ***	362
-Ca	-	316	206	364 ooo
+Ca	-	238 ***	173 *	315 *
<b>Mg</b>				
Control	-	177	247	238
-P	-	279	418	368 oo
+P	-	264	381	340
-Ca	-	293	434	390 ooo
+Ca	-	250 **	365 ***	318 ***
-Mg	-	170	206	231 ooo
+Mg	-	373 ***	593 ***	477 ***
<b>pH</b>				
Control	4.00	3.26	3.10	3.17
-Ca	4.12	3.47	3.19	3.29ooo
+Ca	6.05***	5.60***	5.27***	5.14***
<b>Al</b>				
Control	-	-	-	20
-Ca	-	-	-	17
+Ca	-	-	-	5 ***

\*Symbols following a pair of figures: \*\*\*, ooo =  $P < 0.0001$ , \*\*, oo =  $P < 0.001$ , \*, o =  $P < 0.01$ , for ANOVA (\*) and Repeated Measures Analysis of Variance (o), respectively.

the 24 plots that did not receive P. Important interactions between treatments are mentioned in the text. For all applied nutrients it should be noted that possible increases in the forest floor or mineral soil may have been caused directly, or indirectly through increased returns in root and leaf litter.

#### Factorial experiment

*P- and lime effects.* Of the four elements applied, Ca induced the widest array of effects, especially in the forest floor (Tables 3, 4). In 1986 liming had increased the forest floor pH by ca. 2 units. In all treatments the pH of the forest floor declined in the period 1986-1988 (Table 3). The pH decline of the unlimed forest floor may indicate that soil acidification by nitrification of deposited  $\text{NH}_x$  proceeds in spite of a low pH. More likely, the 1985 thinning may have enhanced nitrification by disturbing the ecosystem (Vitousek et al., 1979). Later disturbances (windfelling and subsequent thinning in 1990) were followed by increased  $\text{NO}_3$  concentrations in the soil solution (Chapter 5). In autumn 1991 the forest floor pH in the Ca plots was 4.15 and that in the control plots had remained stable at 3.15 (Chapter 7). Liming started to raise the pH of

Table 4. Concentrations of  $\text{CaCl}_2$ -extractable elements in 0-25 and 25-50 cm mineral soil of the factorial experiment ( $\text{mg kg}^{-1}$  air-dry soil).

Treatment	Year			
	1986	1987	1988	1989
<b>0-25 cm</b>				
	$\text{NO}_3$			
Control	1.1	0.5	1.2	1.9
-Ca	1.0	0.4	1.0	1.3 000*
+Ca	2.3 ***	0.7 ***	1.9 ***	1.6
	$\text{NH}_4$			
Average	3.7	1.6	1.7	1.4
	K			
+P	6.6	4.6	3.2	4.6
-K	6.9	5.0	3.8	5.0 000
+K	10.4 *	8.8 *	6.2 ***	8.8 ***
	Mg			
Control	2.6	1.1	1.8	1.5
-Ca	3.7	2.6	4.1	3.0 00
+Ca	4.1	3.0	5.3 *	3.6
-Mg	2.6	1.7	2.4	1.9 000
+Mg	5.3 ***	3.8 ***	7.0 ***	4.7 ***
	pH			
Control	4.15	4.34	4.24	4.27
-Ca	4.12	4.28	4.20	4.21
+Ca	4.12	4.29	4.23	4.27 **
	Al			
Control	-	-	-	28.8
-Ca	-	-	-	33.0
+Ca	-	-	-	26.0 *

(continued next page)

both mineral soil layers in 1988. In 1989 the increases were small, but significant (Table 4). Also in 1991 a small, but significant difference in pH (0-22 cm mineral soil) was found between the control- and Ca plots (Chapter 7). It may be assumed that a pH gradient had developed in the 0-25 cm layer, with considerable pH increases in the top layer. Marschner and Wilczynski (1991) found a pH increase from 3.3 to 4.1 in the top 10 cm mineral soil 3 y after liming an Arenosol under Scots pine. It is frequently observed that surface application of lime does not ameliorate subsoil acidity (e.g. Kreutzer et al., 1991; McCray and Sumner, 1990), but the present sandy soil with a low buffer capacity and high permeability evidently permitted discernable pH

changes below a 25-cm depth to take place after liming. It can be questioned, however, whether pH changes of this magnitude are of any biological significance. Moreover, in this pH range Scots pine would probably not respond to a small pH increase. The pH optimum for Scots pine growth has been examined by a number of authors, reviewed by Van den Burg (1981). The reported optima vary between pH(H<sub>2</sub>O) 3.5 and 6.5, and the tolerated pH values between 3 and 7.8, showing that Scots pine is highly tolerant of low pH values.

The annual variations in N concentrations in the forest floor and mineral soil were considerable, especially for NO<sub>3</sub> (Table 3). The reason for this may be the variable circumstances, especially temperature and soil moisture content, before and during the samplings, influencing mineralization and immobilization rates. From the start of the experiment liming altered the N transformations and N distribution of the soil profile. NO<sub>3</sub> was significantly increased due to liming in both soil strata in 1986-1988 (Table 4) and in the forest floor in each year, except 1988

(Table 3). NH<sub>4</sub> was lowered by liming in the forest floor (1986-1989) and unaffected by any treatment in the mineral soil. Extractable organic N was remarkably inert to fertilization and liming in the whole profile: only in 1988, it showed a weakly significant increase in the forest floor due to liming (data not shown). It amounted to 193, 3 and 1 mg kg<sup>-1</sup> in the forest floor and both mineral soil strata, respectively. The amount of inorganic N, N<sub>i</sub> (NO<sub>3</sub> + NH<sub>4</sub>), on an area basis (g N m<sup>-2</sup>) was calculated for each soil compartment, using soil and litter dry matter data. The forest floor mass of the experimental area is about 70 Mg DM ha<sup>-1</sup> and the mineral soil bulk density is ca. 1500 kg m<sup>-3</sup> (Chapter 7). In 1986-1988 the N<sub>i</sub> pool of the forest floor was significantly lowered by liming, while in the mineral soil the reverse was true (Table 5). This can be attributed to the greater mobility of NO<sub>3</sub> compared to NH<sub>4</sub>. Only in 1986 liming increased the N<sub>i</sub> content of the whole soil profile. In 1987 and 1988 the total

Table 4 (continued)

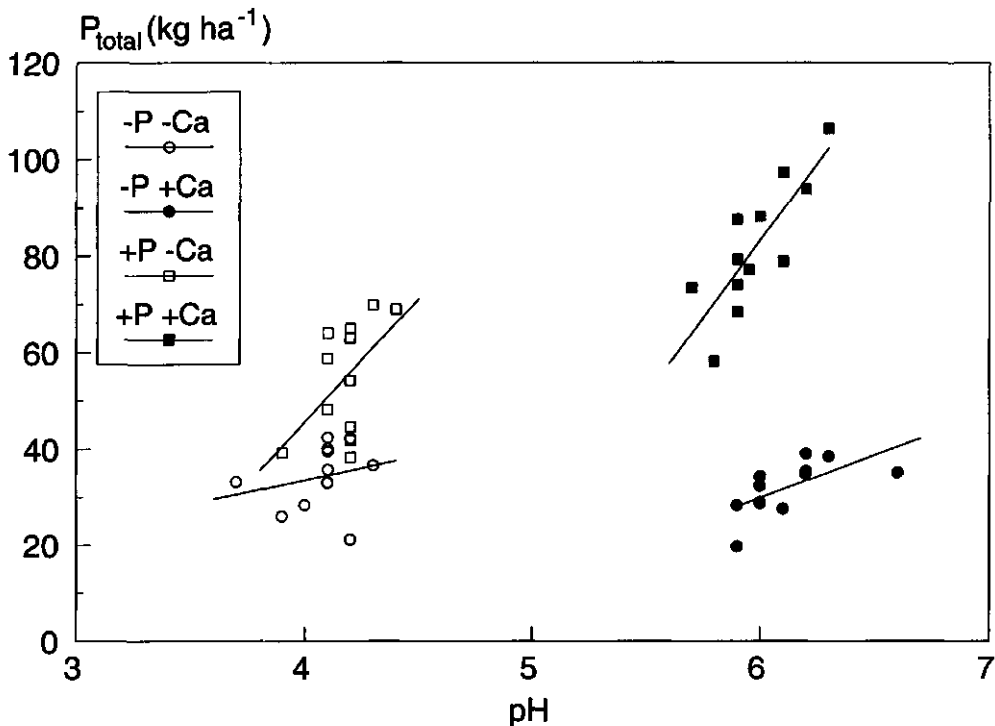
	Year			
	1986	1987	1988	1989
<b>25-50 cm NO<sub>3</sub></b>				
Control	1.1	0.3	0.8	1.9
-Ca	1.1	0.3	0.9	1.6 ooo
+Ca	2.4 ***	0.7 **	1.9 ***	2.0
<b>NH<sub>4</sub></b>				
Average	1.1	0.8	1.1	0.5
<b>K</b>				
Control	3.7	3.6	1.4	1.8
-K	3.4	2.9	1.4	2.5
+K	3.2	3.7	3.1 *	4.9 ***
<b>Mg</b>				
Control	1.2	-	0.9	1.2
-Mg	2.7	-	1.0	1.2
+Mg	2.8	-	3.6 ***	2.4 ***
<b>pH</b>				
Control	4.70	4.62	4.57	4.51
-Ca	4.64	4.57	4.48	4.46
+Ca	4.63	4.56	4.50	4.51 *
<b>Al</b>				
Control	-	-	-	15.4
-Ca	-	-	-	17.9
+Ca	-	-	-	14.4 ***

\*For an explanation of symbols see Table 3.



Table 5. Amounts of  $\text{CaCl}_2$ -extractable inorganic N in the forest floor and mineral soil of the factorial experiment.

Year	Treatment	Inorganic N ( $\text{g m}^{-2}$ ) per soil layer				
		Forest floor	0-25 cm	25-50 cm	0-50 cm	Total
1986	-Ca	1.43	1.68	0.77	2.45	3.88
	+Ca	1.20 ***	2.28 ***	1.32 ***	3.60 ***	4.80 ***
1987	-Ca	1.36	0.72	0.41	1.12	2.48
	+Ca	1.03 ***	0.87 *	0.55 *	1.42 **	2.45
1988	-Ca	1.68	1.04	0.77	1.80	3.48
	+Ca	1.09 ***	1.33 ***	1.09 ***	2.42 ***	3.51
1989	-Ca	1.51	1.05	0.84	1.89	3.40
	+Ca	1.49	1.05	0.91	1.97	3.45

\*\*\* =  $P < 0.0001$ , \*\* =  $P < 0.001$ , \* =  $P < 0.01$ .Fig. 1. The effect of P application and liming on  $P_{\text{total}}$  in the forest floor in 1986 in relation with forest floor pH.

amount of  $N_i$  in the profile was not affected by liming. In 1989 all effects of liming on  $N_i$  had vanished.  $N_i$  was not affected by the other treatments. It should be noted that the  $N_i$  data presented here regard *states*. In a separate experiment N mineralization *rates* were determined (Chapter 7).

Extractable K and Mg in the forest floor were reduced by liming (Table 3). The decrease in extractable Mg by liming occurred in spite of an increased  $Mg_{\text{total}}$

Table 6. "Total" concentrations of elements in the forest floor of the factorial experiment ( $\text{g kg}^{-1}$  DM).

Treatment	Year		
	1986	1987	1988
$N_{\text{total}}$			
Control	13.4	17.1	15.6
-Ca	12.3	16.7	15.9 ooo
+Ca	11.7	15.8 ***	14.3 ***
$P_{\text{total}}$			
Control	0.53	0.74	0.59
-P	0.47	0.66	0.59 ooo <sup>a</sup>
+P	0.98 ***	1.07 ***	0.90 ***
-Ca	0.64	0.75	0.65 ooo
+Ca	0.81 ***	0.98 ***	0.84 ***
$K_{\text{total}}$			
Average	0.77	0.79	0.65
$Ca_{\text{total}}$			
Average	1.15	2.31	1.09
-Ca	1.48	2.23	1.42 ooo
+Ca	20.42 ***	21.88 ***	21.99 ***
$Mg_{\text{total}}$			
Control	0.35	0.31	0.31
-Ca	0.46	0.57	0.60 ooo
+Ca	0.77 ***	0.88 ***	0.86 ***
-Mg	0.47	0.43	0.42 ooo
+Mg	0.75 ***	1.02 ***	1.03 ***

<sup>a</sup>For an explanation of symbols see Table 3.

was applied, while for extractable Mg the reverse was true, indicating that kieserite dissolved at a slower rate when applied after liming. This interaction, however, did not influence Mg concentrations in the mineral soil.

The average values for  $P_{\text{total}}$  in the forest floor (Table 6) are somewhat misleading.  $P_{\text{total}}$  in the forest floor was higher only when lime and P were applied together ( $P \times Ca$  interaction in 1986-1988,  $P < 0.0001$ ). Without P application, liming had no influence on  $P_{\text{total}}$ . In 1987 extractable P in the forest floor was significantly reduced by liming, but in 1989 this effect was reversed (Table 3). The latter liming effects did not interact with P application, which may indicate that the effect of liming on extractable P mainly involved native P. It can be speculated that lime first fixed native P by precipitation and that in 1989 the decreased pH had caused this fixed P to dissolve again. If so, the effects of liming on extractable P apparently were too small to affect  $P_{\text{total}}$  (see below).

concentration in the forest floor after liming (Table 6). Most likely adsorbed K and Mg were exchanged against Ca and subsequently leached, while Mg contained in the lime was not retained in the overabundant presence of Ca. Ca is known to quickly replace other cations on the exchange complex (Mochoge and Beese, 1986). Nitrate, released by nitrification in the forest floor after liming, may have promoted leaching of cations by serving as an accompanying anion. Liming, therefore, may have enhanced K- and Mg leaching from the forest floor. However, it did not markedly influence extractable K- and Mg concentrations in the mineral soil. Extractable Mg (0-25 cm) did tend to be increased by liming, but only in 1988 this effect was significant (Table 4). RMAOV indicates a significant liming effect on extractable Mg for the whole measurement period.  $Ca \times Mg$  interactions for  $Mg_{\text{total}}$  and extractable Mg in the forest floor (consistent throughout the years, but significant with RMAOV only) show that the Mg-effected increase in  $Mg_{\text{total}}$  was higher when both Mg and lime were applied than when only Mg

The implications of liming effects on  $P_{\text{total}}$  are illustrated by calculating the 1986 amounts of  $P_{\text{total}}$  in the forest floor per P and Ca combination and plotting these amounts against pH (Fig. 1). This shows that the amount of  $P_{\text{total}}$  was highest at the +P+Ca plots. The +P+Ca treatments had average additional amounts of 47, 45 and 35 kg P ha<sup>-1</sup> in 1986, 1987 and 1988, respectively, relative to the -P-Ca treatments, while these amounts were only 20, 8 and 5 kg ha<sup>-1</sup> for the +P-Ca treatments. Thus, adding lime and P together prevented P leaching from the forest floor. This is most likely due to a reduced dissolution, and subsequent down-wash, of the applied P fertilizers at a higher pH. A confirmation of this is the fairly good correlation of  $P_{\text{total}}$  with pH at the +P+Ca objects in 1986 (Fig. 1).

$P_{\text{total}}$  in the mineral soil was not affected by P application or liming (Table 7), probably because the total amount of P in the mineral soil (ca. 400 kg ha<sup>-1</sup>) was too large to be noticeably affected by any infiltrated amounts.

P lowered extractable Mg in the forest floor (Table 3). This was probably due to Mg exchanging against Ca contained in the P fertilizers.

Extractable Al was significantly reduced by liming in the whole sampled profile in 1989

(Table 3). Al and pH were negatively correlated. However, in the forest floor there are no linear relationships between pH and Al at the +Ca and -Ca objects separately. In 0-25 cm and 25-50 cm mineral soil, the  $R^2$  values for Al versus pH are 0.25 ( $P < 0.002$ ) and 0.12 ( $P < 0.05$ ), respectively. This may indicate that the processes influencing the amount of CaCl<sub>2</sub>-extractable Al change with depth. At a greater depth pH changes by liming are smaller and the amount of CaCl<sub>2</sub>-extractable Al is probably mainly decreased by exchange against Ca.

*Effects of K- and Mg application.* In the forest floor, K application initially increased extractable K, but this effect turned insignificant in 1989 (Table 3). However, any increase in  $K_{\text{total}}$  in the forest floor by K application was never significant (Table 6). In 1986-1988, K and Mg were applied about 6 mo before sampling (Table 2). The subsequent small and insignificant increases in  $K_{\text{total}}$  in the forest floor indicate that the solubility of K<sub>2</sub>SO<sub>4</sub> is too high and K<sup>+</sup> adsorption too low to effect a durably improved K availability in the forest floor. However, by tree uptake K was incorporated in the system's K cycle and so it remained more available than in the -K plots (Chapters 5 and 6). CaCl<sub>2</sub>-extractable Mg and  $Mg_{\text{total}}$  in the forest floor remained at significantly increased levels after Mg application (Tables 3, 6), although kieserite is also highly soluble.

Unexpectedly, the amounts of K and Mg in the mineral soil were similar. In the 0-25 cm mineral soil layer extractable K and Mg were readily and persistently raised by

Table 7. "Total" concentrations of elements in the 0-25 and 25-50 cm mineral soil layers of the factorial experiment (g kg<sup>-1</sup> air-dry soil).

Layer	Year	$N_{\text{total}}$	$P_{\text{total}}$	$K_{\text{total}}$	$Ca_{\text{total}}$
0-25	1986	0.45	0.11	0.57	0.11
	1987	0.52	0.12	0.56	0.15
	1988	0.34	0.11	0.35	0.20
25-50	1986	0.25	0.14	0.88	0.11
	1987	0.43	0.13	0.71	0.15
	1988	0.33	0.13	0.48	0.18

fertilization from 1986 on (Table 4), the increases corresponding with ca.  $12 \text{ kg ha}^{-1}$  for each element, each year. In the 25-50 cm layer K and Mg were increased in 1988 and 1989 (Table 4) with ca.  $8 \text{ kg ha}^{-1}$  in both years. These amounts were rather constant in time because of the split applications until 1988 (Table 2). The lack of increase in  $K_{\text{total}}$  by K fertilization in the forest floor and the observed extractable amounts of K and Mg suggest that relatively less K than Mg was recovered by the extractions, or that K was quickly transported to deeper soil layers.

### *Liming experiment*

The results of the liming experiment partly duplicate those of the factorial experiment. Therefore, only additional results will be discussed. The significant liming effects that were found in the factorial experiment were not always confirmed by the results of the liming experiment. This may partly be due to the statistically less efficient design of the latter.

The forest floor pH of the limed plots remained at an essentially constant elevated level over the four-year period. Only the 3tL treatment, that received lime in 1985 only, showed a gradual decrease (Table 8), as in the factorial experiment. In each year after 1986, the relationship between forest floor pH and lime rate could be described by an exponential curve. For the 1987 and 1988 data, these curves level off at ca.  $6 \text{ Mg lime ha}^{-1}$ . This indicates that at higher applications than  $6 \text{ Mg ha}^{-1}$  the lime particles reached an equilibrium with the forest floor soil solution. In 1989, the pH reached a plateau at ca.  $12 \text{ Mg lime ha}^{-1}$ . This shift of the maximum pH to higher lime applications indicates that the amount of lime determines when the forest floor pH starts to decline again. Probably a new acidification front starts from above (Kreutzer et al., 1991) and a reduced overall forest floor pH may result from lime dissolution in the top forest floor layer. It is not possible to infer lime dissolution rates from the data. Ca was probably retained by adsorption in the forest floor and, thus, may still be present in large quantities when all lime is dissolved (Kreutzer et al., 1991). The proper parameter for assessing lime dissolution, i.e. bicarbonate content, was not determined.

In 1987 and 1989 the pH of the topsoil was significantly raised by liming (Table 8). In 1987, about 6 months after completion of the highest lime application, the effect matched a sharply increasing exponential curve, but in 1989 the pH increased linearly with lime rate (Table 11). In 1988 and 1989 the pH of the 25-50-cm layer was linearly related with lime level (Tables 8, 11). This shows that on poorly buffering sandy soils high lime applications may have profound effects on the pH of deeper soil horizons. However, applying lime may have several detrimental effects, i.e. loss of organic matter, increased soil heavy-metal concentrations and  $\text{NO}_3$  leaching (Marschner and Wilczynski, 1991; Marschner et al., 1989), and thus should be carried out with caution. Presently, in the Netherlands low lime applications corresponding with  $1500 \text{ kg CaCO}_3 \text{ ha}^{-1}$  are advised to prevent these deleterious effects (Anon., 1990).

Table 8. Concentrations of  $\text{CaCl}_2$ -extractable elements in the forest floor (ff) and mineral soil of the liming experiment ( $\text{mg kg}^{-1}$  DM).

	Year				Year			
	1986	1987	1988	1989	1986	1987	1988	1989
ff	$\text{NH}_4$				$\text{NO}_3$			
Control <sup>a</sup>	170	182	181	191	3.0	9.2	54.0	39.0
3tL	119	131	128	126	37.3	14.2	37.4	83.0
6tL		102	100	86		13.3	44.3	75.3
9tL		119	111	106		12.2	48.7	73.3
18tL		77	99	105		10.3	36.0	73.3
	***	*		**	***b			
	P				K			
Control	-	133	123	99	-	336	233	412
3tL	-	133	103	114	-	246	158	311
6tL		91	85	74		226	160	248
9tL		98	95	75		179	148	244
18tL		57	55	58		197	136	219
		*		***		**		**
	Mg							
Control	-	362	610	471				
3tL	-	333	474	372				
6tL		328	491	365				
9tL		322	420	380				
18tL		290	403	264				
		**	**					
	pH				Al			
Control	4.07	3.61	3.33	3.39	-	-	-	11.8
3tL	5.80	5.59	5.37	5.06	-	-	-	4.3
6tL		5.92	5.71	5.99		-	-	5.6
9tL		6.03	5.82	6.11		-	-	2.0
18tL		6.23	6.03	6.40		-	-	4.9
	***	***	***	***				**
0-25 cm	pH				Al			
Control	4.12	4.24	4.22	4.21	-	-	-	29.8
3tL	4.19	4.31	4.25	4.32	-	-	-	27.1
6tL		4.30	4.19	4.34		-	-	17.9
9tL		4.31	4.21	4.30		-	-	25.2
18tL		4.30	4.30	4.43		-	-	14.5
		**		*				*
25-50 cm	pH				Al			
Control	4.62	4.54	4.48	4.46	-	-	-	29.8
3tL	4.63	4.58	4.55	4.52	-	-	-	27.1
6tL		4.55	4.46	4.56		-	-	17.9
9tL		4.60	4.54	4.57		-	-	25.2
18tL		4.58	4.71	4.65		-	-	14.5
		**						*

<sup>a</sup>PKMg treatment<sup>b</sup>\*\*\* =  $P < 0.001$ , \*\* =  $P < 0.01$ , \* =  $P < 0.05$ .

Table 9. Amounts of  $\text{CaCl}_2$ -extractable inorganic N ( $\text{g m}^{-2}$ ) in the forest floor and mineral soil of the liming experiment.

Year	Treatment	Forest floor	Mineral soil		Total
			0-25	25-50	
1986	Control <sup>a</sup>	1.19	1.50	0.88	3.57
	3tL	1.09	3.44	1.36	5.88
	6tL	0.97	2.60	1.06	4.62
	9tL	1.10	2.78	0.70	4.58
	18tL	1.14	2.66	1.35	5.15
1987	Control	1.32	0.66	0.40	2.37
	3tL	1.00	0.64	0.47	2.11
	6tL	0.79	0.65	0.41	1.84
	9tL	0.90	0.63	0.44	1.97
	18tL	0.60	0.83	0.56	1.98
		*e <sup>b</sup>			
1988	Control	1.61	1.02	0.83	3.46
	3tL	1.14	1.49	1.45	4.07
	6tL	0.99	1.05	0.93	2.96
	9tL	1.09	1.12	1.02	3.23
	18tL	0.93	1.51	1.39	3.83
			*	*	*
1989	Control	1.58	0.93	0.63	3.14
	3tL	1.43	1.03	1.08	3.53
	6tL	1.11	0.83	1.03	2.97
	9tL	1.23	1.20	0.88	3.31
	18tL	1.22	1.18	0.96	3.36

<sup>a</sup>PKMg treatment

<sup>b</sup>\* =  $P < 0.05$ , e = fits an exponential model.

clear. Occasional differences in texture and leaching properties per plot, interfering with the liming effect, should also have affected the concentrations of other nutrients and, hence, are not a likely explanation for these high  $\text{N}_i$  concentrations. Neither is there a reason to assume that the trees absorbed more N at higher lime applications (Chapter 6). The  $\text{N}_{\text{total}}$  concentrations in the forest floor suggest that N loss (possibly due to N mineralization) increased linearly with lime rate (Tables 10, 11), and, hence, do not explain high  $\text{N}_i$  concentrations in the 3tL treatment.

Extractable P in the forest floor was linearly lowered by liming in 1987 and exponentially in 1989 (Tables 8, 11). However, the  $R^2$  value of the latter relationship is low, so the model is not appropriate. In fact, in 1989 extractable P of the 3tL level was equal to, or even higher than that of the control. This agrees with results from the factorial experiment, where liming first lowered extractable P (at the 3tL level), after which it grew higher than that of the -Ca plots (Table 3). It can thus be concluded

Extractable  $\text{NH}_4$  in the forest floor was significantly decreased by liming (Table 8). This effect was significant in 1986 and linear in 1987 and 1989 (Table 11).  $\text{NO}_3$  in the forest floor and 0-25 cm mineral soil was significantly increased by liming in 1986 only. In the mineral soil liming had little influence on both N species.  $\text{NO}_3$  in 0-25 cm and 25-50 cm mineral soil tended to be increased by liming, but the effects were not significant and not apparently related with lime level (Table 8). As a consequence, liming had little influence on the areal amounts of  $\text{N}_i$  (Table 9). In each year the amount of  $\text{N}_i$  in the forest floor was higher in the control than in the liming treatments. However, only in 1987 the effect of liming was significant with an exponential decrease in  $\text{N}_i$  with increasing lime rate. In the mineral soil, the  $\text{N}_i$  pool tended to be increased by liming, but only in 1988 the effect of liming was significant, although it could not be described by polynomial nor by exponential models. Each year, but most distinctly in 1988, the 3tL plots had relatively high amounts of  $\text{N}_i$  in the mineral soil, thus disturbing an otherwise positive linear relationship with lime rate (Table 9). The reason for this is not

that retarded dissolution of applied P, that initially was fixed in the forest floor, only occurred at the lowest liming level, where the pH values gradually decreased (Tables 3, 8).  $P_{\text{total}}$  in the forest floor showed little differences between the lime treatments (Table 10). The tendency of  $P_{\text{total}}$  to decrease at the highest liming level may indicate that at that level added P precipitated in forms that were not fully disclosed by the applied digestion (Materials and methods section).

The concentration of extractable Al in 1989 was markedly affected by lime rate (Table 8). In each soil compartment the Al concentrations decreased linearly with lime level (Tables 8, 11). In the forest floor the correlation between pH and Al was moderate ( $R^2 = 0.35$ ,  $P < 0.05$ ). In 0-25 cm and 25-50 cm mineral soil, Al and pH were clearly negatively correlated ( $R^2 = 0.76$ ,  $P < 0.001$  and  $R^2 = 0.51$ ,  $P < 0.01$ , respectively).

Table 10. "Total" concentrations of N and P in the forest floor ( $\text{g kg}^{-1}$  DM) of the liming experiment ( $\text{mg kg}^{-1}$  DM).

Treatment	Year		
	1986	1987	1988
	$N_{\text{total}}$		
Control <sup>a</sup>	11.1	16.5	16.2
3tL	9.9	15.4	13.6
6tL		15.1	13.6
9tL		13.7	14.1
18tL		12.1	11.4
		***b	**
	$P_{\text{total}}$		
Control	0.67	0.84	0.64
3tL	0.67	0.84	0.64
6tL	0.80	1.12	1.04
9tL		1.21	1.00
18tL		1.18	0.92
		0.85	0.86
			***

<sup>a</sup>PKMg treatment

b\*\*\* =  $P < 0.001$ , \*\* =  $P < 0.01$ .

## Conclusions

Variable effects of liming an acid forest soil were observed. Most of these effects were linear with lime rate and, thus, more effective with increasing lime applications. In the first place, moderate lime applications (i.e. around  $3 \text{ Mg ha}^{-1}$ ) appeared to stimulate N mineralization, whereas higher applications did not cause further increases, but even tended to reduce the mineralization rate. Second, liming may affect the residence time of added P and possibly other nutrients in the forest floor after surface dressing. It was shown in the present experiment that the pH increase effected by liming may temporarily anchor P fertilizers in the forest floor, followed by a gradual release when the pH declines again. Thus, the simultaneous additions of lime and P created a relatively small, but longer-lasting increase in readily available P, which may be profitable in silviculture. Third, the effects usually expected from liming, i.e. reductions in  $\text{H}^+$  and Al concentrations, were observed in the forest floor. They even occurred in the subsoil, which can be attributed to the high permeability and low buffer capacity of the sandy soil.

Table 11. All curve-fitting results with  $R^2$  values > 50% of several response parameters vs. liming rate ( $\text{Mg ha}^{-1}$ ) in the liming experiment. Element dimensions are as used in previous tables.

Element	Year	Soil layer	Exponential models		Linear and polynomial models			
			$R^2$	r-value	$R^2$	Intercept	Linear component	Square component
Al	89	Ff <sup>a</sup>			90.6	10.8	-1.58	0.070
Al	89	0-25			68.1	28.47	-0.774	
Al	89	25-50			93.8	17.4	-1.41	0.049
Ca <sub>total</sub>	87	Ff	74.8	0.905	92.9	2.83	6.37	-0.170
Ca <sub>total</sub>	88	Ff	88.3	0.912	94.8	1.87	6.65	-0.169
EON <sup>b</sup>	87	Ff			70.4	189	-3.22	
EON	88	0-25			76.8	2.53	0.033	
K	87	Ff	74.4	0.739	83.6	330	-25.6	1.01
K	89	Ff	57.6	0.767	83.8	402	-29.7	1.093
Mg	87	Ff			78.9	353	-3.61	
Mg	88	Ff	67.2	0.791	80.9	592	-27.3	0.94
Mg	89	Ff			72.6	440	-9.73	
Mg	89	0-25			66.7	4.21	0.066	
Mg <sub>total</sub>	87	Ff	91.5	0.953	93.9	0.818	0.103	
Mg <sub>total</sub>	88	Ff	93.2	1.019	96.4	0.746	0.112	
NH <sub>4</sub>	87	Ff			77.0	158	-4.94	
NH <sub>4</sub>	89	Ff	74.0	0.597	84.6	182	-17.45	0.742
N <sub>total</sub>	87	Ff	85.3	0.969	89.9	16.3	-0.245	
N <sub>total</sub>	88	Ff	54.3	0.938	79.2	15.3	-0.216	
P	87	Ff			73.3	134	-4.35	
P	89	Ff	59.3	0.956				
pH	87	Ff	98.3	0.614				
pH	87	0-25	69.1	0.028				
pH	88	Ff	96.9	0.614				
pH	88	25-50			69.7	4.46	0.012	
pH	89	Ff	98.6	0.751				
pH	89	0-25			76.4	4.25	0.0099	
pH	89	25-50			64.6	4.48	0.0099	
P <sub>total</sub>	87	Ff			64.1	0.868	0.081	-0.005
P <sub>total</sub>	88	Ff	64.5	0.028				

<sup>a</sup>Forest floor<sup>b</sup>Extractable organic nitrogen.

The above-mentioned effects of liming may be advantageous. However, liming also has possible disadvantages. First, especially at high lime dosages the increase in forest floor pH is long-lasting, and a durably increased N mineralization may be expected. With such gradual N releases, the ecosystem will probably develop a profoundly modified flora (Van Dobben, 1992a) and microflora (Kuyper, 1990). Second, nitri-



fication may be harmful. Since pine trees were shown to more readily absorb  $\text{NH}_4$  than  $\text{NO}_3$  (Chapter 3), liming may be expected to result in an accumulation of soil  $\text{NO}_3$ , which may enhance  $\text{NO}_3$  leaching.

The amount of lime to be applied should be determined by weighing the desired and unwanted effects. In the present experiment, the applied amounts were high, compared with the recommended application of  $1.5 \text{ Mg lime ha}^{-1}$  (Anon., 1990). This amount is advised to avoid adverse effects of liming. However, when a low soil pH or high levels of soluble Al in the subsoil hamper tree performance, higher dosages on highly permeable soils may offset the induced negative effects.

Presently there are no soil analysis-based fertilizer recommendations for K and Mg for mature forests in the Netherlands. Recommendations for K and Mg fertilization are based on foliar analysis. For P a soil concentration  $< 87 \text{ mg P}_{\text{total}} \text{ kg}^{-1}$  is considered deficient for *Pinus* spp. (Anon., 1990). In the present experiment soil  $\text{P}_{\text{total}}$  was higher than this value (Table 7). However, soil testing has not been practised sufficiently to serve as a sound basis for fertilizer recommendations in forestry in the Netherlands. Also in other countries it is reported that foliar analysis and field trials are commonly considered more reliable than soil information (e.g. Jokela et al., 1988). The use of foliar analysis for assessing nutrient requirements is dealt with in Chapter 6.

**Response of a Scots pine (*Pinus sylvestris*) stand to application  
of phosphorus, potassium, magnesium and lime.**

**II. Soil solution composition**

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**Keywords:** forest fertilization, leaching, liming, *Pinus sylvestris*, root zone, soil solution chemistry

### Abstract

In the previous chapter the effects of liming and P, K and Mg fertilization on the extractable and total nutrient concentrations in the forest floor and mineral soil of a Scots pine stand were discussed. The present chapter regards the influence of P, K and Mg addition (as one treatment) and liming on solute concentrations at depths of 30 cm, i.e. in the root zone (monitored September 1989 - April 1992) and 100-150 cm, i.e. below the root zone (April 1991). The latter are compared with previous results. Liming increased  $\text{NO}_3$  concentrations at both depths. At 30 cm this effect of liming gradually vanished during the monitoring period, concurrently with a declining soil solution pH, while an increasing effect of PKMg on  $\text{NO}_3$  developed. Probably it took several years for the nitrifying microflora to adapt to an improved nutrient availability, while the effect of liming was instantaneous. These data show that besides liming, fertilization may increase  $\text{NO}_3$  leaching.  $\text{NH}_4$  was not affected by any treatment. The applied K and Mg compounds were easily soluble, and their soil solution concentrations were increased at both depths in the PKMg-amended plots. Probably slow-release fertilizers would allow for a more efficient incorporation of K and Mg into the system's biomass. Any reducing effects of liming on soluble Al were weak (30 cm) or absent (100-150 cm). Al at 30 cm did not reach concentrations known to be harmful to Scots pine, but Ca:Al ratios were critical in the unlimed plots. No evidence is found for an enhanced cation leaching caused by an increased formation of nitric acid in the limed plots.

### Introduction

The soil solution is the carrier for transport in the soil system (Bolt and Bruggenwert, 1978). Therefore, the dissolved nutrient pool has a major effect on the uptake rate of plant roots (Mengel and Kirkby, 1987; Nye and Tinker, 1977) but also on nutrient leaching (Matzner et al., 1983; Vitousek et al., 1979). Necessarily, to have any effect on plants, fertilization and liming treatments should influence nutrient concentrations in the soil solution, but preferably not to such an extent that leaching is enhanced. The balance between increased uptake and leaching depends on the added amounts and the timing of the application. In agriculture, to optimize nutrient uptake and minimize leaching, split applications have become common practice, and the potential to adjust fertilizer additions more closely to crop demand is still increasing. Fertilization and liming in forestry are applied far less frequently, because they are more laborious than in agriculture (Leek, 1990) and the trees can profit from one application for many

years. Inevitably, if fertilization is aimed at an increased nutrient availability for more than one season, in regions with a precipitation excess nutrients are likely to leach, especially during the dormant season, thus making it difficult to manipulate the balance between uptake and leaching. A possible tool to minimize leaching is the use of slow-release fertilizers, which will improve the incorporation of nutrients in the ecosystem biomass (Anon., 1990) and minimize losses during the vegetative period.

Liming is rarely aimed at increasing Ca availability but mostly at improving chemical and structural soil conditions. However, it may affect the concentrations of many elements in the soil solution by raising the pH, which affects chemical equilibria and microbial activity, and by exchange processes.

Since the added amounts are usually very large compared to fertilization, liming may have a profound influence on the uptake and leaching of nutrients other than Ca.

In a Scots pine forest a fertilization experiment was carried out (Chapter 4), in which the effects of fertilizer and lime applications on the soil solution composition were investigated. Soil moisture in and below the root zone was analyzed to assess the effects on nutrient availability and on possible leaching, respectively.

## Materials and methods

The experimental area is situated near Harderwijk, the Netherlands. The soil is a coarse sandy fluvioglacial deposit covered by a fine drift sand layer of varying depth (10–40 cm) with a weak podzolic soil formation and a litter layer that is distinct from the underlying mineral soil. The water table is at ca. 9 m. The site was planted with Scots pine (*Pinus sylvestris* L.) in 1960.

Initial soil data are presented in Table 1. P, K, Mg and lime were applied to 22 × 25-m plots in a 2<sup>4</sup> factorial complete randomized block design with 3 replicates. These treatments are denoted as P, K, Mg and Ca, respectively (Table 2). For more details, see Chapter 4.

## Sampling

**Root zone.** Starting in September 1989, soil moisture sampling was carried out in the

Table 1. Initial element concentrations in the forest floor, and in the 0–25 and 25–50 cm mineral soil segments.

Element	Soil layer		
	Forest floor (g kg <sup>-1</sup> )	0–25 cm (mg kg <sup>-1</sup> )	25–50 cm (mg kg <sup>-1</sup> )
EON <sup>a</sup>	-	4.6	2.3
NO <sub>3</sub>	-	0.0	0.0
NH <sub>4</sub>	-	4.0	1.1
K	-	5.8	2.1
Mg	-	1.7	0.6
pH	-	4.24	4.56
N-total	15.7	454	263
P-total	0.62	102	142
K-total	0.80	632	728
Ca-total	1.20	69	74
Mg-total	0.33	29	38
Zn-total	-	8	10

<sup>a</sup>Extractable organic nitrogen.

Control-, Ca- and PKMg plots. Since 22 February 1991, also the PKCaMg plots were sampled, resulting in a  $2^2$  experiment derived from the  $2^4$  experiment. For sampling, per plot 4 ceramic high-flow tension cups (Soil Moisture Equipment Corporation) were installed 6 mo in advance at a 30-cm depth, i.e. in the rooting zone. The cups had first been rinsed with dilute HCl

and demi water. At a 30-cm depth, sand of variable texture and low in organic matter is found. For installation a hole was drilled with a gouge at a  $45^\circ$  angle, just above the required depth. The place for the cup was prepared by forcing a PVC tube into the bottom end of the hole, so that the centre of the cup would be 30 cm below the mineral soil surface. A ceramic cup was firmly pressed into this slightly undersized cavity. Next, the soil material was packed back into the hole, restoring the original stratification. The cup was connected with a spaghetti-tube that was plugged into a bottle, which was brought to a suction pressure of ca. 15 KPa (0.15 bar). The bottles were buried under the litter layer to avoid light exposure and warming, and they were changed every 2 or 3 weeks.

*Leaching.* In order to assess the influence of liming and fertilization on the leaching of several elements, in April and May 1991 the soil solution of the 100-150 cm layer mineral soil in the above-mentioned  $2^2$  experiment was sampled. In April, little annual differences in soil water storage are found, making this the best sampling time for comparing different years (Van Grinsven, 1988). In a neighbouring pine stand, De Visser (1990) found that the fine root biomass and fine root density had reached very low values below a 60-cm depth. Hence, elements at a 100-150-cm depth are unlikely to be recovered by root uptake. Nine points per plot were sampled in a regular grid. First, a hole was drilled with an auger to a 100-cm depth. Into this hole a PVC tube was inserted to prevent collapse and contamination by surface-soil material. Next, the 100-150 cm layer was sampled with a smaller auger. In April, 2 experimental blocks were sampled and 1 block at the beginning of May. The soil samples were kept frozen in plastic bags until further handling. The soil solution was collected by centrifugation at 7000 r.p.m. for 15 min using a method similar to that described by Reynolds (1984), and stored frozen until analysis.

Table 2. Amounts and compositions of fertilizers and lime applied in the factorial experiment and at the 3tL level of the liming experiment.

Nutrient	Amount (kg ha <sup>-1</sup> )	Fertilizer	Date of first application
P	25	Rock phosphate	autumn 1985
	25	Triple superphosphate	autumn 1985
K	100 <sup>a</sup>	Potassium sulphate	spring 1986
Mg	100 <sup>a</sup>	Kieserite	spring 1986
Ca	1200	Calcium carbonate <sup>b</sup> (powder, 3000 kg)	autumn 1985

<sup>a</sup>K and Mg applications were split, with additional applications made in the springs of 1987 and 1988, to a total of 100 kg ha<sup>-1</sup> each.

<sup>b</sup>Contained 3.6% MgCO<sub>3</sub> (1% Mg).

### *Analyses and statistics*

Analysis of N species, K and Na was carried out as described previously (Chapter 4). In some 30-cm soil solution samples and in all subsoil solution samples Al, Ca and Mg were measured with an ICP. Occasionally, Mg in the 30-cm soil solution was measured by flame AES.  $\text{Cl}^-$  was measured with a coulometer.  $\text{Cl}^-$  is relatively inert with respect to surface exchange (especially if  $\text{SO}_4^{2-}$  is present) and chemical reactions, and is rather constant in precipitation. Therefore, its concentration fluctuates with, and thus reflects the soil's moisture content. Consequently, element:Cl ratios are more independent of soil moisture content than element concentrations, and these ratios can therefore be used as an index of the amounts of nutrients present in the soil zone extracted by the cup (c.f. Tietema and Verstraten, 1992). However, such estimates are rather rough and were therefore used only for comparisons between treatments.

For statistical analysis SAS software (SAS Institute Inc., 1989) was used. The data were not normally distributed, for which reason medians rather than means were calculated per sampling date and per plot for both sampling depths. The medians were analyzed with the SAS Glm procedure for a correct handling of missing data. A probability level of 5% was denoted as significant. For the 30-cm soil solution data obtained before January 1991, means were separated using the REGWF test as supplied in the SAS Glm procedure. For those obtained later, the  $2^2$  design allowed a more efficient statistical analysis and more often treatment effects appeared to be significant. Still, on many individual sampling dates no significant treatment effects occurred. However, time-plots may show (insignificant) differences that persist for a certain period of time, which qualitatively proves that the treatments did have an influence in that period.

## **Results and discussion**

### *Soil solution composition at 30-cm depth*

*pH.* The pH was variable throughout the season, but did not show any general trend in time, except for the Ca treatment, where a gradual decrease was observed in 1989 and 1990 (Fig. 1). Before January 1991, only for two dates significantly higher pH values for the lime treatment were found; afterwards it frequently occurred that Ca raised, and PKMg reduced the pH significantly. In summer, the pH at the PKMg treatment frequently made a sharp drop. However, these values were based on only one or a few observations, always including those from one sampling cup that extracted relatively large amounts of soil water. The solution pH values were in the same range as the  $\text{pH-CaCl}_2$  values (Chapter 4).

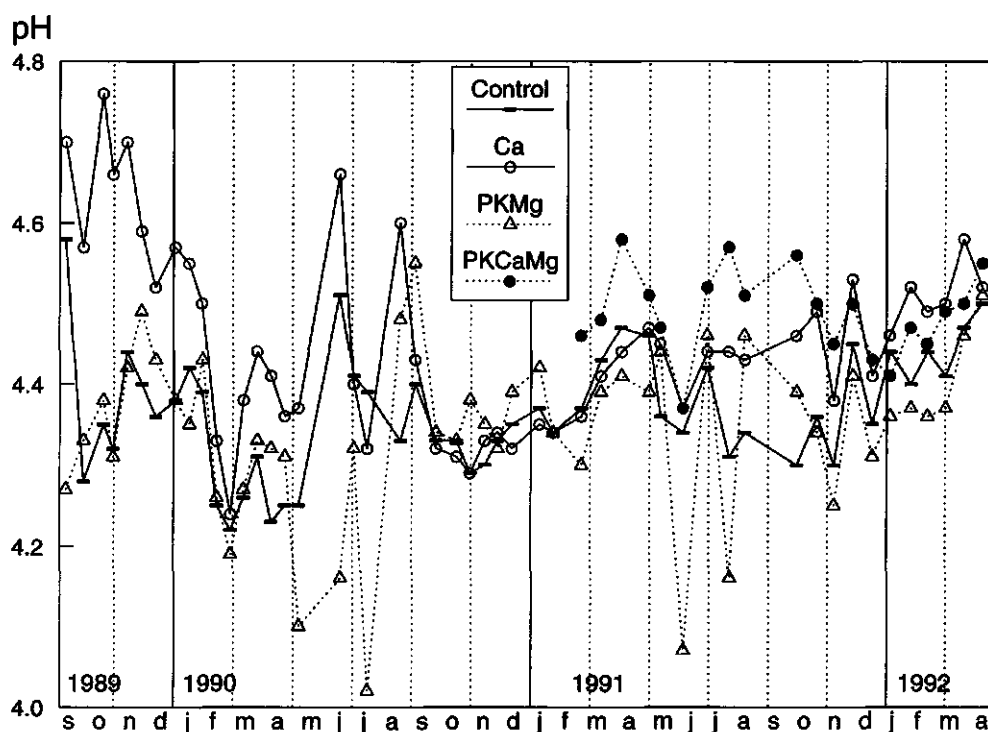


Fig. 1. pH values in the soil solution at 30 cm.

$\text{NO}_3$ . Generally, the  $\text{NO}_3$  concentrations and amounts showed an increasing trend during the sampling period. Only for the Ca treatment a decreasing trend was seen since the end of 1990 (Fig. 2). Each year, peak concentrations occurred in the autumn and winter. This indicates that ammonification and nitrification proceed when plant uptake is reduced during the dormant season, which is likely to cause N leaching to below the root zone. In the vegetative period microbial processes in the topsoil may be inhibited by drought while nutrient uptake from deeper soil layers may still proceed, causing lower  $\text{NO}_3$  concentrations. During the sampling period the  $\text{NO}_3$  peak concentrations grew higher each year and moved to an earlier date. The increasing peak concentrations may be due to a storm and subsequent thinning that occurred on 25 January and in summer 1990, respectively. Vitousek et al. (1979) pointed out that tree harvesting may lead to increased  $\text{NO}_3$  concentrations in the soil solution, if the soil processes that prevent  $\text{NO}_3$  accumulation and transport are saturated. When mineralization is stimulated in canopy gaps and at the same time less  $\text{NH}_4$  is taken up by roots, more substrate is made available to nitrification, and  $\text{NO}_3$  may accumulate. In a situation of a precipitation excess and reduced root uptake,  $\text{NO}_3$  is likely to be subjected to leaching since  $\text{NO}_3$  retention is governed by biological uptake and not by abiotic chemical processes (Johnson et al., 1986). Vitousek et al. (1979) reported increased lysimeter  $\text{NO}_3$  concentrations at least 1 y after making trenches in

three different forests. In the present research, after May 1990  $\text{NO}_3$  concentrations quickly rose to much higher values than in 1989.

The most pronounced effects of liming on  $\text{NO}_3$  were visible in the 1990/91-autumn/winter peaks in concentrations and amounts, but these effects diminished subsequently (Fig. 2). Although liming increased soil solution  $\text{NO}_3$  from the start of the measurement period on, the ecosystem disturbance in 1991 seemed to enhance this effect. The subsequent decline of the liming effect may have been caused by a naturally wearing off of the disturbance effect in combination with the gradual pH decrease at the limed plots (Fig. 1). Mid 1991 for the first time significantly increased  $\text{NO}_3$  concentrations were observed in the PKMg plots. This enhancing effect of PKMg on  $\text{NO}_3$  suggests that the nitrifying microflora developed an ability to profit from an improved nutrient availability. In a large number of acid forest soils Kriebitzsch (1978) found fairly good correlations between availability indices of P and K+Ca and net  $\text{NO}_3$  production, which showed that non-nitrifying soils had a low nutrient availability. However, this does not necessarily mean that supplying nutrients will directly raise nitrification. Sahrawat et al. (1985) found little effect of P addition on in-vitro ammonification and nitrification, and adding P and lime had the same stimulating effect on mineralization as adding lime only. A soil microflora adapted to poor conditions may not have the potential to develop or increase nitrification as a response to a higher nutrient availability, or only do so after a lag-phase. Probably, the sampling period in the present experiment included the end of such a lag-phase.

In several acid forest soils in Germany, Davis (1990) found  $\text{NO}_3$  concentrations that at a 20-40-cm depth ranged from undetectable to ca.  $500 \text{ mmol m}^{-3}$ . Compared to this value, the  $\text{NO}_3$  concentrations in the present research are high, but in the same range as, or lower than those observed by many other workers in western Europe (e.g. Van Breemen et al., 1987; Kreutzer et al., 1991; Marschner et al., 1991). In the Netherlands large quantities of atmospheric N are deposited, so that high  $\text{NO}_3$  concentrations can be expected. The rapid increase in  $\text{NO}_3$  concentrations after a relatively small disturbance as windfelling (see above) also indicates a high N status (Gundersen and Rasmussen, 1990). The term "nitrogen saturation" is often used for such situations, but it is not yet consistently defined (Skeffington, 1990; Tietema, 1992; Wright et al., 1991).

$\text{NH}_4$ .  $\text{NH}_4$  was usually present in low concentrations relative to  $\text{NO}_3$  (Fig. 3). Treatment effects were inconsistent and rarely significant. The peak concentrations of  $\text{NH}_4$  in both summer seasons coincided with dry periods, when only few cups could be sampled. These peaks may be due to increased mineralization during periods with high temperatures, but more likely they are due to water readily running through the dry soil during summer showers. The  $\text{NH}_4$  concentrations are in the same range as those found by Davis (1990) in Germany and New Zealand.

*K and Mg.* The concentrations of K (Fig. 4) and Mg (Fig. 5) were persistently increased due to fertilization up to the last sampling date, which was ca. 4 y after the final



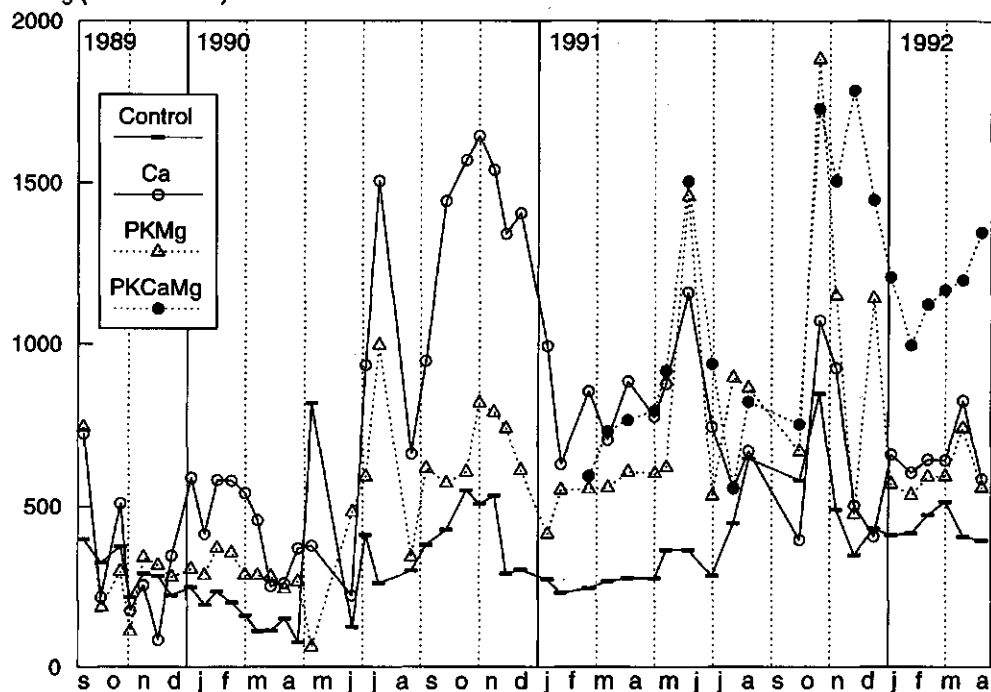
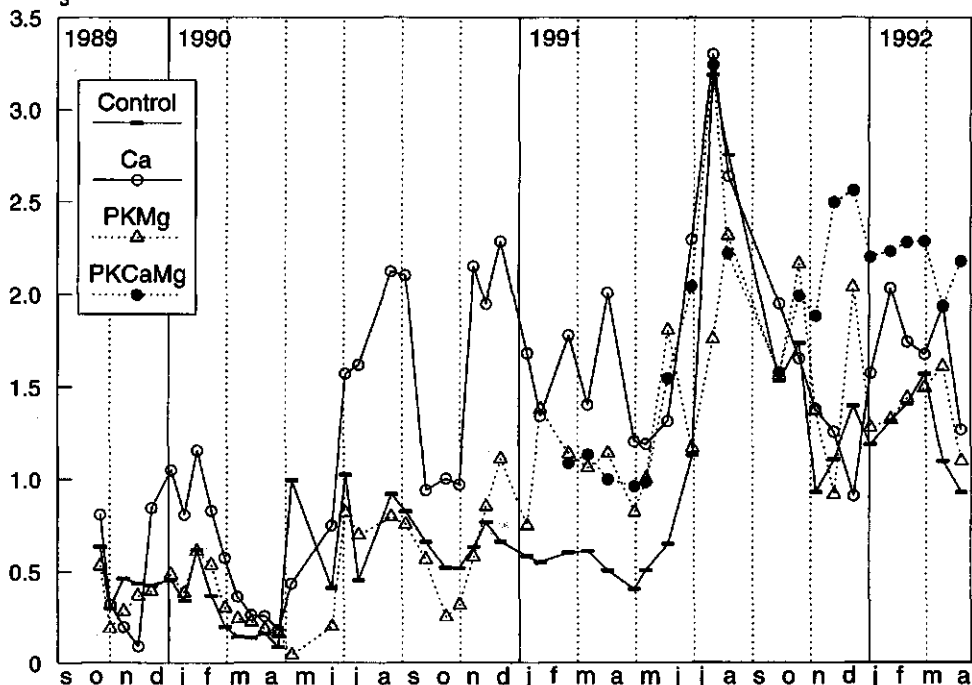
$\text{NO}_3$  (mmol m<sup>-3</sup>) $\text{NO}_3/\text{Cl}$ 

Fig. 2.  $\text{NO}_3$  concentrations in the soil solution at 30 cm; top: concentrations, bottom: estimated indices of amounts.

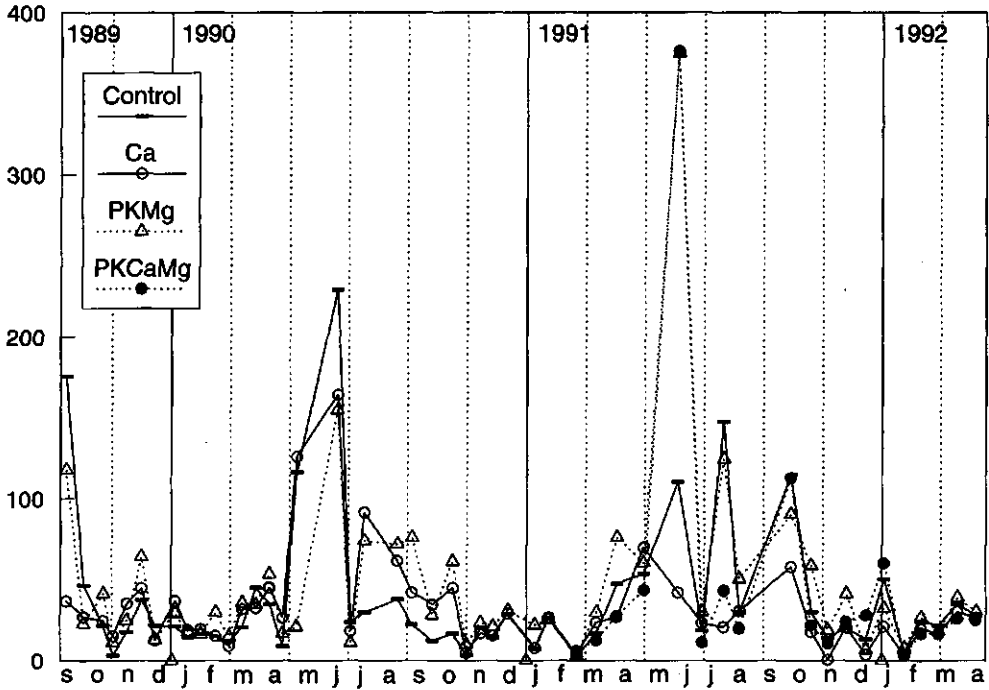
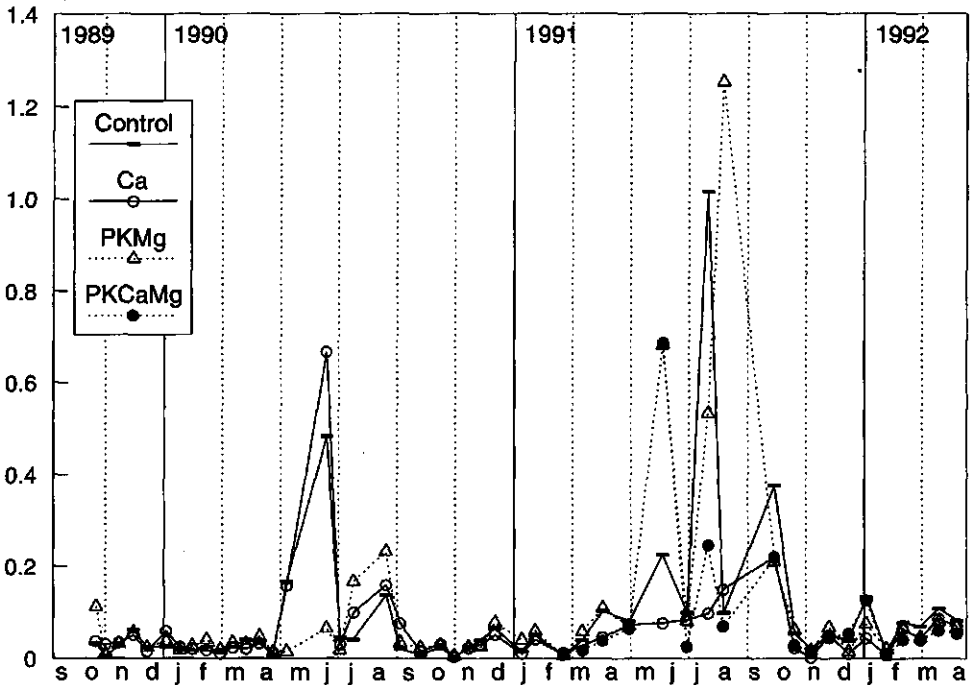
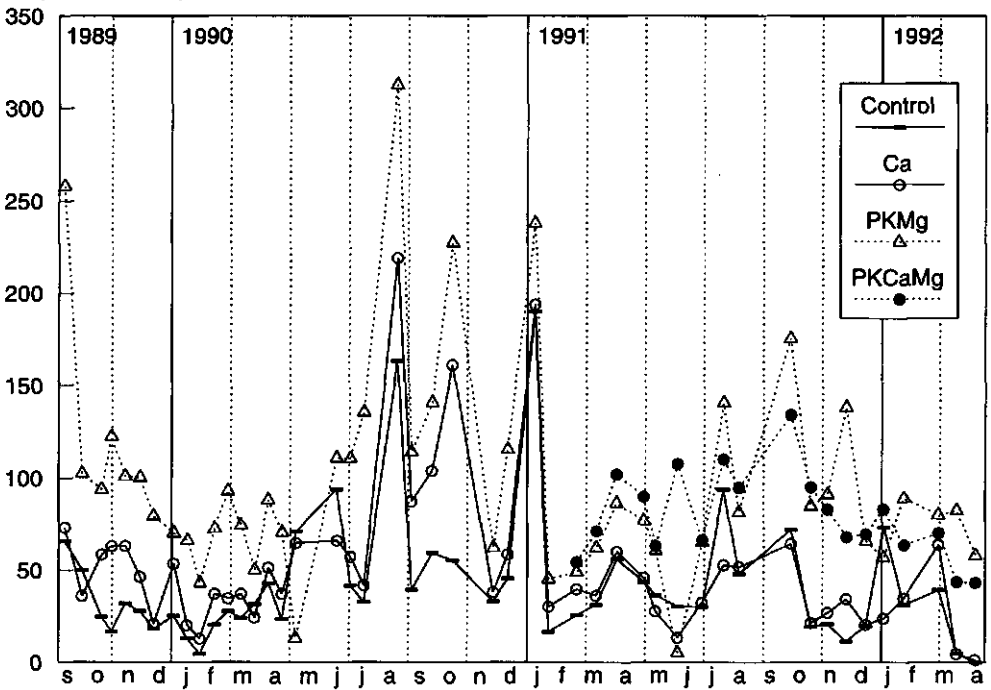
$\text{NH}_4$  ( $\text{mmol m}^{-3}$ )

 $\text{NH}_4/\text{Cl}$ 


Fig. 3.  $\text{NH}_4$  concentrations in the soil solution at 30 cm; top: concentrations, bottom: estimated indices of amounts.

K ( $\text{mmol m}^{-3}$ )



K/Cl

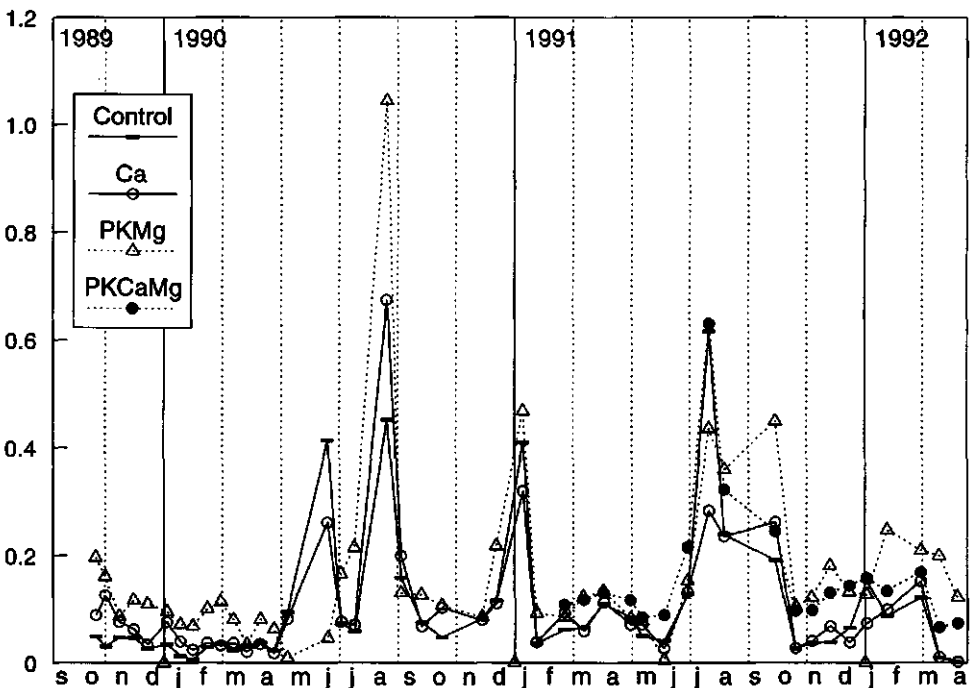
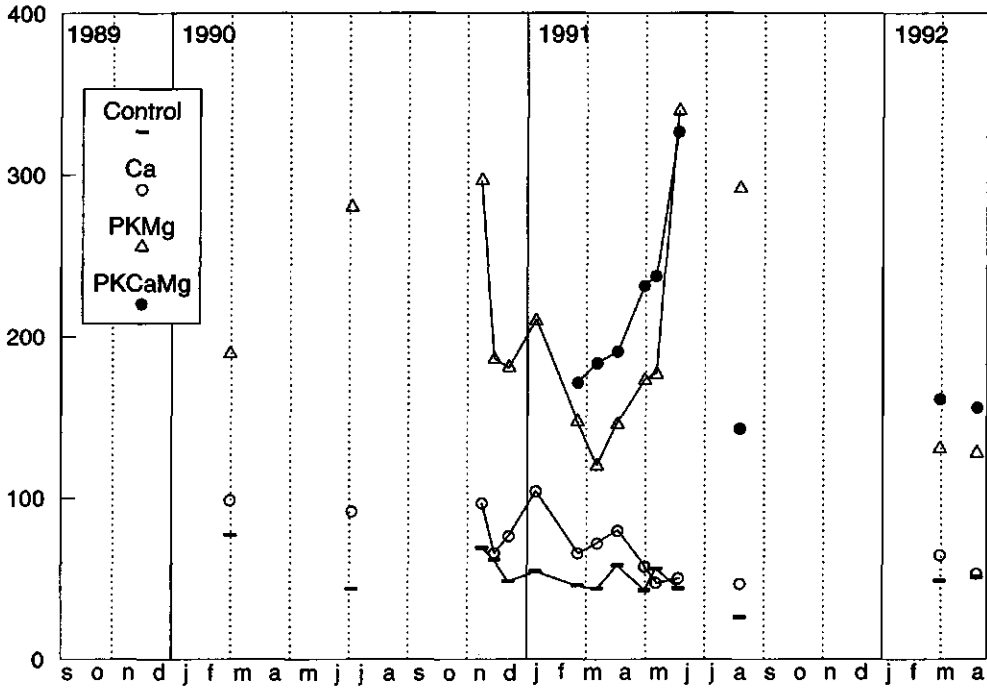


Fig. 4. K concentrations in the soil solution at 30 cm; top: concentrations, bottom: estimated indices of amounts.

Mg ( $\text{mmol m}^{-3}$ )


Mg/Cl

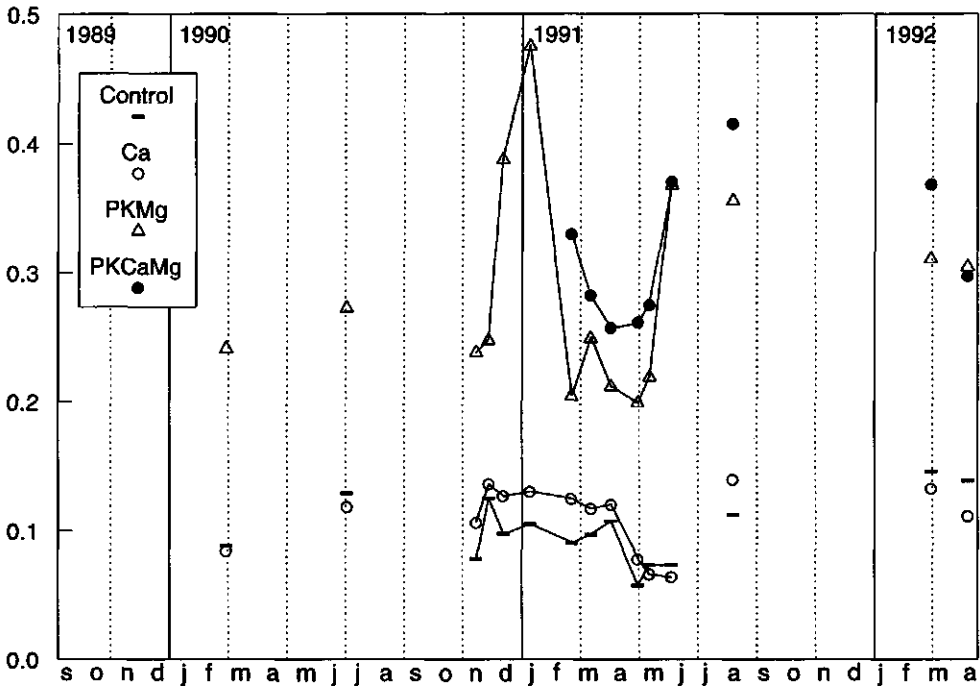


Fig. 5. Mg concentrations in the soil solution at 30 cm; top: concentrations, bottom: estimated indices of amounts.

PKMg application (Table 2). K was applied in a soluble form and seemed to be readily washed out of the forest floor (Chapter 4). Therefore, it is likely that in the long run increased K in the soil solution was caused by K recycling (c.f. Chapter 6), e.g. canopy leaching and K release from needle litter. Because Mg was better retained in the forest floor (Chapter 4), both a direct fertilization effect and a turnover effect may have played a role in sustaining an increased Mg availability.

Table 3. Concentrations of Ca and Al in the soil solution at a 30-cm depth at 3 dates in 1991. The effects of liming on Ca were highly significant. CI ratio = concentration / CI  $\times$  1000.

Element	Treatment	mmol m <sup>-3</sup> at 3 dates			CI ratio at 3 dates		
		14/5	1/7	8/8	14/5	1/7	8/8
Ca	Control	35	21	26	46	142	105
	PKMg	85	54	99	110	164	177
	Ca	592	416	296	951	1944	1123
	PKCaMg	354	493	375	422	973	1072
Al	Control	274	183	189	404	995	808
	PKMg	521	373	546	594	956	638
	Ca	172	149	98	337	749	290
	PKCaMg	289	234	225	230	401	348
Ca/Al	Control	0.1	0.1	0.1			
	PKMg	0.2	0.2	0.3			
	Ca	5.7	4.1	5.5			
	PKCaMg	2.2	3.1	3.9			

*Al and Ca.* The Al concentrations measured in the growing season of 1991 were lowered by liming and increased by PKMg (Table 3), but only at 1/7/91 these effects were significant. Van Grinsven et al. (1989), using a simulation model, predicted that additions of K and Mg (as sulphates) in a December-month would cause dramatically increased Al concentrations in the soil solution at 40 cm in the following year. However, the model was calibrated for a different location. The actual Al peak in Harderwijk may have covered a longer period, which will probably have made it less extreme. In such a case, the eventual effect may be more harmful than that of a short and intense peak. However, the observed Al concentrations (Table 3) are lower than those known to impair Scots pine seedling roots, which are generally mentioned to be  $> 1$  mM, (Arovaara and Ilvesniemi, 1990; Keltjens and Van Loenen, 1989; Eldhuset et al., 1987). Al tolerance may even be higher when ectomycorrhizae are present (Göransson and Eldhuset, 1990), which is the case in the present experiment (Kuyper, 1990). On the other hand, at the unlimed plots the Ca:Al ratios may be critical (Table 3). Ca:Al ratios  $< 0.2$  are suspected to cause root damage in spruce (Ulrich, 1983).

*Soil solution composition at 100-150 cm depth*

In April 1989, Denier van der Gon (1990) analyzed the soil solutions at a 100-150-cm depth in the Control and PKMg treatments and in plots that had been treated with 6 Mg lime ha<sup>-1</sup> and P, K and Mg according to Table 2 (denoted as 6tL). For comparison with the present data, his results were reprocessed. Median instead of mean concentrations per plot were calculated after which significance of treatment effects was assessed using the REGWF test. Therefore, the data given here (Table 4) differ slightly from those published earlier (Denier van der Gon, 1990).

Table 4. Concentrations of elements in the soil solution at a 100-150-cm depth in several experimental plots with Scots pine in April 1989 (from: Denier van der Gon, 1990).

Treatment	mmol m <sup>-3</sup>							
	NH <sub>4</sub>	NO <sub>3</sub>	K	Ca	Mg	Al	Cl	SO <sub>4</sub>
Control	85 a <sup>2</sup>	518 a	53 a	141 a	102 a	151 a	642 a	317 a
PKMg	119 a	422 a	106 a	192 a	466 b	206 a	708 a	832 b
PKMg + 6 Mg lime ha <sup>-1</sup>	27 a	599 a	52 a	646 b	375 b	134 a	623 a	1027 b

<sup>2</sup>Averages followed by a common letter are not significantly different (REGWF test at  $P = 0.05$ ).

Table 5. Concentrations of elements in the soil solution at a 100-150-cm depth in several experimental plots with Scots pine in April/May 1991.

Treatment	mmol m <sup>-3</sup>						
	NH <sub>4</sub>	NO <sub>3</sub>	K	Ca	Mg	Al	Cl
Control	61	250	85	238	110	47	1006
PKMg	84	405	181	312	320	57	1107
Ca	97	826	101	484	173	48	895
PKCaMg	76	669	142	607	306	51	1025

$P$  values per treatment (dashes:  $P > 0.10$ ):

Ca	-	0.008	-	0.0001	-	-	-
PKMg	-	-	0.001	0.003	0.0002	-	-
Ca×PKMg	0.06	-	0.05	-	-	-	-

**NO<sub>3</sub> and NH<sub>4</sub>.** The present observations (Table 5) show increased NO<sub>3</sub> leaching after liming, as was expected (see above). On an average, liming doubled the NO<sub>3</sub> concentrations (Table 5). With an assumed soil moisture content of 10% (w/w), in the 100-150 cm layer of the limed plots compared to the unlimed plots an additional amount of ca. 4.5 kg NO<sub>3</sub>-N ha<sup>-1</sup> was present below the root zone. Taking the present concentrations as annual averages, a precipitation excess of 200 mm y<sup>-1</sup> would effect a NO<sub>3</sub> leaching of ca. 9 and 21 kg N ha<sup>-1</sup> y<sup>-1</sup> in the unlimed and limed plots, respectively. These amounts are lower than those found to leach to a 22-cm depth in an in-situ incubation experiment carried out at the same site (Chapter 7). This, and the fact that the NO<sub>3</sub> concentrations at 30 cm are higher than at 100-150 cm suggest that the

trees absorb  $\text{NO}_3$  from soil segments deeper than ca. 30 cm. Denitrification probably played not more than a minor role in this well-aerated sandy soil (Grofman and Tiedje, 1989). The annual N deposition in the region where the experiment was conducted amounts to approx. 30 kg  $\text{NH}_4\text{-N}$  and 10 kg  $\text{NO}_3\text{-N ha}^{-1}$  (Beier and Rasmussen, 1993). Thus, total N deposition is higher than the estimated quantities of N annually lost due to  $\text{NO}_3$  leaching.  $\text{NH}_4$  leaching could not be estimated, but was probably negligible. In a pine forest in Germany with throughfall depositions of  $\text{NH}_4$  and  $\text{NO}_3$  almost identical to those mentioned above, Van Grinsven et al. (1991) found  $\text{NO}_3$  fluxes at a 1-m depth amounting to ca. 17 kg N  $\text{ha}^{-1} \text{y}^{-1}$ , whereas  $\text{NH}_4$  did not leach to that depth.

In 1989 no effect of liming on  $\text{NO}_3$  was found (Table 4), which was due to the high  $\text{NO}_3$  concentrations in the unlimed plots in 1989, relative to those in 1991 (Table 5). This may be a result of a higher mineralization and/or nitrification, a lower N uptake, or both, in 1988 than in 1990. Because the relatively moist summer conditions in 1988 were favourable for Scots pine growth (Chapter 6), it is likely that tree N uptake was stimulated, rather than reduced. But mineralization and nitrification were probably more stimulated than tree uptake, because microbial processes can more readily respond to changing external conditions. Further, the  $\text{NO}_3$  concentrations at 30 cm showed a pronounced difference between the limed and unlimed plots at the end of 1990 (Fig. 2), which may be a reason that in spring 1991 a liming effect was observed in the subsoil. Marschner et al. (1989) found rather steady  $\text{NO}_3$  concentrations of ca. 140  $\text{mmol m}^{-3}$  at a 2-m depth in the control plot of a Scots pine liming experiment. A sharp increase to ca. 800  $\text{mmol m}^{-3}$  was observed 6 mo after liming and a gradual decrease to ca. 280  $\text{mmol m}^{-3}$  in the subsequent 2 y.

There were no significant effects of PKMg on the  $\text{NO}_3$  concentrations (Table 5) because at the time of sampling these effects just started to appear in the topsoil (see above).

It is unusual to find higher  $\text{NH}_4$  soil solution concentrations in the subsoil than in the topsoil (Table 5, Fig. 3). But the measured  $\text{NH}_4$  concentrations were probably too high, because the filters that were used during the centrifugation contained  $\text{NH}_4$ . However, as this contamination was probably systematic, comparison of treatments is still valid, which shows no significant main effects and a weak  $\text{Ca} \times \text{PKMg}$  interaction (Table 5). The centrifugation procedure of the 1989 samples was similar to that of the present research, and may also have caused a systematic  $\text{NH}_4$  contamination. The data suggest that liming lowered the  $\text{NH}_4$  concentration in 1989 (Table 4), but the effect was not significant (overall F test:  $P = 0.06$ ). Any lowering of  $\text{NH}_4$  in the soil solution by liming is not expected, because liming increases N mineralization, but not the proportion of mineralized N that is nitrified (Chapter 7).

*K and Mg.* K and Mg were significantly increased in the PKMg-amended plots. With the assumptions mentioned above, the estimated additional annual leaching of K and Mg caused by fertilization would amount to 5 and 8 kg  $\text{ha}^{-1}$ , respectively. In 1989

these values were 4 and 18 kg ha<sup>-1</sup>, respectively (recalculated from Denier van der Gon, 1990). The obvious conclusion that Mg is more susceptible to leaching than K is contradicted by previous results (Chapter 4), which suggested a direct and complete loss of added K from the forest floor, while Mg was retained to some extent. With the present data the apparently lower K leaching is hard to explain. It can be speculated that large quantities of K leached deeper than 1.5 m within 1 y (after the final 1988 application). Marschner et al. (1989) found the maximum increase in K concentration in the soil solution at a 2-m depth only 2 mo after K application on a sandy soil. Another explanation may be the larger crop demand for K than for Mg.

*Al and Ca.* The Al concentrations (Table 5) are low when compared to those in Table 4. In the present research a 3-fold dilution was used during the measurements, which may have caused an error. Unfortunately, this can not be verified any more from the original results. However, it is clear that Al was not influenced by any treatment, as was found earlier (Table 4). Marschner et al. (1989) found persistently increased Al concentrations in the soil solution at a 2-m depth in 2 years directly after additions of K<sub>2</sub>SO<sub>4</sub> and lime, whereas those at 50 cm were only slightly raised for ca. 1 year. The Table 4 data were obtained 3 y after the second and last lime application (3 Mg lime ha<sup>-1</sup>) in the 6tL treatment and 1 y after the 3rd and last K and Mg applications, in which time a possibly induced Al flush (Van Grinsven et al., 1989) may have passed beyond the 100-150 cm layer. The unaffected Al concentrations at 100-150 cm suggest that the observed increases at 30 cm in the PKMg plots (see above) are occasional. The seemingly lower Al concentration at the limed plots in Table 4 is not confirmed by later observations (Table 5).

Ca was significantly increased by liming in 1989 and 1991 (Tables 4 and 5). In 1991 the Ca concentration was also increased by PKMg (Table 5). This may be due to exchange processes, which created a Ca front that had not yet reached the 1-m depth in 1989 (Table 4). In 1991, this effect was not found at 30 cm (Table 3), indicating that a new equilibrium had established.

#### *Relationships between cation and anion concentrations*

To examine whether liming may have increased cation mobilization by an increased nitrification, multiple regression analysis of individual cation concentrations on anion concentrations was carried out with log-transformed data. Correlations between cation- and NO<sub>3</sub> concentrations suggest the occurrence of this mobilization process (Mulder and Van Breemen, 1987; Van Breemen and Jordens, 1983).

In most cases positive correlations between cation- and Cl concentrations were found (Tables 6 and 7). These can be attributed to evaporation/dilution effects.

For Mg and especially for K at 30 cm poor correlations with anions were found and liming did not improve correlations with NO<sub>3</sub> (Table 6). Generally, Al and Ca at 30 cm correlated better with Cl and/or NO<sub>3</sub> than did K and Mg. Liming caused Al to



become correlated with  $\text{NO}_3$  (Table 6), indicating that in 1991  $\text{NO}_3$  played a role in Al mobilization in the topsoil of the limed plots. However, the latter was not paralleled in the deeper soil solution, where Al was correlated with  $\text{NO}_3$  irrespective of treatment, or where liming even cancelled this correlation (Table 7). Of the other cations in the soil solution at 100-150 cm only Ca in 1991 was correlated with  $\text{NO}_3$  as a consequence of liming (Table 7). It can thus be concluded that there is no direct evidence of liming to increase leaching of nutrient cations through the formation of nitric acid.

## Conclusions

When Scots pine was treated with P, K, Mg and lime, the concentrations of each of the involved cations was increased in the soil solutions in the root zone. Consequently, the main objective of these applications, an increased nutrient availability and subsequent uptake by the trees, was realized, as was also shown by foliar analysis (Chapter 6). Several treatments increased the soil solution concentrations of K, Ca, Mg,  $\text{NO}_3$  and  $\text{SO}_4$  below the most densely rooted soil zone, indicating that these ions were subjected to leaching. Part of the increased leaching was due to the use of highly soluble K- and Mg sulphates. In the period 1988-1990, ca. 5% and 8-18% of the applied K and Mg, respectively, annually leached to below a 1-m depth. Moreover, K leaching directly following application may have been considerable. Nevertheless, K was successfully incorporated in the biomass and strongly increased the trees' volume growth (Chapter 6). This indicates that the applied amount of K greatly exceeded the uptake potential of the trees, and that little K was necessary for a considerable growth response.

When growth responses occur in a narrow range of nutrient availability, the use of slow-release fertilizers may be appropriate, because they reduce leaching. However, they are not necessarily always the proper choice. In principle, fertilizers should release nutrients at a rate not higher than the expected maximum uptake rate during the growing season. A lower release rate may be beneficial, maintaining a continuous nutrient demand that keeps the nutrient pool in the soil solution at a low level until

Table 6. Results of (multiple) regression of cation on anion concentrations in soil moisture at 30 cm sampled at two dates. Models with overall  $P$  values < 0.05 and  $T$  values < 0.05 for each anion; + : positive correlation; (+) : regression model with slightly smaller  $R^2$  value.

Cation	Period <sup>a</sup>	Treatment	Anions		R <sup>2</sup>
			Cl	NO <sub>3</sub>	
K	1	-Lime	+		0.09
		+Lime	+		0.13
	2	-Lime		+	0.07
		+Lime	+		0.03
	3	-Lime		+	0.02
		+Lime		+	0.06
Mg	1	-Lime		+	0.18
		+Lime	+	(+)	0.37
	2	-Lime	+	+	0.35
		+Lime	+		0.10
	3	-Lime	+	+	0.64
		+Lime	+	+	0.34
Al	May-Aug 1991	-Lime	+		0.56
		+Lime	+	+	0.51
Ca	May-Aug 1991	-Lime	+	+	0.40
		+Lime		+	0.28

<sup>a</sup>1 = Sept. 1989 - April 1990; 2 = April 1990 - April 1991; 3 = April 1991 - April 1992.

Table 7. Results of (multiple) regression of cation on anion concentrations in soil moisture at 100-150 cm sampled at two dates. Models with overall  $P$  values  $< 0.0002$  and  $T$  values  $< 0.05$  for each anion; + : positive correlation; (+) : (multiple) regression model with slightly smaller  $R^2$  value.

Date	Cation	Treatment	Anions			$R^2$
			Cl	$\text{NO}_3$	$\text{SO}_4$	
April 1989	K	Control	(+)		+	0.67
		Limed <sup>a</sup>	+			0.56
	Ca	Control	+		(+)	0.43
		Limed	+		+	0.84
	Mg	Control	+	+ (+)	(+)	0.87
		Limed	+		+	0.90
	Al	Control	(+)	(+) +	+	0.92
		Limed	+	+ (+)	(+)	0.88
	Na	Control	(+)		+	0.62
		Limed	(+)		+	0.57
April 1991	K	-Lime	+	+	nd <sup>b</sup>	0.64
		+Lime <sup>c</sup>	+		nd	0.71
	Ca	-Lime	+		nd	0.50
		+Lime	+	+ (+)	nd	0.53
	Mg	-Lime	+		nd	0.43
		+Lime	+		nd	0.40
	Al	-Lime	+	+	nd	0.55
		+Lime	+		nd	0.67
	Na	-Lime	+		nd	0.92
		+Lime	+		nd	0.91

<sup>a</sup>6000 kg lime ha<sup>-1</sup>

<sup>b</sup>not determined

<sup>c</sup>3000 kg lime ha<sup>-1</sup>

the end of the growing season and, thus, reduces subsequent nutrient leaching. In most cases, this will be accomplished by using slow-release fertilizers, but for fast-growing tree species or in stands before canopy closure easily soluble fertilizers may be necessary to meet the stand's nutrient requirements.

In the year following a heavy storm,  $\text{NO}_3$  concentrations reached maxima in the root zone of the plots that had received lime only (Fig. 2). This suggests that liming may corroborate  $\text{NO}_3$  leaching invoked by physical disturbance.

The often quoted hazard of liming to increase  $\text{NO}_3$  leaching appeared to possibly apply to fertilization as well. The former will occur in the short term, while the latter may be a long-term effect. Increased soil solution  $\text{NO}_3$  concentrations as a result of PKMg additions were measured from mid 1991 on at a 30-cm, but not below a 1-m depth. In spite of an increased nitrification, the estimated annual N leaching was lower than the annual N deposition

for each treatment, indicating that N is retained in the ecosystem and that a situation of "N saturation" is not (yet) reached.

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**Response of a Scots pine (*Pinus sylvestris*) stand to application of phosphorus, potassium, magnesium and lime. III. Foliar nutrient concentrations and stand development**

*Co-author: A. Van Diest*

**Keywords:** fertilizer recommendations, foliar diagnosis, liming, *Pinus sylvestris*, tree and stand measurements

### **Abstract**

In the two previous chapters the effects of liming and P, K and Mg fertilization in 1985-1988 on soil nutrient concentrations in the forest floor and mineral soil of a Scots pine stand were discussed. The present chapter addresses the effects of these treatments on foliar nutrient concentrations and tree growth. Stem volume increments of individual trees were measured, and corresponded with ca.  $16 \text{ m}^3 \text{ ha}^{-1} \text{ y}^{-1}$ . P and K showed increased foliar concentrations instantly after fertilization and were the only elements that significantly increased volume growth in the period 1988-1991. Additional volume growth of individual trees brought about by P and K corresponded with 0.9 and  $2.2 \text{ m}^3 \text{ ha}^{-1} \text{ y}^{-1}$ , respectively. Foliar Mg and Ca were increased later and insignificantly reduced and increased volume growth, respectively. Lime and Mg applications tended to lower foliar N. When absolute foliar concentrations and element ratios were compared to Dutch and German standards, some of these standards appeared to be inappropriate for predicting growth responses to nutrient additions.

### **Introduction**

In Chapter 4 a forest fertilization experiment was described. The present chapter evaluates to which extent Scots pine in this experiment could benefit from the fertilization and liming treatments and whether growth responses were correctly predicted by foliar analysis. The treatment effects on foliar nutrient concentrations, height-, basal area- and volume growth are discussed.

Fertilization responses may be evaluated using 'vitality' parameters like crown density and needle colour (Den Boer and Van den Tweel, 1985). However, in the present study these were not assessed, because it is difficult to do so without bias and it was uncertain if this method would be powerful enough to detect treatment effects. Also, differences in needle colour of Scots pine are most clear in the needles of the leader, which is hard to observe. For these reasons, growth measurements were used to evaluate the effects of fertilization and liming on stand performance.

Foliar analysis is a tool to assess nutrient requirements of crops (Walworth and Sumner, 1987) and forests (Van den Driessche, 1974; Evers, 1986). At present, the interpretation of total nutrient concentrations is well-documented, while alternatives are still developing. For the evaluation of foliar nutrient concentrations of Scots pine German (Hüttel, 1986) standards are available. In the Netherlands, standards were developed later (Anon., 1990), based on both Dutch and Northwest European experi-

ments. According to these standards, at a 'low' concentration stunted growth and visible deficiency symptoms may occur, and stress tolerance is low. At a 'normal' level the nutrient concentration is sufficient to support adequate growth, fertilization does not result in profitable additional yields and recovery may be expected after exposure to extreme circumstances. If the concentration of a nutrient is 'high' a further increase in its concentration will not stimulate and may even reduce growth, while a reduced stress tolerance may result from an excess of this nutrient, or from induced deficiencies of other nutrients.

The extent to which nutrient availability is balanced can be assessed by considering nutrient ratios, rather than absolute concentrations (Walworth and Sumner, 1987). Ingestad (1979) reported optimum nutrient proportions with respect to N for several tree species. According to preliminary Dutch standards, partly based on Ingestad's, several nutrient ratios are classified as 'low', 'normal' or 'optimal', not differentiating for species (Anon., 1990). The 'optimal' range of the mineral nutrient:nitrogen ratios approximately agrees with those proposed by Ingestad (1979). At an 'optimal' mineral nutrient:nitrogen ratio no growth response can be expected from addition of the nutrient.

The present research evaluates the above-mentioned foliar analysis-based growth prognoses as applied to *P. sylvestris*.

## Materials and methods

### *Fertilization and needle analysis*

The experimental area is a Scots pine stand, planted in 1960. In 1985, foliar nutrient concentrations were analyzed prior to fertilization (Table 1). P, Ca and Mg were rated 'deficient', K 'sufficient', and N more than 'sufficient' by German standards (Hüttl, 1986; Table 2a). By Dutch standards (Anon., 1990; Table 2b,c), initial foliar P and Ca would have rated 'low' and N, K and Mg 'normal'. The ratios of P, K and Mg to N and the K/Mg ratio were evaluated as 'normal', and the K/Ca ratio higher than 'optimal' (Table 2b,c).

P, K and Mg fertilizers and lime (Table 3) were applied in a 2<sup>4</sup> design (factorial experiment) and at 5 liming rates (0, 3, 6, 9 and 18 Mg lime ha<sup>-1</sup>, denoted as 3tL, 6tL, etc.) with full PKMg application (liming experiment). In autumn 1985, all lime treat-

Table 1. Nutrient concentrations in 1985 autumn foliage of Scots pine (before starting the experiments).

Nutrient	Unit	Needle age	
		0.5 y	1.5 y
N	g kg <sup>-1</sup>	17.9	18.5
P		1.13	1.07
K		5.49	4.73
Ca		1.11	2.36
Mg		0.76	0.54
Zn	mg kg <sup>-1</sup>	47	43
B		18	28
Cu		3.7	3.6

Table 2. Evaluation of nutrient concentrations in Scots pine. A. German standards (Hüttl, 1986); B. Dutch standards; C. nutrient mass ratios according to Dutch standards (Anon., 1990).

A. German standards	Nutrient g kg <sup>-1</sup>	Deficient supply <	Sufficient supply	Good supply >
	N	13 - 14	14 - 16	16
	P	1.2 - 1.3	1.3 - 1.5	1.5
	K	4.0 - 4.5	4.5 - 6.0	6.0
	Ca	1.0 - 2.0	2.0 - 3.0	3.0
	Mg	0.7 - 0.8	0.8 - 1.0	1.0
B. Dutch standards	Nutrient g kg <sup>-1</sup>	Low <	Normal range	High >
	N	14.0	14 - 18	18.0
	P	1.4	1.4 - 1.7	1.7
	K	5.0	5.0 - 7.0	7.0
	Ca	(2.0)	(≥ 2.0)	
	Mg	0.7	0.7 - 1.0	1.0
C. Nutrient mass ratios	Ratio	Low	Normal	Optimal
	P/N × 100	< 5	5 - <10	10-14
	K/N × 100	< 25	25 - <50	50-100
	Mg/N × 100	< 5	5 - <10	10
		Low	Normal	Optimal
	K/Ca	< 0.5	0.5 - 1.0	1.0 - 3.5
		Normal	High	Very high
	K/Mg	1 - 7 to 9	7 to 9 - 12	> 12

ments received 3 Mg lime ha<sup>-1</sup>. Three, 6 and 9 Mg ha<sup>-1</sup> were applied to the 6tL, 9tL and 18tL treatments, respectively, in autumn 1986. Finally, 6 Mg ha<sup>-1</sup> was applied to the 18tL treatment in spring 1987. The individual plot size was 22 × 25 m. Within these plots, 5-m-wide boundary zones were observed in which no sampling and measurements were carried out.

In October or November, i.e. in the dormant season, of 1986 to 1989, needle samples were taken from the fifth whorl from the top, in the light-exposed part of the canopy. Per plot needles were collected from one dominant tree, and in 1989 from 10 dominant trees. The current year's and previous year's needles were sampled. These needles are denoted as 0.5-y and 1.5-y needles, respectively, after their approximate age at the time of sampling. Older needles were not present. The needle samples were dried in a forced-draft oven at 70°C and ground. Subsamples were digested in a H<sub>2</sub>SO<sub>4</sub>, salicylic acid, H<sub>2</sub>O<sub>2</sub> medium, catalyzed by selenium. In the digests N and P were measured colorimetrically, K, Ca and Na by atomic emission and Mg by atomic absorption spectrometry. Zn, B and Cu were determined occasionally and appeared to be sufficient and unaffected by treatments. Therefore, these elements are not discussed in this chapter.

Table 3. Amounts and compositions of fertilizers and lime applied in the factorial experiment and at the 3tL level of the liming experiment.

Nutrient	Amount (kg ha <sup>-1</sup> )	Fertilizer	Date of first application
P	25	Rock phosphate	autumn 1985
	25	Triple superphosphate	autumn 1985
K	100 <sup>a</sup>	Potassium sulphate	spring 1986
Mg	100 <sup>a</sup>	Kieserite	spring 1986
Ca	1200	Calcium carbonate <sup>b</sup> (powder, 3000 kg)	autumn 1985

<sup>a</sup>K and Mg applications were split, with additional applications made in the springs of 1987 and 1988, to a total of 100 kg ha<sup>-1</sup> each.

<sup>b</sup>Contained 3.6% MgCO<sub>3</sub> (1% Mg).

### Stand properties

In 1985 thinning was carried out, aimed at an equal basal area per plot (23.4 m<sup>2</sup> ha<sup>-1</sup> ± 10%). Thus, the factorial and the liming experiment were thinned to 2127 and 2324 trees ha<sup>-1</sup>, respectively, in the autumn of 1985. In 1991 the stockings had declined to 1762 and 2051 trees ha<sup>-1</sup>, respectively, by natural mor-

tality and mostly by storm damage (25 January, 1990) and subsequent thinning. The storm did not strike treatments selectively. Therefore, during the whole measurement period the reductions in number of trees did not significantly differ per treatment (tested with a binomial distribution). Average heights (and the averages of dominant height per plot) in 1985 were 10.3 (12.4) and 12.8 (15.0) m in 1991.

Starting in 1985, tree height and diameter at breast height (DBH) were measured every winter until 1987 and 1989, respectively. In winter 1991 both entities were recorded again. All trees within the net plots were measured. DBH was measured with a tree diameter tape. To prevent bias, each tree was marked at a 1.3-m height. If there was an old whorl at that height, the diameters 15 cm above and below that point were measured and averaged. Tree height was measured with a telescoping height pole (1985-1987) or with a Blume-Leiss altimeter (1991). Compatibility of both methods was checked. The highest point above the ground was measured, which, therefore, did not necessarily correspond to the actual stem length. Top shoot length in the years 1983-1985 was estimated in 1985 by measuring the heights of the three whorls below the top shoot. Total top growth from 1983 to 1985 was used as a covariate in testing treatment effects on height increment. In 1991 it was not feasible any more to infer the heights in 1988-1990, as the visibility of lower whorls was reduced by the increased tree height.

The DBH and height data were used to estimate the stem volumes of individual trees according to

$$V = \text{DBH}^{1.82075} \times H^{1.07427} \times e^{-2.88085}$$

where V is the stem volume (dm<sup>3</sup>), DBH the diameter at breast height (cm) and H the tree height (m) (Dik, 1984). The results of these calculations were used to assess if, when, and to what extent fertilization effected an additional volume growth. The annual basal area and volume increments per plot were calculated by summing the individual increments of those trees that were alive at the end of the examined time

Table 4. Foliar nutrient concentrations in 0.5-y old and 1.5-y old needles of the factorial experiment ( $\text{mg kg}^{-1}$  DM).

Treatment	Year				Year			
	1986	1987	1988	1989	1986	1987	1988	1989
<b>0.5-y needles</b>								
	N				P			
Control	16.3	19.1	18.2	23.3	1.11	1.36	1.14	1.33
-P	16.2	18.2	18.1	20.9	1.05	1.37	1.20	1.40 ooo
+P	16.1	18.1	18.6	21.2	1.26 ***	1.66 ***	1.40 ***	1.69 ***
-K	15.7	18.1	18.3	21.5	1.16	1.53	1.30	1.56
+K	16.6	18.1	18.4	20.6	1.14	1.50	1.31	1.52
-Ca	16.3	18.3	18.7	21.5 o	1.23	1.52	1.32	1.52
+Ca	16.0	17.9	18.1	20.6	1.06 **	1.50	1.29	1.56
-Mg	16.5	18.3	18.6	21.4 oo	1.17	1.50	1.28	1.53
+Mg	15.9	18.0	18.1	20.7	1.14	1.53	1.32	1.56
	K				Ca			
Control	5.03	6.35	5.85	5.71	0.72	1.55	1.10	1.49
-P	5.52	6.52	6.02	6.24	0.95	2.17	1.87	2.03 o
+P	5.86	6.53	6.08	6.31	1.04	2.39	2.28	2.19
-K	5.32	6.13	5.58	5.75 ooo	0.94	2.18	2.08	2.06
+K	6.05 **	6.93 ***	6.52 **	6.81 ***	1.05	2.38	2.07	2.15
-Ca	5.77	6.70	6.35	6.50	0.94	1.92	1.60	1.36 ooo
+Ca	5.60	6.36	5.76	6.05 *	1.06	2.64 ***	2.55 ***	2.85 ***
-Mg	5.70	6.59	6.00	6.31	0.91	2.18	2.04	2.11
+Mg	5.67	6.47	6.10	6.25	1.09	2.39	2.11	2.11
	Mg							
Control	0.73	0.81	0.72	0.72				
-P	0.76	0.88	0.85	0.78				
+P	0.77	0.91	0.88	0.82 *				
-K	0.75	0.89	0.89	0.83				
+K	0.78	0.90	0.84	0.77 ***				
-Ca	0.75	0.90	0.86	0.75				
+Ca	0.78	0.90	0.87	0.86 ***				
-Mg	0.75	0.85	0.78	0.75 ooo				
+Mg	0.78	0.94 *	0.95 **	0.85 ***				

(Continued next page)

interval. Thus, as an example, for the volume increment in the years 1988-1991 the volume increase between autumn 1987 and autumn 1991 of each tree that was alive in 1991 was calculated. This approach was used because the amounts withdrawn in-between by (self-) thinning were not known, so the volume increment of a plot as a whole could not be calculated.

Relative growth rates of the stem volume and basal area (denoted as  $\text{rgr}_v$  and  $\text{rgr}_b$ , respectively) of each time interval were also calculated on an individual tree basis, and averaged per plot.

### Statistics

For calculations and statistical tests SAS software (SAS Institute Inc., 1990) was used. In the factorial experiment only effects with  $P < 0.01$  were considered to be significant (Chapter 4).



Table 4. (continued)

Treatment	Year				Year			
	1986	1987	1988	1989	1986	1987	1988	1989
<b>1.5-y needles</b>	<b>N</b>				<b>P</b>			
Control	17.7	20.0	18.8	22.0	0.97	1.23	0.85	1.32
-P	18.0	19.0	16.1	20.1 ooo	1.01	1.19	0.83	1.41 ooo
+P	17.0	17.1 **	15.8	19.3 *	1.08	1.31 *	0.93 *	1.63 ***
-K	17.3	17.9	16.1	20.0	1.04	1.27	0.88	1.52
+K	17.7	18.3	15.8	19.5	1.04	1.23	0.88	1.52
-Ca	17.3	18.2	16.6	20.1	1.08	1.27	0.89	1.50
+Ca	17.7	18.0	15.3 *	19.3 *	1.01	1.23	0.86	1.53
-Mg	17.6	18.5	16.5	19.8	1.03	1.25	0.88	1.49
+Mg	17.4	17.6	15.4 *	19.6	1.05	1.25	0.87	1.54
	<b>K</b>				<b>Ca</b>			
Control	4.40	5.66	4.46	4.67	1.64	1.46	2.63	1.61
-P	4.98	5.37	5.00	5.33	1.99	2.10	3.61	3.04
+P	4.86	5.62	5.11	5.77 ***	1.84	2.24	4.03	3.47
-K	4.65	5.16	4.44	5.11 ooo	1.81	2.05	3.95	3.16
+K	5.19	5.83 *	5.68 ***	6.00 ***	2.02	2.29	3.69	3.35
-Ca	4.86	5.62	5.04	5.54	1.82	1.93	3.18	2.24 ooo
+Ca	4.99	5.37	5.07	5.57	2.01	2.41 *	4.46 ***	4.28 ***
-Mg	4.92	5.58	4.97	5.43	1.77	1.95	3.72	3.07
+Mg	4.92	5.41	5.15	5.68	2.06	2.39 *	3.92	3.44
	<b>Mg</b>							
Control	0.52	0.55	0.55	0.52				
-P	0.57	0.59	0.74	0.57				
+P	0.54	0.61	0.76	0.59				
-K	0.55	0.58	0.80	0.60				
+K	0.56	0.61	0.69	0.56				
-Ca	0.53	0.59	0.70	0.55				
+Ca	0.58	0.61	0.79	0.60 *				
-Mg	0.54	0.58	0.68	0.54 oo				
+Mg	0.57	0.62	0.82	0.62 ***				

\*Symbols following a pair of figures: \*\*\*, ooo =  $P < 0.0001$ , \*\*, oo =  $P < 0.001$ , \*, o =  $P < 0.01$ , for ANOVA (\*) and Repeated Measures Analysis of Variance (o), respectively.

In the liming experiment effects with  $P < 0.05$  were denoted as significant. For the 1986 data all liming levels were equal. For the data acquired later, polynomial and exponential curve-fitting was carried out. Polynomial models with significant linear and/or quadratic and insignificant lack-of-fit contrasts were maintained. Exponential curve-fitting was carried out with Genstat's Fitcurve directive (Rothamsted Experimental Station, 1990). Exponential models were preferred to polynomial models if the former had higher  $R^2$  values.

If appropriate, results of repeated measures analysis of variance (RMAOV) of treatment effects will be presented (Moser et al., 1990), only if no significant year  $\times$  effect interactions were found.

## Results

### *Foliar concentrations*

**Factorial experiment.** The needle concentrations quickly responded to fertilization and liming (Table 4). Already in 1986, after the first fertilizer applications in spring, the P and K concentrations of the 0.5-y needles were significantly increased. P in the 0.5-y needles in 1986 was lowered by liming (Table 4). The effects of P and lime application on foliar P were not interactive, except for a significant  $P \times Ca$  interaction on P in the 1.5-y needles in 1986, showing that P was raised only in the absence of lime.

In 1987, when the split dosages of K and Mg were not yet completed, all applied nutrients had significantly increased their corresponding nutrient concentrations in the 0.5-y needles. In 1989 these effects were strongly significant (Table 4).

Starting in 1987, P, K and Ca application significantly increased the concentrations of the corresponding nutrients in the 1.5-y needles. Not until 1989 was Mg in the 1.5-y needles significantly increased by Mg application (Table 4).

The K concentration of the 0.5-y needles was significantly reduced by liming in 1989. This may be related with the observed lime-induced reduction in K concentrations (total and extractable) of the forest floor. However, K in the soil solution was not markedly influenced by liming (Chapter 5). A K-Ca uptake antagonism may have played a role.

Ca in the 0.5-y needles was slightly raised by P (Table 4), which increase was significant ( $P < 0.01$ ) in RMAOV only. This may be an effect of Ca being the accompanying cation of the applied P (Table 2).

Mg in the 0.5-y needles was influenced by the applications of all major nutrients in 1989: P, Ca and Mg applications significantly increased and K application significantly reduced foliar Mg concentrations (Table 4). The Mg increase effected by liming is easily explained by the Mg content of the applied lime (Table 2), thus compensating a possible Ca-Mg uptake antagonism. The Mg increase by P application cannot be attributed to soil processes (Chapter 4). It may have a physiological basis (Truog et al., 1947). The reduced Mg concentration induced by K may be due to K inhibiting Mg uptake (Mengel and Kirkby, 1987) or to Mg being diluted by a K-induced additional volume growth (see below). However, such a dilution, if occurring, should probably have affected other nutrient concentrations as well.

N in the 0.5-y needles was not significantly affected by any treatment in individual years (Table 4). RMAOV, however, showed that N in the 0.5-y needles was significantly reduced by Ca and by Mg applications. Ca and Mg interactively lowered 0.5-y foliar N ( $P = 0.008$ , RMAOV). N in the 1.5-y needles was significantly reduced by P application in 1987 and 1989. In both needle classes foliar N gradually increased during the measurement period (Table 4). The ratio of N in 0.5-y over that in 1.5-y needles, which is usually  $> 1$  if N supply is not excessive, increased in the control plots from 0.93 in 1986 to 1.07 in 1989. Each year, this ratio was increased by P and

Table 5. Foliar nutrient concentrations in 0.5-y old and 1.5-y old needles of the liming experiment ( $\text{g kg}^{-1}$  DM).

Treatment	Year				Year			
	1986	1987	1988	1989	1986	1987	1988	1989
<b>0.5-y needles</b>								
	N				P			
Control <sup>a</sup>	16.1	18.1	20.1	21.2	1.35	1.64	1.57	1.71
3tL	15.5	17.1	17.2	20.5	1.16	1.54	1.29	1.64
6tL		17.6	17.5	20.3		1.56	1.37	1.58
9tL		17.2	17.6	21.3		1.85	1.28	1.61
18tL		17.7	18.2	20.3		1.64	1.24	1.57
	K				Ca			
Control	6.14	6.88	7.33	6.69	1.29	2.41	2.42	1.79
3tL	6.33	6.49	6.26	6.35	1.46	3.21	3.45	3.19
6tL		6.89	5.92	6.31		3.34	4.37	3.72
9tL		7.09	6.71	6.28		3.28	3.10	3.55
18tL		6.74	5.83	6.13		3.32	3.66	3.24
						ab		*
	Mg							
Control	0.81	1.04	1.05	0.86				
3tL	0.80	0.94	0.87	0.83				
6tL		0.94	1.11	0.90				
9tL		0.90	0.89	0.94				
18tL		0.97	0.83	0.88				
<b>1.5-y needles</b>								
	N				P			
Control	15.7	16.6	15.0	19.3	1.12	1.32	0.97	1.70
3tL	18.4	17.1	15.6	18.3	1.04	1.22	0.90	1.58
6tL		17.3	13.3	17.6		1.30	0.88	1.49
9tL		15.5	14.6	19.4		1.17	0.82	1.56
18tL		18.1	15.2	18.3		1.28	0.85	1.53
		*						*
	K				Ca			
Control	5.08	6.05	5.90	6.27	2.10	2.63	3.56	2.69
3tL	5.10	5.33	5.70	5.68	2.30	2.99	4.97	4.52
6tL		6.62	5.67	6.00		2.94	5.57	5.24
9tL		5.84	5.81	5.90		3.40	4.92	4.91
18tL		6.36	5.12	5.71		3.45	5.73	4.75
							**	**
	Mg							
Control	0.56	0.66	0.76	0.62				
3tL	0.53	0.58	0.62	0.57				
6tL		0.64	0.86	0.62				
9tL		0.66	0.83	0.66				
18tL		0.66	0.69	0.57				

<sup>a</sup>PKMg treatment\*\*\* =  $P < 0.01$ , \* =  $P < 0.05$ .

Table 6. All curve-fitting results with  $R^2$  values > 50% of several response parameters vs. liming rate ( $\text{Mg ha}^{-1}$ ) in the liming experiment. Element dimensions as used in previous tables.

Foliar nutrient	Year	Needle age (y)	Exponential models		Linear and polynomial models			
			$R^2$	r-value	$R^2$	Intercept	Linear component	Square component
Ca	87	0.5	59.8	0.474	67.7	2.55	0.162	-0.007
Ca	88	1.5	59.1	0.615	79.6	3.87	0.269	-0.009
Ca	89	0.5	62.0	0.513	72.5	1.99	0.353	-0.016
Ca	89	1.5	63.5	0.529	89.1	2.98	0.443	-0.019
K	89	0.5			50.1	6.53	-0.026	
P	88	0.5			62.8	1.45	-0.014	
P	88	1.5			69.6	0.928	-0.006	
P	89	1.5			74.0	0.675	-0.030	0.001

unaffected by K, Mg and lime, indicating that the P-supplied trees utilized N more efficiently.

Evaluation of nutrient concentrations in the 0.5-y needles by the standards given in Table 2 shows that N was 'high' since 1987. P in the 0.5-y needles was increased to 'normal' in the +P plots since 1987. K and Mg remained in the 'normal' range since 1986, both with and without fertilization. The Ca concentrations remained 'low' in the -Ca plots and were increased to 'normal' by liming since 1987.

The P/N and K/N mass ratios were always in the normal range, although each year the P/N ratio was significantly increased by P application and in 1987-1989 the K/N ratio by K application. The Mg/N ratio was significantly increased by Mg application in 1987-1989, but remained 'low', or at best reached the lower part of the 'normal' range. It was always < 0.06, which is the optimal Mg/N ratio according to Ingestad (1979). K/Ca was significantly reduced to the 'optimal' range by liming in 1987-1989. K/Mg was 'normal' or 'high' (but always < 9) throughout the whole period, although in 1987-1989 it was significantly reduced by Mg application and significantly increased by K application in 1988-1989.

*Liming experiment.* Also in the liming experiment the foliar concentrations quickly responded to the applications (Table 5). The Ca concentrations in 1986 were anomalously low, as in the factorial experiment, and did not show treatment effects, but in 1987-1989 the Ca concentrations of the 0.5-y needles were increased by liming. In 1987 and 1989 the linear and quadratic components of the liming effect were significant, and polynomial models fitted better than exponential models (Table 6). In 1988 and 1989 the Ca concentrations of the 1.5-y needles were significantly increased by liming, also according to polynomials. From the fitted curves it could be inferred that 0.5-y foliar Ca concentrations peaked at 12 and 11  $\text{Mg lime ha}^{-1}$ , in 1987 and 1989, respectively. For Ca in both needle classes RMAOV showed significant liming effects over 4 years.

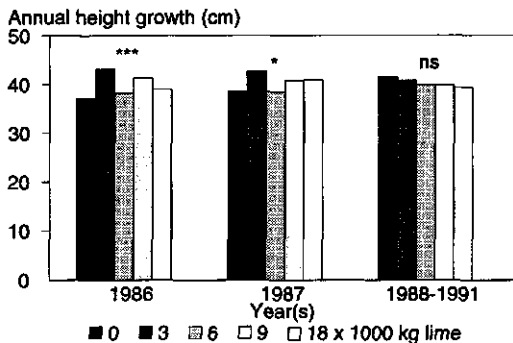


Fig. 1. Annual height growth of Scots pine as influenced by various lime applications (liming experiment); ns: not significant; \*:  $P < 0.05$ , \*\*\*:  $P < 0.001$ .

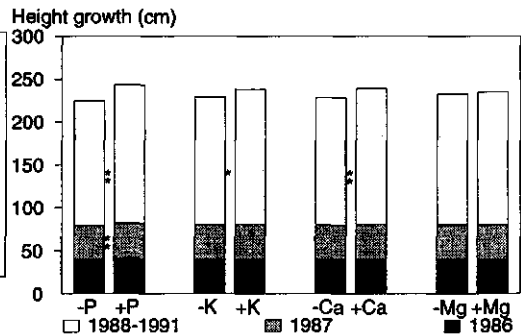


Fig. 2. Height growth of Scots pine as influenced by applications of P, K, Mg and lime (Ca) (factorial experiment); ns: not significant; \*:  $P < 0.01$ , \*\*:  $P < 0.0001$ .

There were only a few effects of liming on other nutrient concentrations. Any influence of the additional amount of Mg applied by liming was probably nullified by the standard Mg application. In 1987 liming significantly raised the N concentrations of the 1.5-y needles, but the effect was not consistent: the 9tL treatment had a relatively low N concentration (Table 5). Liming consistently reduced the N concentrations in 0.5-y needles, as was also observed in the factorial experiment. This effect was not significant in individual years, but RMAOV showed a significant liming effect over 4 years ( $P = 0.02$ ).

In 1988 P in the 0.5-y and 1.5-y needles was linearly decreased by liming. In 1989 P in the 1.5-y needles was lowered by liming, with a linear and quadratic correlation with liming level (Table 5).

The K/Ca mass ratio was lowered by liming in 1988 and 1989, with a reduction of the same magnitude for all liming rates. The K/Mg ratio was not influenced by liming.

#### *Mensurational data - factorial and liming experiments*

The height measurement results are controversial for the factorial and liming experiment. In the liming experiment there was an immediate treatment effect, that vanished after 1989 (Fig. 1), whereas in the factorial experiment there was a delayed response (Fig. 2). The height increments in the liming experiment had no apparent relationship with the liming levels, which hampers the interpretation of underlying mechanisms. In 1987 in the factorial experiment, height growth was significantly increased by P and in 1988-1991 by P, K and lime, with a significant  $P \times K \times \text{Mg}$  interaction. This interaction expresses that Mg caused an additional height growth only when both P and K were not applied.

The annual relative volume growth rate,  $\text{rgr}_v$ , in the factorial experiment was not

Table 7. Annual relative growth rates ( $\times 10,000$ ) of basal areas ( $\text{rgr}_b$ ) and cone volumes ( $\text{rgr}_v$ ) in the factorial experiment.

Treatment	$\text{rgr}_b$ per period					$\text{rgr}_v$ per period		
	1986	1987	1988	1989	1990-1991	1986	1987	1988-1991
-P						1001	960	893
+P						996	1003	941 **
-K	627	645	642	417	626	982	974	890
+K	661	655	691 *	422	657	1014	989	944 *
-Ca						984	979	898
+Ca						1013	984	936
-Mg						998	999	920
+Mg						999	965	913

\*\* =  $P < 0.01$ .Table 8. Annual relative growth rates ( $\times 10,000$ ) of basal areas ( $\text{rgr}_b$ ) and cone volumes ( $\text{rgr}_v$ ) in the liming experiment.

Treatment	$\text{rgr}_b$ per period					$\text{rgr}_v$ per period		
	1986	1987	1988	1989	1990-1991	1986	1987	1988-1991
Control <sup>a</sup>	632	608	658	425	634	976	954	977
3tL	692	661	783	431	701	1095	1044	977
6tL	702	705	691	488	680	1056	1051	979
9tL	634	761	729	419	685	1031	1123	970
18tL	672	655	711	482	652	1020	1006	954

<sup>a</sup>PKMg treatment

significantly affected by any nutrient, up to and including 1987 (Table 7). In the period 1988-1991, P and K significantly increased  $\text{rgr}_v$  (Table 7;  $P = 0.006$  and  $0.003$ , respectively). There were no significant interactions between treatments. The volume increments of individual trees expressed on an area basis were  $15.2$ ,  $16.3$  and  $17.5 \text{ m}^3 \text{ ha}^{-1} \text{ y}^{-1}$  in 1986, 1987 and 1988-1991, respectively, and the additional volume growth brought about by P and K in 1988-1991 corresponded with  $0.9$  and  $2.2 \text{ m}^3 \text{ ha}^{-1} \text{ y}^{-1}$ , respectively. In the latter period the actual increases in standing crop in the field were smaller, because of the storm and thinning in 1990. The actual standing stem volumes were  $135$ ,  $150$ ,  $167$  and  $218 \text{ m}^3 \text{ ha}^{-1}$  in 1985, 1986, 1987 and 1991, respectively.

The annual relative basal area growth rate,  $\text{rgr}_b$ , was raised in the +K plots, which effect was significant in 1988 only (Table 7). The basal area is directly related to the stem volume, so it is likely that the strongest K effect on volume growth occurred in 1988, two years after the first fertilizer applications. The other treatments did not significantly affect  $\text{rgr}_b$ .

There were no significant treatment effects on  $\text{rgr}_v$  and  $\text{rgr}_b$  in the liming experiment (Table 8). However, the  $\text{rgr}$ 's showed a tendency to peak around the 6tL level.

## Discussion

### *Needle concentrations*

The reduced foliar N concentrations following liming may result from the lowered  $\text{NH}_4/\text{NO}_3$  ratios of extractable N (Chapter 4) and N in the soil solution at a 30-cm depth (Chapter 5) at the limed plots. Scots pine absorbs  $\text{NH}_4$  better than  $\text{NO}_3$  from a mixed N source (Chapter 3).

The reduction in 0.5-y foliar P by liming which occurred in 1986 only (Table 4), corresponds with an initial liming-effected reduction of extractable P in the forest floor, gradually turning into higher extractable P concentrations in 1989 (Chapter 4). On the other hand, the lime-independent increase in foliar P by P application (Table 4) corresponds with the lime-independent increase in extractable (available) P by P application (Chapter 4).

The Ca concentrations in both needle classes were extremely low in 1986 (Tables 4, 5). Re-analysis showed that these values were not due to inaccuracy. Moreover, the low values of the 0.5-y needles in 1986 are followed by low values in the 1.5-y needles in 1987. Annual variation in Ca concentrations is not uncommon. Variation of the same magnitude as found in the present experiment was found by e.g. Leaf et al. (1970) in red pine (*P. resinosa*) and Atterson (as cited by Van den Driessche, 1974) in lodgepole pine (*P. contorta*). In the former case the variation was related to precipitation. Van den Burg and Kiewiet (1989) reported a steady decrease in Scots pine foliar Ca since 1956 in a high  $\text{NH}_x$ -deposition area in the Netherlands and Sauter (1991) since 1978 in northern Bavaria (with rapidly increasing foliar N since ca. 1980). In the latter case foliar Ca showed a minimum value in 1984, after which there was a gradual increase until 1988. In the Netherlands foliar Ca levels were low in the same period, which was possibly caused by root damage (J. van den Burg, pers. commun.).

The responses of foliar concentrations to the various liming rates were always better described by polynomial than by exponential models (Table 6). The Ca concentrations showed negative quadratic responses to liming rate, with maxima at 11 to 12 Mg lime  $\text{ha}^{-1}$  for the 0.5-y needles. The decline of foliar Ca concentrations at higher lime applications can not easily be explained. Liming lowered  $\text{H}^+$  and Al in the forest floor and mineral soil (Chapter 4), so the circumstances for root growth and, thus, Ca uptake by young root tips (Mengel and Kirkby, 1987) were increasingly favourable at higher liming levels. At the recommended lime application of 1.5 Mg  $\text{ha}^{-1}$  (Anon., 1990) the 0.5-y foliar Ca concentrations would have been 2.8 and 2.5 g  $\text{kg}^{-1}$  in 1987 and 1989, respectively, which is sufficient for Scots pine (Table 2).

### *Stand development*

The controversial findings concerning the height increments in the factorial and the liming experiment may be related to the higher stem density of the latter (possibly

stimulating height growth and its responses). However, the inconsistent relationship with liming rates, and the absence of any response in 1988-1991, when the first liming response was observed in the factorial experiment, suggest that the observed differences are not related to the treatments.

The use of  $\text{rgr}$  values assumes exponential growth. Exponential growth of individual trees was likely, as (1) diameter growth from 1985 to 1991 was highly linear (87 % of the trees had  $R^2$  values  $> 0.90$  in linear regression of DBH on year,  $R^2$  increasing with DBH). Tree-ring analysis on 20 trees in a control plot showed that since 1978 the radial growth had been remarkably constant at ca.  $1.5 \text{ mm y}^{-1}$  (data not shown); and (2)  $\text{rgr}_b$  and  $\text{rgr}_v$  had been reasonably constant throughout the measurement period (Tables 6, 7).

After canopy closure a stand is less responsive to fertilization than before. However, thinning can be seen as a temporal return to the stage before canopy closure. In that stage biomass aggradation is not completely balanced by litter fall and there is greater competition for soil nutrients (Miller, 1981). The 1985 thinning, therefore, probably enabled the stand to respond to fertilization. Such a reasoning is supported by the  $\text{rgr}$  being essentially constant throughout the measurement period. Trees in a fully occupied stand would exhibit a more linear, or even declining, annual volume increment.

Theoretically, a second growth response could have been expected after the storm and thinning in 1990 when part of the canopy was opened. However, as shown, the data indicate that the strongest response occurred in 1988 (Table 7), i.e. before thinning. Spiecker (1991) found that the greatest fertilization and liming responses occurred in favourable years. 1988 was a very favourable year for Scots pine, with a cool and cloudy summer probably preventing drought stress. This is reflected in a relatively high  $\text{rgr}_b$ , especially in the fertilized plots (not the control plots). The low  $\text{rgr}_b$  in 1989 is probably due to the extremely dry and sunny weather in May, followed by a relatively dry summer.

In the period 1990-1991,  $\text{rgr}_b$  recovered from the depression in 1989, despite very dry periods in both summer seasons. However, no fertilization effects were observed. Probably, the 1990 thinning stimulated basal area growth, in spite of the unfavourable weather conditions. The absence of any significant fertilizer effect on basal area growth after canopy opening in 1990 might suggest that at that time the growth potential was governed more by light interception than by nutrient availability.

In the present experiment there was no obvious relationship between foliar nutrient concentrations in any year and the  $\text{rgr}_v$  in 1988-1991 (as examined by computer-aided selection of multiple regression models). In the liming experiment, foliar Ca concentrations and the relative growth rates showed similar optimum curves versus liming rate, but there was no positive correlation between foliar Ca and growth rates.  $\text{Rgr}_v$  and  $\text{rgr}_b$  in 1986 were negatively correlated with the 1.5-y foliar Ca concentrations of that year. This suggests that high growth rates in the previous year reduced foliar Ca by dilution. After the start of the experiment such an effect was eliminated by an



increased Ca availability.

#### *Foliar analysis and fertilizer recommendations*

Van den Driessche (1974) stated that in silviculture annual variations in nutrient concentrations may limit the value of foliar analysis as a diagnostic tool, because the situation in any one year may not be representative. In the present research, the 1985 foliar analysis correctly reflected the trees' nutritional status, because throughout the experimental period the foliar nutrient concentrations in the control plots remained in the same ranges as found in 1985. Only the rating for N changed, as it increased to  $> 18 \text{ g kg}^{-1}$  (i.e. 'high') after 1986. Also, the nutrient ratios in the control plots remained in the same ranges as in 1985. However, the classification of the ranges may have to be reevaluated. German (Hüttl, 1986) and Dutch (Anon., 1990) standards agreed upon foliar K being sufficient and P and Ca deficient, whereas Mg was rated deficient by the former and normal by the latter. Most remarkably, the K/Ca ratio was very high. Therefore, if the rating in Table 2 is correct, it may be surprising that besides P, K increased volume growth, and that lime did not. However, in 1985 the N/K ratio was not higher than 'normal', leaving room for a growth response to K application. Moreover, foliar K and P concentrations were the first to increase after fertilization, while Ca and Mg responded later (Table 4). This suggests that there was a crop demand for P and K, which was correctly predicted for P, but not consistently for K. The fact that a highly mobile and a highly immobile nutrient showed the first responses does not support the assumption that mobility determines the response time.

Additional volume growth due to liming is usually to be expected after a few years. Spiecker (1991) reports a long-lasting effect of liming on volume growth of Norway spruce starting about 5 years after lime application. Growth responses to liming of Scots pine can be negative or positive, but are usually small (Van den Burg, 1985, 1986). Ca deficiency is usually not caused by a deficient Ca bioavailability, but by a disability of the plant to absorb Ca. The elimination of this disability, e.g. root damage due to acidification, will take longer than the actual improvement in Ca availability.

The lack of growth response to Mg application suggests that in the present situation the Dutch standards are more valid than the German ones. Mg in the soil solution at a 30-cm depth was most significantly increased by Mg application until the final measurements in spring 1992 (Chapter 5). However, the consistently 'low' Mg/N ratio would indicate that Mg sufficiency was not evident. Mg uptake may have been hampered by drought occurring in the growth seasons of 1989, 1990 and 1991. Al may also have inhibited Mg uptake. In the unfertilized plots it may have done so by a low Mg:Al ratio and in the fertilized plots by an increased absolute Al concentration (Chapter 5). Huang and Bachelard (1993) found that *Pinus radiata* shoot and root concentrations of Mg decreased with increasing Al concentrations in nutrient solutions.

## Conclusions

Nitrogen is usually the nutrient limiting plant growth most. In a situation of ample nitrogen supply through atmospheric deposition, growth rates may be high in spite of developing deficiency syndroms or malfunctioning (Nihlgård, 1985). Consequently, growth responses to fertilization with mineral nutrients may be small, and not necessarily indicative of tree vitality.

Assuming that growth responses to nutrient additions are related to nutrient demand, evaluation of foliar nutrient concentrations in 1985, according to standards listed in Table 2, did not always lead to correct conclusions regarding the forest's nutritional status. In this experiment the largest increase in volume growth,  $2 \text{ m}^3 \text{ ha}^{-1} \text{ y}^{-1}$ , was observed after K application, although foliar K concentrations of unfertilized trees were rated 'sufficient'. However, the K/N ratio was 'normal', while only at an 'optimal' ratio no fertilization effects are to be expected. This shows that nutrient ratios may more precisely indicate nutrient deficiencies than do concentrations. Foliar P and Mg ratings correctly predicted that the stand's growth would increase after P and not after Mg application, respectively. However, the additional growth caused by P application was small:  $0.9 \text{ m}^3 \text{ ha}^{-1} \text{ y}^{-1}$ , while the rating 'low' suggested that fertilization was likely to result in a profitable growth increase. From this short-term experiment it can not be judged whether such growth increases are profitable.

Liming did not give a growth response, in spite of a rating 'deficient' for foliar Ca and improved Ca concentrations after liming. This does not necessarily mean that the rating for Ca should be corrected, since the response may be delayed. It may be deemed positive that liming reduced foliar N concentrations in this area with high N deposition, because such a reduction may lower the susceptibility to plagues and diseases (Nihlgård, 1985). The reduction in foliar N is likely associated with a lowered  $\text{NH}_4/\text{NO}_3$  ratio of total available N. Such a lowering may inhibit N absorption by pine trees absorbing  $\text{NH}_4$  better than  $\text{NO}_3$  from a mixed N source (Chapter 3). However, liming did promote N mineralization (Chapter 7), and thus the possibility of  $\text{NO}_3$  leaching. A reduced N accumulation in biomass will further corroborate this effect.

As the interpretation of foliar analysis for healthy trees is still uncertain, this method has limited value as an indicator or predictor of forest damage. Several other indicators were proposed for that aim, e.g. leaf surface properties (Cape et al., 1989; Turunen and Huttunen, 1990), xylem sap flow velocity (Čermák and Kučera, 1990) and needle water potential (Maier-Maercker and Koch, 1991). Techniques to understand forest ecosystems by remote sensing are developing (e.g. Hagman, 1985; Wessman et al., 1988a,b) and may become powerful tools to assess forest vitality.

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**Nitrogen mineralization and  $H^+$  transfers in the forest floor and mineral soil of a Scots pine (*Pinus sylvestris* L.) forest, as measured by the ion exchange resin core technique**

*Co-authors: M.L. van Beusichem and A. van Diest*

**Keywords:** ammonification, in-situ incubation, ion exchange resin, liming, mineralization, nitrification, nitrogen, *Pinus sylvestris*, soil acidification

## Abstract

In a mature Scots pine stand subjected to high  $\text{NH}_4^+$  deposition the role of soil N transformations in  $\text{H}^+$  transfers was determined. Soil cores were used of which both ends were closed with layers of ion exchange resin (IER) to prevent influx and efflux of ions. The effect of liming on N transformations and the resulting soil acidification was investigated in 7 incubation periods of each ca. 8 wk. Because of its high mobility  $\text{NO}_3$  accumulated in both IER layers at the expense of that in the forest floor and mineral soil. Annually, both  $\text{NO}_3$  and  $\text{NH}_4$  accumulated in the soil cores as a whole, yielding total mineralized amounts of 40 and 77 kg N  $\text{ha}^{-1} \text{y}^{-1}$  in the control and limed plots, respectively. In both treatments ca. 65% of mineralized N was nitrified. Total acidification due to N transformations amounted to ca. 1.2 mol  $\text{ha}^{-1} \text{y}^{-1}$  in the control and limed plots. Isolated forest floor material has insufficient capacity to buffer such acid inputs, but in the field its buffering capacity is maintained by mass turnover. Liming reduced the amount of C in the forest floor, but not its mass, because of an increased mixing with mineral particles.

## Introduction

To quantify the overall proton budget of an ecosystem's nitrogen cycle, several nitrogen transformation rates must be known. In an experiment discussed elsewhere (Chapter 3) the effect of nitrogen uptake by trees on soil pH was estimated. It was shown that trees may acidify their rhizosphere by absorbing ammonium in larger amounts than nitrate. The extent to which this source of acidification may be balanced or corroborated by net N mineralization or immobilization is investigated in the present research.

The rate of N mineralization can be assessed by several techniques, but the most common procedure is to measure the concentration of the inorganic N components in a substrate at the beginning and at the end of a defined period of time. Increases and decreases in inorganic N ( $\text{N}_i$ , i.e.  $\text{NH}_4 + \text{NO}_3$ ) are defined as net mineralization and immobilization, respectively, the occurrence of which depends on the balance between gross ammonification, nitrification and immobilization. Besides the specific properties of the substrate and its microflora the transformation rates are governed by the incubation circumstances. In the laboratory these circumstances can be controlled, but the transformation rates can not directly be translated to the field situation. In-vitro incubations, therefore, may detect causal relationships between transformation rates and external conditions, while actual transformation rates should

be assessed by in-situ techniques. Laboratory results, then, help to identify mechanisms that may have led to observations made in the field.

The development of in-situ techniques for determining N mineralization in soil is marked by attempts to keep the circumstances in the incubated soil close to those in the surrounding soil. This mainly pertains to the length of the incubation period and the degree of soil disturbance. A reduced incubation time prevents artifacts caused by progressive changes in the substrate. Sequential sampling (Eno, 1960) is a way to measure mineralization during a long period of time with a reduced incubation time. Nadelhoffer et al. (1984) applied this method in an essentially unchanged way. They used polyethylene (PE) bags filled with soil that were left in the field. PE bags are permeable to  $CO_2$  and  $O_2$ , but not to water. Soil disturbance was inevitable, and a further drawback of this method is the elimination of ambient moisture fluctuations. An advantage is the possibility to determine the initial and final concentrations of elements in the same sample. Raison et al. (1987) demonstrated a methodology of sequential sampling with soil cores. This technique had been described before by Adams and Attiwill (1986). The use of soil cores instead of polyethylene bags allowed incubation of undisturbed soil samples. Similar methods were used by Becquer et al. (1990). The incubation interval had to be relatively short to prevent leaching of nitrate, or, alternatively, the cores should be covered to prevent downward water transport. This method has some drawbacks: still, the moisture content in the confined soil can not fluctuate as in the environment, and the initial concentrations must be measured in separate samples. Even when the latter is done in samples taken close to the incubated soil, an additional source of variability is introduced.

Adams et al. (1989) discussed the core technique mentioned above and found it applicable under certain specified conditions. In the contained soil plant uptake is prevented, while ammonification, nitrification and immobilization are assumed to proceed at the same rates as in the surrounding soil. Therefore, variations in ammonium and nitrate concentrations are caused by microbial processes (and deposition) only and the net rates of these processes can be estimated. Adams et al. (1989) observed that the applicability of the technique depends on the validity of these assumptions and concluded that its validity decreased with increasing length of the containment period. On the other hand, the incubation period should be long enough to yield detectable mineralization.

DiStefano and Gholz (1986) modified the soil core technique by confining the soil column between two layers of ion exchange resin (IER). Thus, water flow through the column is allowed to proceed as in the surrounding soil. The top IER layer prevents atmospheric deposition of ions into the soil, and leached ions are trapped in the bottom IER layer. This technique prevents accumulation of mineralization products and a concomitant possibility of re-immobilization, thus allowing longer containment periods (Binkley and Hart, 1989). In the present research a modified IER core method was used to quantify N mineralization and nitrification in a mature Scots pine stand.

## Materials and methods

### *Site*

The study area is situated near Harderwijk, the Netherlands. The soil is a coarse sandy fluvioglacial deposit covered by a fine drift sand layer of varying depth (10-40 cm) with a weak podzolic soil formation and a forest floor that is distinct from the underlying mineral soil. The site was planted with Scots pine (*Pinus sylvestris* L.) in 1960. In 1985 a forest fertilization experiment was started, including applications of P, K, Mg and lime in a 2<sup>4</sup> factorial lay-out with 3 replicates (Chapter 4). N mineralization was investigated in the control plots and the limed plots only. The latter had received 3000 kg CaCO<sub>3</sub> (powder) in autumn 1985.

### *Sampling technique*

For the incubation sharp-edged PVC tubes (thickness 2.5 mm, length 30 cm, inner diameter 45 mm) were used. The tubes were perforated with 20 holes ( $\phi$  3 mm) to allow for some moisture equilibration with the surrounding soil, without mass flow taking place. In the top part of the tube larger holes were made in which a rod could be inserted to pull the tube out of the soil.

IER cores were prepared by inserting IER bags in the top- and bottom ends of the incubated tubes. The bags were manufactured by sewing together two discs of nylon netting (mesh-size 50  $\mu$ m). In each bag a small hole was left open to allow insertion of a PVC brace and filling with IER, after which the hole was closed. The braces consisted of 0.5-cm high PVC rings (sawn from the same tubing as used for the sampling cores) in which 2-cm openings were cut. Thus, a brace could be squeezed together and, after insertion into the tube, could coil back, thus pressing the IER bag against the wall (Fig. 1). By this construction the bags could easily be inserted and removed again and subsequently be extracted and regenerated for following incubation periods (damage during handling and degeneration of the resin by drying limited the number of periods in which the bags could be used). One layer of IER consisted of two bags that were filled with a strongly acid cation exchanger (on top) and a strongly basic anion exchanger (Merck Ion Exchanger I and III, respectively). After the incubation period each such pair of bags (air-dry) was extracted with 100 mL 1 M KCl. Regeneration was carried out by rinsing the cation- and anion IER bags several times in 4% HCl or 3% NaOH, respectively, and subsequently in deionized water. Resin completely saturated with K and Cl, respectively, could also have been used for a new incubation, but in that case the exchange of cations in the top layer would have caused an unwanted input of a nutrient cation (K) into the incubated soil. In case an effect of K on the investigated transformations is not anticipated, a single layer of a mixed-bed ion exchanger can be used in combination with KCl extraction.

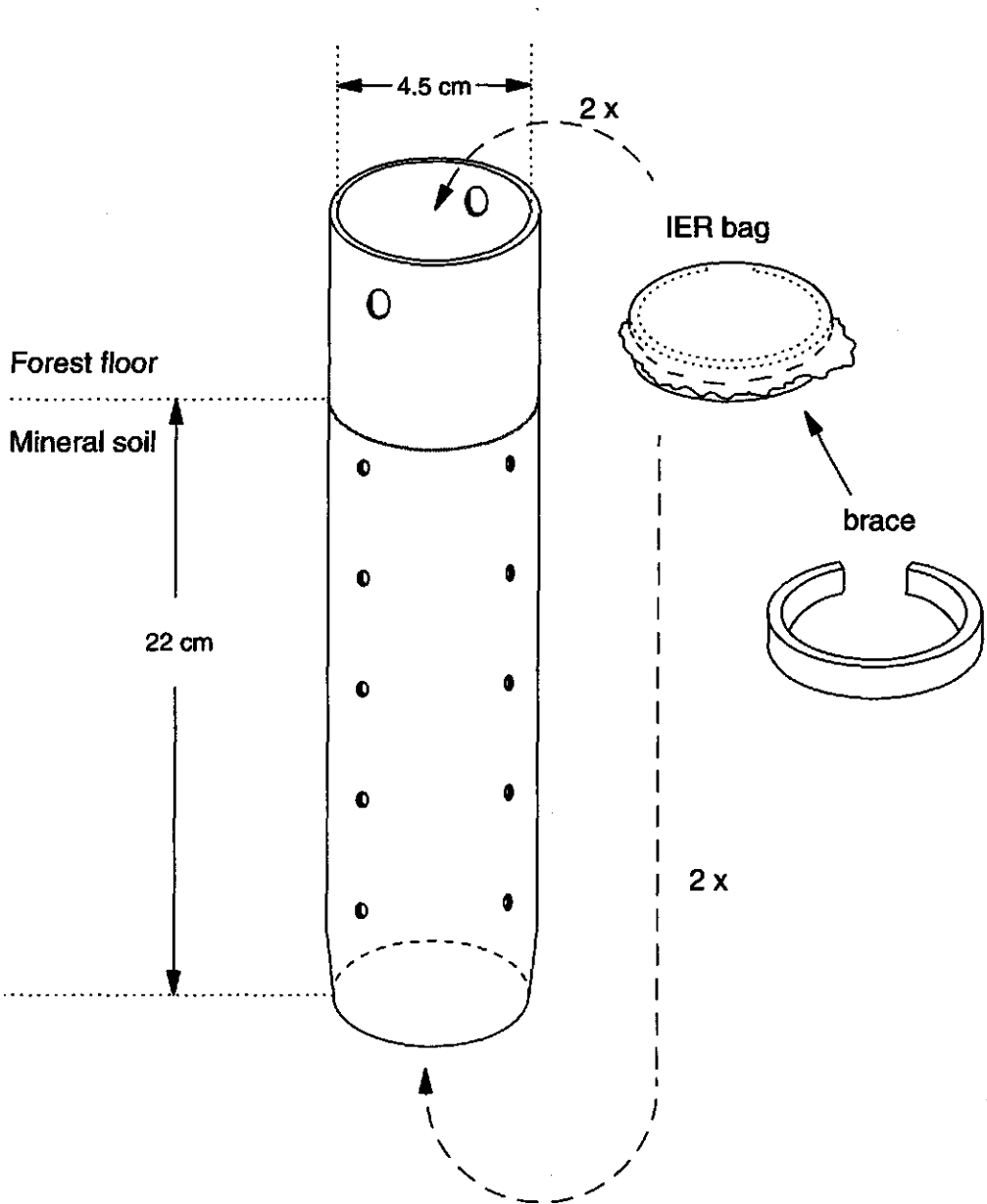


Fig. 1. Construction of an IER core for in-situ incubation of undisturbed forest soil



### Methodology

In each plot 4 sampling spots were chosen to be used throughout the research period. At the start of each incubation period (Table 1) at each sampling spot two tubes were driven into the forest floor and mineral soil to a 22-cm depth below the forest floor-mineral soil boundary, leaving ample space above the forest floor for placement of IER bags. Of every 2 tubes one was removed directly and taken to the laboratory for analysis (denoted as b-core), while the other one (denoted as i-core) was prepared as IER core by replacing 1 cm of soil in the bottom by two IER bags and placing two IER bags on top of the enclosed forest floor (Fig. 1). After 8-week periods, new cores were inserted as closely as possible (ca 20 cm) to the previous i-cores.

This procedure was repeated every 8 weeks (48 days for the second period) during one year. The sampling dates are denoted  $T_0$ - $T_7$  (Table 1). The change in amounts of N species per  $m^2$  ( $\Delta N$ ) in the forest floor and mineral soil during an incubation period  $T_{(n)}$  to  $T_{(n+1)}$  were calculated for each sampling spot separately as the difference in  $NH_4$  or  $NO_3$  between the i-cores at  $T_{(n+1)}$  and the b-cores at  $T_{(n)}$ . The amounts of N trapped in the bottom bags at  $T_{(n+1)}$  were added to the former to yield the total accumulation of each N species in the whole column during the incubation period. These amounts will be referred to as 'column totals'. The  $H^+$  accumulation caused by net mineralization is calculated by subtracting the amounts of  $NH_4$ -N formed by net ammonification from the amounts of  $NO_3$ -N formed by net nitrification. Nitrification produces 2 mol  $H^+$  per mol N nitrified, but it is preceded by ammonification, which consumes 1 mol  $H^+$  per mol N.

### Length of the incubation periods

The incubation length of 8 wk was a compromise between the necessity to reduce the period of containment as much as possible and available labour. Adams et al. (1989) found that N mineralization measured by in-situ incubations in Eucalyptus forests was not linear in time when the incubation period was longer than 1 wk. However, Kriebitzsch (1987) found linear increases of  $N_i$  in 8-wk in-vitro incubations in soils with lower mineralization rates. Before the present research, a preliminary study with

Table 1. Starting dates and codes of the incubation intervals.

Code	Sampling date	Incubation interval (days)	Number of interval used in text
T0	19-Mar-91		
T1	14-May-91	56	1
T2	01-Jul-91	48	2
T3	26-Aug-91	56	3
T4	21-Oct-91	56	4
T5	16-Dec-91	56	5
T6	10-Feb-92	56	6
T7	06-Apr-92	56	7
		— +	
		384	

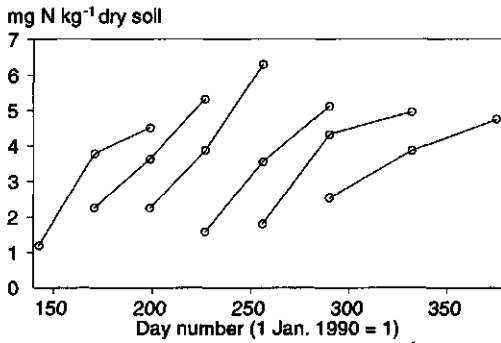


Fig. 2. Inorganic N concentrations ( $\text{mg kg}^{-1}$ ) in 25 cm mineral soil incubated for 4 or 8 wk in non-IER cores.

overlapping incubation periods was carried out in a control plot, using non-IER cores. The accumulation of  $N_i$  (dominated by  $\text{NH}_4$ ) in the mineral soil was linear in 8-wk periods (Fig. 2). IER-cores reduce the risk of accumulation and reimmobilization of  $N_i$ . Therefore, the 8-wk incubation periods used in the present research probably did not cause serious artifacts.

### Analysis

After collection the cores were stored at  $4^\circ\text{C}$ . As soon as possible, usually within 2 days, the soil was pushed out of each core and subdivided in litter and mineral soil. The bottom 1 cm of each b-core was discarded. The mineral soil was sieved over a 2-mm sieve. Litter was dried at  $30^\circ\text{C}$  in a forced-draft oven, and ground. All fractions were weighed and the gravimetric moisture content of the  $< 2$  mm soil fraction was determined. Subsamples of the  $< 2$  mm soil fraction (3 g field-moist material) and the ground litter (1.5 g) were extracted with 30 mL 0.01M  $\text{CaCl}_2$ . In the extracts  $\text{NH}_4$  and  $\text{NO}_3$  and pH were measured as described elsewhere (Chapter 4).  $\text{NH}_4$  and  $\text{NO}_3$  in the KCl extracts from the IER bags (see above) were measured colorimetrically on a continuous-flow system. In the forest floor material of the  $T_4$  b-cores total N, P, K, Ca and Mg were measured as before (Chapter 4) and organic C using the Kurnies method.

To conform with previous routine analyses (Chapter 4) 0.01 M  $\text{CaCl}_2$  was chosen as extractant. This electrolyte is thought to extract the available soil nutrient pool (Houba, 1990). Probably the absolute amounts of nutrients extracted with e.g. 1 M KCl would have been higher, especially in the forest floor. The differences between i-cores and b-cores, however, might have remained in the same range, as a stronger electrolyte would have extracted more of the adsorbed pool, that probably was essentially the same for the b- and the i-cores.

### Titration curve

To get an impression of the impact of acidification on the soil pH, titration curves of the limed and untreated forest floor and soil material were made. Various quantities of 1 M HCl in 1 M KCl (forest floor) or 0.02 M HCl in 0.01 M  $\text{CaCl}_2$  (soil) adding up to 50 ml were added to 2.5-g subsamples from the pooled b-core material of the forest floor sampled at  $T_4$  and of air-dried soil sampled at  $T_7$ . The maximum acid additions corresponded with ca. 180 years of total acidification due to N transformations and

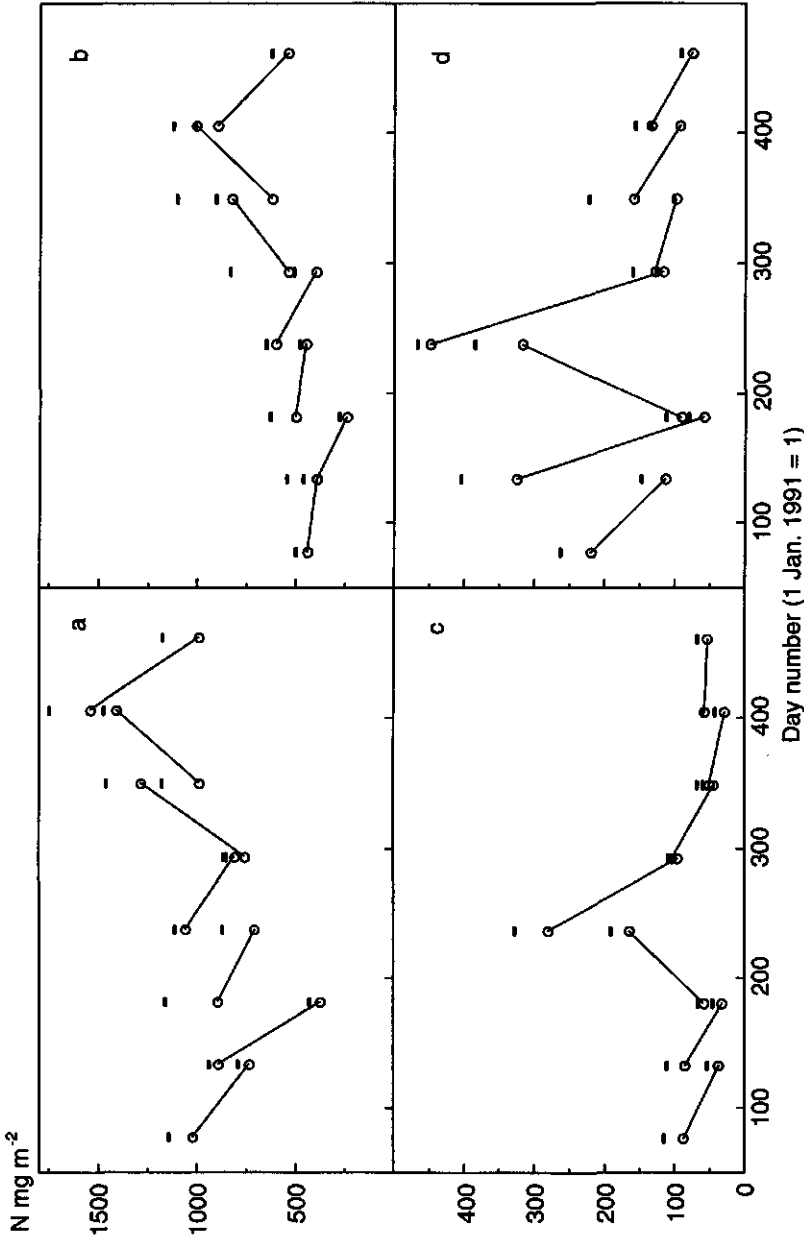


Fig. 3. Changes in amounts of  $\text{CaCl}_2$ -extractable inorganic N ( $\text{mg N m}^{-2}$ ) in the untreated and limed forest floor during 7 successive incubation periods. a  $\text{NH}_4$  control; b  $\text{NH}_4$  lime; c  $\text{NO}_3$  control; d  $\text{NO}_3$  lime. Dashes indicate standard errors of mean ( $n = 3$ ).

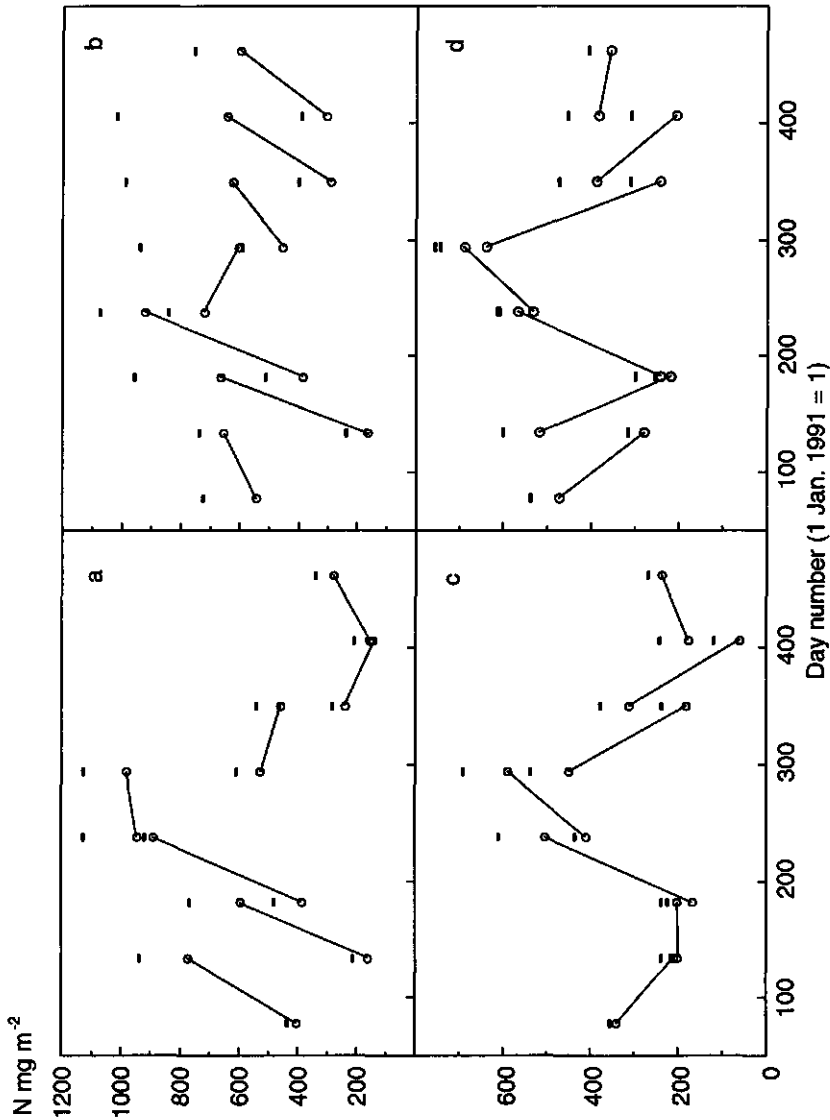


Fig. 4. Changes in amounts of  $CaCl_2$ -extractable inorganic N ( $mg\ N\ m^{-2}$ ) in 22 cm mineral soil of untreated and limed plots during 7 successive incubation periods. a  $NH_4^+$  control; b  $NH_4^+$  lime; c  $NO_3^-$  control; d  $NO_3^-$  lime. Dashes indicate standard errors of mean ( $n = 3$ ).

deposition/leaching proceeding at the current rate, if this were to be neutralized by the forest floor only *or* by the mineral soil only. Thus, because of the low forest floor mass relative to that of the soil, the forest floor samples received larger acid quantities per mass unit. The suspensions were shaken for 2 hours a day during 1 week, after which the pH was measured.

### *Calculations*

For statistical calculations SAS software was used (SAS Institute Inc., 1990). For each incubation period,  $\Delta\text{NH}_4$ ,  $\Delta\text{NO}_3$  and  $\Delta\text{N}_i$  were averaged over the four sampling spots per plot. For the whole sampling period these entities were totalled per sampling spot. By maintaining the same sampling spots throughout the experiment, the plot average of the sums per sampling spot could be calculated. Taking random sampling spots for each incubation period would have required a calculation of the sum of plot averages. The former procedure reduces variability in total  $\Delta\text{N}$ . The averages per plot were used in ANOVA to test the significance of liming effects and of the difference between  $\text{NH}_4$  and  $\text{NO}_3$  within one treatment. For ions intercepted by the top IER layer medians per plot were calculated, as these data showed a number of outliers.

## **Results**

### *General patterns*

The amounts of  $\text{NH}_4$  in the forest floor and the mineral soil were larger than those of  $\text{NO}_3$ , both for the control and the lime treatment (Figs. 3, 4). This is commonly found in the research area (Chapter 4). The forest floor and mineral soil showed a large seasonal variability in  $\Delta\text{N}$  (Figs. 3, 4). In many cases the amounts of each N species decreased (Table 2). In the forest floor net accumulation of  $\text{NH}_4$  occurred in periods 5 and 6 only, i.e. in early winter. Forest floor  $\text{NO}_3$  accumulated in the 3rd period only, which was in mid summer (Table 2). The mineral soil showed a net accumulation of  $\text{NH}_4$  in most cases, decreases only occurring in the colder season, whereas soil  $\text{NO}_3$  only accumulated in the warmer season (Table 2). As a consequence, of both N forms in each layer only  $\text{NH}_4$  in the mineral soil showed a net annual accumulation (Table 2). In very few cases only a weak relationship could be found between  $\Delta\text{N}$  in the forest floor and  $\Delta\text{N}$  in the mineral soil. These relationships were mostly positive, which means that accumulations of N in the mineral soil could not be explained by N influx from the forest floor.

The amounts of  $\text{NH}_4$  trapped in the bottom resin layers were of the same order of magnitude as those accumulated in the mineral soil.  $\text{NO}_3$  accumulation in the bottom resin was usually much higher than the combined losses from the forest floor and mineral soil (Table 2). Therefore, for each incubation interval there always was a net

Table 2. Net accumulation of  $\text{NH}_4\text{-N}$ ,  $\text{NO}_3\text{-N}$ ,  $\text{N}_i$  ( $\text{NH}_4 + \text{NO}_3$ ) and  $\text{H}^+$  ( $\text{mg m}^{-2}$ ) in a Scots pine forest during 7 incubation intervals covering ca. 1 year. Underlining indicates that  $\text{NH}_4$  is significantly different from  $\text{NO}_3$  within treatment.

Soil layer	N species	Treatment	Incubation interval <sup>a</sup>							Whole period
			1	2	3	4	5	6	7	
Forest floor (a)	$\text{NH}_4$	Control	-286	-516	-184	-252	528	421	-550	-840
		Limed	-46	-152 **	-54	-205	287	388	-357	-140
	$\text{NO}_3$	Control	-50	-53	107	-185	-59	-21	-4	-264
		Limed	-106 **	-267	226	-332 *	-30	-66	-58	-633 *
	$\text{N}_i$	Control	-336	-569	-77	-437	469	400	-553	-1104
Soil (b)	$\text{NH}_4$	Limed	-152	-419	172	-537	257	322	-416	-773
		Control	370	433	504	37	-70	-95	124	1304
	$\text{NO}_3$	Limed	111	503	534	-116	170	353	292	1846
		Control	-130	0	336	180	-267	-252	61	-72
	$\text{N}_i$	Limed	-194	-298 **	325	157	-396	-182 **	-28	-616
Bottom resin (c)	$\text{NH}_4$	Control	240	433	840	217	-337	-347	185	1232
		Limed	-83	204	859	41	-225	171	264	1230
	$\text{NO}_3$	Control	64	137	156	111	191	102	91	853
		Limed	116	143	117	158	233	168 *	190	1126
	$\text{N}_i$	Control	39	201	326	350	447	318	458	2138
Column total (a+b+c)	$\text{NH}_4$	Limed	631	331	348	1076	889 *	532 *	683 ***	4491 ***
		Control	103	338	482	461	638	421	549	2991
	$\text{NO}_3$	Limed	748	475	465	1234	1122	699	874	5617 ***
		Control	148	53	477	-104	649	429	-335	1317
	$\text{N}_i$	Limed	181	494	597	-163	690	908	125	2832 *
$\text{H}^+$ accumulation	$\text{NH}_4$	Control	-141	148	768	344	121	45	516	1802
		Limed	332	-234 *	899	902	463	284 *	597	3243 **
	$\text{NO}_3$	Control	7	202	1245	241	770	474	180	3119
		Limed	512	260	1496	738	1154	1192	721	6074 **
	$\text{N}_i$	Control	-21	7	21	32	-38 #	-27	61	35 #
		Limed	11	-52	22 #	76	-16	-45	34	29 #

<sup>a</sup>For dates, see Table 1.

<sup>b</sup>Liming effect per N species: \* =  $P < 0.1$ , \*\* =  $P < 0.05$ , \*\*\* =  $P < 0.01$ , # = significantly different from 0 ( $P < 0.05$ ).

$N_i$  accumulation in the whole column and a net annual accumulation of  $NH_4$ ,  $NO_3$  and  $N_i$  was observed (Table 2). The high  $NO_3$  accumulation in the bottom resin caused the column totals of  $NO_3$  to approach those of  $NH_4$ , and no significant differences were found between the annual column accumulations of  $NH_4$  and  $NO_3$ . Therefore, the annual  $H^+$  accumulations were small, but significantly different from zero (Table 2). Most of the N mineralization occurred from mid summer until mid winter (periods 3-6, Table 2), i.e. partly in the dormant season.

### Liming effects

Six years after lime application, the limed plots still had significantly higher forest floor- and soil pH( $CaCl_2$ ) values. The average forest floor pH values of the b-cores at the limed and the untreated plots were 4.15 and 3.11, respectively. These values were 4.16 and 4.04 for the mineral soil. Any pH differences between the b- and i-cores within one treatment were not significant. The pH at  $T_4$  and pH values measured in previous autumn seasons (Chapter 4), show that the forest floor pH of the limed plots progressively declines, while that of the control plots is stable (Fig. 5). In the mineral soil the annual variation in autumn-pH values obscures any discernible trend.

The amount of forest floor C at  $T_4$  was lower in the limed than in the control plots. However, the forest floor masses were identical for both treatments (Table 3). This indicates that at the limed plots more mineral particles were mixed into

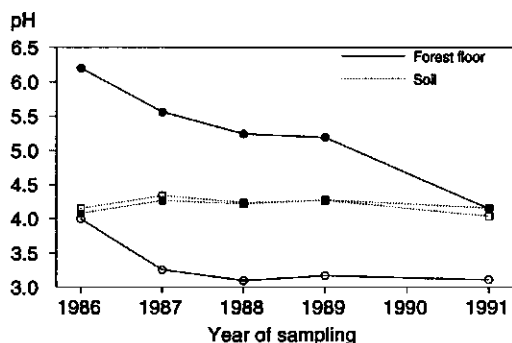


Fig. 5. Autumn pH- $CaCl_2$  values in the forest floor and mineral soil in the period 1986-1991. Open symbols: control; solid symbols: limed.

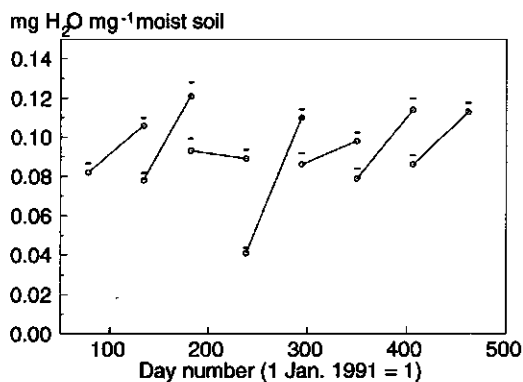


Fig. 6. Changes in gravimetric soil water content (g water/g 105°C dry soil) in 22 cm mineral soil during 7 successive incubation periods.

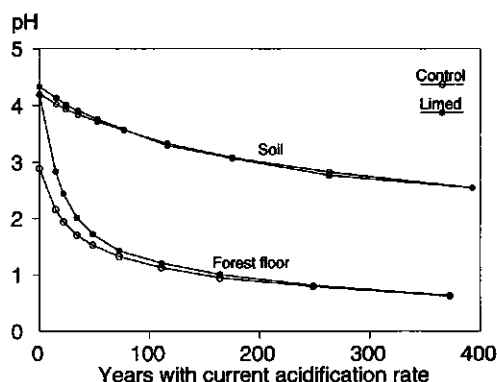


Fig. 7. pH in suspensions of forest floor and soil material equilibrated for 1 week in 1 M KCl + various quantities of 1 M HCl (forest floor) or in 0.01 M  $CaCl_2$  + various quantities of 0.02 M HCl (soil). The acid applications are expressed in number of years with the current acidification rate.

Table 3. Forest floor mass, total nutrient concentrations and element ratios (g g<sup>-1</sup>) in the forest floor at 21/10/91, and C percentages in 1986 and 1991

Treatment	Mass g m <sup>-2</sup>	C g m <sup>-2</sup>	Nutrient concentrations (g kg <sup>-1</sup> )					C/N	C/P	N/P
			1986	1991	N	P	K	Ca		
Control	6990	2701	37	38	13.1	0.57	0.56	1.03	30	704
Limed	6870	1778**	32	28***	10.8	0.47	0.63	5.75***	26	581

Liming effect: \*\* =  $P < 0.05$ , \*\*\* =  $P < 0.01$ 

the forest floor by a stimulated faunal activity. In 1986, 1 y after treatment, forest floor C was not significantly lowered by liming (Table 3). Total nutrient concentrations in the forest floor, except Ca, were not significantly changed by liming, neither were the C/N, C/P and N/P ratios (Table 3).

Annually N<sub>i</sub> was released from the forest floor with no significant differences between both treatments, but liming increased the fraction of NO<sub>3</sub> lost (Table 2). Liming occasionally produced a significantly higher accumulation of NH<sub>4</sub> or NO<sub>3</sub> in the bottom IER, but annually only the accumulation of NO<sub>3</sub> in the bottom IER was significantly increased by liming (Table 2). The annual column totals of NH<sub>4</sub>, NO<sub>3</sub> and N<sub>i</sub> accumulations were significantly increased by liming (Table 2). Both at the control and at the liming treatment the proportion of NO<sub>3</sub> in the total annual N<sub>i</sub> accumulation was about 50% and, as a consequence, the annual H<sup>+</sup> accumulations in both treatments were small and approximately identical (Table 2).

#### Deposition effects

The top IER layer intercepted atmospheric deposition (Table 4), but probably also a part of the NO<sub>3</sub> produced in the forest floor (see Discussion). The interception in the top IER was quite uniform throughout the year, except for relatively high NO<sub>3</sub> values in the 3rd period. The soil moisture contents (MC) tended to increase during incubation, in spite of the holes in the PVC cores (Fig. 6). On an average the MC's of the b- and i-cores were 0.08 and 0.11 (g H<sub>2</sub>O g<sup>-1</sup> moist soil), respectively.

#### Titration curve

The forest floor pH quickly dropped with increasing additions of acid, whereas that of the mineral soil decreased gradually (Fig. 7). This difference is partly due to the larger quantities of acid added per mass unit of forest floor material. The limed forest floor neutralized an additional amount of ca. 0.18 mmol H<sup>+</sup> g<sup>-1</sup> relative to the control. This would correspond with 12.7 kmol ha<sup>-1</sup> acid neutralizing capacity that is left from the 1985 lime application, or ca. 11 times the current annual acid load due to N mineralization (Table 5). Correspondingly, Figure 7 shows that theoretically the pH of the limed forest floor would reach that of the control in 14 to 15 years. The differences in H<sup>+</sup> neutralizing capacity



Table 4.  $\text{NH}_4$  and  $\text{NO}_3$  ( $\text{mg m}^{-2}$ , medians of 4 values) and their sum in the top IER layers originating from atmospheric deposition and possibly from  $\text{NO}_3$  diffusion from the forest floor (see text)

Nitrogen species	Treatment	Incubation interval <sup>a</sup>							Sum	Possibly diffused amounts
		1	2	3	4	5	6	7		
$\text{NH}_4\text{-N}$	Control	331	394	352	526	289	515	548	2955	
	Limed	338	369	324	664	450	528	505	3178	
$\text{NO}_3\text{-N}$	Control	93	193	653	211	341	176	278	1946	894
	Limed	247	284	620	408	575	230	277	2639	1587
$\text{N}_i$	Control	424	586	1006	737	630	691	826	4901	
	Limed	585	653	944	1072	1024	758	782	5817	

<sup>a</sup>For dates, see Table 1.

between the control and limed soil are too small, relative to the total amounts of  $\text{H}^+$  neutralized, to be conclusive.

## Discussion

### *N fluxes within the column*

Reduction of  $\text{N}_i$  during incubation (Table 2) would usually be attributed to immobilization. However, in this research any decrease of  $\text{N}_i$  in the forest floor or mineral soil may be due to a combination of immobilization, exchange between both soil compartments and diffusion into an IER layer, which processes can not be discriminated. Before the experiment, it was considered to also insert IER bags between the forest floor and the mineral soil, to be able to quantify N fluxes between both soil layers. However, this idea was abandoned, to avoid disturbance of the forest floor, which might have altered the availability of energy-rich substrate for soil organisms (Salonius, 1987) and, thus, microbial transformation rates.  $\text{NH}_4$  leaching from the forest floor may have promoted the observed accumulation of  $\text{NH}_4$  in the mineral soil.

### *N fluxes into the IER layers, additional nitrification*

The amounts of N annually intercepted in the top IER (Table 4) probably exceeded throughfall deposition. In a neighbouring Scots pine stand ca. 30 kg  $\text{NH}_4\text{-N ha}^{-1}$  and ca. 10 kg  $\text{NO}_3\text{-N ha}^{-1}$  are deposited annually (Beier and Rasmussen, 1993). T-testing showed that only the intercepted amounts of  $\text{NO}_3$  significantly exceeded this amount of throughfall deposition. The excesses probably diffused from the forest floor into the top IER layer and, thus, should be attributed to nitrification (Table 4). Diffusion of  $\text{NO}_3$  into the top IER is likely because the anion exchanger was in contact with the forest floor and  $\text{NO}_3$  is relatively mobile. Consequently, total nitrification and total N mineralization were corrected (Table 5). It is import-

ant to know the origin of NO<sub>3</sub> in the top IER layer in view of the net H<sup>+</sup> accumulation that is related to the N cycle. The H<sup>+</sup> accumulation in the soil due to net influx or efflux of N can be calculated by the charge balance ( $\text{NH}_{4,\text{in}} - \text{NH}_{4,\text{out}} + (\text{NO}_{3,\text{out}} - \text{NO}_{3,\text{in}})$ , after Van Breemen et al. (1983). This means that an external NO<sub>3</sub> input may reduce H<sup>+</sup>

accumulation. On the other hand, an internal NO<sub>3</sub> accumulation due to nitrification, has an acidifying effect. Adopting the above-mentioned throughfall quantities and the resulting additional nitrification for the present research, the net H<sup>+</sup> accumulation was corrected (Table 5). For the corrected data the effect of liming was tested. Liming had no significant effect on the additional nitrification and total H<sup>+</sup> production, nor on H<sup>+</sup> production from deposition and from total mineralization, separately, but total nitrification and mineralization were significantly increased by liming (Table 5).

As mentioned, the resin layers may have served as a sink for diffusion of ions from the soil and forest floor. This was the original aim of using IER bags in the field for assessing nutrient availability (Lundell, 1989; Binkley and Matson, 1983). It would add to the benefit of using IER cores, when besides the possibility of monitoring a natural downward ion flux the IER would also (partly) simulate the sink function of absorbing roots.

#### *Liming effects on the litter accumulation/decomposition balance*

The amounts mineralized in the limed plots probably approximate the annual N returns by litter fall. De Visser (1990) found an average N return of 56 kg N ha<sup>-1</sup> y<sup>-1</sup> in the untreated plots of a nearby experiment, which amount is intermediate between those at other locations (Table 6). The variation in litter fall fluxes per location corresponds with local differences in throughfall N fluxes. In the present research, litter N return was probably equal for both treatments, as the N concentration of ca. 1.5 y-old needles (that will fall within 1 year) at the limed plots tended to be lower than that of the unlimed plots (Chapter 6). Assuming the above-mentioned amount of litter fall, the estimated mineralization rates of ca. 40 and 77 kg N ha<sup>-1</sup> y<sup>-1</sup> at the control and lime treatments, respectively (Table 5), suggest that organic matter is accumulated in the former and depleted at the latter. The lower forest floor C content at the limed than at the untreated plots (Table 3) confirms the occurrence of a different forest floor accumulation/decomposition regime for both treatments. From the present data no estimates of the organic matter decomposition rates per soil layer can be derived, because the net results of N immobilization and leaching can not be distinguished. In the long run, the limed plots may return to litter accumulation for two reasons. First, the forest floor pH will decrease again, reducing mineralization.

Table 5. Nitrification and mineralization (NH<sub>4</sub> + NO<sub>3</sub> accumulation) during 384 days incubation, corrected for NO<sub>3</sub> diffusion into the top IER layer.

Process involved	mg N or H <sup>+</sup> m <sup>-2</sup> per treatment		P
	Control	Limed	
Mineralization	4013	7662	0.03
Nitrification	2696	4830	0.01
H <sup>+</sup> production	99	143	ns

Table 6. Annual litter fall N returns in several Scots pine forests.

Location	Age (years)	Year of sampling	Litter fall N-flux kg ha <sup>-1</sup> y <sup>-1</sup>	Reference
Tongbersven (NL)	ca. 35	1984/85	80	Van Dobben et al. (1992b)
Gerritsfles (NL)	ca. 60	1984/85	72	Van Dobben et al. (1992b)
Scotland	46	1977	37	Lim and Cousens (1986)
Harderwijk (NL)	35	1988	59	De Visser (1990)
		1989	74	
		1990	34	
Average Harderwijk			56	

Second, the organic matter loss may stop when humus formed under acid circumstances is depleted and more stable humus compounds are produced by improved pH and Ca availability (Van Breemen and Brinkman, 1978; Seibt and Wittich, 1977).

Clearly, forest floor mass alone gives insufficient information on the changes in the organic C pool: the mineral particles mixed into the forest floor perfectly complemented the organic mass loss caused by liming. This may also have played a role in research carried out by Berg (1986), who found that liming did not affect forest floor mass loss rate, but profoundly altered the soil fauna, part of which influenced the forest floor. Mixing of mineral particles into material incubated in litter bags may also occur, which makes mass loss data unreliable. Marschner and Wilczynski (1991) found a forest floor C percentage that steadily declined from 27% to 17% in 3 y after liming a Scots pine forest, although not leading to a significantly lower C pool. Still, they conclude that there is strong evidence for an increased humus loss caused by liming, which may partly be due to an increase in the water-soluble C fraction. However, an increase in water-soluble organic substances by liming was not observed in previous work (Chapter 4), which showed that the concentration of extractable organic N in forest floor and mineral soil extracts was not influenced by liming.

In the present research the sharp boundary between the forest floor and the mineral soil was not noticeably affected by liming. Makeschin (1991) found that liming improved bioturbation of the forest floor and mineral soil of a *Picea abies* stand, causing the sharp boundary between both layers, typical of the control plots, to disappear. However, Kreutzer et al. (1991) found that the humus stratification in the same experiment was not disturbed 5 y after liming. They observed a low pH (pH-KCl ≈ 3) in the bottom part of the limed forest floor, presumably causing a locally suppressed earthworm activity. In addition to a higher pH, an increased faunal activity due to liming in the present research may have been an important factor increasing N mineralization in the present experiment.

#### *Withdrawal of N*

Although in the limed plots N in litter fall and N mineralization were found to be

balanced (Tables 5 and 6), this not necessarily means that N is fully recycled. Annually, a maximum of ca. 35 kg N ha<sup>-1</sup> may be stored in ca. 7000 m<sup>3</sup> stem wood volume (Chapters 3 and 6), most of which will eventually be withdrawn from the ecosystem. Besides that, it was shown that NO<sub>3</sub> leaching to below a 1-m depth occurred in the control and more so in the limed plots (Chapter 5).

#### *pH and moisture*

The changes in CaCl<sub>2</sub>-extractable  $H^+$  during incubation were not correlated with and were far smaller than the net  $H^+$  production from ammonification and nitrification in the forest floor and mineral soil (Table 2). This indicates that the  $H^+$  resulting from ammonification and nitrification was buffered.

It was observed previously (Chapter 5) that the effect of liming on NO<sub>3</sub> concentrations in the soil solution at a 30-cm depth gradually disappeared after spring 1991, while fertilization with P, K and Mg started to increase soil solution NO<sub>3</sub> since mid 1991.

The soil moisture content may influence the mineralization rate (Wilhelmi and Rothe, 1990; Clarholm et al., 1981). However, in the present research the moisture content probably did not seriously affect the N transformations: there was no correlation between the incubation cores' initial MC, final MC or average MC, on the one hand, and ammonification, nitrification (or their sum) or NO<sub>3</sub><sup>-</sup>, NH<sub>4</sub><sup>-</sup> and N<sub>i</sub> concentrations per sampling spot, on the other hand. The lack of correlation between MC and NO<sub>3</sub> concentrations of the i-cores indicates that no noticeable denitrification occurred at these MCs.

#### *Titration curve*

The sharp pH drop in the forest floor, as suggested in Figure 7, will probably not occur in reality, even though the applied acid only represented the acidification due to N transformations. It was found previously (Chapter 4) that the forest floor pH is stable at about 3.15. The probable cause is that litter fall continuously supplies the forest floor with fresh organic matter, and decomposition of organic matter prevents long exposure to acid loads for parts of the forest floor. De Vries et al. (1990) compiled some data on pools and residence times of elements in forest ecosystems. They found residence times of Scots pine forest floor mass in litter bags ranging from 2.4 to 4.2 y, depending on humus type and climate, in which time the pH will drop at most ca. 10% (Fig. 7). The significance of the titration curve lies in the fact that it indicates that the buffer capacity of the forest floor material itself is easily overestimated. Its buffering capacity is mainly a result of turnover dynamics. This means that profound disturbances such as clearcutting, that usually promote nitrification and reduce the supply of fresh organic matter, may cause severe pH drops in the forest floor.

In the field the pH decrease in the mineral soil will probably be slowed down by weathering processes that take much longer than the 1-wk titration period, and by the input of buffering substances in forest floor leachate.

## Conclusions

In the present research during in-situ incubation of the forest floor and 22 cm mineral soil of a Scots pine forest stand, soil acidification due to net ammonification and nitrification amounted to  $1.2 \text{ kmol ha}^{-1} \text{ y}^{-1}$  (Table 5). Previous results (Chapter 3) revealed that under the current circumstances prevailing in the Netherlands also tree growth in Scots pine stands may cause soil acidification, which on the basis of a pot experiment was estimated to amount to ca.  $3 \text{ kmol ha}^{-1} \text{ y}^{-1}$ . Such acidification stems from a larger uptake of ammonium than nitrate, thus leading to an excess of cation-over-anion uptake.

This form of soil acidification in forests in general must have been a common phenomenon before intensive livestock production in the Netherlands and other European countries became responsible for an additional influx of ammonium into forest soils. However, in the original situation the ammonium becoming available to the tree roots originated predominantly from the ammonification process in which the conversion of organically bound amino-N requires the consumption of  $1 \text{ mol H}^+$  per mol  $\text{NH}_4$  formed. Thus, uptake and ammonification were balanced in terms of soil acidification.

In the present situation, with high atmospheric ammonium inputs, a large part of the ammonium absorbed by tree roots is not provided by the acid-consuming ammonification process, and thus will cause soil acidification (Binkley and Richter, 1987). Moreover, the excessive availability of ammonium will enhance the naturally occurring excess cation-over-anion uptake and the consequent  $\text{H}^+$  excretion by tree roots.

The total availability of ammonium in Dutch forest soils presently exceeds the uptake capacity of the trees, thus leaving ammonium available to the nitrification process. An increased nitrate production may promote nitrate leaching, especially when nitrate is not preferentially absorbed by roots. The latter is true e.g. for Scots pine (Boxman and Roelofs, 1988; Chapter 3), Douglas fir (Kamminga-Van Wijk, 1993; Gijsman, 1991) and Norway spruce (Marschner et al, 1991; Ingestad, 1979). The strong acidification due to nitrification could become partly offset by the growth of nitrophilous species. They absorb nitrate preferentially and consequently, they exhibit an excess anion-over-cation uptake and thus consume  $\text{H}^+$ . Moreover, such species reduce the risk of nitrate leaching. However, many nitrophilous species (e.g. several Urticaceae and Rubiaceae) are generally not appreciated as contributors to a forest vegetation.

In the present research, several effects of liming on N transformations were

observed. First, liming increased net N mineralization but had no effect on the proportion of mineralized N that was nitrified. Both in the control and the limed plots ca. 65% of the mineralized N consisted of nitrate (Table 5), which suggests that the mineralization rate greatly determined the nitrification rate. This may be related to findings of De Boer et al. (1989), who hypothesized that in an acid environment ammonifying microorganisms can release the nitrification potential of acid-sensitive nitrifiers through a mechanism not yet understood. It is well established now that nitrification takes place at low pH values, although it is usually increased by liming (Killham, 1990). Second, liming increased the H<sup>+</sup> accumulation generated by N transformations. Liming may, however, have various effects on acidifying processes. It may reduce the acidification exerted by nutrient uptake because it lowers the ammonium/nitrate ratio in the soil solution and, thus, the excess of cation- over anion uptake (Chapter 3). On the other hand, by an improved pH liming may increase root density in the forest floor (Murach, 1988), where roots will be exposed to higher ammonium concentrations than in the mineral soil and thus show a higher H<sup>+</sup> excretion.

When liming is necessary, it should be applied frequently, depending on the decline rate of the forest floor pH. A constantly improved pH and Ca availability may promote the formation of stable humus compounds, which may reverse an initial loss of organic matter, and, thus, in the long run reduce the risk of an increased nitrate leaching by liming. But it should be recognized that a high pH will promote nitrification of atmospheric ammonium inputs and that choosing for frequent liming may imply choosing for a different ecosystem (Van Dobben et al., 1992a).

### Acknowledgements

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# 8

## **General discussion**

## Introduction

In Chapter 1 two investigations were presented pertaining to the effects of an enhanced  $\text{NH}_4$  deposition on forests. First it was investigated whether by an increased uptake of  $\text{NH}_4$  trees may increasingly acidify their rhizosphere, and whether the N mineralization process compensates or corroborates this acidification. In this general discussion, the data presented in the previous chapters will be used to quantify N transformation-related  $\text{H}^+$  budgets in the forest system, both for untreated and for limed Scots pine.

The second question pertains to the possibilities of forest fertilization and liming to alleviate negative effects of increased  $\text{NH}_4$  depositions. It will be discussed below whether the experiences from the present field experiment contribute to an increased understanding of these possibilities.

## N transformations related to $\text{H}^+$ turnover

### *Nitrogen uptake by trees*

In a pot experiment, three-year-old Scots pine trees appeared to acidify their rhizospheres by absorbing  $\text{NH}_4$  more readily than  $\text{NO}_3$  from the soil solution. To obtain an impression of acidification due to tree uptake under field conditions, the acidification found in this pot experiment was extrapolated without modifying soil solution  $\text{NH}_4/\text{NO}_3$  ratios or plant N concentrations to those which may be expected in the field (Chapter 3). Moreover, it was assumed that N uptake of mature trees was reflected by N required for the growth of new biomass. Sapwood generally has higher N concentrations than heartwood (De Visser, 1992; Lim and Cousens, 1986), but part of the N uptake needed for the growth of sapwood is provided by N retranslocation within the tree and, thus, does not draw upon soil sources. The same holds for the growth of needles.

Retranslocation can be large when compared to soil sources (Helmisaari, 1992), but it may not be necessary to determine retranslocation fluxes between various tree components. When it is reasonable to assume steady N concentrations in biomass, the above-ground N accumulation may be considered to equal the amounts in litter fall and those withdrawn by timber harvest. For a calculation of the actual N uptake in the investigated stand these amounts were estimated on the basis of data pertaining to mature trees. This approach provides a better approximation of acidification due to N uptake by trees in the field.

Litter fall was not determined in the present research, but in a neighbouring Scots pine stand N returns in litter averaged  $56 \text{ kg ha}^{-1} \text{ y}^{-1}$ , measured over 3 years (P. de Visser, 1990). The annual variability of litter fall fluxes was considerable (see Chapter 7, Table 5), but N uptake is probably more constant. This value of 56 kg was taken



as an estimate of N uptake for growth of needles, cones, etc., both for the control and for the limed plots. The observed lower needle N concentrations in the limed plots of the present research (Chapter 6) may have caused a lower litter-N return in the limed than in the control plots. However, this possible difference could not be accounted for because litter fall of limed Scots pine was not measured. In the neighbouring stand stem wood N concentrations were  $1.3 \text{ g kg}^{-1}$ . (T. Benfenati, unpubl.). Net bark increment was considered negligible. The wood increment value as measured in Chapter 6 was adopted, which implied the assumption that all stem wood production contributes to soil acidification. The latter is true in case of frequent timber harvesting, but also when no wood is removed a progressive biomass accumulation in stem wood will take place (Miller, 1984).

The  $\text{NH}_4/\text{NO}_3$  uptake ratio was found to be 7 times the  $\text{NH}_4/\text{NO}_3$  ratio in the soil solution (Chapter 3). Choosing a realistic value for the latter is, however, rather arbitrary. As discussed in Chapter 3, in the mineral soil this ratio is likely to show a strong variability with depth. Therefore, a gradient may exist from tree-induced acidification in the topsoil to proton consumption in the subsoil. Moreover, the root distribution also varies with depth. The integrated result of these variable parameters is difficult to estimate. Therefore, simply the average soil solution  $\text{NH}_4/\text{NO}_3$  ratios at a 30-cm depth in the growing seasons (April-September) of 1990 and 1991 were taken (see Chapter 5). These values were 0.40 and 0.16 in the control and limed plots, respectively. Unfortunately, estimates of acidification due to N uptake are strongly influenced by the soil solution  $\text{NH}_4/\text{NO}_3$  ratio values.

#### *N mineralization*

The amounts of  $\text{NH}_4$  and  $\text{NO}_3$  annually released by ammonification and nitrification were determined with in-situ incubation as described in Chapter 7. The N accumulation during 384 days of sequential incubation in the control and limed plots corresponded with releases of 13 and 27  $\text{kg NH}_4\text{-N ha}^{-1} \text{ y}^{-1}$  and 26 and 46  $\text{kg NO}_3\text{-N ha}^{-1} \text{ y}^{-1}$ , respectively. It should be noted that these values pertain to one year of measurements, while N mineralization probably shows a large annual variability.

#### *N accumulation*

According to the data mentioned above, throughfall input of  $\text{N}_i$  exceeds output due to leaching. Thus, in the soil to a 1-m depth N accumulation would amount to ca. 31 and 19  $\text{kg ha}^{-1} \text{ y}^{-1}$  for the control and limed plots, respectively. However, the amounts of available  $\text{N}_i$  in the forest floor and the top 50-cm mineral soil layer (Chapter 4, Table 5) and those of  $\text{N}_{\text{total}}$  did not clearly increase in the period 1986 to 1989. There may be several reasons for this discrepancy. First, throughfall and mineralization in 8-wk incubation intervals pertain to N accumulation in inorganic form.  $\text{N}_i$  not absorbed by trees will in the long run be assimilated by microflora. The resulting

change in soil organic N probably is too small to cause discernible changes in the total amounts of N in the forest floor and 50 cm mineral soil (ca. 4000 kg ha<sup>-1</sup>). Second, the preliminary estimates of N leaching may have been too low. Van Grinsven et al. (1991) determined N fluxes more accurately in a pine forest in Germany with throughfall quantities identical to those in Harderwijk, and found NO<sub>3</sub> fluxes at a 1-m depth amounting to ca. 17 kg N ha<sup>-1</sup> y<sup>-1</sup>, i.e. almost twice the value estimated in the control plots of the present research. Third, a build-up of N storage in the roots may occur. Fourth, N accumulation may have been prevented by denitrification. However, as argued before (pages 70 and 109), any considerable denitrification was not expected to occur.

Although the discrepancy mentioned above may well be explained by some of these arguments, the occurrence of N accumulation in areas subjected to high N depositions is not unlikely. N accumulation was found in other forests subjected to increased N immissions. Van Grinsven et al. (1991) (see above) found accumulations of ca. 30 kg N ha<sup>-1</sup> y<sup>-1</sup>. Van Breemen et al. (1987) assumed an annual N accumulation of ca. 20 kg ha<sup>-1</sup> to have proceeded over a 25-year period in several oak/birch forest floors.

#### *Fate of deposited NH<sub>4</sub>*

The uptake and mineralization data mentioned above provide enough information on most fluxes to allow the calculation of an H<sup>+</sup> budget for the ecosystem as a whole. However, in the present research throughfall deposition was intercepted by IER, so that ammonification was the main NH<sub>4</sub> source of nitrification taking place in incubation cores. Therefore, it still is an open question to which extent deposited NH<sub>4</sub> is nitrified and contributes to soil acidification. Nitrification of deposited NH<sub>4</sub> is not preceded by the acid-consuming ammonification process. Therefore, it may be the major cause of soil acidification (Van Breemen et al., 1983).

Because soil NH<sub>4</sub> originates from two sources, ammonification and throughfall, it is difficult to measure the rate of nitrification of atmospheric NH<sub>4</sub>. A direct measurement was attempted by Stams and coworkers (1990, 1991), who applied <sup>15</sup>NH<sub>4</sub> to oak/birch forest soils in the field and in vitro. The amounts of applied NH<sub>4</sub> nitrified in incubated mineral soil samples varied from 4% in an acid soil to 65% in a calcareous soil (Stams et al., 1990), but acid leaf litter in columns developed very high nitrification rates (Stams and Marnette, 1990). In contrast, of <sup>15</sup>NH<sub>4</sub> applied to a Scots pine forest floor only 10-20% reached the mineral soil as NO<sub>3</sub> (Stams, unpublished). In these experiments, large amounts of <sup>15</sup>N were incorporated in organic matter, which hampered a proper evaluation of the fate of NH<sub>4</sub> in throughfall under field conditions. To illustrate the importance of nitrification of deposited NH<sub>4</sub> it was assumed in the present investigation that 30% and 60% of NH<sub>4</sub> in throughfall were nitrified in the control and limed plots, respectively. It is reasonable to assume that liming increases this process.

## H<sup>+</sup> budget

### N transformations

*Importance of various H<sup>+</sup> sources - untreated Scots pine.* In Fig. 1 the N fluxes as mentioned above are depicted. The most important difference from the presentation in Chapter 2 is the distinctive use of two arrows for the nitrification process, according to its sources: ammonification or deposition. Such a distinction is more intelligible in view of the H<sup>+</sup> budget.

In untreated Scots pine, N uptake, as estimated from the soil solution NH<sub>4</sub>/NO<sub>3</sub> ratios at a 30-cm depth, is more acidifying than N mineralization and the assumed nitrification of atmospheric NH<sub>4</sub> (Table 1). Mineralization caused acidification because 65% of the NH<sub>4</sub> from ammonification was nitrified (c.f. Eqs. 1 and 2, Chapter 1). However, this value of 65% implies that per mol mineralized N only 0.15 mol H<sup>+</sup> is produced. Hence the H<sup>+</sup> production from mineralization is relatively low. When it is correct that 30% of the annual deposition of 2.14 kmol NH<sub>4</sub> ha<sup>-1</sup> is nitrified, 1.29 kmol H<sup>+</sup> ha<sup>-1</sup> would be formed by this process, making it a more important source of H<sup>+</sup> than N mineralization, but a less important one than N uptake (Table 1).

The fact that N uptake and mineralization both produce acidity is probably largely caused by N deposition. In most temperate forests and especially plantations not subjected to anthropogenic N depositions, N is the most limiting plant nutrient (Keeney, 1980) and, therefore, it is efficiently preserved. In such a situation, small N inputs and outputs occur, N uptake and mineralization are in balance and, thus, N transformations play a minor role in the overall H<sup>+</sup> cycling of an ecosystem (Van Breemen and Jørgensen, 1983). The present situation of high NH<sub>4</sub> inputs probably enhances H<sup>+</sup> production through both N uptake and N mineralization. N uptake as

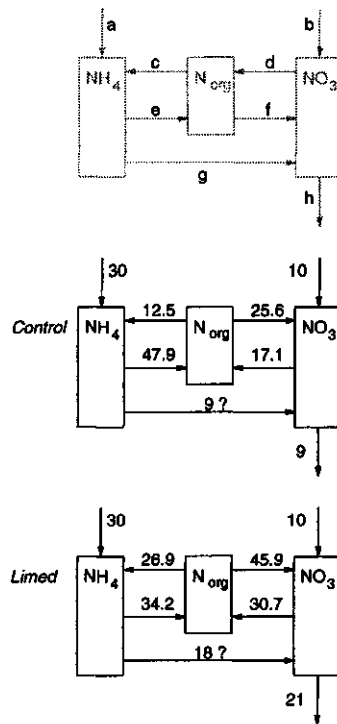


Figure 1. Main external and internal inorganic N fluxes (kg ha<sup>-1</sup> y<sup>-1</sup>) in untreated and limed plots with Scots pine. Vertical arrows are neutral with respect to the H<sup>+</sup> budget; horizontal arrows indicate a production (right-pointed) or consumption (left-pointed) of 1 or 2 mol H<sup>+</sup> per mol N, according to their length.

- a. deposition of NH<sub>4</sub>
- b. deposition of NO<sub>3</sub>
- c. ammonification
- d. NO<sub>3</sub> uptake
- e. NH<sub>4</sub> uptake
- f. nitrification preceded by ammonification
- g. nitrification of deposited NH<sub>4</sub>
- h. NO<sub>3</sub> leaching

Table 1. Annual acidification due to N transformations in untreated and limed Scots pine plantations.

Source	H <sup>+</sup> production (kmol ha <sup>-1</sup> y <sup>-1</sup> )	
	Untreated	Limed
Mineralization	0.94	1.36
Uptake	2.20	0.25
	— +	— +
	3.14	1.61
Estimated nitrification of throughfall NH <sub>4</sub>	1.29	2.57
Total	4.42	4.18

source of H<sup>+</sup> may be enhanced because more NH<sub>4</sub> is absorbed by roots than in a natural ecosystem. Acidification due to mineralization may be enhanced because decomposition of N-rich substrates produces more NH<sub>4</sub> than can be absorbed by plants, thus making more NH<sub>4</sub> available to the nitrification process.

*Effects of liming.* The uptake pattern of limed Scots pine appears to be nearly neutral with respect to the H<sup>+</sup> budget (Table 1). On the other hand, the rates of ammonification and nitrification were equally increased by liming, and thus a larger amount of H<sup>+</sup> was produced by mineralization than in the control treat-

ment. However, total H<sup>+</sup> production due to N uptake and mineralization in the limed plots is about half that in the control plots (Table 1). It can thus be concluded that liming may not only alleviate soil acidification by a direct pH effect, but also by reducing the H<sup>+</sup> production of plant roots. This indirect effect of liming may become stronger when a nitrophilous vegetation develops under the influence of a continuously increased NO<sub>3</sub> availability. NH<sub>4</sub> emissions are to be lowered in the near future, so that acidification owing to direct nitrification of deposited NH<sub>4</sub> will decrease, and less NO<sub>3</sub> is expected to leach. Also, when after a possible reduction of atmospheric N inputs NO<sub>3</sub> originating from the mineralization process is more effectively absorbed by plant roots and thus prevented from leaching, less acidification will take place. However, liming may also have several adverse effects (see page 50) and the probably inevitable profound change in the ecosystem's floristic composition may not be wanted.

### Nutrients other than N

The data of Chapter 3 offer an opportunity to calculate the acidification due to root uptake of all major nutrients. The uptake of nutrients other than N was highly independent of the NH<sub>4</sub>/NO<sub>3</sub> availability ratio (Table 2, Chapter 3), so that it could be calculated that this uptake caused a production of 0.284 mol H<sup>+</sup> per mol N absorbed. With the current N uptake this would imply an additional soil acidification of ca. 1.3 kmol ha<sup>-1</sup> y<sup>-1</sup>. However, the extent to which this amount is compensated for by mineralization was not determined.

## Forest fertilization and liming

Forest fertilization and liming have been suggested as possible tools to alleviate negative effects of acid or acidifying deposition, under the premises of judicious applications (Evers and Hüttel, 1990). The question can be raised whether or not the results of the present experiment can be used in this contemplation. As mentioned in Chapter 1, any unfavourable condition of the forest used in the present investigation did not explicitly result from an increased  $\text{NH}_4$  deposition or other sources of pollution. Therefore, it is difficult to draw direct conclusions about the alleviating effects of forest fertilization and liming. However, the following remarks can be useful.

### *Foliar analysis and growth responses*

Until today, fertilization and liming in the present investigation did not result in improved tree performance visible to the naked eye, as reported for some 'diagnostic fertilization trials' in Germany. Therefore, the less conspicuous volume growth responses were used to evaluate the effects of fertilization and liming.

It was questioned before (Chapter 6) whether or not growth responses to fertilization and liming are a reflection of tree vitality. Life processes in the plant culminate in the formation of biomass, and, thus, growth usually indicates good health or 'vitality'. However, the growth rates of trees in the present experiment amounted to  $16 \text{ m}^3$  stem wood increment  $\text{ha}^{-1} \text{ y}^{-1}$ , which is very high for Scots pine. De Vries et al. (1990) mention an average growth rate of  $7.1 \text{ m}^3 \text{ ha}^{-1} \text{ y}^{-1}$  for Scots pine on favourable sites. The high growth rate in the present experiment is probably due to an increased N availability, but is obviously not a reflection of high availability of other nutrients (see Chapter 6 and below). Such growth rates may not indicate a high degree of tree vitality, but rather suggest that negative effects of an increased N availability may develop in the near future. Any observed nutrient deficiencies will probably not be mitigated, but will rather aggravate as long as high growth rates persist, irrespective of their causes (e.g. depleted soil sources, drought, root damage). Hofman (1990) observed that Scots pine initially increased its growth rate as a response to increased N availability, but eventually succumbed to negative effects of N deposition, in particular to depletion of C reserves needed for N detoxification. However, increased growth after application of mineral nutrients may indicate that a situation of balanced nutrition has been restored. If so, growth may be used as a parameter to quantify positive effects of fertilization and liming.

The interpretation of foliar nutrient concentrations and ratios according to Dutch standards is based on three aspects of plant nutrition (Anon., 1990). The first is whether the observed concentration or nutrient ratio is sufficient to support good growth, and whether growth responses may be expected after addition of the considered nutrient. Second, it is investigated whether or not the nutrient supply is

balanced, and third, the expected recovery from several forms of stress is evaluated. Of these aspects the first and third are operational ones, while the second one is difficult to define. However, unbalanced nutrition will probably cause growth reductions and/or reduced stress tolerance.

The results obtained in the present experiment showed that foliar analysis may not always be interpreted correctly when growth responses are concerned. Quick growth responses to additions of P and K were found, although only foliar P was deficient, while the foliar K concentration and K/N ratio indicated that K fertilization was not necessary. On the other hand, Mg availability was considered sufficient on the basis of foliar analysis and, correspondingly, Mg application did not influence growth. Foliar Ca concentrations were improved by liming, but were not (yet?) accompanied by growth increases (Chapter 6). More experiments should be carried out to investigate the effect of fertilization on foliar nutrient concentrations and growth responses. However, at present there is probably no budget (nor a vision) for such investigations in the Netherlands. Yet, in the 'Long-Range Planning in Forestry' it is intended to increase the forested area in the Netherlands and to make the Netherlands more self-supporting with respect to wood supply (Hinssen, 1991). When, as a consequence, forest fertilization would gain a wider interest, fertilizer recommendations could further be developed provided foresters would be stimulated to frequently carry out some stand measurements and foliar analyses. The present fertilizer recommendations heavily lean on foliar analysis, to be performed once. When fertilization is carried out, it seems recommendable and feasible to frequently measure the effects on diameter growth and about 5 years after application those on foliar chemical composition.

#### *Fertilization and nitrification*

An infrequently mentioned side-effect of fertilization found in the present experiment was a stimulation of nitrification, after a 3-year lag phase, in plots amended with P, K and Mg (page 63). It may well be that when N is not the limiting nutrient the addition of other nutrients stimulates the microflora, including autotrophic nitrifiers. However, it is not generally found that applications of mineral nutrients with or without N increases nitrification. DiStefano and Gholz (1989) concluded that P availability had little apparent effect on net N transformations in the surface soil of slash pine plantations. Mai and Fiedler (1986) reported that adding P and K alone to a Norway spruce forest soil did not markedly influence the microflora. In these studies, N applications increased nitrification, but independent of the additions of other nutrients. More research on the effects of mineral nutrients on nitrification in a situation of a large potential N supply should clarify in which cases forest fertilization would not be recommendable.

*Is there a role for forest fertilization ...*

Probably long after N depositions will have been reduced to levels lower than the so-called critical loads, ecosystems that are presently exposed to high N depositions will contain more N than they originally did. Therefore, they may continue to be potential sources of  $\text{NO}_3$  leaching and be characterized by soil acidification and an unbalanced nutrient supply. In N-enriched regions, production forests may effectively prevent  $\text{NO}_3$  leaching if they are enabled to accumulate N at a rate similar to the N mineralization rate. This implies the maintenance of high growth rates, which may require fertilization with nutrients other than N. Such an approach would be in line with the present policy in forestry in the Netherlands (see above).

As long as substantial  $\text{NH}_3$  emissions remain, forests may serve as buffer zones to protect other ecosystems against atmospheric depositions. Productive coniferous tree species, e.g. Douglas fir, would be most suitable for this purpose.

Also in the restoration of oligotrophic ecosystems, fertilization may play an important role. When ecosystems are to be deprived of excessive N, frequent removal of biomass may be contemplated, and even litter raking has been suggested (Klap and Schmidt, 1993). However, in doing so, not only N will be removed, and it may prove necessary to replenish nutrients other than N, lest a situation of unbalanced nutrient supply is maintained. In an age of unwanted eutrophication it sounds blasphemous and paradoxical to promote nutrient additions to restore oligotrophic ecosystems. However, it may be more effective to temporarily maintain productive biotopes and to frequently remove biomass, than to wait until ecosystems with low growth rates have disposed of their excessive N in a natural way. In this context, biomass removal should be preferred to litter removal, as the latter would increase the risk of desiccation (P. de Visser, pers. comm.). One requirement to be made is that the removed biomass consists of useful wood or fibres, to prevent another contribution to the growing pile of organic refuse.

*... and for liming ?*

The above-mentioned positive effects of liming on soil acidification (i.e. a less acidifying root uptake pattern) and on acidity do not warrant an unbridled use of lime and similar substances in forests. Several hazards of liming are well known and have been pointed out earlier (page 50). In addition, when K deficiencies play a role, a Ca/K uptake antagonism may be very harmful, and strong stimulation of ammonification may have adverse effects as well, in view of the fact that  $\text{NH}_4$  is known to inhibit K uptake (Mengel and Kirkby, 1987).

Another reason for limiting the use of liming is that liming is usually not very effective in eliminating mineral soil acidity, especially not when a forest floor is present. Mixing lime and topsoil is very laborious and thus expensive, and moreover it will strongly activate mineralization. Special attention should therefore be paid to

the development of substances that increase mineral soil pH and pAl without drastically increasing forest floor pH. Such substances should, other than limestone meal or granules, easily permeate the forest floor and topsoil. Gypsum ( $\text{CaSO}_4$ ) meets the latter requirement, but it only improves the Ca/Al ratio and not pH. Moreover, it may displace considerable amounts of base cations (Oates and Caldwell, 1985), which may aggravate the removal of such cations by  $\text{NH}_4$ . Phosphogypsum and especially hydrofluorogypsum proved to effectively remove Al from acid subsoil material in columns and the latter also raised the pH. However, very large quantities of gypsum were required to reduce Al to the same extent as lime did in an incubation test (Oates and Caldwell, 1985).

Potentially promising compounds may be Ca fulvates. Coal-derived Ca fulvate was found to moderately increase the pH throughout 80-cm soil columns. It also brought Ca into the profile, more effectively than  $\text{CaSO}_4$  and Ca-EDTA did, while  $\text{CaCO}_3$  and  $\text{Ca(OH)}_2$  did not provide Ca to deeper soil zones (Van der Watt et al., 1991). However, native dissolved organic substances are frequently found to prevail in the topsoil, thus making it as yet unclear whether Ca fulvates (or humates) will be able to permeate soils under field conditions (W. Van Riemsdijk, pers. comm.). In addition, at present such substances have not yet been sufficiently tested and are too expensive to have any practical meaning.



## Summary

### Introduction

Since the 1950s, in the Netherlands and several other western European countries an intensified livestock production system has developed. As a consequence, the quantities of  $\text{NH}_x$  ( $\text{NH}_3$  and  $\text{NH}_4^+$ ) emitted into the atmosphere have increased. However, atmospheric  $\text{NH}_x$  is eventually returned to the soil surface, which occurs through dry and wet deposition. In forests, dry deposition on leaf surfaces is enhanced by several processes, resulting in average deposited amounts of  $30 \text{ kg NH}_4\text{-N ha}^{-1} \text{ year}^{-1}$ . In addition, annually  $10 \text{ kg NO}_3\text{-N ha}^{-1}$  is deposited. Possible negative effects of increased  $\text{NH}_4$  depositions are, first, soil acidification and, second, an imbalanced plant nutrition in the form of (relative) mineral nutrient deficiencies.

The former effect may be caused by nitrification of atmospheric  $\text{NH}_4$ , or by an increased excess of cation-over-anion uptake by tree roots. When more (or less) cations than anions are absorbed, plants maintain their internal charge balance by net excretion of  $\text{H}^+$  (or  $\text{OH}^-$ ) ions. Therefore, an increased uptake of  $\text{NH}_4^+$  may increase the roots'  $\text{H}^+$  production.

Relative or absolute nutrient deficiencies may occur when N availability is excessively high relative to that of the mineral nutrients, or through increased mobilization and leaching of base cations, respectively.

This thesis presents two lines of research, pertaining to these possible negative effects of increased  $\text{NH}_x$  depositions in the Netherlands. The first one deals with the question whether or not nutrient uptake of trees can be considered an important source of soil acidification, relative to other  $\text{H}^+$  sinks and sources. The second line of research was aimed at obtaining more understanding of forest fertilization and liming as possible tools to ameliorate acid-rain-induced nutritional disorders and soil acidification.

Scots pine (*Pinus sylvestris* L.) was chosen as experimental tree species, because of its predominant contribution to the forested area in the Netherlands and other western European countries. A pot experiment was conducted with 3-y-old trees, and a forest fertilization experiment was carried out in a mature Scots pine stand in Harderwijk, the Netherlands.

### *N uptake pattern of Scots pine, N mineralization and soil acidification*

The  $\text{H}^+$  production due to nutrient acquisition of plant roots can indirectly be assessed by measuring the uptake of cations and anions. The  $\text{H}^+$  production is calculated as the difference between the absorbed charge equivalents of cations and anions ( $C_a - A_a$ ). Most cations and anions are absorbed by plant roots in one distinct form. Therefore, their uptake charge balance can straightforwardly be derived from plant analysis. However, N can be taken up as  $\text{NH}_4^+$  or as  $\text{NO}_3^-$ . These N sources can not be distinguished when assimilated to organically bound N. However, a distinction can be made when one of the N sources is labelled with the stable nitrogen isotope  $^{15}\text{N}$ , while the other one is applied in its natural  $^{14}\text{N}$  form. To investigate the feasibility of this approach, in a pilot-study a method to measure the uptake of  $^{15}\text{NH}_4$  or  $^{15}\text{NO}_3$  was

tested with larch (*Larix kaempferi*) trees. In a modified form, this method was used to investigate the uptake pattern of 3-y-old Scots pine trees. In a pot experiment N was supplied in three  $\text{NH}_4\text{-N}/\text{NO}_3\text{-N}$  ratios, 3:1, 1:1 and 1:3, either as  $^{15}\text{NH}_4 + ^{14}\text{NO}_3$  or as  $^{14}\text{NH}_4 + ^{15}\text{NO}_3$ . After these applications, the  $\text{NH}_4/\text{NO}_3$  ratios in the soil solution were monitored. At each application ratio, Scots pine appeared to show an  $\text{NH}_4/\text{NO}_3$  uptake ratio 7 times wider than that in the soil solution. It could be estimated that Scots pine exhibits an acidifying uptake pattern as long as the contribution of nitrate to the N nutrition is lower than 70%. For the three application ratios this contribution was always < 70%.

These data were used to estimate acidification occurring under field conditions. For the three application ratios  $\text{H}^+$  production due to wood growth was estimated to range from 2.5 to 4.2  $\text{kmol ha}^{-1} \text{y}^{-1}$ , and that due to wood and needle growth from 3.6 to 6.2  $\text{kmol ha}^{-1} \text{y}^{-1}$ . However, for these estimates the acidification found in the pot experiment was adopted without modifying soil solution  $\text{NH}_4/\text{NO}_3$  ratios or plant N concentrations according to those which may be expected in the field. For more realistic estimates, N concentrations in the soil solution as found in the root zone of the field trial in Harderwijk (see below) were combined with N uptake data of mature Scots pine trees. Thus, the acidification due to nutrient uptake for above-ground growth appeared to be lower, i.e. 2.20 and 0.25  $\text{kmol ha}^{-1} \text{y}^{-1}$ , for untreated and limed plots, respectively. The lower value for limed plots was caused by higher  $\text{NO}_3$  concentrations in the soil solution of the limed soil, thus reducing the excess cation-over-anion uptake and the associated  $\text{H}^+$  excretion of tree roots.

Besides nutrient uptake, nitrification is an acidifying process as well, whereas ammonification is acid-consuming. Ammonification consumes 1 mol  $\text{H}^+$  per mol  $\text{NH}_4$  produced. Nitrification, if preceded by ammonification, produces 1 mol  $\text{H}^+$  per mol  $\text{NO}_3$  produced. The combined result of both processes, the formation of inorganic N from organic N, is referred to as N mineralization. Depending on the rates of these microbial N transformations, acidification due to nutrient uptake may either be (partly) compensated for or be corroborated. To investigate these rates, an in-situ incubation experiment was carried out in the control and limed plots of the field experiment. PVC tubes were driven into the forest floor and the top 22-cm mineral soil layer, and left in the field. This was done in seven subsequent incubation periods of each 7 or 8 weeks (384 days total). For each period, the changes in the amounts of  $\text{NH}_4$  and  $\text{NO}_3$  in the tubes were measured. To prevent the input of atmospheric N and N leaching, the undisturbed soil column in each tube was confined between two layers of ion exchange resin (IER). Annually, in the control and limed plots 38 and 73  $\text{kg N ha}^{-1}$  were mineralized, respectively. In both treatments approx. 65% of  $\text{NH}_4$  from the ammonification process was nitrified. Therefore, mineralization was a slightly acidifying process. The annual acidification in the control and lime treatments amounted to 0.9 and 1.4  $\text{kmol H}^+ \text{ha}^{-1}$ , respectively; these amounts were not significantly different. Thus, acidification owing to nutrient uptake and mineralization adds up to 3.14 and 1.61  $\text{kmol H}^+ \text{ha}^{-1} \text{y}^{-1}$  for the untreated and limed Scots pine plots, respectively. The acidification due to direct nitrification of deposited  $\text{NH}_4$  was not determined, but may be considerable because through this process 2 mol  $\text{H}^+$  is produced per mol  $\text{NH}_4$  nitrified.

In situations of low atmospheric N inputs N is effectively retained in most ecosys-

tems. This implies that N is eventually taken up in the form in which it is released by N mineralization. Hence, acidification due to N transformations would probably be negligible.

### *Field experiment*

In order to alleviate potential nutrient deficiencies as a result of high N depositions, in 1985-1988 a Scots pine forest (planted in 1960) was limed and fertilized with P, K and Mg in a 2<sup>4</sup> factorial design and in a separate experiment with 5 liming levels and basic dressings of P, K and Mg. Liming induced many effects on the mineral soil and especially on the forest floor. It proved to be responsible for the loss of extractable K and Mg from the forest floor, probably by exchange against Ca. The residence time of added P and possibly Mg in the forest floor was increased by liming, probably due to a reduced solubility of the added fertilizers (triple superphosphate and kieserite). Single applications of 3000 kg lime ha<sup>-1</sup> in 1985 increased the forest floor pH to 6.2. In the subsequent 5 years, the pH gradually declined to 4.2. In the same period, the pH of the untreated forest floor declined from 4.0 to 3.1. Five years after application, lime had caused a small, but significant pH increase to a 50-cm depth. Extractable Al in the forest floor and mineral soil was lowered by liming. Until 1989, inorganic N in the forest floor was lowered and that in the mineral soil was raised by liming. This could be attributed to the formation of NO<sub>3</sub> in the limed plots, which is more mobile than NH<sub>4</sub>. Added K (potassium sulphate) was poorly retained in the forest floor and probably substantial quantities quickly leached to soil layers deeper than 50 cm.

The soil solution composition at a 30-cm depth was monitored from autumn 1989 until spring 1992. In spring 1991, the soil solution at a depth of 1-1.5 m was analyzed. The applied K and Mg compounds were easily soluble, and hence the soil solution concentrations of K and Mg were increased at both depths in the PKMg-amended plots. Liming increased Ca and left Al unaffected at both depths. The Ca/Al ratios were low (i.e. around 0.2) in the root zones of the unlimed plots, which may impair root functioning. The NO<sub>3</sub> concentrations at 30 cm were high in winter and lower in summer. NO<sub>3</sub> was increased by liming at both depths. The largest increase at 30 cm occurred in the autumn/winter of 1990/91, i.e. a few months after windfelling and thinning. Increased NO<sub>3</sub> concentrations in the soil solution are commonly observed after ecosystem disturbance. The present research shows that liming may enhance such an effect. The additions of P, K and Mg also increased NO<sub>3</sub>, but this effect only started 2½ year after the last applications. This finding suggests that the nitrifying microflora gradually developed an ability to profit from an improved nutrient availability.

Stem volume increments, measured at all individual trees, amounted to 16 m<sup>3</sup> ha<sup>-1</sup> y<sup>-1</sup>, which is high for Scots pine. It is likely that high N depositions increased tree growth. These high growth rates suggest that negative effects of an increased N availability may develop in the near future. However, increased growth after application of mineral nutrients may indicate that a situation of balanced nutrition has been restored, so that growth may be used as a parameter to quantify positive effects of fertilization and liming.

In the period 1988-1991, fertilization with P and/or K increased stem volume growth

by 0.9 and 2.2 m<sup>3</sup> ha<sup>-1</sup> y<sup>-1</sup>, respectively, while Mg and/or lime had no effect. Correspondingly, P and K were the first nutrients to show increased foliar concentration after fertilization. However, foliar analysis at the start of the experiment indicated that in fact the P supply was insufficient while that of K was sufficient. This discrepancy may have two causes. First, the foliar-analysis-based conclusions concerning a forest's nutritional status may not yet be fully accurate, or second, growth responses may not correctly reflect changes in the nutritional status.

### *Future perspectives*

Probably long after N depositions will have been reduced according to presently stated prescriptions, ecosystems that have been exposed to high N depositions will contain more N than they originally did. Therefore, they may continue to be potential sources of NO<sub>3</sub> leaching and be characterized by soil acidification and an unbalanced nutrient supply. In N-enriched regions, production forests may effectively prevent NO<sub>3</sub> leaching if they are enabled to accumulate N at a rate similar to the N mineralization rate. This condition requires the maintenance of high growth rates. When oligotrophic biotopes are to be restored, excessive N must be removed. This may most effectively be carried out by temporarily maintaining productive vegetations from which biomass is frequently removed. Because high growth rates require a sufficient and balanced nutrition, for both situations fertilization with nutrients other than N may be necessary.

Liming may be applied to improve soil pH and reduce Al concentrations. However, liming may have several adverse effects, e.g. increased soil heavy-metal concentrations, profound floristic changes and an increased N mineralization and NO<sub>3</sub> leaching. Moreover, lime (CaCO<sub>3</sub>) is not very effective in improving deeper soil horizons. Special attention should therefore be paid to the development of substances that increase mineral soil pH and pAl without drastically increasing forest floor pH.

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## Samenvatting

### *Introductie*

Sinds de vijftiger jaren heeft zich in Nederland en andere westeuropese landen intensieve veehouderij ontwikkeld. Als gevolg daarvan werden toenemende hoeveelheden NH<sub>x</sub> (NH<sub>3</sub> en NH<sub>4</sub><sup>+</sup>) uitgestoten in de atmosfeer. Uiteindelijk wordt dit NH<sub>x</sub> echter weer teruggebracht naar het aardoppervlak. Dit gebeurt door droge en natte depositie. In bossen wordt door een aantal processen de droge depositie op blad- en naaldoppervlakken verhoogd. Het resultaat is een gemiddelde jaarlijkse doorval-neerslag (door het kronendak) van 30 kg NH<sub>4</sub>-N ha<sup>-1</sup>. Daarnaast komt er jaarlijks een hoeveelheid van ca. 10 kg NO<sub>3</sub>-N ha<sup>-1</sup> naar beneden. De verhoogde neerslag van NH<sub>4</sub>

kan negatieve effecten hebben. Ten eerste kan bodemverzuring in de hand worden gewerkt. Ten tweede kan de overmaat aan stikstof een onevenwichtig nutriëntenaanbod en relatieve (t.o.v. N) of absolute nutriëntentekorten veroorzaken.

Het eerstgenoemde effect kan veroorzaakt worden door nitrificatie van atmosferisch  $\text{NH}_4$ , of door een toegenomen overmaat aan kationen t.o.v. anionen die door de boomwortels worden opgenomen. Wanneer er meer, resp. minder kationen worden opgenomen dan anionen, moet de boomwortel zijn interne ladingsbalans in stand houden door netto excretie van  $\text{H}^+$ , resp.  $\text{OH}^-$ -ionen. Daardoor kan een toegenomen opname van  $\text{NH}_4^+$  de  $\text{H}^+$  uitscheiding (= verzuring) door wortels verhogen.

Relatieve, respectievelijk absolute nutriëntentekorten kunnen ontstaan wanneer de beschikbaarheid van stikstof overmatig hoog is vergeleken met die van de minerale nutriënten, of door mobilisatie en uitspoeling van kationen.

Dit proefschrift beschrijft twee lijnen van onderzoek die te maken hebben met de bovengenoemde negatieve effecten van een toegenomen  $\text{NH}_4$ -neerslag in Nederland. De eerste betreft de vraag of nutriëntenopname door bomen een belangrijke bron van bodemverzuring is, vergeleken met andere processen die  $\text{H}^+$  produceren of consumeren. De tweede onderzoekslijn had als doel meer inzicht te verschaffen in de mogelijkheden die bosbemesting en -bekalking bieden om inadequate voeding en bodemverzuring als gevolg van zure regen te verhelpen.

Voor de experimenten werd grove den (*Pinus sylvestris*) gekozen, omdat dit de dominante boomsoort is in Nederland en diverse andere westeuropese landen.

#### *N-opnamepatroon van grove den, N-mineralisatie en bodemverzuring*

De  $\text{H}^+$ -productie die veroorzaakt wordt door nutriëntenopname van plantewortels kan indirect worden bepaald door de opname van kat- en anionen te meten. De  $\text{H}^+$ -productie wordt gelijk gesteld aan het verschil tussen de opgenomen ladingsequivalenten van kat- en anionen ( $C_a - A_a$ ). De meeste kat- en anionen worden in één bepaalde vorm opgenomen, zodat de ladingsbalans van hun opname direct volgt uit plantanalyse. Echter, N kan opgenomen worden als  $\text{NH}_4^+$  of als  $\text{NO}_3^-$ . Deze twee N-bronnen kunnen niet meer onderscheiden worden wanneer ze geassimileerd zijn tot organisch N. Ze kunnen echter wel onderscheiden worden wanneer één van beide wordt gemerkt met de stabiele stikstofisotoop  $^{15}\text{N}$ , terwijl de andere aangeboden wordt in de natuurlijke  $^{14}\text{N}$ -vorm. Om de bruikbaarheid van deze methode te toetsen, werd met lariks (*Larix kaempferi*) een vooronderzoek uitgevoerd om de opname van  $^{15}\text{NH}_4$  of  $^{15}\text{NO}_3$  te bepalen. Deze methode werd in gewijzigde vorm gebruikt om het opnamepatroon van drie-jarige grove-den-bomen te onderzoeken. In deze potproef werd N aangeboden in drie  $\text{NH}_4\text{-N}/\text{NO}_3\text{-N}$ -verhoudingen, 3:1, 1:1 en 1:3, of als  $^{15}\text{NH}_4 + ^{14}\text{NO}_3$  of als  $^{14}\text{NH}_4 + ^{15}\text{NO}_3$ . Na deze toedieningen werden regelmatig de  $\text{NH}_4/\text{NO}_3$ -verhoudingen in het bodemvocht gemeten. Bij iedere toedieningsverhouding bleek grove den N op te nemen in een  $\text{NH}_4/\text{NO}_3$ -verhouding die ca. 7 keer zo groot is als die in het bodemvocht. Op grond hiervan kon geschat worden dat grove den een verzurend opnamepatroon heeft als het aandeel van  $\text{NO}_3$  aan de totale N-voeding lager is dan 70%. Bij de drie toedieningsverhoudingen was dit aandeel altijd lager dan die waarde.

Deze gegevens werden gebruikt om een schatting te maken van de verzuring die in

het veld optreedt. Voor de drie toedieningsverhoudingen werd de verzuring als gevolg van hout-aanwas geschat op  $2,5 - 4,2 \text{ kmol ha}^{-1} \text{ jaar}^{-1}$ . De verzuring veroorzaakt door houtaanwas en naalddgroei lag tussen  $3,6$  en  $6,2 \text{ kmol ha}^{-1} \text{ jaar}^{-1}$ . Voor deze schattingen werd echter de verzuring in de potproef vertaald naar de veldsituatie, zonder de  $\text{NH}_4/\text{NO}_3$ -verhoudingen in het bodemvocht en de N-concentraties in de plant aan te passen aan waarden die in het veld verwacht mochten worden. Voor meer reële schattingen werden de bodemvocht- $\text{NH}_4/\text{NO}_3$ -verhoudingen in de wortelzone in de veldproef te Harderwijk (zie onder) gecombineerd met N-opnamegegevens van volwassen grove den. Zodoende bleek de verzuring als gevolg van bovengrondse groei lager uit te vallen, n.l.  $2,20$  en  $0,25 \text{ kmol H}^+ \text{ ha}^{-1} \text{ jaar}^{-1}$ , voor resp. onbehandelde en bekalkte proefveldjes. De lagere verzuring in de bekalkte veldjes wordt veroorzaakt door de hogere  $\text{NO}_3$ -concentratie in het bodemvocht, waardoor de overmaat opgenomen kationen t.o.v. anionen en daarmee de  $\text{H}^+$ -excretie door de wortels verlaagd wordt. Hieruit blijkt derhalve dat bekalking niet alleen de zuurgraad van een bodem verbetert, maar ook de verzuringssnelheid kan verlagen.

Naast nutriëntenopname is ook nitrificatie een verzurend proces, terwijl ammonificatie zuur neutraliseert. Ammonificatie consumeert  $1 \text{ mol H}^+$  per mol gevormde  $\text{NH}_4$ ; nitrificatie, indien voorafgegaan door ammonificatie, produceert  $1 \text{ mol H}^+$  per mol gevormde  $\text{NO}_3$ . Het resultaat van deze beide processen, de vorming van anorganisch N ( $\text{NH}_4$  en  $\text{NO}_3$ ) uit organisch N, wordt stikstofmineralisatie genoemd. Afhankelijk van de snelheid van deze N-transformaties kan de verzuring a.g.v. nutriëntenopname (gedeeltelijk) gecompenseerd worden, of juist worden aangevuld. Om de snelheid van N-mineralisatie te onderzoeken werd een in-situ-incubatie-experiment uitgevoerd in de onbehandelde en de bekalkte veldjes van de proef in Harderwijk. Hiervoor werden PVC-buizen in de strooisellaag en de bovenste  $22 \text{ cm}$  minerale grond geslagen en vervolgens voor een bepaalde tijd achtergelaten in het veld. Dit werd gedaan in opeenvolgende perioden van  $7$  of  $8$  weken, tot een totaal van  $384$  dagen. Voor elk van deze perioden werden de veranderingen in  $\text{NH}_4$ - en  $\text{NO}_3$ -concentraties in de buizen gemeten. Input van N door atmosferische depositie en N-verlies door uitspoeling werden voorkomen door de grondkolom in te sluiten tussen twee lagen van ionenwisselaar. De hoeveelheden N die jaarlijks werden gemineraliseerd in de onbehandelde en bekalkte veldjes bedroegen  $38$ , resp.  $73 \text{ kg N ha}^{-1}$ . In beide behandelingen werd ongeveer  $65\%$  van  $\text{NH}_4$  uit het ammonificatieproces genitrificeerd, zodat N-mineralisatie een licht verzurend effect had. Deze verzuring bedroeg resp.  $0,9$  en  $1,4 \text{ kmol ha}^{-1} \text{ jaar}^{-1}$ , in de onbehandelde en bekalkte veldjes, welke bedragen niet significant van elkaar verschilden. De verzuring a.g.v. mineralisatie en nutriëntenopname komt hiermee op  $3,14$  en  $1,61 \text{ kmol ha}^{-1} \text{ jaar}^{-1}$ , voor resp. de onbehandelde en bekalkte veldjes. De mate waarin doorval- $\text{NH}_4$  wordt genitrificeerd is niet bepaald. Dit kan echter een aanzienlijke bron van verzuring zijn, omdat in dat geval  $2 \text{ mol H}^+$  wordt geproduceerd per mol gevormde  $\text{NO}_3$ .

Wanneer de input van N via neerslag laag is, wordt in de meeste ecosystemen N zeer effectief vastgehouden. Dit houdt in dat N uiteindelijk wordt opgenomen in de vorm waarin het gemineraliseerd is, zodat de verzuring a.g.v. N-transformaties waarschijnlijk verwaarloosbaar klein is.

## Veldonderzoek

In 1985 werd een veldonderzoek gestart in een aanplant (1960) van grove den in Harderwijk, met als doel het opheffen van potentiële nutriëntengebreken a.g.v. hoge N-depositie. In de periode 1985-1988 werden P, K, Mg en kalk toegediend in een  $2^4$ -factoriële proef en in een aparte kalktrappenproef met PKMg-basisbemesting.

Bekalking sorteerde veel effecten in de minerale grond en vooral in de strooisellaag. Kalk verwijderde extraheerbaar K en Mg uit de strooisellaag, waarschijnlijk door uitwisseling tegen Ca. De verblijftijd van toegediend P en Mg werd verhoogd door bekalking, en dit was waarschijnlijk een gevolg van een verlaagde oplosbaarheid van de toegediende P- en Mg-meststoffen (tripelsuperfosfaat en kieseriet). Een eenmalige bekalking met  $3000 \text{ kg CaCO}_3 \text{ ha}^{-1}$  verhoogde de pH in de strooisellaag tot 6,2. In de daarop volgende 5 jaar nam de pH weer geleidelijk af tot 4,2. De pH in de strooisellaag van de onbehandelde veldjes nam in diezelfde periode af van 4,0 tot 3,1. Vijf jaar na toediening veroorzaakte kalk een kleine, maar significante toename van de pH in de minerale grond tot een diepte van 50 cm. Bekalking verlaagde extraheerbaar Al in de strooisellaag en minerale grond. Tot 1989 was in de bekalkte veldjes de hoeveelheid anorganisch N in de strooisellaag lager en die in de minerale grond hoger dan in de onbekalkte veldjes. Dit kon toegeschreven worden aan de vorming van  $\text{NO}_3$  in de bekalkte veldjes, dat mobieler is dan  $\text{NH}_4$ . Toegediend K (kaliumsulfaat) werd slecht vastgehouden in de strooisellaag. Er zijn aanwijzingen dat een groot deel ervan zeer snel uitspoelde naar diepten beneden de 50 cm.

Vanaf september 1989 tot en met april 1992 werd de bodemvochtsamenstelling op 30 cm diepte regelmatig gemeten. Daarnaast werd in het voorjaar van 1991 het bodemvocht op een diepte van 1 tot  $1\frac{1}{2}$  m geanalyseerd. De toegediende K- en Mg-meststoffen waren goed oplosbaar, zodat de bodemvochtconcentraties van K en Mg op beide diepten verhoogd werden door PKMg-bemesting. Bekalking verhoogde de Ca-concentratie maar had geen invloed op Al op beide diepten. De Ca/Al-verhoudingen waren laag (d.w.z. rond 0,2) in de wortelzone van de onbekalkte veldjes, hetgeen het functioneren van wortels kan belemmeren. De  $\text{NO}_3$ -concentraties waren hoog in de winter en laag in de zomer.  $\text{NO}_3$  werd op beide diepten verhoogd door bekalking. De grootste toename vond plaats in de herfst en winter van 1990/91, enkele maanden na het optreden van stormschade in januari 1991 en daaropvolgende dunning. Verhoogde  $\text{NO}_3$ -concentraties worden vaak waargenomen na verstoring van een ecosysteem. Het huidige onderzoek laat zien dat bekalking zo'n effect zou kunnen versterken. Ook bemesting met P, K en Mg verhoogde de  $\text{NO}_3$ -concentratie, maar dat pas  $2\frac{1}{2}$  jaar na de laatste toediening. Deze waarneming doet vermoeden dat de nitrificerende microflora geleidelijk het vermogen ontwikkelde om te profiteren van een verhoogde nutriëntenbeschikbaarheid.

De stamhout-aanwas van alle individuele bomen in het proefveld werd gemeten. Deze bedroeg  $16 \text{ m}^3 \text{ ha}^{-1} \text{ jaar}^{-1}$ , hetgeen zeer hoog genoemd mag worden voor grove den. Waarschijnlijk heeft de verhoogde N-depositie de boomgroei gestimuleerd. Deze hoge groeisnelheid doet vermoeden dat in de nabije toekomst negatieve effecten van de verhoogde N-beschikbaarheid zullen gaan optreden. Een positieve groeirespons op nutriëntentoeënningen wijst echter op een herstelde nutriëntenbalans. In dat geval zou groei als een parameter mogen dienen om positieve effecten van bemesting en

bekalking te kwantificeren.

Bemesting met P en/of K verhoogde in de periode de houtaanwas met resp. 2,2 en 0,9 m<sup>3</sup> ha<sup>-1</sup> jaar<sup>-1</sup>, terwijl Mg en/of kalk daar geen effect op hadden. In overeenstemming hiermee waren P en K de eerste nutriënten die na bemesting een verhoogde concentratie in de naalden lieten zien. Naaldanalyse bij de start van het onderzoek wees er echter op dat de P-voorziening wel onvoldoende was, maar die van K voldoende. Deze tegenstrijdigheid kan twee redenen hebben. Allereerst zouden de uitspraken, die op basis van bladanalyse worden gedaan over de voedingstoestand van het bos nog niet geheel accuraat kunnen zijn. Ten tweede bestaat de mogelijkheid dat de groeiresponsen geen exacte weerslag waren van veranderingen in de voedings-toestand.

### *Toekomstperspectief*

Zelfs wanneer de depositie van N verlaagd zou worden tot onder de nu gestelde normen, zullen nadien de huidige N-belaste ecosystemen waarschijnlijk nog lange tijd meer N bevatten dan de oorspronkelijke hoeveelheid. Daardoor zullen ze potentiële bronnen van NO<sub>3</sub>-uitspoeling blijven en mogelijk gekenmerkt worden door bodemverzuring en een onevenwichtig nutriëntenaanbod. In N-verrijkte gebieden kunnen produktiebossen effectief NO<sub>3</sub>-uitspoeling voorkomen, wanneer ze in staat zullen zijn om evenveel N op te nemen als er beschikbaar wordt gemaakt door mineralisatie. Dit vereist het in stand houden van een hoge groeisnelheid. Wanneer men weer oligotrofe biotopen wil creëren, moet de overmaat aan N verwijderd worden. Misschien is de meest effectieve methode hiervoor het tijdelijk in stand houden van een produktieve vegetatie, waar regelmatig nuttige biomassa aan onttrokken kan worden. Beide situaties zouden bereikt kunnen worden door bemesting met minerale nutriënten, want hoge groeisnelheden vereisen een toereikend en gebalanceerd nutriëntenaanbod.

Bekalking zou toegepast kunnen worden om de zuurgraad te verbeteren en Al-concentraties te verlagen. Echter, bekalking kan ook nadelige effecten hebben, zoals een toename van concentraties van zware metalen, grondige veranderingen in de floristische samenstelling en toegenomen mineralisatie en NO<sub>3</sub>-uitspoeling. Bovendien is kalk (CaCO<sub>3</sub>) niet erg doeltreffend in het verbeteren van diepere bodemhorizonten. Daarom moet de aandacht speciaal uitgaan naar het ontwikkelen van materialen die wel de pH en pAl in de minerale grond kunnen verbeteren, zonder echter de pH van de strooisellaag drastisch te verhogen. Eventueel geschikte stoffen zouden sommige gips-bijprodukten kunnen zijn, ware het niet dat daar zeer grote hoeveelheden van toegediend zouden moeten worden, of calciumfulvaten. Over de toepasbaarheid van deze laatste, dure, stoffen is echter nog weinig bekend.



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## Levensloop

Gert Arnold werd op 9 januari 1959 te Den Haag geboren, als tweede zoon van H.P. Arnold (bedrijfseconoom) en A.B. Arnold-Tigelaar. In 1978 behaalde hij het Athe-neumdiploma aan het Groen van Prinsterercollege in Den Haag en verhuisde naar Wageningen om aan de Landbouwhogeschool een studie Bodemkunde (N-33) te beginnen. Na één jaar wedstrijdroeien besloot hij de roeivereniging Argo vaarwel te zeggen om zijn betrokkenheid bij de christelijke studentenbeweging de Navigators verder uit te kunnen bouwen. In 1987 rondde hij zijn studie af met als hoofdvak bodemvruchtbaarheid en bijvakken landbouwplantenteelt, voorlichtingskunde en wiskundige statistiek.

Vlak daarvoor trouwde hij met Trice Bolt en verhuisde naar Groningen, waar zij geneeskunde studeerde. In afwachting van een baan op zijn eigen vakgebied volgde hij een informatica-opleiding en verrichtte als vrijwilliger enige redactionele werkzaamheden voor het tijdschrift 'Plant and Soil'. Dat laatste is hij tot op heden blijven doen.

In 1988 werd hij aangesteld als Assistent in Opleiding aan de vakgroep Bodemkunde en Plantenvoeding van de Landbouwuniversiteit te Wageningen. Het onderzoek dat in dat kader werd verricht resulteerde in dit proefschrift.

In 1991 ontvingen Trice en Gert een dochtertje, Jacqueline.