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## Physiological modulation of the vitality of Scots pine trees by atmospheric ammonia deposition

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## 2. Foliar uptake of gaseous ammonia <sup>2</sup>

### 2.1. Abstract

Young saplings of *Pinus sylvestris* L. were exposed to gaseous  $\text{NH}_3$  at 53 or  $105 \mu\text{g m}^{-3}$  for one year in open-top chambers. Saplings received  $^{15}\text{N}$ -labelled  $(\text{NH}_4)_2\text{SO}_4$  via the soil. To examine the importance of foliar N uptake, changes in the concentration of total and labelled N in the needles were followed. Increases in needle biomass and N concentration were found in trees exposed to  $\text{NH}_3$ , confirming that atmospheric  $\text{NH}_3$  acted as a N fertilizer.  $\text{NH}_3$  had a greater and quicker effect than  $(\text{NH}_4)_2\text{SO}_4$ : compared with the growth in ambient air, the N concentration in the needles exposed to  $\text{NH}_3$  had increased by 49% in four months, while the increase after the highest N-fertilization ( $200 \text{ kg N ha}^{-1} \text{ y}^{-1}$ ) was only 8%. The small contribution of  $\text{NH}_4^+$  fertilization to the total N concentration was not due to a deficient N uptake: the  $^{15}\text{N}$  concentration in the needles increased significantly with time. On the other hand,  $\text{NH}_3$  uptake in shoots may have had a negative effect on the  $\text{NH}_4^+$  root uptake. The relation between plant N and atmospheric  $\text{NH}_3$  concentration was non-linear and possible reasons for this observation are discussed. Fumigation with  $\text{NH}_3$  significantly

decreased the ratios of K/N and P/N, showing that fumigation disrupted the nutrient balance.

### 2.2. Introduction

Deposition of atmospheric N has increased during the last decades, especially in the Netherlands (Asman et al., 1988; Maas, 1991). It is probably an important cause of forest decline in Europe. Conifers and hardwoods planted on soils originally poor in available N, are in the most difficult situation (Van Breemen & Van Dijk, 1988). The  $\text{NH}_y$  deposition ( $\text{NH}_3 + \text{NH}_4^+$ ) on the forests in the Netherlands is higher than average over the country as a whole: 80 compared with 47  $\text{kg N ha}^{-1} \text{ y}^{-1}$  (Houdijk & Roelofs, 1991) and N deposition is assumed to be implicated in the damage to forest ecosystems (Boxman et al., 1986; Heinsdorf, 1991; Nihlgård, 1985; Roelofs et al., 1985; Van Dijk et al., 1990). Scots pine (*Pinus sylvestris* L.) is the most common species in boreal forests and its vitality is low nearly all over Central and Western Europe. Heij & Schneider (1991), Horn et al. (1989), Temmerman &

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<sup>2</sup> Published as Pérez-Soba M, Van der Eerden LJM. 1993. Nitrogen uptake in needles of Scots pine (*Pinus sylvestris* L.) when exposed to gaseous ammonia and ammonium fertilizer in the soil. *Plant and Soil* 153: 231-242.

Coosemans (1988) and Van Dijk & Roelofs (1988) found that Scots pine with yellow needles have a very high N concentration of 2.8 to 3% dry weight, far above the 1.8% considered optimal for production (Von Heinsdorf & Krauß, 1991). They concluded that this is caused by excess atmospheric N entering the tree through roots and shoots.

Hutchinson et al. (1972) and Porter et al. (1972) found that leaves are efficient sinks for gaseous  $\text{NH}_3$ . Later reports (Dueck et al., 1991; Lalissee & Bardin, 1981; Van der Eerden et al., 1990; Whitehead & Lockyer, 1987) confirmed that atmospheric  $\text{NH}_3$  can be used by the plant as a source of N. Ammonia can be absorbed through the cuticle but it mainly enters the plant via the stomata (Hosker & Lindberg, 1982). Van Hove et al. (1989) found that only 3% of the  $\text{NH}_3$  absorbed by leaves of *Phaseolus vulgaris* L. entered via the cuticle while the remainder passed via the stomata. The dominant role of stomata in  $\text{NH}_3$  absorption was also found in conifers (Van Hove et al., 1992). N absorption takes place in two phases: first it is stored and then it is metabolized (Lalissee & Bardin, 1981), in which  $\text{NH}_3$  is incorporated into organic N via the glutamine synthetase-glutamate synthase pathway (Mifflin & Lea, 1976). These organic N compounds are partly transported from the needles via the phloem to the roots where they can meet the N requirement (Lemon & Van Houtte, 1980; Rowland, 1986; Rowland et al., 1987).

Cycling-organic N compounds play a major role in regulation of nitrate uptake (Cooper et al., 1986; Rowland et al., 1987). We suggest that direct foliar uptake of  $\text{NH}_3$  will increase the N concentration in the needles and transport to the roots where it will reduce the N uptake.

The object of our experiment was to test this hypothesis: the incorporation of needle N from  $\text{NH}_3$  fumigation was measured as well as N uptake by fertilizing with  $(\text{NH}_4)_2\text{SO}_4$ . We chose  $\text{NH}_4^+$  instead of  $\text{NO}_3^-$  as the N source, because conifer seedlings and mycorrhizas show preference for  $\text{NH}_4^+$  (Boxman & Roelofs, 1988; Finlay et al., 1989a; Kamminga-van Wijk, 1991; Zedler et al., 1986).  $\text{SO}_4^{2-}$  was chosen because this is an important anion in precipitation (Heij & Schneider, 1991). Scots pine trees were exposed to gaseous  $\text{NH}_3$  in open-top chambers and received  $^{15}\text{N}$ -labelled  $(\text{NH}_4)_2\text{SO}_4$  via the soil. The changes in N concentrations of the needles were followed for a year so that seasonal changes in N uptake in relation to the N requirement for growth were recorded. In addition, we studied the influence of  $\text{NH}_3$  on the nutrient balance and water demand implied by earlier experiments (Van der Eerden & Pérez-Soba, 1992; Van der Eerden et al., 1992) and the nutrient composition and water potential of the needles were examined.

### 2.3. Materials and methods

Table 2.1. Design of the experiment in which *Pinus sylvestris* L. trees were exposed to ambient air (AA) and AA supplemented with 46 or 98  $\mu\text{g m}^{-3}$   $\text{NH}_3$  and fertilized with  $(\text{NH}_4)_2\text{SO}_4$  (enriched with  $^{15}\text{N}$ ) over one year.

$\text{NH}_3$ fumigation ( $\mu\text{g m}^{-3}$ )	$(\text{NH}_4)_2\text{SO}_4$ fertiliza- tion ( $\text{kg N ha}^{-1} \text{y}^{-1}$ )
7 (AA)	0 50 100 200
53	0 50
105	0 50

#### Plant material and soil type

Two-year-old Scots pine trees (*Pinus sylvestris* L.) of the same provenance were potted in nutrient-poor acidic sandy soil in 10 l pots. The soil had a  $\text{pH}(\text{CaCl}_2)$  of 5.2 and a nutrient composition of 0.20 % dry weight total N,  $6.94 \mu\text{g g}^{-1} \text{NH}_4^+$ ,  $9.43 \mu\text{g g}^{-1} \text{NO}_3^-$ ,  $1.31 \mu\text{g g}^{-1} \text{P}$  and  $24.9 \mu\text{g g}^{-1} \text{K}$ . After planting, the trees were kept outdoors for 11 months until the start of the experiment, which ran from August 1988 till August 1989.

#### Fumigation and fertilization

In August 1988, we chose seventy-two trees of similar height and with about the same number of branches. We divided them at random into eight groups, each with nine replicates, and placed them in open-top chambers. For one year, four of the groups were exposed to ambient air (AA) and the other four to AA supplemented with 46 or 98  $\mu\text{g m}^{-3}$   $\text{NH}_3$ . The annual mean concentration of  $\text{NH}_3$  in AA was  $7 \mu\text{g m}^{-3}$ . A description of the chambers and  $\text{NH}_3$

fumigation is given by Dueck (1990). Table 2.1 is a summary of the experimental design.

Once a month, a  $(\text{NH}_4)_2\text{SO}_4$  solution enriched in  $^{15}\text{N}$ , ( $^{15}\text{N}$  abundance of 2.260 atom % compared with a natural abundance of 0.366 atom %) was applied as a fertilizer to the soil, at N concentrations equivalent to 50, 100 and 200  $\text{kg N ha}^{-1} \text{y}^{-1}$  (hereafter referred to as  $\text{kg N}$ ) to a number of the groups exposed to AA or to 53 and 105  $\mu\text{g m}^{-3}$   $\text{NH}_3$  (Tab. 2.1). We chose a fertilization of 50  $\text{kg N}$  because it is close to the average deposition in the Netherlands. The  $^{15}\text{N}$  enriched  $(\text{NH}_4)_2\text{SO}_4$  solution was supplied to field capacity to prevent loss of fertilizer. Hence, there were no losses of seepage water. Since other vegetation competes strongly for the fertilizer, we weeded once in the winter and once a month from April onwards. We watered the trees when necessary to prevent a low soil water status that could make the fertilization useless or even harmful (Van Breemen & Van Dijk, 1988).

#### Assessment of parameters in the needles

At the end of the experiment we harvested the needles and sorted them into current-year and one-year-old needles. After oven-drying them at  $75^\circ\text{C}$  for 24 h, we weighed and ground them, and determined total N (Carlo Erba Elemental Analyzer). We also dry-ashed samples at  $600^\circ\text{C}$  and measured the concentrations of P, Ca and Mg, using a Technicon Auto Analyzer and K by flame spe-

trophotometry. We determined the  $^{15}\text{N}$  atom % abundance by mass spectrometry using the method described by Dueck et al. (1991). To determine the seasonal variations in N concentration and dry-weight percentage (% fresh weight) in the needles, five times during the experiment we took samples of approx. 0.5 g fresh weight. Samples were taken from the central branch at the top of the crown because the N concentration in the needles significantly varies according to their position in the crown (Evers et al., 1991). We measured the current-year and one-year-old top shoots at maximum length.

We derived the following formula to estimate the respective contributions of  $\text{NH}_3$  fumigation and  $(\text{NH}_4)_2\text{SO}_4$  fertilization to the needle N concentration:

$$\Delta\text{N} = \Delta\text{N}_{\text{NH}_3} + \Delta\text{N}_{(\text{NH}_4)_2\text{SO}_4} \quad (1)$$

where:

$\Delta\text{N}$  = increase in N concentration (% dry-weight) due to the  $\text{NH}_3$  treatment, calculated by subtracting the N concentration in the needles of trees exposed to AA from the N concentration in the needles exposed to AA supplemented with  $\text{NH}_3$  and fertilized with  $(\text{NH}_4)_2\text{SO}_4$ .

$\Delta\text{N}_{\text{NH}_3}$  = increase in N concentration due to gaseous  $\text{NH}_3$

$\Delta\text{N}_{(\text{NH}_4)_2\text{SO}_4}$  = increase in N concentration due to uptake by the roots from the  $\text{NH}_4^+$  fertilizer and transport to the needles, calculated as follows:

Table 2.2. Needle biomass (g DW) and length increment of the current-year central shoot with respect to the previous year (% previous year length) of *Pinus sylvestris* L. exposed to ambient air (AA), 53 and 105  $\mu\text{g m}^{-3}$   $\text{NH}_3$  and fertilized with  $(\text{NH}_4)_2\text{SO}_4$  ( $\text{kg N ha}^{-1} \text{y}^{-1}$ ). Means ( $n = 9$ ) in each column with the same letter are not significantly different at  $\alpha = 0.05$ .

$\text{NH}_3$ fumigation	$(\text{NH}_4)_2\text{SO}_4$ fertilization	Needle biomass		Shoot length increment
		Current year	One year-old	
AA	0	20.7 <sup>a</sup>	11.3 <sup>a</sup>	38.6 <sup>a</sup>
AA	50	18.3 <sup>a</sup>	10.8 <sup>ab</sup>	58.7 <sup>a</sup>
AA	100	20.1 <sup>a</sup>	10.4 <sup>ab</sup>	69.8 <sup>a</sup>
AA	200	28.6 <sup>b</sup>	10.7 <sup>ab</sup>	82.8 <sup>a</sup>
53	0	31.8 <sup>b</sup>	6.8 <sup>bc</sup>	64.3 <sup>a</sup>
53	50	31.3 <sup>b</sup>	4.8 <sup>c</sup>	67.4 <sup>a</sup>
105	0	32.8 <sup>b</sup>	5.9 <sup>c</sup>	57.4 <sup>a</sup>
105	50	34.1 <sup>b</sup>	8.7 <sup>abc</sup>	56.8 <sup>a</sup>

$$\frac{a-b}{c-b} \times (\% \text{ N needles})$$

(2)

where a, b and c are, respectively, the atom %  $^{15}\text{N}$  in the needle sample, in the air and in the added  $\text{NH}_4^+$ . We made the following assumptions in the calculation of (2):  $^{14}\text{N}$  and  $^{15}\text{N}$  behave similarly in the tree, the natural difference in  $^{15}\text{N}$ -percentage between AA and needles was negligible (Vose, 1980) and the needles did not lose N. By substitution of (2) in (1)  $\Delta\text{N}_{\text{NH}_3}$  can be calculated. The incorporation rate of total N into the needles by  $\text{NH}_4^+$  fertilization was calculated from (2).

#### Water potential

We measured the water potential in the needles using the pressure chamber described by Scholander et al. (1965).

#### Statistics

We evaluated needle biomass, the concentration of N, Ca, Mg, P, K,  $^{15}\text{N}$  and the uptake rate of total N from the fertilizer with analyses of variance. We performed a square root transformation to stabilize the variance of the  $^{15}\text{N}$  content and the data on the rate of uptake of total N from the fertilizer. After the analyses of variance we used an LSD-test for testing pairwise differences.

Water potential data were studied by analyses of variance according to a split-plot design, with

Figure 2.1. Changes in total N concentration (% DW) with time in needles of *Pinus sylvestris* L. exposed to ambient air and fertilized with  $(\text{NH}_4)_2\text{SO}_4$  ( $\text{kg N ha}^{-1} \text{ y}^{-1}$ ). Each bar diagram represents the mean of nine measurements and the vertical lines represent the LSD at  $\alpha = 0.05$  for each harvest.

fertilization on whole plots and time on subplots.

## 2.4. Results

### Shoot growth

We measured growth in terms of needle biomass production and increase in length of the central shoot compared to that in the previous year (Table 2.2). Data concern the biomass of the remaining needles. The biomass of previous year's needles (one-year-old) decreased significantly after  $\text{NH}_3$  fumigation (loss of needles), while the production of current-year needles showed a significant increase after  $\text{NH}_3$  fumigation and after the largest fertilization,

200 kg N. The increase in length of the central shoot was greater with respect to that of the previous year after fumigation and fertilization,

although differences were not significant.

There was a general increase in the dry weight percentage with time

Figure 2.2. Changes in total N concentration (% DW) with time in needles of *Pinus sylvestris* exposed to ambient air, to 53 and to 105  $\mu\text{g m}^{-3}$   $\text{NH}_3$  and not fertilized (a) or fertilized with  $(\text{NH}_4)_2\text{SO}_4$  to 50 kg N  $\text{ha}^{-1}$   $\text{y}^{-1}$  (b). Each bar diagram represents the mean of nine measurements and the vertical lines represent the LSD at  $\alpha = 0.05$  for each harvest.



Table 2.3.  $^{15}\text{N}$  concentration (atom % excess) and uptake rate of total N ( $\text{mg N g}^{-1}$  needle FW month $^{-1}$ ) by  $(^{15}\text{NH}_4)_2\text{SO}_4$  fertilization (1.894 atom % excess  $^{15}\text{N}$ ) into one-year-old needles of *Pinus sylvestris* exposed to ambient air (AA), to 53 and to 105  $\mu\text{g m}^{-3}$   $\text{NH}_3$  and fertilized with  $(^{15}\text{NH}_4)_2\text{SO}_4$ . Contrast between different fumigation (AA, 53 and 105  $\mu\text{g m}^{-3}$   $\text{NH}_3$ ) and fertilization (50, 100 and 200  $\text{kg N ha}^{-1} \text{y}^{-1}$ ) treatments, and their interactions with time were tested by analyses of variance. \*,  $P < 0.05$ ; \*\*,  $P < 0.01$ ; \*\*\*,  $P < 0.001$ ; ns, not significant.

NH <sub>3</sub> fumigation	(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> fertilization	$^{15}\text{N}$				N uptake rate			
		Dec	Feb	May	Aug	Dec	Feb	May	Aug
AA	50	0.006	0.020	0.057	0.063	0.009	0.021	0.039	0.027
AA	100	0.007	0.051	0.108	0.145	0.013	0.058	0.069	0.051
AA	200	0.068	0.190	0.371	0.291	0.144	0.237	0.278	0.155
53	50	0.004	0.017	0.044	0.044	0.008	0.027	0.044	0.027
105	50	0.006	0.019	0.043	0.040	0.015	0.039	0.056	0.025
Analyses of variance									
Time		***				***			
NH <sub>3</sub> fumigation		ns				ns			
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> fertilization		***				***			
Time x fumigation		ns				ns			
Time x fertilization		*				**			

from August 1988 to August 1989 (no data given), that was only interrupted in the spring. In August 1989, the dry weight percentage of the one-year-old needles was significantly higher in the fumigated trees than in the non-fumigated ones, while in the current-year needles, the dry weight percentage was significantly lower in the fumigated trees.

#### Changes in total N concentration in needles

The N concentration of the one-year-old needles was not significantly affected by  $(\text{NH}_4)_2\text{SO}_4$  fertilization, except with 200  $\text{kg N}$  in August 1989 (Fig. 2.1). It increased very

significantly ( $P < 0.001$ ) with  $\text{NH}_3$  fumigation, both with or without 50  $\text{kg N}$  (Figs. 2.2a and 2.2b). Interestingly, the N concentration of one-year-old needles of unfertilized trees was not much different for the two  $\text{NH}_3$  fumigation treatments; for fertilized trees, the higher  $\text{NH}_3$  fumigation resulted in a higher N concentration from August 1988 till May 1989. No significant interaction was found between  $\text{NH}_3$  fumigation and  $(\text{NH}_4)_2\text{SO}_4$  fertilization. The tendency found for current-year needles was the same as found for one-year-old needles, a l

Table 2.4.  $^{15}\text{N}$  concentration (atom % excess) and uptake rate of total N ( $\text{mg N g}^{-1}$  needle FW  $\text{month}^{-1}$ ) by  $(\text{NH}_4)_2\text{SO}_4$  fertilization (1.894 atom % excess  $^{15}\text{N}$ ) into current-year needles of *Pinus sylvestris* exposed to ambient air (AA), to 53 and to 105  $\mu\text{g m}^{-3}$   $\text{NH}_3$  and fertilized with  $(\text{NH}_4)_2\text{SO}_4$ . Needles emerged in May 1989. Contrast between different fumigation (ambient, 53 and 105  $\mu\text{g m}^{-3}$   $\text{NH}_3$ ) and fertilization (50, 100 and 200  $\text{kg N ha}^{-1} \text{y}^{-1}$ ) treatments was tested by analyses of variance. \*\*\*,  $P < 0.001$ .

$\text{NH}_3$ fumigation	$(\text{NH}_4)_2\text{SO}_4$ fertilization	$^{15}\text{N}$	N uptake rate
AA	50	0.160	0.243
AA	100	0.276	0.386
AA	200	0.543	0.877
53	50	0.094	0.153
105	50	0.095	0.170
Analyses of variance			
$\text{NH}_3$ fumigation		***	***
$(\text{NH}_4)_2\text{SO}_4$ fertilization		***	***

though its statistical significance was less.

#### Changes in $^{15}\text{N}$ concentration in needles

Data on one-year-old and current-year needles are shown in tables 2.3 and 2.4, respectively. In one-year-old needles,  $^{15}\text{N}$  concentration increased with time in all the  $(\text{NH}_4)_2\text{SO}_4$  fertilization treatments. This increase was more than proportional: the results suggest a curve of the second order in which the  $^{15}\text{N}$  concentration proportionally increased with the square of the exposure time.

The contribution of 50  $\text{kg N}$  to the  $^{15}\text{N}$  concentration did not differ significantly between fumigated and non-fumigated trees, although it was lower in the fumigated trees. There was a very clear response to  $\text{NH}_4^+$  fertilization, with the highest  $^{15}\text{N}$  concentration observed with the highest concentration of fertilizer. We found a significant interaction between time and fertilization. Current-year needles, formed during the experimental period, had a significantly higher ( $P < 0.001$ )  $^{15}\text{N}$  concen-

Table 2.5. Contribution (%) of  $\text{NH}_3$  fumigation (FU) and  $(^{15}\text{NH}_4)_2\text{SO}_4$  fertilization (FE) to the increase in N concentration in needles of *Pinus sylvestris* exposed to ambient air supplemented with  $\text{NH}_3$  (53 and 105  $\mu\text{g m}^{-3}$ ) and fertilized with  $(\text{NH}_4)_2\text{SO}_4$ . Increase with respect to N concentration after exposure to ambient air.

$\text{NH}_3$ fumigation	$(\text{NH}_4)_2\text{SO}_4$ fertilization	One-year-old needles								Current-year needles	
		Dec 1988		Feb 1988		May 1988		Aug 1988		Aug 1988	
		FU	FE	FU	FE	FU	FE	FU	FE	FU	FE
53	50	99.3	0.7	97.3	2.7	90.0	10.0	91.6	8.4	82.1	17.9
105	50	99.3	0.7	97.9	2.1	82.0	18.0	92.5	7.5	81.2	18.8

tration than one-year-old needles despite the shorter exposure time (Tables 2.3 and 2.4).

The results were the same as in one-year-old needles, but more significant effects of fumigation were observed.

#### Changes in rate of uptake of fertilizer N by needles

The uptake rate of fertilizer N into the needles indicated a pattern of response similar to that of  $^{15}\text{N}$  content (Tables 2.3 and 2.4).

#### Incorporation of N from fumigation and fertilization

The contribution of  $\text{NH}_3$  fumigation and  $(\text{NH}_4)_2\text{SO}_4$  fertilization to the N concentration of the needles differed significantly (Table 2.5). In one-

year-old needles, approximately 90% of the increase in N concentration was due to  $\text{NH}_3$  fumigation and about 10% to the 50 kg N fertilization. In the current-year needles the percentage contribution from fumigation was 80% compared to 20% from fertilization.

#### Macronutrient composition

Table 2.6 shows the P, K, Mg and Ca composition of one-year-old and current-year needles. One-year-old needles had lower P, K and Mg and higher Ca concentrations than current-year needles. Specifically the K % dry weight was reduced in proportion to the  $\text{NH}_4^+$  fertilization.

The concentrations of all macronutrients in current-year needles were lower in the  $\text{NH}_3$  fumigated

Figure 2.3. Changes in water potential (MPa) of needles of *Pinus sylvestris* exposed to ambient air and fertilized with  $(\text{NH}_4)_2\text{SO}_4$  ( $\text{kg N ha}^{-1} \text{ y}^{-1}$ ), on the 17th August 1989. LSD at  $\alpha = 0.05$  for all pairwise comparisons.

than in non-fumigated treatments while the total amounts (expressed in mg of dry-weight) were higher. In current-year needles the K % dry weight was not so much affected by  $\text{NH}_4^+$  fertilization as in one-year-old needles.

#### Water potential

The water potential of needles was not affected by  $\text{NH}_3$  fumigation (no data given). It decreased significantly with N fertilization during the warmest part of the day (Fig 2.3). The daily course of the water potential showed the usual drop during day time from 5:00 to 17:00 h, whereafter recovery occurred from 17:00 to 20:00 h. The drop in potential was strongly increased by the 100 and 200 kg N application, although full recovery to the control levels occurred between 17:00 and 20:00 h.

Table 2.6. Chemical composition of needles of *Pinus sylvestris* collected after one year exposure to ambient air (AA) and 53 and 105  $\mu\text{g m}^{-3}$   $\text{NH}_3$  and fertilization with  $(\text{NH}_4)_2\text{SO}_4$ . In the first column the chemical composition is given in  $\text{mg g}^{-1}$  DW, in the second column relative to N and in the third column in total amount expressed in mg DW.

NH <sub>3</sub> fumigation	(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> fertiliza- tion	Chemical composition											
		mg g <sup>-1</sup> DW				% of N (N = 100)				Total amount (mg)			
		P	K	Mg	Ca	P	K	Mg	Ca	P	K	Mg	Ca
One-year-old needles													
AA	0	0.93	2.25	1.40	4.41	9.27	22.43	13.96	43.97	11	25	16	50
AA	50	0.89	1.70	1.71	5.21	8.79	16.73	16.83	51.43	10	18	18	56
AA	100	0.89	1.63	1.55	4.64	8.74	16.04	15.25	45.80	9	17	16	48
AA	200	0.83	1.52	1.42	4.62	6.84	12.49	11.71	38.09	9	16	15	49
53	0	0.82	1.34	1.37	4.14	6.18	10.06	10.32	31.16	6	9	9	28
53	50	0.65	1.29	1.31	3.96	4.62	9.13	9.31	28.11	3	6	6	19
105	0	0.99	1.32	1.28	4.03	7.23	9.64	9.34	29.38	6	8	8	24
105	50	0.76	1.56	1.23	4.18	5.35	11.03	8.72	29.61	7	14	11	36
Current-year needles													
AA	0	0.89	2.85	1.70	2.70	8.88	28.34	17.00	27.03	18	59	35	56
AA	50	1.25	2.68	2.08	2.84	10.78	23.06	17.93	24.48	23	49	38	52
AA	100	1.37	2.58	2.11	2.61	12.84	24.22	19.85	24.55	27	52	42	52
AA	200	1.13	2.31	1.70	2.20	9.15	18.74	13.82	17.85	32	66	49	63
53	0	0.77	1.98	1.73	2.30	6.70	17.22	15.04	20.00	24	63	55	73
53	50	0.92	1.72	1.75	2.25	7.38	13.83	14.11	18.10	29	54	55	70
105	0	1.06	2.26	1.80	2.26	8.41	17.90	14.25	17.90	35	74	59	74
105	50	0.91	2.14	2.26	2.34	6.65	15.74	17.90	17.17	31	73	56	80

## 2.5. Discussion

A year of fertilization had hardly any effect on either the needle biomass or the N concentration in the needles. Contribution of the fertilizer N to the total N concentration in the needles varied from 2 to 15% depending

on the treatment. The small size of this contribution could have several reasons. It might be explained by the distribution of fertilizer N in the tree: in general, roots and trunk have the highest proportion and needles and leaves the lowest (Lalisse & Bardin, 1981; Paavilainen, 1973; Porter et al., 1972). Another reason could be the late moment at which

we applied the fertilizer (August 1988): the N content of the needles can only be increased during the year of the fertilization when fertilizer is applied at the start of the growing season (Paavilainen, 1973). Differences in the shoot-root ratio offer another explanation; Van Dijk et al. (1990) observed that a treatment with  $480 \text{ kg N ha}^{-1} \text{ y}^{-1}$  applied in rainwater to young coniferous trees increased the shoot-root ratio and decreased the biomass of fine roots and the number of mycorrhizas hence increasing the sensitivity to drought. This hypothesis is supported by the water potential results (Fig. 3): the lowest water potential occurred after 100 and 200 kg N fertilization, in the afternoon. This finding agrees with the conclusion of De Kam et al. (1991) who also found a decrease in the water potential with an annual fertilization of 150 and 300 kg N ha<sup>-1</sup>. We confirmed that the lower water potential of the fertilized needles was not due to a higher transpiration resulting from the higher needle biomass. We did not find a decrease in <sup>15</sup>N uptake in fertilized trees (Tables 2.3 and 2.4), indicating that differently from water uptake, the roots still functioned well in N uptake.

In agreement with Paavilainen (1973) current-year needles had a greater proportion of fertilizer N than one-year-old needles, possibly caused by N translocation from one-year-old to current-year needles and/or a more active transport of <sup>15</sup>N from the roots to current-year needles. With the emergence of current-

year needles in May the fertilizer N uptake rate by one-year-old needles decreased till the end of the experiment in August. The stimulation of needle biomass in current-year needles (Table 2.2) and the increase in N concentration with time (Fig. 2.2) in trees fumigated with NH<sub>3</sub>, confirm that atmospheric NH<sub>3</sub> is used as a foliar supply of N. The NH<sub>3</sub>-induced increases in N concentration were not caused by stimulation of the N uptake by the roots (Tables 2.3 and 2.4). Gaseous NH<sub>3</sub> did not increase the uptake of NH<sub>4</sub><sup>+</sup> by the one-year-old needles and it even depressed it in the current-year needles. Needles fumigated with NH<sub>3</sub> had a N concentration above 1.3% (Fig. 2.2), a level considered to be sufficient for growth (Van den Burg, 1985); non-fumigated needles were N-deficient (Fig. 2.1). We conclude that uptake of NH<sub>4</sub><sup>+</sup> is reduced at the root surface or on the xylem loading when needles get enough N from the gaseous NH<sub>3</sub>, which evidently yields a signal to the roots. In the same way, N deficiency in the non-fumigated needles also might provide a signal to the roots to increase the uptake or the transport of N derived from the NH<sub>4</sub><sup>+</sup> fertilizer (see the <sup>15</sup>N concentration and N uptake rate of the needles in Tables 2.3 and 2.4). The results of Cooper et al. (1986) were similar: when the N supply to the root via the phloem by foliar feeding was increased, the N uptake by the root from the soil was decreased. Therefore, the N status of the shoot may control the N uptake in the roots. Clarkson (1986) suggested

that this shoot-root signal could be a gradient of organic (perhaps specific amino acids) and inorganic compounds. Farquhar et al. (1980) and Lemon & Van Houtte (1980) found that for ambient concentrations above the "compensation point" of  $\text{NH}_3$  ( $0.5\text{--}5.0 \mu\text{g m}^{-3}$ ), at which net  $\text{NH}_3$  uptake occurs,  $\text{NH}_3$  was deposited in a linear proportion to ambient  $\text{NH}_3$  concentrations, in *Zea mays* L. and fields of *Glycine max* L. and *Medicago sativa* L., respectively. Van Hove et al. (1987) found that  $\text{NH}_3$  flux into leaves increases linearly with concentrations up to  $400 \mu\text{g m}^{-3}$ . When Porter et al. (1972) and Whitehead & Lockyer (1987) fumigated corn seedlings and Italian ryegrass, respectively, with  $\text{NH}_3$  after isolating the soil-root system from the air in the chamber and fertilizing it with  $\text{NO}_3^-$ , they found a linear increase in the total plant N as the  $\text{NH}_3$  concentration increased. In the present long-term experiment we did not find a linearity between plant N and atmospheric  $\text{NH}_3$  in the needles: there was no significant difference between several parameters such as needle biomass (Table 2.2), total N (Fig. 2.2) and  $^{15}\text{N}$  concentrations and fertilizer N uptake rate by needles (Tables 2.3 and 2.4) after fumigation with  $53 \mu\text{g m}^{-3} \text{NH}_3$  and  $105 \mu\text{g m}^{-3} \text{NH}_3$ . The lack of linearity could be due to the N concentration reached in both fumigations: after 4 months exposure to  $\text{NH}_3$ , this was close to 2% considered the threshold for damage (Focke, 1991). Hence, in our experiment,  $53 \mu\text{g m}^{-3}$

$\text{NH}_3$  was already optimal for needle biomass production. The "excess" N provided by  $105 \mu\text{g m}^{-3} \text{NH}_3$  may form part of the cycling-N (Cooper et al., 1986) and/or be stored in other parts of the tree than the needles (Bowden et al., 1989; Lalissee & Bardin, 1981). Considering the  $\text{NH}_3$  compensation point (max.  $5 \mu\text{g m}^{-3} \text{NH}_3$ ), we do not expect loss of  $\text{NH}_3$  from the needles by volatilization at the concentrations used. In conclusion, linearity between  $\text{NH}_3$  flux and  $\text{NH}_3$  concentration is limited to short-time studies, since the optimal N concentrations in leaves are soon reached.

Heinsdorf (1991) and Houdijk & Roelofs (1991) found a positive correlation between intensive livestock breeding, a major source of atmospheric  $\text{NH}_3$ , and loss of needles. We also found a lower needle biomass in the one-year-old needles of the trees exposed to  $\text{NH}_3$ , implicating that  $\text{NH}_3$  accelerates their fall, thus counteracting the uptake of extra N. The shorter leaf life means an increased production of litter which, because of its high N/C ratio, has a high mineralization rate. Needle fall accelerates the cycling of N and the leaching of N to the ground water.

In four months, the N concentration in the needles exposed to  $\text{NH}_3$  increased by 49% compared with ambient air, while the increase caused by 200 kg N ammonium fertilization was only 8%. This observation implies that in our experiment,  $\text{NH}_3$  fertilized faster than  $(\text{NH}_4)_2\text{SO}_4$ . Van der Eerden et al.

(1992) also found a more marked effect with  $\text{NH}_3$  than  $\text{NH}_4^+$  on the N concentration in the needles of *Pseudotsuga menziesii* [Mirb.] Franco, after 13 weeks exposure to  $180 \mu\text{g m}^{-3} \text{NH}_3$  and 500 and  $2500 \mu\text{mol l}^{-1} (\text{NH}_4)_2\text{SO}_4$ . On the one hand, direct foliar uptake of  $\text{NH}_3$  can be more efficient for the trees than  $\text{NH}_4^+$  uptake through the roots, because competition with soil microorganisms and leaching to the ground water are avoided (Bowden et al., 1989). On the other hand foliar uptake of  $\text{NH}_3$  is disadvantageous for the acid-base regulation of the needle (Raven, 1988).

The general increase in the dry-weight percentage of needles with time was probably caused by an increase in dry matter (as is commonly found in Scots pine forests) due to an increase in the starch content of one-year-old needles during spring and early summer (Ericsson, 1979). In August, we found a significantly higher dry-weight percentage in one-year-old needles fumigated with  $\text{NH}_3$ . This could be explained by a lower water content in the needles since  $\text{NH}_3$  increases the shoot-root ratio (Van der Eerden & Pérez-Soba, 1992). Therefore more drought stress in the summer months may be expected.

During the experiment all the treatments showed the same pattern: accumulation of Ca and Mg and a decrease of K and P down to levels of deficiency. The higher contents of P, K, Mg and Ca found in the current-year needles exposed to  $\text{NH}_3$  could be explained by the higher

needle biomass of the fumigated needles but also by the translocation of nutrients from one-year-old needles. Translocation may be caused by faster abscission of dying needles in the fumigated trees and the higher nutrient values of living one-year-old needles compared with those of the current-year needles (Mohren, 1987).  $\text{NH}_3$  fumigation significantly decreased the ratios K/N and P/N. These changes in nutrient balance have been related to high N concentration in shoots such as those found by Van der Eerden et al. (1992) after fumigating *Pseudotsuga menziesii* [Mirb.] Franco saplings with  $180 \mu\text{g m}^{-3} \text{NH}_3$ .

Toxic effects of  $\text{NH}_3$  should always be considered due to the ability of the needles to absorb  $\text{NH}_3$  even when the trees are well supplied with N from the soil (Porter et al., 1972; Whitehead & Lockyer, 1987). Toxicity of  $\text{NH}_3$  will depend on concentrations of N in the ambient air, plant and soil. The  $\text{NH}_3$  concentrations used in our experiment, about 50 and  $100 \mu\text{g m}^{-3}$ , are higher than the mean concentrations in the highest  $\text{NH}_3$ -polluted parts of The Netherlands ( $25\text{-}40 \mu\text{g m}^{-3}$ ) and comparable to measurements in Germany made by Von Heinsdorf & Krauß (1991), which were  $45\text{-}65 \mu\text{g m}^{-3}$ . Therefore, an increased emission of  $\text{NH}_3$  into the atmosphere, will result in a higher  $\text{NH}_3$  uptake by the trees which K/N ratio will decrease, increasing the sensitivity of plants to frost (Dueck et al., 1991) and drought (Van der Eerden et al.,



1991). Hence, plants will be damaged more easily.

The present results are relevant for forest management as they indicate a considerable contribution of atmospheric  $\text{NH}_3$  to the N concentration in the needles (Table 2.5) which need to be taken into account in forest fertilization experiments. Extending these findings to crops, atmospheric  $\text{NH}_3$  deposition should be estimated in any soil fertilization in order to reduce the N dosage.