6. Interactive effects of gaseous ammonia, ozone and elevated carbon dioxide

6.1. Abstract

Four-year-old saplings of Scots pine (Pinus sylvestris L.) were exposed for 11 weeks in controlled-environment chambers to charcoal-filtered air, or to charcoal-filtered air supplemented with NH₃ (40 µg m⁻³), O₃ (110 µg m⁻³ during day/ 40 µg m⁻³ during night) or NH₃ + O₃. All treatments were carried out at ambient (350 µl l⁻¹) and at elevated CO₂ concentration (700 µl l⁻¹). Total tree biomass, mycorrhizal infection, net CO₂ assimilation (Pₙ), stomatal conductance (gₛ), transpiration of the shoots and NH₃ metabolism of the needles were measured. In ambient CO₂ (1) gaseous NH₃ decreased mycorrhizal infection, without significantly affecting tree biomass or N concentration and it enhanced the activity of glutamine synthetase (GS) and glutamate dehydrogenase (GDH) in one-year-old needles; (2) ozone decreased mycorrhizal infection and the activity of GS in the needles, while it increased the activity of GDH; (3) exposure to NH₃ + O₃ lessened the effects of single exposures to NH₃ and O₃ on reduction of mycorrhizal infection and on increase in GDH activity. Similar lessen effects on mycorrhizal infection as observed in trees exposed to NH₃ + O₃ at ambient CO₂, were measured in trees exposed to NH₃ + O₃ at elevated CO₂. Exposure to elevated CO₂ without pollutants did not significantly affect any of the parameters studied, except for a decrease in the concentration of soluble proteins in the needles. Elevated CO₂ + NH₃ strongly decreased root branching and mycorrhizal infection and temporarily stimulated Pₙ and gₛ. The exposure to elevated CO₂ + NH₃ + O₃ also transiently stimulated Pₙ. The possible mechanisms underlying and integrating these effects are discussed. Elevated CO₂ clearly did not alleviate the negative effects of NH₃ and O₃ on mycorrhizal infection. The significant reduction of mycorrhizal infection after exposure to NH₃ or O₃, observed before significant changes in gas exchange or growth occurred, suggest the use of mycorrhizal infection as an early indicator for NH₃ and O₃ induced stress.

6.2. Introduction

A realistic prediction of the effects of elevated atmospheric carbon dio-
(Norby et al., 1992) as a result of changes in carbon allocation, mycorrhizal infection and carbon-nitrogen relationships. An increase in the root/shoot ratio is frequently observed under limiting conditions of nutrients and/or water, but it is absent under non-limiting conditions (Stulen & Den Hertog, 1993). An interesting question is whether elevated CO$_2$ may counteract the decrease in net photosynthesis induced in trees by O$_3$ (Dizengremel et al., 1994) and enhance the stimulating effects of NH$_3$ on photosynthesis (Van der Eerden & Pérez-Soba, 1992; Van Hove et al., 1992). Ozone can also interact with NH$_3$, although little is known about the effects of this combination on growth of trees (Van der Eerden et al., 1993b; Van Hove & Bossen, 1994; Dueck, pers. commun.). In addition, elevated CO$_2$ might reduce O$_3$ and NH$_3$ uptake, since both gases are mainly absorbed through the stomata, and elevated CO$_2$ reduces stomatal conductance (Jarvis, 1989).

The above-mentioned possible effects of interactions between elevated CO$_2$, O$_3$ and NH$_3$ on tree growth, were studied in the following experiment. Scots pine saplings were exposed to NH$_3$, O$_3$ or the combination of NH$_3$ + O$_3$. All exposures were carried out at ambient and/or at elevated CO$_2$ concentrations, for a period of 11 weeks in controlled-environment chambers. The NH$_3$ concentration used was similar to the mean concentration observed in the more severely NH$_3$-polluted regions in The Netherlands.
The concentration of O$_3$ applied was close to that in the Dutch atmosphere from May to September (70-90 µg m$^{-3}$; Tonneijck, 1989). Special attention was paid to root development, including mycorrhizal infection, since NH$_3$ generally favours shoot growth above root growth (Fangmeier et al., 1994) and O$_3$ also increases the shoot/root ratio (Cooley & Manning, 1987). In addition, gas exchange (net CO$_2$ assimilation and transpiration), stomatal conductance, N metabolism in one-year-old needles (activities of GS and GDH) and concentration of soluble proteins were studied; physiological and biochemical characteristics of plants often are sensitive indicators of stress (Wolfenden & Mansfield, 1991).

6.3. Materials and Methods

Plant material and soil type
In January 1992 three-year-old Scots pine (Pinus sylvestris L.) saplings were potted into nutrient-poor acidic sandy soil in 10 l pots. The initial soil nutrient contents were 0.10% total N, 0.30% P$_2$O$_5$, 0.29% K$_2$O, with pH(CaCl$_2$) 4.8. All trees were 60-80 cm in height. In February they were placed in a heated glasshouse in order to break bud dormancy.

Exposure conditions
In April, saplings were placed into 8 controlled-environment exposure chambers, described by Dueck et al. (1992a); 5 saplings per chamber. At this time, the spring flush of the trees was underway. Temperature was 20°C during the day (16 h) and 15°C at night (± 0.5°C). Relative humidity was maintained at 75% ± 3% during day and night. Photon fluence density at 60 cm height above the pots was 430 (± 6.7) µmol m$^{-2}$ s$^{-1}$ (PAR), supplied by mercury (HPL-N) and sodium (SON-T) lamps.

Ambient air was ventilated via charcoal filters (1.5 s contact time) through each chamber (3.3 m$^3$) at a rate of 7 m$^3$ min$^{-1}$ (wind velocity ca. 1 m s$^{-1}$). If required, CO$_2$, NH$_3$ and O$_3$ were added to the charcoal-filtered air, singly or in combination, including a control treatment with only charcoal-filtered air. Elevated CO$_2$ was applied at 700 µl l$^{-1}$; NH$_3$ at 40 µg m$^{-3}$; and O$_3$ at 110 µg m$^{-3}$ (9-h mean d$^{-1}$) and 40 µg m$^{-3}$ (15-h mean d$^{-1}$). Ozone was generated from pure oxygen with a Sorbios generator. Concentrations of O$_3$ and NH$_3$ were sequentially monitored by an ultraviolet absorption analyser (model 8810) and a chemiluminescent nitrogen oxide analyser (model 8840), preceded by a thermo-convertor (model 8750), all from Monitor Labs. Carbon dioxide was monitored with an infrared gas analyser (Kipp 3300). There was no chamber replication since trial experiments showed a variation lower than 3 and 15% in the climatological and fumigation conditions, respectively (Mooi & Jolink, 1989).

Gas exchange measurements
Seven days after introduction of the trees in the fumigation chambers (allowing acclimation to differences in light intensity and air flow), gas exchange was determined. Net $\text{CO}_2$ assimilation ($P_n$), stomatal conductance ($g_s$) and transpiration of the shoots were measured after 7, 29 and 56 days of fumigation in a 0.25 l leaf cuvette with a LiCor 6200. Gas exchange measurements were always performed on the same group of one-year-old needles at ambient photon flux densities (360-460 $\mu\text{mol m}^{-2} \text{s}^{-1}$).

Needle area was calculated by sampling 96 needle pairs and measuring their individual projected areas with a Delta-T leaf area meter. A mean ratio of 1:2.25 (Dueck, pers. commun.) was used to convert projected to actual needle area. The individual needle pairs were oven-dried (50°C for 48 h) and weighed. The total needle area was estimated with the observed linear relationship between needle area and dry weight ($R^2 = 0.91$).

Determination of growth parameters and nitrogen metabolism
At the end of the experiment (22 June 1992) current-year needles, one-year-old needles, woody parts and roots were harvested separately and the fresh weight of all needles per tree was determined. Trees were harvested when the soil moisture content was at field capacity (0.10-0.20 MPa). After oven-drying at 75°C for 24 h, needles, wood and roots were weighed and needles were finely ground for N and C analysis. Nitrogen and carbon concentration were measured as $\text{NO}_2$ and $\text{CO}_2$, respectively, following combustion in oxygen and helium at 1000 °C, by gas chromatography with a Carlo Erba Elemental Analyser.

Whole-root systems were carefully washed, soaked in a metaphosphate solution (Saterson & Vítousek, 1984), washed again and stored at 4°C until further processing. Coarse (≥2 mm) and fine (<2 mm) roots were separated, and subsamples of approximately 1 g were taken from the fine root fraction to determine root length and branching with a gridline intersect method (Ambler & Young, 1977). The mycorrhizal infection was calculated as the percentage of mycorrhizal root tips with respect to total root tips. Roots were then oven-dried (48 h at 105°C) and weighed.

For determination of soluble protein concentration and GS and GDH activities (Pérez-Soba et al., 1994a), one-year-old needles (ca. 10 pairs per tree) were harvested in four trees per chamber, before and at the end of the fumigation.

Statistics
Data were tested for homogeneity with Barlett's test and were ln- and arcsin-transformed, if necessary. A three-way analysis of variance (Sokal & Rohlf, 1981) followed by an LSD-test for testing pairwise differences, was performed to test all parameters except for fine roots biomass. Data on fine roots biomass
were analysed with the Wilcoxon’s signed rank test, since they were not homogeneous after ln-transformation. The least significant difference between filtered air at ambient CO\textsubscript{2} (FA\textsubscript{a}) and the other treatments was calculated for $\alpha = 0.05$.

### Table 6.1. Biomass production (g DW) of Pinus sylvestris saplings.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Fine roots</th>
<th>Coarse roots</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ambient CO\textsubscript{2}</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FA\textsubscript{a}</td>
<td>4.1 ± 0.7</td>
<td>8.7 ± 2.2</td>
</tr>
<tr>
<td>NH\textsubscript{3}</td>
<td>4.6 ± 0.4</td>
<td>8.3 ± 1.0</td>
</tr>
<tr>
<td>O\textsubscript{3}</td>
<td>5.5 ± 0.3</td>
<td>8.6 ± 1.6</td>
</tr>
<tr>
<td>NH\textsubscript{3} + O\textsubscript{3}</td>
<td>5.7 ± 1.4</td>
<td>10.0 ± 2.5</td>
</tr>
<tr>
<td>Elevated CO\textsubscript{2}</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FA\textsubscript{e}</td>
<td>7.0 ± 1.4</td>
<td>12.8 ± 2.7</td>
</tr>
<tr>
<td>NH\textsubscript{3}</td>
<td>8.9 ± 3.1</td>
<td>11.5 ± 2.3</td>
</tr>
<tr>
<td>O\textsubscript{3}</td>
<td>7.5 ± 2.4</td>
<td>10.5 ± 3.0</td>
</tr>
<tr>
<td>NH\textsubscript{3} + O\textsubscript{3}</td>
<td>7.4 ± 2.3</td>
<td>11.2 ± 2.3</td>
</tr>
</tbody>
</table>

There were no statistically significant differences between fumigation treatments and FA\textsubscript{a} at P<0.05, irrespective of whether fumigation was carried out at ambient or at elevated CO\textsubscript{2}.

### 6.4. Results

#### Biomass production

Biomass production of current-year and one-year-old needles, wood and fine and coarse roots were not statistically significant affected by exposure of the saplings to any fumigation treatment. There was a trend for fine roots biomass to be higher under elevated CO\textsubscript{2} (Table 6.1); however, this trend was not statistically significant due to the large variance between trees at elevated CO\textsubscript{2}, which was ten times higher compared with the variance at ambient CO\textsubscript{2}.

#### Root characteristics

The number of root tips per root cm (root branching) was decreased by 81% in elevated CO\textsubscript{2} + NH\textsubscript{3} as compared to filtered air at ambient CO\textsubscript{2} (Fa\textsubscript{a}). Elevated CO\textsubscript{2} + NH\textsubscript{3} decreased root branching more than elevated CO\textsubscript{2} or NH\textsubscript{3} separately, showing an interaction (P=0.05) between both gases on root branching (Table 6.2).

The percentage of mycorrhizal tips with respect to total root tips (mycorrhizal infection) was strongly reduced by exposure of the saplings to NH\textsubscript{3} or O\textsubscript{3}, at ambient as well as at elevated CO\textsubscript{2}. A significant interaction (P=0.007) between NH\textsubscript{3} and O\textsubscript{3} in the combined treatment was observed with respect to mycorrhizal infection: the latter was much higher and close to the value in FA trees, especially at elevated CO\textsubscript{2}.

The number of mycorrhizal tips in the total root system showed the same reduction by NH\textsubscript{3} and O\textsubscript{3} observed in mycorrhizal infection,
except for O₃ at elevated CO₂; in the latter treatment, mycorrhizal number did not differ significantly from the value in FAₐ. Combined exposure to NH₃ and O₃ at ambient CO₂ increased the mycorrhizal number, showing a significant interaction (P=0.02) between NH₃ and O₃. The root weight ratio (RWR, ratio of the root biomass to the total tree mass) and specific root length (SRL, ratio of the total root length to the total root biomass) did not differ significantly between the fumigation treatments and FAₐ.

Nitrogen and carbon concentration in one-year-old needles
Eleven weeks of exposure to NH₃, O₃, NH₃ + O₃, at ambient or at elevated CO₂ did not significantly affect the concentrations of N and C in one-year-old needles. Nitrogen concentration ranged from 2.1 to 2.6% and C concentration ranged from 50 to 51%.

Table 6.2. Root characteristics: root weight ratio (RWR), specific root length (SRL, cm mg⁻¹), root branching (root tips cm⁻¹), mycorrhizal infection (% of mycorrhizal tips respect to total root tips) and mycorrhizal number in the total root system of Pinus sylvestris saplings. Saplings were exposed for 11 weeks to charcoal-filtered air at ambient CO₂ (FAa) and elevated CO₂ (FAe), and to both treatments supplemented with NH₃, O₃ or NH₃ + O₃. Means ± SE are given (n = 5). Means within the same column followed by the same letter are not significantly different at α = 0.05. Statistics on mycorrhizal number are based on seven treatments, excluding elevated CO₂ + NH₃.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>RWR</th>
<th>SRL</th>
<th>Root branching</th>
<th>Mycorrhizal infection</th>
<th>Mycorrhizal number</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Ambient CO₂</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FAₐ</td>
<td>0.12 ± 0.01a</td>
<td>3.6 ± 0.4a</td>
<td>0.27 ± 0.02ab</td>
<td>48.6 ± 32.2a</td>
<td>608 ± 55b</td>
</tr>
<tr>
<td>NH₃</td>
<td>0.14 ± 0.01a</td>
<td>3.8 ± 0.8a</td>
<td>0.29 ± 0.03a</td>
<td>1.8 ± 0.8b</td>
<td>108 ± 79a</td>
</tr>
<tr>
<td>O₃</td>
<td>0.12 ± 0.01a</td>
<td>2.8 ± 0.3a</td>
<td>0.28 ± 0.03a</td>
<td>1.1 ± 0.7b</td>
<td>65 ± 42a</td>
</tr>
<tr>
<td>NH₃ + O₃</td>
<td>0.12 ± 0.01a</td>
<td>3.8 ± 0.4a</td>
<td>0.28 ± 0.06a</td>
<td>36.3 ± 18.4a</td>
<td>2229 ± 169c</td>
</tr>
<tr>
<td><strong>Elevated CO₂</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FAₑ</td>
<td>0.14 ± 0.01a</td>
<td>2.9 ± 0.2a</td>
<td>0.17 ± 0.04b</td>
<td>18.0 ± 9.2a</td>
<td>490 ± 198ab</td>
</tr>
<tr>
<td>NH₃</td>
<td>0.15 ± 0.02a</td>
<td>3.2 ± 0.3a</td>
<td>0.05 ± 0.01c</td>
<td>0.1 ± 0.0b</td>
<td>0 ± 0</td>
</tr>
<tr>
<td>O₃</td>
<td>0.13 ± 0.01a</td>
<td>3.4 ± 0.2a</td>
<td>0.26 ± 0.03ab</td>
<td>2.2 ± 1.4b</td>
<td>261 ± 163ab</td>
</tr>
<tr>
<td>NH₃ + O₃</td>
<td>0.14 ± 0.02a</td>
<td>3.2 ± 0.4a</td>
<td>0.26 ± 0.03ab</td>
<td>17.8 ± 8.4a</td>
<td>566 ± 256b</td>
</tr>
</tbody>
</table>
Gas exchange in one-year-old needles
After 7 days of exposure, net CO$_2$ assimilation ($P_n$) was significantly higher in needles exposed to elevated CO$_2$ + NH$_3$ and to elevated CO$_2$ + NH$_3$ + O$_3$, compared with saplings exposed to FA$_a$ (Table 6.3). After 56 days, $P_n$ was still higher in both treatments than in FA$_a$, but not significantly.

After 7 days of exposure, the effects of elevated CO$_2$ on $g_s$ differed in the presence and absence of NH$_3$; in the presence of NH$_3$, the response of $g_s$ was 94% larger than in the absence of NH$_3$. However, after 56 days exposure there were no significant differences between the two exposures due to a strong decrease of $g_s$ under elevated CO$_2$ + NH$_3$. Thus, the stimulation of $g_s$ by elevated CO$_2$ in the presence of NH$_3$ was only transient.
There were no significant differences in transpiration between the treatments and FA after 7 or 56 days of exposure.

Soluble protein concentration, GS and GDH activities in one-year-old needles
The changes from week 0 to week 11 in soluble protein concentration, GS and GDH activities in needles were calculated as described by Pérez-Soba et al. (1994a). After 11 weeks of exposure, the soluble protein concentration in one-year-old needles was decreased in all treatments except in the combination of elevated CO$_2$ with NH$_3$ (Fig. 6.1a). In this treatment the concentration was unchanged and it showed a significant interaction ($P<0.05$) between CO$_2$ and NH$_3$. The general decrease in concentration was significantly larger in needles exposed to elevated...
CO₂ (33%) than in needles exposed to ambient CO₂ (8%).

The specific activity of GS (µmol γ-glutamylmonohydroxamate h⁻¹ mg⁻¹ protein) in needles increased in all treatments except in needles exposed to O₃ in ambient CO₂, in which GS activity was significantly decreased by 7% (Fig. 6.1b). The general increase in GS activity was significantly larger in trees exposed to NH₃ in ambient CO₂ (76%) than in trees exposed to FA (32%). The specific activity of GS in needles, exposed to the combinations of elevated CO₂, NH₃ and O₃ did not exhibit any significant interactions.

Figure 6.1c shows that the specific activity of GDH in needles was increased in all treatments, excluding exposures of saplings to NH₃ + O₃ in ambient CO₂ and to NH₃ in elevated CO₂. In these treatments, the GDH activity was decreased by 8 and 22%, respectively, and it showed a significant interaction (P<0.05) between NH₃ and O₃ and elevated CO₂ and NH₃. The increase in GDH activity in the needles exposed to O₃ both in ambient and elevated CO₂ was significantly larger (115 and 98%, respectively) than in FA (26%).

6.5. Discussion

In the present experiment, mycorrhizal infection was strongly decreased by exposure of saplings to NH₃ and O₃. The decrease affected not only the percentage of mycorrhizal tips with respect to total root tips but also the mycorrhizal number in the total root system. The observed decrease in mycorrhizal growth induced by NH₃ is in agreement with the observations of Van der Eerden et al. (1992), who fumigated Pseudotsuga menziesii with 180 µg m⁻³ for 14 weeks, and Dueck et al. (unpublished), who fumigated Pinus sylvestris with 40 µg m⁻³ NH₃ for 7 weeks. Reduction of the occurrence of carpophores of mycorrhizal fungi has been found in the field, where a significant negative correlation between the NH₃ deposition and the number and dry weight production of carpophores was observed in old stands of P. sylvestris (Termorshuizen & Schaffers, 1991). These authors suggested that NH₃ decreased the carbohydrate supply from the tree to the mycorrhizas by reducing carbohydrate production, increasing respiration or decreasing C transport to roots. However, we did not observe significant decreases in net CO₂ assimilation or biomass production that could indicate a reduction in carbohydrate production. We also did not observe decreases in C allocation to the roots (measured as RWR and fine root biomass), that could reduce the fraction of photosynthate available for mycorrhiza. A reduction in the formation of ectomycorrhiza by O₃ has been also observed.
Figure 6.1. Changes in (a) soluble protein concentration, (b) glutamine synthetase specific activity, and (c) glutamate dehydrogenase specific activity in one-year-old needles of Pinus sylvestris saplings. Saplings were exposed for 11 weeks to charcoal-filtered air at ambient CO$_2$ (350 µl l$^{-1}$, FA$_a$), to elevated CO$_2$ (700 µl l$^{-1}$, FA$_e$), and to both treatments supplemented with NH$_3$ (40 µg m$^{-3}$), O$_3$ (110 µg m$^{-3}$) or NH$_3$ + O$_3$ (40 + 110 µg m$^{-3}$). Changes are relative to the values at week 0. n = 4. Significant differences between treatments and filtered ambient air are indicated by * (P<0.05). Mean (± SD) values at week 0 for FA$_a$, NH$_3$, O$_3$, NH$_3$ + O$_3$, FA$_e$, elevated CO$_2$ + NH$_3$, elevated CO$_2$ + O$_3$, and elevated CO$_2$ + NH$_3$ + O$_3$, respectively; soluble protein (mg g$^{-1}$ FW) 11.0 ± 1.8, 11.8 ± 2.3, 10.4 ± 0.9, 10.7 ± 0.7, 12.6 ± 2.0, 9.4 ± 1.4, 11.4 ± 0.9 and 9.1 ± 0.9; GS activity (µmol h$^{-1}$ mg$^{-1}$ protein) 12.6 ± 2.2, 10.5 ± 1.4, 13.3 ± 2.1, 14.1 ± 3.5, 11.2 ± 3.1, 12.8 ± 4.2, 9.2 ± 2.4 and 11.1 ± 2.6; GDH activity (µmol h$^{-1}$ mg$^{-1}$ protein) 2.3 ± 0.6, 2.0 ± 0.3, 1.7 ± 0.3, 4.1 ± 1.0, 2.7 ± 0.9, 4.0 ± 1.1, 2.2 ± 0.7 and 3.8 ± 0.3.
before in Pinus strobus (Reich et al., 1986) and Pinus taeda (Meier et al., 1990). The effect of O$_3$ on mycorrhiza must be explained by changes in plant metabolism since O$_3$ does not enter the soil surface but is immediately degraded to oxygen molecules (Turner et al., 1973). In the present study, no significant changes on net CO$_2$ assimilation, shoot and root biomass production and RWR were observed. This suggests that the reduction in mycorrhizal infection might be caused by an O$_3$ modification in root exudates (qualitatively and quantitatively), as suggested by Lefohn (1992), rather than by a decrease in C allocation to the roots (Adams et al., 1990; Górrissen et al., 1991). Exposure to O$_3$ changed the non-structural carbohydrate content of Pinus taeda seedlings (Meier et al., 1990).

Gaseous NH$_3$ may increase the activity of foliar GS, which is associated with the assimilation of NH$_3$ into glutamine and subsequently into amino acids and proteins (Pérez-Soba et al., 1994a,b). Gaseous NH$_3$ may also enhance current-year needle biomass and net CO$_2$ assimilation rate (Van der Eerden & Pérez-Soba, 1992; Van Hove et al., 1992). In our present experiment, eleven weeks of exposure to 40 µg m$^{-3}$ NH$_3$ increased the GS activity in one-year-old needles but, in discrepancy with former experiments, did not increase N concentration nor soluble protein concentration. Gaseous NH$_3$ also did not enhance the net CO$_2$ assimilation rate and current-year needle biomass. The contrasting results between experiments are probably associated with the N status of the needles prior to the fumigation:

1. if the N concentration is lower than optimal for growth (experiment of Van der Eerden & Pérez-Soba, 1992), NH$_3$-derived N may alleviate N deficiency; the limiting N concentration in the needles will increase, which is associated with an increase in net CO$_2$ assimilation rate (Brix, 1981), resulting in extra needle biomass production.

2. if the N concentration is supraoptimal for growth (present experiment), more NH$_3$-derived N may be reallocated from the needles to other tree parts with larger N demands. Net CO$_2$ assimilation will not be further stimulated and needle growth will not be enhanced.

Exposure to O$_3$ resulted in an increased activity of GDH in the needles as earlier observed in Picea abies, fumigated for five years with concentrations of O$_3$ ranging from 30 to 180 µg m$^{-3}$ (Bender et al. 1990). This increase in GDH activity was possibly induced by an increased demand of carbohydrates in shoots by O$_3$ (Amthor, 1988): GDH catalyses the breakdown of glutamate to supply carbon skeletons in case of carbohydrate deficiency (Lea et al., 1992; Robinson et al., 1991). This result supports the use of GDH as an early indicator of O$_3$ stress, when neither visible injury nor effects on gas exchange are detectable (Jäger, 1982). The activity of GS in one-year-old needles was signif-
significantly decreased by exposure to O$_3$ at ambient CO$_2$, which might be due to a decreased reassimilation of N in preference of formation of phenolic complexes, which are deposited in O$_3$ exposed plants (Jones et al., 1994; Tingey et al., 1976). Additionally, exposure to O$_3$ may cause malfunctioning of chloroplasts (Lefohn, 1992) and thus, decrease the chloroplastic fraction of GS.

Needle GDH activity was not enhanced by exposure to the combination of NH$_3$ with O$_3$ (at both CO$_2$ levels), contrary to O$_3$ alone, suggesting that 11 weeks exposure to NH$_3$ + O$_3$ caused less stress than the single exposure to O$_3$. Following exposure to NH$_3$ + O$_3$ (day and night concentrations of 49+83 and 49+44 µg m$^{-3}$), shoots of Pseudotsuga menziesii showed a transient increase in the rate of photosynthesis in the period from 4 to 8 weeks and thereafter a decline till week 19 (Van Hove & Bossen, 1994). In our study, the increase of P$_n$ in shoots exposed to NH$_3$ + O$_3$ (only significant at elevated CO$_2$) occurred in the first week of exposure and it lasted till week 8. This increase in P$_n$ may result in a temporary increase in C flux from shoots to roots and thus fulfil the C demand of mycorrhizas. This may explain the observed higher mycorrhizal infection and mycorrhizal number in the combined exposure to NH$_3$ + O$_3$.

The responses of photosynthesis and root systems to elevated CO$_2$ are not consistent among tree species and vary with experimental conditions, phenological state of the tree and its morphological characteristics (Eamus & Jarvis, 1989; Stulen & Den Hertog, 1993). In the present study P$_n$ was not significantly increased under elevated CO$_2$, thus extra dry matter was not significantly produced. Distribution of dry matter between shoots and roots (estimated as RWR) and within the root system (estimated as fine and coarse root biomass and root branching) were also not significantly influenced by elevated CO$_2$. The large variance of data on fine roots biomass at elevated CO$_2$ when compared with ambient CO$_2$, is probably explained by the large genetic diversity of forest trees in order to tolerate environmental stress factors (Gregorius, 1989). Exposure to elevated CO$_2$ also did not significantly affect mycorrhizal infection. Data in the literature on the effect of elevated CO$_2$ on mycorrhiza are scarce and conflicting. The number of mycorrhizal tips per unit root length was twice larger after 6 weeks exposure to 700 µl l$^{-1}$ compared to 360 µl l$^{-1}$, whereas difference was no longer observed in Pinus echinata seedlings after 24 weeks exposure (O’Neill et al., 1987). They suggested that the extra C fixed under elevated CO$_2$ tended to counteract the costs of mycorrhizal establishment and that the lack of difference at 24 weeks was the result of prolonged growth in the containers. However, mycorrhizal density in Pinus echinata seedlings grown in pots was still increased by elevated CO$_2$ after 34 weeks exposure (Norby et al., 1987). These two experiments
were performed with seedlings younger than one year, which total root biomass was up to 25 times smaller than the total root biomass of the 4-year-old saplings that we used in our experiment. Mycorrhizal infection was not affected in Gossypium hirsutum plants exposed to 550 µl l⁻¹, despite the larger root systems (Runion et al., 1994). The lack of significant changes in the present study was not caused by a constrained rooting in the pots (Arp, 1991): we used 10 l pots, which were large enough for 11 weeks of growth. Stomatal conductance was also not significantly changed after 56 days, which differs from the observation of a 10-60% decrease in gs after prolonged exposure to elevated CO₂ (Eamus & Jarvis, 1989). Stomatal sensitivity to CO₂ may be decreased by a high water content in the plant (Conroy et al., 1986) and by high photon flux densities (Tolley & Strain, 1985). In our study, the water content of trees under elevated CO₂ was similar to that of FA₄ trees (data not shown) and the gas exchange measurements were done at low ambient photon flux density (360-460 µmol m⁻² s⁻¹). A significant decrease in foliar soluble protein concentration after exposure to elevated CO₂ has been observed previously (Cure et al., 1988); it might be caused by a decrease in Rubisco protein concentration (Rowland-Bamford et al., 1991; Tissue et al., 1993).

Elevated CO₂ in the presence of NH₃ stimulated transiently Pn. However, elevated CO₂ did not counteract the decrease in mycorrhizal infection caused by NH₃, indicating that the extra photosynthates were not invested in the mycorrhizal association. Root branching was strongly reduced in elevated CO₂ + NH₃, and this may have led to less favourable conditions for infection by mycorrhizal fungi (Gorissen et al., 1993).

The serious foliar nutrient imbalance observed in Dutch forests (Van Dijk & Roelofs, 1988) is often thought to be caused by soil-mediated NH₄⁺ (Gorissen et al., 1993; Van Dijk et al., 1990), but our experiments show that atmospheric NH₃ also induces an increase in needle N concentration (Pérez-Soba et al. 1994a,b), and a decrease in mycorrhizal infection (this experiment), which in turn will reduce nutrient uptake by the tree (Marschner & Dell, 1994). The significant reduction in mycorrhizal infection already after 11 weeks exposure to NH₃ or O₃ suggests that mycorrhizal infection can be used as an early indicator of NH₃ or O₃ stress, when neither visible injury nor effects on biomass or gas exchange are noticed.