Atmospheric NH3 deposition, S and N metabolism in curly kale
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Chapter 6.

Changes in growth and nutrient uptake in *Brassica oleracea* L. exposed to atmospheric ammonia

Abstract
Plant shoots form a sink for NH$_3$ and are able to utilize it as N source. NH$_3$ was used as a tool to investigate the interaction between foliar N uptake and root N uptake. To what extent NH$_3$ can contribute to the N budget of the plant or can be regarded as a toxin, was investigated in relation to its concentration and the N supply in the root environment. *Brassica oleracea* L. was exposed to 0, 4 and 8 µl l$^{-1}$ NH$_3$, with and without nitrate in the nutrient solution. Growth, N compounds, nitrate uptake rate, soluble sugars and cations were measured. In nitrate-sufficient plants, biomass production was not affected at 4 µl l$^{-1}$ NH$_3$, but reduced at 8 µl l$^{-1}$ NH$_3$. In nitrate-deprived plants, shoot biomass was increased at both concentrations, but root biomass decreased at 8 µl l$^{-1}$ NH$_3$. The measured nitrate uptake rates agreed well with the plant’s N requirement for growth. In nitrate-sufficient plants, nitrate uptake at 4 and 8 µl l$^{-1}$ NH$_3$ was reduced by 50 and 66 %, respectively. The present data do not support the hypothesis that NH$_3$ toxicity is caused by a shortage of sugars or a lack of capacity to detoxify NH$_3$. It is unlikely that amino acids, translocated from the shoot to root, are the signal metabolites involved in the down regulation of nitrate uptake, since no relation was found between changes in nitrate uptake and root soluble N content of NH$_3$ exposed plants.
Introduction

Roots of higher plants are able to take up inorganic N ions, as nitrate and/or ammonium from the soil (Peuke et al., 1998a,b; Britto et al., 2001; Glass et al., 2002). In addition, plant shoots can also take up NH$_3$ from the atmosphere. The foliar uptake of NH$_3$ is determined by the diffusive conductance of the stomata, since the internal (mesophyll) resistance to this gas is low due to its high solubility and rapid dissociation into NH$_4^+$ in the water phase of the mesophyll cells (Hutchinson et al., 1972; Fangmeier et al., 1994). The foliar uptake of NH$_3$ shows diurnal variation and is dependent on water status, temperature, light intensity, internal CO$_2$ concentration, nutrient availability, ontogeny and developmental stage of the plant (Hutchinson et al., 1972; Rogers and Aneja, 1980; Husted and Schjoerring, 1996; Schjoerring et al., 1998).

The foliarly absorbed NH$_3$ may be metabolized by the glutamine synthetase/glutamate synthase cycle (Lea and Miflin, 1974; Pérez-Soba et al., 1994, Pearson and Soares, 1998) and NH$_3$ exposure may result in an increase in total N content and N-containing metabolites (Van Dijk and Roelofs, 1988; Pérez-Soba and Van der Eerden, 1993; Clement et al., 1997; Gessler et al., 1998, Barker, 1999) and changes in glutamine synthetase (GS) and nitrate reductase (NR) (Pérez-Soba et al., 1994; Pearson and Soares, 1998). NH$_3$ is potentially phytotoxic and high atmospheric levels may negatively affect growth and alter the shoot to root ratio (Van der Eerden and Pérez-Soba, 1992; Pérez-Soba et al., 1994). NH$_3$ toxicity has been related to a shortage of soluble sugars, a lack of capacity to detoxify NH$_3$, nutrient imbalances due to cation release (Fangmeier et al., 1994; Krupa, 2003) and effects on acid-base balance (Yin and Raven, 1997). There is little information on interactions between foliar uptake of atmospheric NH$_3$ and N uptake by the roots. Pérez-Soba and Van der Eerden (1993) concluded from experiments with Pinus sylvestris exposed to 0.075 µl 1$^{-1}$ NH$_3$ that 90 % of the increase in needle N was derived from the atmospheric N source, and that root N uptake was reduced. Clement et al. (1997) exposed winter wheat (Triticum aestivum L.) to 1 and 2 µl 1$^{-1}$ NH$_3$ and found a reduction in nitrate uptake rate of 8.0 and 13.8 % respectively. Gessler et al. (1998), using concentrations of 0.05 µl 1$^{-1}$ NH$_3$, on seedlings of beech (Fagus sylvatica L.), described a reduction of 35 % in nitrate uptake rate by the roots.

It needs to be established to what extent the foliar uptake of atmospheric NH$_3$ may contribute to the plant’s N requirement for growth and whether NH$_3$ exposure affects N uptake by the root. To our knowledge, no experimental studies are available in which the impact of atmospheric NH$_3$ concentrations on net nitrate root uptake was actually measured in relation to changes in plant growth. The aim of the present study was
threefold. Firstly, the impact of atmospheric NH$_3$ on root net nitrate uptake rate of *Brassica oleracea* was assessed. *B. oleracea* was chosen as a suitable species because of its high RGR, its preference for nitrate (Pearson and Stewart, 1993) as well as its tendency towards sensitivity to NH$_4^+$ as a member of the Brassicaceae (Britto and Kronzucker, 2002). The experiments were carried out at two atmospheric NH$_3$ concentrations (4 and 8 µl l$^{-1}$), based on preliminary experiments with a range of concentrations which showed that growth of *B. oleracea* was not affected at 4 µl l$^{-1}$ NH$_3$, but reduced at 8 µl l$^{-1}$ NH$_3$ (Chapter 5; Castro *et al.*, 2006a). In nitrate uptake experiments the effects of 4 and 8 µl l$^{-1}$ NH$_3$ on biomass production, relative growth rate, dry matter partitioning, nitrogenous compounds and nitrate reductase activity were measured simultaneously. Since changes in nitrate uptake rate can be caused by changes in RGR of the plant (Ter Steege *et al*., 1998) the effect on sulfate uptake was also measured, in order to be able to distinguish between direct NH$_3$- and growth-related effects. In order to establish whether the measured nitrate uptake rates were of physiological significance, nitrate uptake rates were compared with the plant’s N requirement for growth, calculated from the RGR and the plant’s nitrogen content. The second aim was to investigate to what extent atmospheric NH$_3$ can contribute to the N requirement of the plant, in the absence of nitrate in the root environment. Thirdly, the effect of both NH$_3$ concentrations on soluble sugars, N compounds and cations was measured, to assess whether changes in these parameters could be related to NH$_3$ toxicity.

**Results**

*NH$_3$ exposure and plant growth*

The effect of NH$_3$ on biomass production depended on the atmospheric concentration, and differed between nitrate-sufficient and nitrate-deprived plants (Table 1). Exposure of nitrate-sufficient plants to 4 µl l$^{-1}$ NH$_3$ had no effect on shoot and root biomass production (and RGR) of the nitrate-sufficient plants. However, both shoot and root biomass production (and RGR) of nitrate-sufficient plants was significantly reduced at 8 µl l$^{-1}$ NH$_3$. Exposure of nitrate-deprived plants to 4 and 8 µl l$^{-1}$ NH$_3$ resulted in a significantly higher shoot biomass production (and RGR of the plants), however, it was lower than that of nitrate-sufficient plants (at 0 µl l$^{-1}$ NH$_3$). The effects of NH$_3$ on root biomass differed from those found for the shoot. A concentration of 4 µl l$^{-1}$ NH$_3$ had no effect on root biomass, while exposure to 8 µl l$^{-1}$ NH$_3$ resulted in a significantly lower root biomass production of nitrate-sufficient as well as nitrate-deprived plants.
Shoot dry matter content (DMC) increased with increasing NH$_3$ concentration in nitrate-sufficient plants, but decreased in nitrate-deprived plants (Table 1). In the control plants shoot DMC of the nitrate-deprived plants was much higher than that of the nitrate-sufficient plants. Root DMC was overall significantly lower than the shoot DMC and not affected by NH$_3$ exposure and nitrate nutrition.

Increasing NH$_3$ concentrations significantly increased the shoot to root ratio (S/R) of nitrate-sufficient plants (Table 1), due to a relatively higher shoot biomass production and lower root biomass production. The same effect on S/R was found for nitrate-deprived plants, though the absolute values were lower in comparison to nitrate-sufficient plants.

**Table 1.** The impact of NH$_3$ exposure and nitrate nutrition on growth of *Brassica oleracea*. Plants were exposed to NH$_3$ for 1 week. Shoot (Biomass S) and root biomass production (Biomass R) were calculated by subtracting the final weight from the initial weight and expressed on a fresh weight basis (g FW); Dry matter content of the shoot (DMC S) and of the root (DMC R) (% of fresh weight); S/R, shoot to root ratio calculated on a fresh weight basis; RGR, relative growth rate, calculated over a week period on a plant basis (S+R, g g$^{-1}$ plant day$^{-1}$). Data represent the mean of 2 experiments, with 3 measurements per experiment with 3 plants in each (±SD). Values followed by different letters are statistically different at p<0.01 (Student’s *t*-test).

<table>
<thead>
<tr>
<th></th>
<th>0 µl l$^{-1}$ NH$_3$</th>
<th>4 µl l$^{-1}$ NH$_3$</th>
<th>8 µl l$^{-1}$ NH$_3$</th>
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</thead>
<tbody>
<tr>
<td><strong>Biomass (S)</strong></td>
<td></td>
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<tr>
<td>+ NO$_3^-$</td>
<td>1.51±0.15c</td>
<td>1.56±0.31c</td>
<td>1.07±0.34b</td>
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<td>- NO$_3^-$</td>
<td>0.55±0.09a</td>
<td>1.04±0.15b</td>
<td>0.95±0.14b</td>
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<tr>
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<td></td>
<td></td>
</tr>
<tr>
<td>+ NO$_3^-$</td>
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<td>0.32±0.09b</td>
<td>0.18±0.06a</td>
</tr>
<tr>
<td>- NO$_3^-$</td>
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<td>0.46±0.14b</td>
<td>0.20±0.04a</td>
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<td><strong>DMC (S)</strong></td>
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<tr>
<td>+ NO$_3^-$</td>
<td>9.6±0.9a</td>
<td>10.4±0.6a</td>
<td>12.8±0.9b</td>
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<tr>
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<td>17.2±0.4c</td>
<td>10.4±0.6a</td>
<td>12.6±0.7b</td>
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<td><strong>DMC (R)</strong></td>
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<tr>
<td>+ NO$_3^-$</td>
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<td>6.7±0.9a</td>
<td>6.0±0.6a</td>
</tr>
<tr>
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<td>6.4±0.8a</td>
<td>6.5±0.6a</td>
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<tr>
<td><strong>S/R</strong></td>
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<td></td>
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<tr>
<td>+ NO$_3^-$</td>
<td>3.4±0.4b</td>
<td>4.1±0.6b</td>
<td>1.7±0.5a</td>
</tr>
<tr>
<td>- NO$_3^-$</td>
<td>1.4±0.3a</td>
<td>1.7±0.5a</td>
<td>6.3±0.9c</td>
</tr>
<tr>
<td><strong>RGR (S+R)</strong></td>
<td>0.21</td>
<td>0.22</td>
<td>0.16</td>
</tr>
</tbody>
</table>

*NH$_3$ exposure and total N, nitrate and free amino acid content*

NH$_3$ exposure resulted in a clear increase in shoot N content, in particular the soluble N fraction, in both nitrate-sufficient and nitrate-deprived plants, but did not affect nitrate content in either of the NH$_3$ treatments (Fig. 1a). In all treatments, root N content was
significantly lower than that of the shoot (Fig. 1a,b). Root N content of the nitrate-deprived plants exposed to 8 µl l⁻¹ NH₃ was significantly lower than at 0 and 4 µl l⁻¹.

**Fig. 1.** The impact of NH₃ exposure on nitrate, soluble and insoluble nitrogen content of the shoot (a) and the root (b) and on the free amino acid content of the shoot of *Brassica oleracea* (c). Plants were exposed to 0, 4, 8 µl l⁻¹ NH₃ for 1 week. Nitrate sufficient- and nitrate-deprived treatments are given in dark and light-grey bars, respectively. The various N compounds are given within each bar, from top to bottom. Data represent the mean of 2 experiments, with 3 measurements per experiment with 3 plants in each. Standard deviations and statistics treatment refer to total N content (a,b). Different letters indicate significant differences at p<0.01 (Student’s *t*-test).

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Compared to the control plants, exposure to 4 µl l⁻¹ NH₃ resulted in a 1.6-fold increase in free amino acids in the shoot (Fig. 1c). At 8 µl l⁻¹ NH₃, an 18.5-fold increase was found for the nitrate-sufficient plants, and a 17-fold increase for the nitrate-deprived plants (Fig. 1c). Hence, the main effect of NH₃ exposure on N compounds is a considerable increase in the free amino acid content.

**NH₃ exposure and nitrate reductase activity**

Nitrate reductase activity (NRA) was measured by both *in vivo* (Fig. 2a) and *in vitro* assays (Fig. 2b,c). NH₃ exposure had no significant effect on *in vivo* NRA in the shoot of nitrate-sufficient plants, but lead to a significant increase at 8 µl l⁻¹ in the nitrate-deprived plants, although the values were very low (Fig. 2a). NH₃ exposure significantly decreased *in vitro* NRA, expressed on a fresh weight basis (Fig. 2b). When the *in vitro* NRA was expressed on a soluble protein basis, however, the effect of NH₃ was no longer significant (Fig. 2c), showing that the effect of NH₃ on *in vitro* NRA activity was mainly the result of changes in protein content of the extracts. The value found with the *in vivo* assay for the control, calculated on a 24-h basis, (96 µmol g⁻¹ FW day⁻¹) was in the same range as the calculated N requirement (70 µmol g⁻¹ FW day⁻¹). A similar finding was observed for Plantago *in vivo* and it is a better estimate of the actual nitrate reduction than *in vitro* Stulen *et al.* (1981b).

**NH₃ exposure and nitrate and sulfate uptake rate**

Long-term net nitrate uptake rate, measured over a 7-day period, decreased upon NH₃ exposure (Fig. 3a). The uptake rates at 4 and 8 µl l⁻¹ NH₃ were decreased by 50 and 66 %, respectively, of the control value (0 µl l⁻¹ NH₃). Short-term net nitrate uptake rate, measured with ^1⁵^N-KNO₃ over a 2-hour period, showed a similar effect of NH₃ (Fig. 4), although the absolute values were lower than those of the long-term measurements. The values at 4 and 8 µl l⁻¹ NH₃ were decreased by 48 and 80 % of the control value, respectively.

Net sulfate uptake rate at 0 and at 4 µl l⁻¹ NH₃ did not differ. Exposure to 8 µl l⁻¹ NH₃ resulted in a significant 1.7-fold decrease (Fig. 3b).

In order to establish whether the measured uptake rates were of physiological significance, the nitrate uptake rate needed to cover the plant's nitrogen requirement on a whole plant basis, and was estimated on the basis of RGR times the plant’s N content, for the nitrate-sufficient plants. The estimated nitrogen requirement was 70 µmol g⁻¹ FW
plant day$^{-1}$) which was within the range of measured uptake rate over a 7-day period (Fig. 3).

**Fig. 2.** The impact of NH$_3$ exposure on *in vivo* (a) and *in vitro* (b,c) nitrate reductase activity (NRA) in the shoot of *Brassica oleracea*. Plants were exposed to 0, 4, 8 µl l$^{-1}$ NH$_3$ for 1 week. *In vivo* NRA was determined in the shoot (a). Nitrate-sufficient and nitrate-deprived treatments are given in dark- and light-grey bars, respectively. *In vitro* NRA (b,c) was expressed on a fresh weight (b) and a soluble protein (c) basis. For *in vivo* NRA measurements, data represent the mean of 2 experiments, with 3 measurements per experiment with 3 plants in each (± SD); for *in vitro* NRA measurements data represent the mean of 3 measurements with 3 plants in each (± SD). Different letters indicate significant differences at p<0.01 (Student’s *t*-test).
Fig. 3. The impact of NH₃ exposure on net nitrate (a) and net sulfate (b) uptake rate. Nitrate-sufficient plants were exposed to 0, 4, 8 µl l⁻¹ NH₃ for 1 week. Fig. 3a represents data on long-term nitrate uptake rate measurement over a week period and expressed as µmol g⁻¹ FW plant day⁻¹. Fig. 3b the long term sulfate uptake, over a week period, as µmol g⁻¹ FW day⁻¹ (for experimental details see Chapter 2). Data represent the mean of 2 experiments, with 3 measurements per experiment, with 3 plants in each (± SD). Different letters indicate significant differences at p<0.01.

NH₃ exposure and total sulfur content
In nitrate-sufficient plants, shoot total S content decreased at 4 and 8 µl l⁻¹ NH₃ (Fig. 5a). Root total S content was unaffected upon NH₃ exposure, for both nitrate treatments (Fig. 5b).

NH₃ exposure and soluble sugar content
In nitrate-sufficient plants shoot soluble sugar content was unaffected by NH₃ exposure (Fig. 6a). In nitrate-deprived plants an accumulation of soluble sugars was found at 0 µl l⁻¹ NH₃. In the roots of nitrate-deprived plants a significant decrease at 8 µl l⁻¹ NH₃ was observed (Fig. 6b).
Fig. 4. The impact of NH₃ exposure on the short-term $^{15}$NO₃⁻-net nitrate uptake rate measurement, over 2-hour period ($\mu$mol g⁻¹ FW plant day⁻¹). Data represents the mean of 3 measurements, with 3 plants in each (± SD). Different letters indicate significant differences at $p<0.01$ (Student’s $t$-test).

Fig. 5. The impact of NH₃ exposure on total sulfur content in the shoot (a) and in the roots (b). Plants were exposed to 0, 4, 8 $\mu$l l⁻¹ NH₃ for 1 week. Nitrate-sufficient and nitrate-deprived treatments are given in dark- and light-grey bars, respectively. Data represent the mean of 2 experiments, with 3 measurements per experiment with 3 plants in each (±SD). Different letters indicate significant differences at $p<0.01$ (Student’s $t$-test).
Fig. 6. The impact of NH$_3$ exposure on the soluble sugar content in shoot (a) and roots (b). Plants were exposed to 0, 4, 8 µl l$^{-1}$ NH$_3$ for 1 week. Nitrate-sufficient and nitrate-deprived treatments are given in dark and light-grey bars, respectively. Data represent the mean of 2 experiments, with 3 measurements per experiment with 3 plants in each (± SD). Different letters indicate significant differences at p<0.01 (Student’s $t$-test).

$NH_3$ exposure and cation content

In nitrate-sufficient plants a decrease in shoot K$^+$ content was only found at 4 µl l$^{-1}$ NH$_3$ (Fig. 7a). The effect of atmospheric NH$_3$ on shoot K$^+$ content was more pronounced in nitrate-deprived plants, where a gradual decrease from 0 to 8 µl l$^{-1}$ NH$_3$ was found (Fig. 7a). In the roots the only significant effect of NH$_3$ on K$^+$ content was found at 4 µl l$^{-1}$ NH$_3$ in nitrate-deprived plants (Fig. 7b). Shoot nor root Na$^+$ content were significantly affected by exposure to NH$_3$ (Fig. 7c,d) in either of the nitrate treatments.

Exposure to NH$_3$ had a more drastic effect on the content of divalent cations, Ca$^{2+}$ and Mg$^{2+}$, especially in the shoot (Fig. 7e,g). Exposure to 4 and 8 µl l$^{-1}$ NH$_3$ of the nitrate-sufficient plants resulted in a significant decrease compared to the control.
Fig. 7. The impact of NH₃ exposure on cation content in the shoot (a,c,e,g) and in the roots (b,d,f,h). Plants were exposed to 0, 4, 8 µl l⁻¹ NH₃ for 1 week. Nitrate-sufficient and nitrate-deprived treatments are given in dark and light-grey bars, respectively. Data represent the mean of 3 measurements with 3 plants in each (± SD). Different letters indicate significant differences at p<0.01 (Student’s t-test).
In the shoot of nitrate-deprived plants exposed to 4 µl l⁻¹ NH₃ a significant decrease of 22 and 40 % for Ca²⁺ and Mg²⁺ contents, respectively, compared to the control plants was found. At 8 µl l⁻¹ NH₃ the decrease in Ca²⁺ and Mg²⁺ content was 57 and 53 %, respectively. Root Ca²⁺ content showed a significant decrease at 8 µl l⁻¹ NH₃ in both nitrate treatments, but the decrease was less than observed for the shoot (Fig. 7f). For root Mg²⁺ content significant differences were found between nitrate-sufficient and nitrate-deprived plants at 0 µl l⁻¹ NH₃ (Fig. 7h).

**Discussion**

*To what extent does NH₃ exposure affect plant growth?*

Previous experiments with *B. oleracea* exposed to 0, 2, 4, 6 and 8 µl l⁻¹ NH₃ (Chapter 5, Castro *et al.*, 2006) and the present results (Table 1) revealed that *B. oleracea* can use atmospheric NH₃ for growth up to a concentration of 4 µl l⁻¹ NH₃. Higher NH₃ concentrations are toxic because biomass production, especially of the roots, is reduced. Even though the NH₃ concentrations used in this study were much higher than generally found in polluted areas (Fangmeier *et al.*, 1994; Stulen *et al.*, 1998; Krupa, 2003), *B. oleracea* apparently can use an NH₃ concentration of 4 µl l⁻¹ as a nutrient (Table 1). Since at 8 µl l⁻¹ NH₃ shoot as well as root biomass production was severely impaired both in the presence and absence of nitrate in the nutrient solution, the present results with *B. oleracea* do not support the concept of nitrate-ammonium synergism, in which the toxicity of NH₄⁺ may be alleviated by a simultaneous supply of nitrate (Britto and Kronzucker, 2002). At 8 µl l⁻¹ NH₃ a significant increase in S/R ratio was found, which is in agreement with other data (Table 1; Van der Eerden, 1982; Van der Eerden and Pérez-Soba, 1992; Pérez-Soba *et al.*, 1994). Since the experimental set-up of the present experiments prevented formation of NH₄⁺ in the nutrient solution, the negative effect on root biomass production must have been mediated by a signal from the shoot as a result of the foliar uptake of atmospheric NH₃.

*What is the impact of NH₃ exposure at a metabolic level?*

One of the best-documented effects of the foliar uptake of atmospheric NH₃ on metabolism is the increase in total N and N-containing metabolites, such as amino acids (Van der Eerden, 1982; Van Dijk and Roelofs, 1988; Fangmeier *et al.*, 1994; Pérez-Soba *et al.*, 1994; Clement *et al.*, 1997; Gessler *et al.*, 1998). The present results showed that the increase in the total N content was mainly due to an increase in the soluble N fraction (Fig. 1a). At 8 µl l⁻¹ NH₃ *B. oleracea* was still able to detoxify atmospheric NH₃ as
shown by the dramatic increase in the free amino acid content in the shoot (Fig. 1c), which most likely is the effect of the reduction in growth at this concentration.

In the experiments of Pearson and Soares (1998) with bean (*Phaseolus vulgaris* L.), exposed to 4.7 µl l⁻¹ NH₃ for one hour, a significant decrease in *in vivo* leaf NRA was found. Amino acids, as end products of NH₃ assimilation, have been suggested as inhibiting the enzyme (Pearson and Stewart, 1993). Our experiments, in which plants were exposed for 1 week, showed that 4 µl l⁻¹ NH₃ had no effect on *in vivo* NRA in the shoot. Only at 8 µl l⁻¹ a reduction in *in vivo* NRA was found (Fig. 2a). This effect cannot be ascribed to a change in nitrate concentration, since the nitrate pool was unaffected upon exposure to NH₃ (Fig. 1a). Changes in *in vivo* NRA may be caused by changes in the amount of functional enzyme, and/or the endogenous supply of NADH to the enzyme (De Kok *et al*., 1986). There was only a slight effect of 8 µl l⁻¹ NH₃ on *in vitro* NRA (Fig. 2b,c), therefore it was unlikely that the effect on *in vivo* NRA was primarily due to a change in amount of enzyme. Apparently, at 8 µl l⁻¹ NH₃ the decrease in *in vivo* NRA might be due to a change in endogenous NADH supply (De Kok *et al*., 1986) and/or via modulation of the catalytic activity of the existing NR protein (Kaiser *et al*., 1992).

*To what extent does NH₃ exposure affect nitrate uptake by the root?*

In the present experiments nitrate uptake rate on a plant basis was measured in two ways, in a long-term experiment, in which nitrate depletion from the solution was measured over a 7-day period, and in a short-term experiment, over a 2-hour period. Comparison of the measured uptake rates of the nitrate-sufficient control plants (0 µl l⁻¹ NH₃, Fig. 3a) with the N requirement for growth (N flux; RGR times the plant’s nitrogen content, De Kok *et al*., 2000, 2002a) showed that long-term uptake rate corresponded with the N requirement for growth, and can therefore be considered as physiologically relevant. The uptake rate in the short-term experiment (Fig. 4) was lower than the N requirement for growth. The difference may be caused by differences in uptake rate during the day and/or night. Exposure to 4 and 8 µl l⁻¹ NH₃ resulted in a reduction of nitrate uptake rate (long-term uptake experiment) of 50 and 66 %, respectively. A similar trend was found in the short-term uptake experiment.

At nitrate-sufficient conditions, 4 µl l⁻¹ NH₃ had no effect on sulfate uptake rate, but decreased nitrate uptake rate, so that the effect of NH₃ can be considered direct, and not mediated through changes in growth. A significant decrease in sulfate uptake rate (Fig. 4) and a concomitant decrease in total S content (Fig. 5a,b) were only found at the toxic concentration of 8 µl l⁻¹ NH₃, when RGR was decreased. The effect of NH₃ on nitrate and sulfate uptake rate at this concentration, therefore, can be considered to be indirect,
and growth-related. This is in accordance with the observation that changes in nitrate uptake rate are driven by changes in RGR of the plant (Ter Steege et al., 1998).

Exact comparison between the present data and literature data is difficult, because of differences in growth conditions, which may have resulted in differences in RGR, different atmospheric NH₃ concentrations and exposure times. Clement et al. (1997) exposed winter wheat (Triticum aestivum L.) to 1 and 2 µl l⁻¹ NH₃, for one week, and found a reduction of nitrate uptake rate of 8 and 13.8 % respectively, at relatively low light conditions. These plants had a rather low RGR (0.08 g g⁻¹ day⁻¹). Gessler et al. (1998) exposed seedlings of beech (Fagus sylvatica L.) to 0.05 µl l⁻¹ NH₃, and found a reduction of 35 % in cumulative root nitrate uptake over the last 24 h of the exposure. In these experiments, no data was presented on RGR or physiological relevance of the measured uptake rates.

The mechanisms underlying the regulation of nitrate uptake by the roots as well as the signal metabolites involved were dealt with in detail by Imsande and Touraine (1994) and Touraine (2004). Phloem-translocated amino acids acting as a regulatory signal between shoot and root appear to be the explanation for a down-regulation of nitrate uptake, under steady-state conditions, at a whole plant level (Muller and Touraine, 1992; Imsande and Touraine, 1994; Gessler et al., 1998). The present data, however, showed no relation between the effect of NH₃ on nitrate uptake rate and total root soluble N content (amino acids and amides). Although shoot soluble N content and free amino acid content were increased at both NH₃ concentrations, in the root no significant changes in soluble N content were found. However, a change in a specific amino acid cannot be ruled out.

To what extent can atmospheric NH₃ contribute to the plant’s N requirement for growth? From theoretical calculations with spinach, which contained an organic N content of 250 µmol g⁻¹ plant fresh weight and had a S/R ratio of 3.4, it was estimated that an atmospheric NH₃ concentration of 2 µl l⁻¹ might be sufficient for the N requirement of a plant with a RGR of 0.05 g g⁻¹ day⁻¹, and might contribute to 50 % of the total N requirement at a RGR of 0.2 g g⁻¹ day⁻¹ (Stulen et al., 1998). In a series of experiments in which sunflower plants were grown in the absence of N in the root environment, plants were able to grow on 2 µl l⁻¹ NH₃ as a sole N source (Faller, 1972).

From estimations based on previous data (see Chapter 5, Castro et al., 2006) and the present data it is obvious that at 4 µl l⁻¹ NH₃ the foliar N uptake might contribute up to 65-70 % of the total N requirement of B. oleracea. From the data on nitrate-deprived plants it was evident that at 4 and 8 µl l⁻¹ NH₃ indeed was utilized as a N source for
growth, however, plant biomass production was less than that of plants at nitrate-sufficient conditions. Furthermore, it was observed that in nitrate-sufficient plants the toxic effects of NH$_3$ started to prevail at > 4 µl l$^{-1}$. Apparently, there was no clear-cut transition in the level and rate of metabolism of the foliar absorbed NH$_3$ and the phytotoxicity upon exposure at 4 and 8 µl l$^{-1}$.

Is NH$_3$ toxicity related to a lack of detoxification capacity or a lack of carbohydrates?

A lack of detoxification capacity, occurring when uptake exceeds the capacity to assimilate NH$_4^+$ via the GS/GOGAT pathway, and a lack of carbon skeletons for the formation of organic N compounds, are considered as primary causes of NH$_3$ toxicity (Van der Eerden, 1982; Fangmeier et al., 1994; Krupa, 2003). The present data do not support these hypotheses. At 8 µl l$^{-1}$ NH$_3$, considered a toxic concentration, the organic N content was higher than in plants exposed to the non-toxic concentration of 4 µl l$^{-1}$ NH$_3$ (Fig. 1), which shows that assimilation into N-compounds was still possible at 8 µl l$^{-1}$ NH$_3$. It is therefore highly unlikely that the toxic effect of 8 µl l$^{-1}$ NH$_3$ can be explained by a lack of capacity to assimilate NH$_4^+$ formed in the mesophyll upon exposure to atmospheric NH$_3$.

Exposure to NH$_3$ did not decrease shoot soluble sugar content at any concentration (Fig. 6). The 2-fold higher soluble sugar content in nitrate-deprived compared to the nitrate-sufficient plants at 0 µl l$^{-1}$ NH$_3$ disappeared upon exposure to 4 and 8 µl l$^{-1}$ NH$_3$. This can be explained by the fact that the accumulated soluble sugars in the nitrate-deficient plants were used as C-acceptors for NH$_4^+$ and structural growth in the presence of an atmospheric N source. This conclusion is corroborated by the changes in DMC (Table 1). The present experiments, therefore, do not support the hypothesis that NH$_3$ toxicity is related to a lack of availability of carbon skeletons. This conclusion is corroborated by findings from other authors. In a similar experimental set-up as the present one, the impact of 1 and 2 µl l$^{-1}$ NH$_3$ on shoot soluble sugar and starch contents in winter wheat was investigated by Clement et al. (1997). Soluble sugar content was not significantly affected by NH$_3$, while plants exposed to 1 µl l$^{-1}$ NH$_3$ even had a slightly higher starch content. In a different experimental set-up in which NO$_3^-$ and NH$_4^+$ containing solutions (20 mM) were applied to the foliage of *Ricinus communis*, grown without NO$_3^-$ in the pedosphere, Peuke et al. (1998a) found that the flow of C in the whole plant as well as the increment of C in different plant organs were similar for both foliar N-treatments. Similar results were found for plants grown on NH$_4^+$ in the nutrient solution. Lang and Kaiser (1994) showed that sugar levels in NH$_4^+$-grown barley plants were unchanged, or even increased, and concluded that the observed growth impairment
was not related to impaired carbohydrate supply. Walch-Liu et al. (2001) concluded from experiments in which the effects of NO$_3^-$ and NH$_4^+$ on tobacco were investigated that inhibitory effects on shoot and root growth could not be related to limitations in the N or C status of the plants, or to NH$_4^+$ toxicity.

Are changes in cations related to NH$_3$ toxicity?

It is generally stated that atmospheric NH$_3$ may lead to a release of inorganic cations as K$^+$, Ca$^{2+}$ or Mg$^{2+}$, leading to nutrient imbalances (Van der Eerden and Pérez-Soba, 1992; Wollenweber and Raven, 1993; Fangmeier et al., 1994; Pérez-Soba et al., 1994; Britto and Kronzucker, 2002). Experimental data differ between a change and no effect, and accordingly, caution should be taken in making comparisons between results since NH$_3$ concentrations and exposure times are rather different.

The present data showed hardly any changes in K$^+$ content. Under nitrate-sufficient conditions, a decrease in shoot K$^+$ content was observed when NH$_3$ acted as a nutrient (4 µl l$^{-1}$), but no change in K$^+$ content when NH$_3$ was toxic (8 µl l$^{-1}$, Fig. 7). In contrast, Wollenweber and Raven (1993) stated that the increase in K$^+$ content was the most striking effect in *Lolium perenne*, exposed to 0.4 - 1.8 µl l$^{-1}$ NH$_3$.

The effect of NH$_3$ exposure on the divalent cations was more pronounced. Ca$^{2+}$ and Mg$^{2+}$ contents of both shoot and roots were decreased by NH$_3$, independent of the nitrate supply (Fig. 7). Van Dijk and Roelofs (1988) and Van der Eerden and Pérez-Soba (1992) observed shifts in the N/K, N/Ca and N/Mg ratios but no decrease in K$^+$, Ca$^{2+}$, Mg$^{2+}$ content, in needles of *P. sylvestris*, after exposure to 0.08-0.4 µl l$^{-1}$ NH$_3$ for 3 months. In the experiments of Peuke et al. (1998b), in which *Ricinus communis* plants were sprayed with NO$_3^-$ and NH$_4^+$ solutions (20 mM), a strong decrease in Mg$^{2+}$ in NH$_4^+$-sprayed plants compared to the NO$_3^-$-sprayed plants was observed. Lang and Kaiser (1994) also found a drastic decrease (90 %) in Mg$^+$ content of roots of barley cultivated on NH$_4^+$ in the nutrient solution. The decrease in Mg$^+$ content, however, was not sufficient to impair the energy status as the ATP and carbohydrate concentrations. In the present experiments the effect of NH$_3$ on Mg$^{2+}$ contents was much less, and did not differ much between plants exposed to a non-toxic (4 µl l$^{-1}$) or a toxic (8 µl l$^{-1}$) concentration. It is therefore highly unlikely that in the present experiments cation imbalances are the cause of the toxic effect of NH$_3$.

Concluding remarks

From the results presented in this paper it is evident that atmospheric NH$_3$ can act as nutrient, since it may contribute considerably to the total N requirement of *B. oleracea,*
at high levels, and in the absence of nitrogen in the nutrient solution. The concentration at which NH₃ changes from being a nutrient to a toxin is not clear-cut, since NH₃ can still be metabolized when growth is already affected. The physiological basis of the phytotoxicity of atmospheric NH₃ is still largely unknown.