Atmospheric NH3 deposition, S and N metabolism in curly kale
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Chapter 9.

General Discussion

Nutrient requirement of plants

The uptake of nutrients by the roots and their further assimilation in the plant is highly regulated and is generally in tune with the nutrient requirement for biomass production with time (De Kok et al., 2002a, 2005). The nutrient requirement varies between species and it may strongly vary at different developmental stages of the plant (vegetative growth, seed production). The overall plants' nutrient requirement \( \text{Nutrient}_{\text{requirement}} \) can be estimated as follows (De Kok et al., 2002a):

\[
\text{Nutrient}_{\text{requirement}} (\mu\text{mol g}^{-1}\text{plant day}^{-1}) = \text{RGR} (\%\text{day}^{-1}) \times \text{Nutrient}_{\text{content}} (\mu\text{mol g}^{-1}\text{plant})
\]

where RGR represents the relative growth rate and \( \text{Nutrient}_{\text{content}} \) of the total plant tissue. The RGR can be estimated as follows:

\[
\text{RGR} = \frac{(\ln W_2 - \ln W_1)}{(t_2 - t_1)}
\]

where \( W_1 \) and \( W_2 \) represent the total weight (g) at time \( t_1 \) and \( t_2 \), respectively, and \( t_2 - t_1 \) the time interval (days) between harvests. The rate of nutrient uptake by the roots necessary to meet the plants' nutrient requirement for growth can be estimated as follows (De Kok et al., 2005):

\[
\text{Nutrient}_{\text{uptake}} (\mu\text{mol g}^{-1}\text{root day}^{-1}) = \text{Nutrient}_{\text{requirement}} (\mu\text{mol g}^{-1}\text{plant day}^{-1}) \times (S/R_{\text{ratio}} + 1)
\]

where \( S/R_{\text{ratio}} \) represents the shoot (S) to root (R) biomass partitioning of the plant.

Is Brassica oleracea a species with a high sulfur requirement?

Brassicaceae (see Box 1) are generally considered to have a high sulfur requirement for growth. From the current study it is evident that Brassica oleracea cultivars had a sulfur requirement of approximately 10 \( \mu\text{mol g}^{-1}\text{plant fresh weight day}^{-1} \) (Chapter 3,
Castro et al., 2003). For comparison, at optimal growth conditions the sulfur requirement of different crop species ranges from 2 to 10 µmol g⁻¹ plant fresh weight day⁻¹ (De Kok et al., 2002, 2005). In general, the major proportion of the sulfur in plants is present in its reduced form in the protein fraction. Brassicaceae species are characteristic for the widespread occurrence of secondary sulfur compounds viz. glucosinolates (Schnug, 1990, 1993), though their content accounted only for a small proportion of the total sulfur fraction. In the shoot of the B. oleracea cultivars their content ranged from 1 to 2 % and 10 to 23 % on a total and organic sulfur basis, respectively (Chapter 4, Castro et al., 2004). Young B. oleracea plants contained a very high sulfate content, which accounted for 70 to 88 % of the total S content in the different cultivars (Chapter 3, Castro et al., 2003). It was proposed that for B. oleracea the organic sulfur content instead of the total sulfur content might be a better parameter for the estimation the sulfur requirement for structural growth (Chapter 3, Castro et al., 2003). Furthermore, it is evident that when the total sulfur requirement of B. oleracea would be corrected for the high sulfate content, the sulfur requirement for structural growth would not substantially differ from most other species in general.

Sulfate deprivation generally results in multiple adaptive responses facilitating enhanced sulfate uptake efficiency on a whole plant basis. Upon deprivation of B. oleracea there was fast induction of expression of the sulfate transporters and sulfate uptake capacity by the roots. More prolonged sulfate deprivation generally results in changes of shoot/root biomass partitioning in favor of that of the root (Stuiver et al., 1997; Yang et al., 2003, 2005; Buchner et al., 2004; Table 1) and altered root morphology (Chapter 7). In this context, the nature of high sulfate levels in B. oleracea needs further to be evaluated, since it might have significance in buffering the sulfur availability in the adaptation to variation of sulfate supply/uptake by the root. In general, it is presumed that the bulk of the sulfate present in the plant is localized in the vacuole and that in contrast to nitrate, sulfate would not be 'stored' but 'wasted' in the vacuole, because the rate constant for the vacuolar exchange or turnover would be very slow (Bell et al., 1990; Cram 1990; Clarkson et al., 1993). From studies on wheat and purple bean it became evident that sulfate present in the vacuole in these species was too immobile to temporary replace the sulfate taken up by the root as sulfur source for growth under sulfur stress. However, it has become obvious that in contrast to these species upon sulfate deprivation B. oleracea is able to remobilize and utilize vacuolar sulfate from both root and shoot as sulfur source for growth (Yang et al., 2003, 2005; Buchner et al., 2004). B. oleracea even benefited from the high amounts of sulfate present in the tissue and plants became more rapidly nitrogen deficient upon nitrate deprivation than sulfate
deficient upon sulfate deprivation (Chapter 8). Sulfate deprivation resulted in a strongly enhanced expression of the Group 1 sulfate transporters, which are responsible for the primary uptake of sulfate by the root, and of the Group 2 sulfate transporters, which are involved in the vascular transport. Moreover, deprivation also resulted in a strongly enhanced expression of Group 4 sulfate transporters in roots, stems/petioles and leaves of B. oleracea (Buchner et al., 2004). The latter transporters may be localized in the tonoplast and may be responsible for the transport of the sulfate from the vacuole to the cytoplasm (Buchner et al., 2004). A high vacuolar exchange and remobilization and assimilation of the abundant sulfate are also supported by the observation that both young and old sulfate-deprived leaves hardly contained any sulfate after prolonged sulfate deprivation (Table 1). Still the root (and cotyledons) contained detectable levels of sulfate, however, it remains to be questioned to what extent it originates from remobilized sulfate from the leaves, since it is assumed that root vacuoles have a higher rate constant for the exchange of sulfate than leaf vacuoles (Bell et al., 1990). Similar to previous observations there appears not to be a strict mutual regulation in the uptake of sulfate and nitrate, since the content of the latter was hardly affected by sulfate deprivation; it slightly increased in all leaves and stem/petioles, it was decreased in the cotyledons, whereas it was unaffected in the root (Table 1).

Table 1. Impact of sulfate deprivation on biomass and sulfate content of Brassica oleracea.

After germination plants were grown on 25 % Hoagland solution for 7 days and subsequently transferred to a 25 % Hoagland solution with and without sulfate for 10 days. Data represent the mean of 3 measurements with 6 plants in each (±SD).

<table>
<thead>
<tr>
<th></th>
<th>Fresh weight (g)</th>
<th>Sulfate (µmol g⁻¹ FW)</th>
<th>Nitrate (µmol g⁻¹ FW)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+S</td>
<td>-S</td>
<td>+S</td>
</tr>
<tr>
<td>4th Leaf</td>
<td>0.41 ± 0.11</td>
<td>0.16 ± 0.04</td>
<td>21 ± 4</td>
</tr>
<tr>
<td>3rd Leaf</td>
<td>0.57 ± 0.09</td>
<td>0.24 ± 0.03</td>
<td>32 ± 1</td>
</tr>
<tr>
<td>2nd Leaf</td>
<td>1.07 ± 0.10</td>
<td>0.55 ± 0.07</td>
<td>40 ± 1</td>
</tr>
<tr>
<td>1st Leaf</td>
<td>0.79 ± 0.02</td>
<td>0.66 ± 0.03</td>
<td>41 ± 3</td>
</tr>
<tr>
<td>Stems + petioles</td>
<td>0.93 ± 0.10</td>
<td>0.55 ± 0.05</td>
<td>12 ± 0</td>
</tr>
<tr>
<td>Cotyledons</td>
<td>0.54 ± 0.03</td>
<td>0.50 ± 0.05</td>
<td>36 ± 0</td>
</tr>
<tr>
<td>Root</td>
<td>0.73 ± 0.11</td>
<td>0.70 ± 0.12</td>
<td>14 ± 1</td>
</tr>
<tr>
<td>S/R ratio</td>
<td>5.95 ± 0.39</td>
<td>3.85 ± 0.50</td>
<td></td>
</tr>
</tbody>
</table>
The impact of atmospheric NH₃ is ambiguous since it is both nutrient and toxin

The data in Chapters 5 and 6 showed that *B. oleracea*, a species with a relatively high relative growth rate, was able to use atmospheric NH₃ concentrations up to 4 µl l⁻¹ N for growth and metabolism. The extent to which NH₃ could contribute to the N budget of the plant was dependent on the presence or absence of nitrate in the root environment. Atmospheric NH₃ concentrations up to 4 µl l⁻¹ NH₃, therefore, could be considered as nutrient. Although a concentration as high as 8 µl l⁻¹ NH₃ could contribute to the N budget of the plant, growth was affected and leaf injuries were visible at this concentration. Taken together, these results lead to the conclusion that the concentration at which atmospheric NH₃ changes from being a nutrient to a toxin is not clear-cut, since NH₃ can act as nutrient as well as toxin at the same concentration (Chapter 6).

Several authors have considered that a lack of detoxification capacity, occurring when NH₃ uptake exceeds the capacity to assimilate NH₄⁺, and a shortage of carbon skeletons for the formation of organic N compounds, are the primary causes for NH₃ toxicity. Moreover, nutrient imbalances due to cations release have been related to NH₃ toxicity (Van der Eerden et al., 1982; Wollenweber and Raven, 1993; Fangmeier et al., 1994; Krupa, 2003).

The results presented in Chapter 6 showed that even at a concentration as high as 8 µl l⁻¹ NH₃, when plant growth was affected, *B. oleracea* was able to increase the total N pool. At the same time, NH₃ exposure did not affect soluble sugar content. A lack of detoxification capacity and a shortage of carbon skeletons for the formation of organic N compounds, therefore, can be excluded as possible cause of NH₃ toxicity in *B. oleracea*. Nutrient imbalances due to the release of cations as primary cause of NH₃ toxicity are also unlikely, since changes in the pool of cations were only found for Mg²⁺. The physiological basis of the phytotoxicity of atmospheric NH₃, therefore, is still largely unknown.

Two further questions can be raised from the data presented in this thesis: i) Is the nitrate uptake by the root tuned to the NH₃ taken up by the shoot, inferring an efficient shoot to root signaling, and ii) To what extent is NH₃ toxicity related to experimental conditions?

In Chapter 5 it was shown that at an NH₃ concentration as low as 2 µl l⁻¹, there was already a significant increase in the total N pool, when nitrate was simultaneously supplied to the roots. Either the foliarly absorbed NH₃ is hardly utilized for structural growth, or in *B. oleracea* there is a poor shoot to root signaling in the tuning of the rate of metabolism of NH₃ taken up by the shoot to the nitrate uptake rate in the root. Studies
on the interaction between atmospheric and pedospheric sulfur nutrition of *B. oleracea* also showed a similar poor shoot to root signalling in regulation of the sulfate uptake (Buchner et al., 2004; Hawkesford and De Kok, 2006).

The proposed pathways and effects of NH$_3$ in *B. oleracea* are represented in Fig. 1.

![Proposed pathways and effects of NH$_3$ in B. oleracea](image)

**Fig. 1.** Proposed pathways and effects of NH$_3$ in *B. oleracea* (after Fangmeier et al., 1994; Krupa, 2003 adapted; see also Fig. 2, Chapter 1). Parameters in bold, effects found in Chapters 5 and 6. Parameters in grey, not measured. Dashed crosses, no support for the proposed effects. Dashed box, no clear distinction between primary and secondary effects.

The data presented in this thesis was based on laboratory experiments, with its inherent constraints, in which the NH$_3$ concentrations used were several orders of magnitude higher, and the exposure periods much shorter than in most field situations. Therefore, comparisons between data from laboratory studies and field experiments should be made carefully.

The current NH$_3$ levels in field conditions range from 0.03 to 1.2 µl l$^{-1}$, the latter as a peak level (Chapter 5). Since it is unlikely that these levels would negatively affect growth of *B. oleracea* under field conditions, one may conclude that *B. oleracea* is able to cope with levels of NH$_3$ exceeding peak levels, most likely because of its rather high nitrogen requirement and high RGR.
Box 1:
Evolution and phylogeny of Brassicaceae
The Brassicaceae members grow well in a diversity of habitats that comprise saline environments: salt marsh, costal dunes as well as gypsum soils and areas in the vicinity of S-emitting sources such as volcanoes (Ernst, 1990). Brassicaceae have migrated extensively to assume a worldwide distribution; being concentrated in the northern temperate regions, the Mediterranean and the mountains of southwest Central Asia (Hall et al., 2002). The modern Brassica sp. likely evolved from an ancient hexaploid (Hall et al., 2002). Several species of Brassica are of economic importance: B. rapa (turnip), B. juncea and B. nigra (mustard), B. oleracea (broccoli, Brussels sprouts, cabbage cauliflower, kale) and B. napus (rapeseed) are constituents of the Brassica Triangle. The Brassica triangle depicted an example of complexity of the poliploid genomes. This model is based on three diploid species: B. rapa (A genome), B. oleracea (C genome) and B. nigra (B genome) and their allopolyploid (merging of two distinct genomes to generate a single, new species) derivatives B. juncea (AB), B. napus (AC) and B. carinata (BC) (Soltis and Soltis, 2000).

Two of the diploids B. oleracea and B. rapa are sister in taxa within a larger “Rapa/Oleracea” lineage. However, the third diploid B. nigra is distantly related to the “Rapa/Oleracea” lineage and appears as part of a second clade of Brassica (the “Nigra” lineage) (Soltis and Soltis, 2000). This information is not only useful for comparative genome analysis, and therefore phylogenetic purposes, but also in interpreting biochemical data like glucosinolate composition in different Brassica species (Rask et al., 2000).

“Brassica triangle”. It exhibits the relationship between cultivated diploid and allotetraploid species of Brassica. Capital letters represent the genome type and n represents the chromosome number (Soltis and Soltis, 2000).
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