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Ammonia stomatal compensation point of young oilseed rape leaves during dark/light cycles under various nitrogen nutritions

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ABSTRACT

The plant can be a source or a sink of ammonia (NH_3) depending on its nitrogen (N) supply, metabolism and on the background atmospheric concentrations. Thus plants play a major role in regulating atmospheric NH_3 concentrations. For a better understanding of the factors influencing the NH_3 stomatal compensation point, it is important to analyse the dynamics of leaf NH_3 fluxes. The relationship between the leaf NH_3 fluxes and the leaf apoplast ammonium and nitrate concentrations, N nutrition and the light and dark periods was studied here.

We designed an experiment to quantitatively assess leaf-atmosphere NH_3 exchange and the stomatal compensation point and to identify the main factors affecting the variation of NH_3 fluxes in oilseed rape. We tested day and night dynamics as well as the effect of five different N treatments. Two experimental methods were used: a dynamic open flux chamber and extraction of the apoplastic solution.

Chamber measurements showed that there was a good correlation between plant NH_3 fluxes and water fluxes. Compensation points were calculated by two different methods and ranged between 0.8 and 12.2 $\mu g\, m^{-3}$ NH_3 (at 20 °C) for the different N treatments. Apoplastic solution measurements showed that there was no significant differences in the apoplastic NH_4^+ concentrations ($[NH_4^+]_{apo}$) extracted in dark and light periods for the same N treatment. Statistical analysis also showed that $[NH_4^+]_{apo}$ was correlated with $[NH_4^+]$ in the nutrient solution and weakly correlated with $[NO_3^-]$. Apoplast NH_4^+ concentrations ranged between 0.1 and 2.1 mM, bulk tissue NH_4^+ concentrations between 3.9 and 6.6 mM and xylem concentrations between 2.4 and 6.1 mM depending on the N supply.

Calculated NH_3 emission potential from the extraction measurements were over-estimated when compared with the value calculated from chamber measurements. Errors related to chamber measurements included separation of the cuticular and stomatal fluxes and the calculation of total resistance to NH_3 exchange. Errors related to the extraction measurements included assessing the amount of cytoplasmic contamination. We do not have another method to assess the NH_3 stomatal compensation point and the choice between these two measurement techniques should depend on the scales to which the measurements apply and the processes to be studied.

1. Introduction

Ammonia (NH_3) participates in the soil and atmosphere nitrogen cycle. It also plays a major role in the nitrogen (N) metabolism of plants. Grasslands and agricultural crops can be either a source or a sink of NH_3, depending on the difference between atmospheric NH_3 concentration and the so-called NH_3 compensation point of the plant. They therefore have a significant impact on the regulation of atmospheric NH_3 concentrations. The stomatal compensation point for NH_3 (χ_s) is defined as the atmospheric NH_3 concentration for which there is no exchange between the leaf and the atmosphere in dry conditions (when the cuticular adsorption/desorption can be neglected) (Flechard, 1998). In theory, χ_s is also the gaseous NH_3 concentration in thermodynamic equilibrium with the aqueous NH_3 in the apoplastic solution (solution surrounding the cells) (Farquhar et al., 1980).

A more complete characterisation of the compensation point in relation to different plant compartments and developmental stages, N supply, management practices, and plant species is crucial for a better understanding of NH_3 deposition at both local and regional scales. It is also important for comprehending how the plant influences χ_s. Moreover, most NH_3 exchange models developed so far do not account for the N metabolism of plants and use a constant value for the compensation point. For a better understanding of the factors driving the NH_3 stomatal compensation point value, it is important to analyse the dynamics of leaf NH_3 fluxes in relation to the ammonium (NH_4^+) and nitrate (NO_3^-)

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concentrations in different leaf compartments in addition to N supply and the light status.

Previous studies showed the variability of NH_3 emissions in relation to the developmental stage of the plant (Francis et al., 1997; Mattsson and Schjoerring, 2003). It has also been suggested that emissions are related to N reallocation, when N is transported as NH_4^+ in the xylem sap (Schjoerring et al., 1993a,b). Other studies also showed a large response of NH_4^+ concentration in xylem sap and apoplastic NH_4^+ to photosynthesis, respiration and glutamine synthetase (GS) inhibition (Mattsson and Schjoerring, 1996a; Yin et al., 1996). Apoplastic pH and NH_4^+ concentrations were also directly dependent on the N supply to the plant (Mattsson et al., 1998; Van Hove et al., 2002).

The canopy NH_3 compensation point (χ_c) can be inferred from measurement of vertical fluxes and concentrations of NH_3, over large fields (e.g. Flechard, 1998), as well as in cuvettes by finding at which concentration the flux is zero (Husted and Schjørring, 1995b). Some studies showed the effectiveness of these methods to assess the NH_3 compensation point for different species, environmental conditions, and its response to environmental parameters and plant nutrition (Mattsson and Schjoerring, 1996b, 2003).

Another technique exists and it is based on the determination of leaf apoplastic NH_4^+ concentration and pH to assess χ_s, by means of extraction of the apoplastic fluid with successive infiltration and centrifugation of leaf segments (Husted and Schjoerring, 1995a). This technique has been successfully applied to several plants in the field (Husted et al., 2000). However, the extraction technique is subject to uncertainties regarding possible regulation of apoplastic pH and NH_4^+ by the plant during the extraction (Hill et al., 2001; Schjoerring et al., 2002).

We designed an experiment to quantitatively assess leaf-atmosphere NH_3 exchange in relation to N nutrition in oilseed rape (*Brassica napus* L.). Oilseed rape is an expanding crop that requires high levels of N fertilization. We studied this crop because it is widely grown and likely to have significant environmental impact and because it has relatively large leaves making the measurements technically easier for both the extraction and the chamber method. We tested dark and light dynamics as well as five N treatments. Two experimental systems were used: (i) the dynamic open flux chamber, and (ii) measurements of apoplast, xylem and whole leaf solution extractions. We compared results from the two methods and tried to explain probable reasons for any discrepancy.

2. Materials and methods

2.1. Plant material

Seeds of oilseed rape (*Brassica napus* L. 'Capvert') were germinated in a small greenhouse for 1 week and then transferred to an aerated nutrient solution. Seed germinations were spread out over several weeks to give the same aged plants for every series of measurements. Plants were grown in phytotrons with average day/night temperatures of $22.0 \pm 0.9/18.0 \pm 0.6$ °C, day length of 12 h (from 12:00 till 00:00), average photosynthetic photon flux densities (PPFD) of 392 ± 108 μmol m^{-2} s^{-1} and average relative humidity of 74 ± 6% during the day and 92 ± 4% at night. It should be noted that PPFD variability was due to spatial distribution of light inside the phytotron; plants were weekly rotated to diminish the effect on growth.

All plants were grown in an aerated nutrient solution with 10 mM NO_3^- and 2 mM NH_4^+ (Lesaint and Coïc, 1982) for 4 weeks before separating them into the different N treatments for 1 week. Aeration of the nutrient solution was performed using an air pump delivering approximately 6 L min^{-1}. On average plants had eight leaves after 4 weeks and were in a vegetative stage.

The pH of the nutritive solution was measured weekly and adjusted to a value of 6 ± 0.3 by addition of NaOH or HCl. The different N treatments are adaptations of the Coïc–Lesaint solution (Lesaint and Coïc, 1982) with the following N concentrations:

- 0N
- 5 mM NH_4^+
- 10 mM NO_3^-
- 5 mM NO_3^- + 1 mM NH_4^+
- 10 mM NO_3^- + 2 mM NH_4^+

For extraction measurements, day extractions were done after 4 h of illumination, whereas dark extractions were done after 9 h of darkness.

2.2. Extraction measurements

2.2.1. Extraction of apoplastic solution

A slightly modified version of the vacuum infiltration technique described by Husted and Schjoerring (1995a) was used to determine apoplastic pH and NH_4^+ concentration. The top most developed leaf of the oilseed rape plant was cut and the central petiole was removed. The remaining leaf was then washed with de-ionized water and blotted dry with laboratory paper. The leaves were then weighed and infiltrated with 50 μM indigo carmine solution (kept at 4 °C) in a 60 mL syringe with a series of pressure/vacuum for 5 min. Hill et al. (2001) and Lohaus et al. (2001) showed that infiltration with indigo carmine instead of sorbitol solution had no significant effect on pH or NH_4^+ measurements. The infiltrated leaves were then blotted dry with clean absorbent paper towel. Leaves were left to equilibrate for 15 min according to Nielsen and Schjoerring (1998). The apoplastic solution was extracted by centrifugation at $2000 \times g$ for 10 min at 4 °C. The duration and centrifugation force were determined after prior tests on oilseed rape leaves for cytoplasm contamination. The contamination tests were done by measuring the activity of malate dehydrogenase (MDH) in apoplast extracts relative to bulk tissue extracts for six repetitions as described by Husted and Schjoerring (1995a). The contamination was less than 3.3 ± 1.5% of MDH activity in apoplast extract relative to bulk tissue extract. The dilution of the extracted solution was measured using a spectrometer at wavelength 595 nm (iEMS reader, Labsystems). The extracted solution was then frozen in liquid N and stored at −18 °C before further analysis.

2.2.2. Extraction of bulk tissue solution

Leaf segments were cut into small pieces and ground in liquid N until we obtained a homogenous powder using a pestle and mortar. A weighed sample was then put in a 1.5 mL tube together with 1 mL of ultra pure water. The tube was shaken for 15 min and then centrifuged at $2000 \times g$ for 20 min. The supernatant (clear solution on top) was then retrieved, frozen and stored at −18 °C for further analysis. The remaining leaf parts were dried and analysed for total N and carbon content.

2.2.3. Extraction of xylem sap

Xylem sap was collected from the cut surface at the shoot/root junction under 'root pressure' (Schurr and Schulze, 1995). Immediately after detopping the plant 0.5 cm below the shoot stump, it was rinsed with de-ionized water and blotted with absorbent tissue to remove contaminants from cut cells. After discarding approximately 50 mm^3 of sap, each cut surface was blotted again and silicon tubing was fitted over the stump. Sap flowing from the tubing was collected, frozen and stored at −18 °C for further analysis.