UPTAKE AND ASSIMILATION OF ATMOSPHERIC NO_2 - N BY SPRUCE NEEDLES (*Picea abies*): A FIELD STUDY

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Abstract. NO_2 enters spruce needles by gas exchange through the stomata. Nitrate formed from NO_2 is reduced in the cytosol by nitrate reductase (NR), the rate limiting enzyme of the nitrogen assimilatory pathway. A linear relationship was found between the nitrate reductase activity (NRA), NO_2 concentration and the amount of N incorporated into amino acids and proteins, so that NRA was suggested as an estimate of NO_2-uptake. In the present field study, 50 spruce trees (*Picea abies*) have been selected, which grow in a natural habitat in a NO_2 concentration gradient in a forest crossed by a highway which is a major NO source. At part of the sites, the microclimatic conditions have been recorded, so that common models of local gas exchange of the needles could be used to estimate stomatal uptake of NO_2. NRA was investigated as a function of radiation and stomatal uptake on the day before needle sampling. Close to the highway NRA was permanently elevated with a maximum in summer. As with the laboratory results, a linear relationship between stomatal uptake and NRA was found. Total N - content of current year shoots was not affected by the additional N-source provided by airborne NO_2. The present study shows that the gas exchange models are consistent with the physiological reactions of spruce needles on a local level and therefore contribute to the validation of calculations of NO_2 dry deposition to spruce forests.

Key words: NO_2, dry deposition, gas exchange, picea abies, spruce forests, nitrate reductase, nitrogen assimilation

1. Introduction

Dry deposition of reduced or oxidized nitrogen compounds to nutrient-limited ecosystems such as forests may represent a significant contribution to the nutrition of plants in these systems and has been discussed as a cause of forest decline in Europe (Nihlgård, 1985, Schulze, 1989, Manderscheid and Jäger, 1993).

The pathway of deposition of NO_2 to plants is mainly stomatal, i.e. by gas exchange (Hanson and Lindberg, 1991, Duyzer and Fowler, 1994). Most plants are able to assimilate incorporated NO_2 (Rowland *et al.*, 1987, Wellburn, 1990, Nussbaum *et al.*, 1993). In most conifers, nitrate is mainly reduced in the roots, and nitrogen is transported to the shoots in the form of amino acids (Manderscheid and Jäger, 1993). Nevertheless, NO_2 at concentrations between 3 and 160 $\mu g\ m^{-3}$ induces nitrate reductase, the first and rate limiting enzyme of nitrate reduction, in spruce needles (von Ballmoos *et al.*, 1993, Wingsle *et al.*, 1987, Norby *et al.*, 1989). The response time of induction was one to three days, the resulting level of nitrate reductase activity (NRA) was linearly related to the NO_2 concentration, and the uptake rates were linearly related to the stomatal conductivity (Thoene *et al.*, 1991, Rondon *et al.*, 1993). These results, suggesting NRA as a monitor for NO_2 uptake by the needles, had been obtained from laboratory fumigations, and only a few studies have tried to assess the relevance of NRA induction and NO_2-uptake in the field (Smirnoff *et al.*, 1984, Egger *et al.*, 1989).

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2. Materials and methods

Ten spruce sites with six individual trees each (*Picea abies* [L.] Karst.) between 10 and 20 years old were selected in a mixed forest (*Galio odorati - Fagetum typicum* on pseudo - gley) in the Swiss Plateau (480 m a.s.l.). This forest area is crossed by a highly frequented highway heading towards north giving rise to a NO_2-gradient on a transect to it. The sites included forest edge, clear cutting, and understory positions east (E1-E5) and west of the lane (W1-W5). Local NO_2 concentrations were obtained at every site as two-weekly means using passive sampling devices. Four of the sites were equipped with sensors for temperature, relative humidity and PAR controlled by two data logger stations.

Needle sampling and subsequent analysis of NRA was conducted in the morning of 4 dates in July and August 1994. Sensitive passive sampling devices similar to those described by Nishikawa *et al.* (1986), had been exposed 24 hours before to get the daily mean NO_2 concentration at every site. In the same period, PAR was determined 4 times at every shoot selected for sampling using a hand-held PAR-sensor. Preceding this extended field experiment, NRA had been measured monthly for 3 years (1987-1989) in the same region at two sites with 6 trees each in a distance of 10 m and 1300 m from the highway.

NRA was determined *in vitro* according to the procedure described by von Ballmoos *et al.*, 1993. Briefly, the cell-free assay of the needle homogenates was incubated under saturating availability of substrate (nitrate) and cosubstrate (NADPH), and the production of nitrite was measured spectrophotometrically. Seasonal dependence of NRA was measured with NADH as cosubstrate. NRA is referred to the protein content determined with BSA as a standard and given as nmol mg_{prot}^{-1} min^{-1}.

Sampling of needles for analysis of total N was performed in November 1994. 1.5 g fresh weight needles were cut from the twig, dried for 24 hours at 80°C, immersed in liquid nitrogen and homogenized using a dismembrator for 2 min at maximum speed. Root-free soil samples from the topmost 5 cm were dried at 105 °C and ground with mortar and pestle and sieved through a 1 mm mesh. N-content in the plant and soil material was measured by an elemental analyzer (Forschungszentrum Seibersdorf, Austria). The projected leaf area of the fresh needles was measured with an area meter and converted to total surface by multiplying by 2.65 (Oren *et al.*, 1986).

The time-resolved data of temperature, relative humidity and PAR were incorporated into a model of local shoot gas exchange by calculating stomatal conductivities according to Baldocchi *et al.* (1987), Wesely (1989), and Jarvis (1976), and using spruce-specific parameters provided by Grace *et al.* (1975). Stomatal uptake was calculated as the mean of the product of conductivity and NO_2 concentration on the day before needle sampling. To estimate the total seasonal stomatal uptake of NO_2, the uptake rate was integrated from April (after bud break) until October. Statistical results were obtained with a statistics software package.

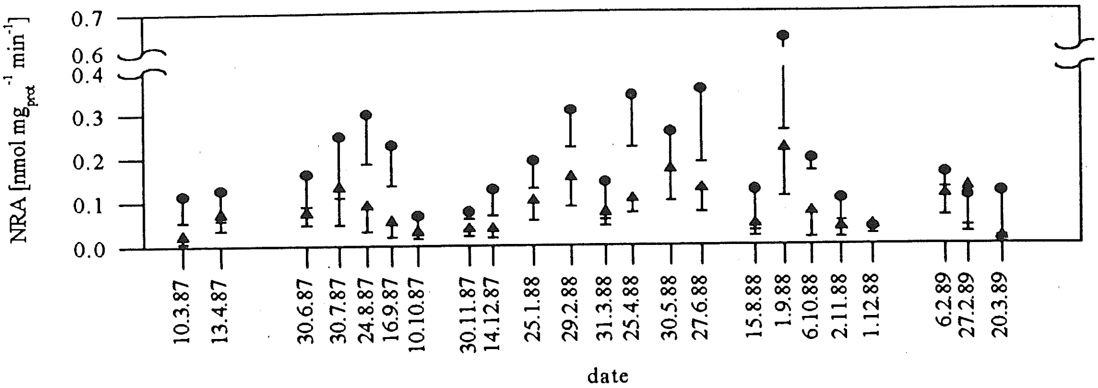

Fig. 1 Seasonal dependence of nitrate reductase activity (NRA) in needles of 25-year-old spruce trees growing at 10 m (●) and 1300 m (▲) distance from the highway measured once each month 1987-1989. Means ± SD of 6 trees are presented. The differences between the two sites were significant at $p<0.05$ except at 13.4.87, 10.10.87, 30.5.88, 15.8.88, 1.12.88, and 27.2.89.

3. Results

The mean NO_2 concentrations (average of two-weekly means of April to October) decreased almost logarithmically with the distance from the highway from 50 μg m^{-3} at 8 m to 10 μg m^{-3} at 900 m. The slight asymmetry observed between the sites east and west of the highway directed towards north were consistent with the prevailing winds from westerly directions during this period (data from a nearby meteorological station, SMI, Switzerland). NO_2 was the only relevant quantity giving rise to a gradient in this transsect. The nitrogen content of the uppermost soil layer densely rooted by spruce was homogeneous (0.30 wt% ± 0.05) throughout the sites. Correspondingly, no significant variation of the composition of the ground vegetation with the distance from the highway was observed.

NRA in trees at 10 m from the lane turned out to be significantly increased throughout the year compared to the control site at 1300 m, with a minimum in November and December (Fig. 1). High NRAs in early autumn have been repeatedly observed in other years and other regions (data not shown).

NO_2 uptake during the day before needle sampling was expected to be the most important factor determining current NRA. In Fig. 2, measured NRA of needles sampled on 4 different days in summer 1994 are compared to PAR measured locally and to mean stomatal uptake of NO_2 derived from NO_2 concentrations and the stomatal response to the micrometeorological conditions. The results show that NRA responds to both influx of NO_2 and PAR on a short time scale. The average NRA of all measurements at each site was typically higher at the sites with higher PAR. But also on these sites, NRA was low after a day with a low uptake rate (3.8.94).

A two dimensional linear regression analysis including the measurements from all 60 trees delivered a significant ($p<0.02$, $n=182$) linear correlation of NRA with PAR and stomatal uptake with a standard error of estimate of 0.057 nmol mg_{prot}^{-1} min^{-1}. In order to establish the link to the laboratory fumigations obtained with potted trees in climate controlled chambers, the measurements fulfilling the condition 100 μE m^{-2} s^{-1} < PAR < 400 μE m^{-2} s^{-1} were selected and displayed in Fig. 3 as function of the stomatal uptake of NO_2-N. Mean PAR of this ensemble was 208 μE m^{-2} s^{-1} compared to 220 μE m^{-2} s^{-1} in

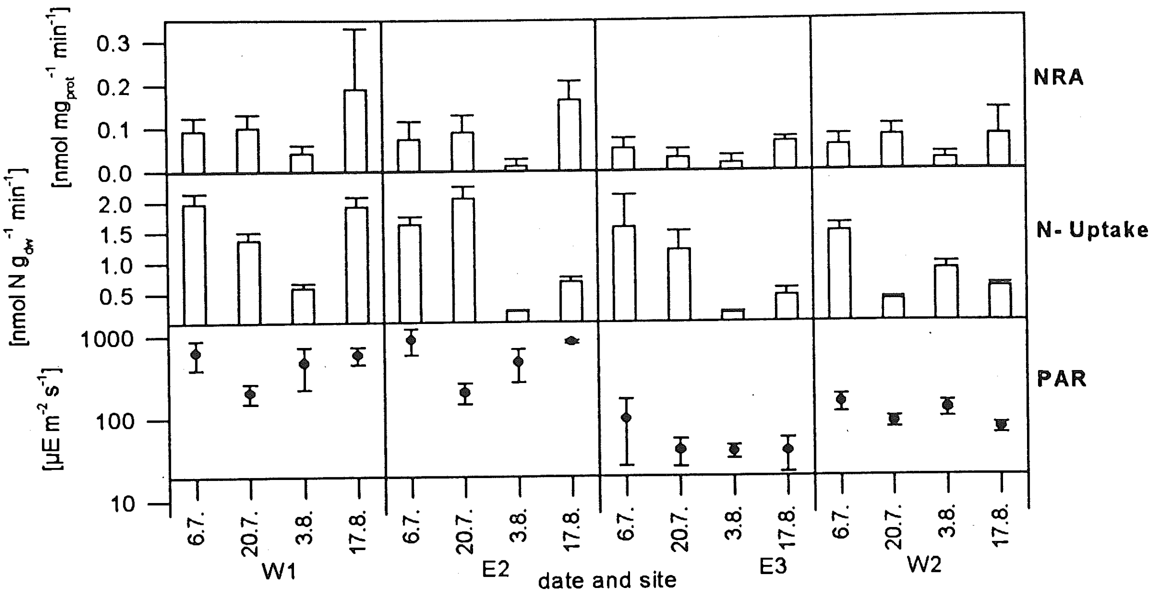

Fig. 2 Nitrate reductase activity (NRA), PAR and calculated stomatal uptake of NO_2-N on the day prior to needle sampling at the fully equipped sites W1, W2, E3 and E2. Means ± SD of 6 trees at four sampling dates in 1994 are presented.

the laboratory study reported by von Ballmoos *et al.* (1993). The linear relationship was confirmed ($p<0.01$, N=64), but the slope of the increase was lower in the field samples.

Finally, the question arose of whether the additional nitrogen assimilated from airborne NO_2 led to an increase in the nitrogen content or needle dry weight in the current year shoots. No correlation was found between the integrated stomatal uptake of NO_2-N in the period of April to October 1994 and the N-content. Total nitrogen content was 1.82 wt% ± 0.17, the protein content was 43.1mg g^{-1} ± 4.9 (fresh weight), and the mean dry weight per needle was 2.02 mg ± 0.64. The latter tended to increase (not significant) within the range of the integrated seasonal stomatal uptake between 1 and 3 mg N g_{dw}^{-1}.

4. Discussion

The presented results confirm that the increase in NRA is also a significant effect under field conditions with realistic NO_2 concentrations and without any impacts from chambers or fumigation equipment. The seasonal dependence (Figure 1) reflects the annual course of stomatal NO_2-uptake (data not shown) and of general activity of nitrogen metabolism with a minimum in winter (Pietiläinen *et al.*, 1991, Manderscheid and Jäger, 1993). The influence of HNO_3, which also induces NRA in conifers (Norby *et al.*, 1989), was not relevant in this study where the gradient was clearly dominated by NO_2. HNO_3 may have contributed to the background at the sites with low NO_2 concentrations.

Under laboratory conditions, a linear relationship between NRA, NO_2 concentration and NO_2 uptake was obtained for spruce (Nussbaum *et al.*, 1993, von Ballmoos *et al.*, 1993, Thoene *et al.*, 1991). The variations of NO_2 uptake with relative humidity could be related to stomatal behaviour affected by the vapour pressure deficit. PAR seemed to influence NRA and NO_2-uptake both by regulation of stomatal aperture and by defining the level of induction of extractable NRA (Lillo, 1994) at a given NO_2 concentration. Correspondingly, the 24-hour record of local meteorological conditions and NO_2 concentration giving rise to the estimated stomatal uptake and PAR were the main factors controlling

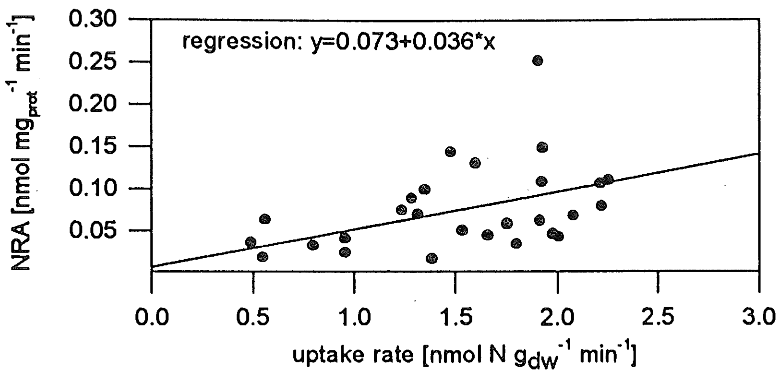

Fig. 3 Linear relation between nitrate reductase activity (NRA) and stomatal uptake of NO_2-N for all shoots exposed to 100 $\mu E\ m^{-2}\ s^{-1} < PAR < 400\ \mu E\ m^{-2}\ s^{-1}$ on the day prior to needle sampling corresponding to conditions during laboratory fumigations. R=0.46, significance of relation $p<0.01$ (n=64).

NRA in this study (Figure 2). The estimates of NO_2-N uptake were based on a gas exchange model frequently used for modelling dry deposition of NO_2 to spruce forests (Duyzer and Fowler, 1994), so that the present results may be regarded as local validation of the parametrisation used. Jarvis (1976), who provided part of the parameters used in this study, pointed to the large variation of gas exchange in relation to light, humidity and temperature found in the field, and estimated that only about 70% of the variation could be explained by a similar model. So far, the scatter in NRA presented here is consistent with the expectations, which in part have to be taken into account even for cloned spruce trees. Replacing the actual time course of NO_2 concentrations by mean values and basing the calculation of NO_2-input on one day only may have further contributed to the large variations of current NRA found in this study.

The difference between NRA levels in the field and the laboratory may be explained by different availability of nitrogen and water in the soil (Sarjala, 1991) and the longer light periods in the laboratory fumigations. The question of whether nutrient availability not only affected enzyme activity but also NO_2-N-uptake itself was not addressed here.

Although the estimated seasonal uptake was larger than 10% of the N-content in the needles, no accumulation of nitrogen was observed in the needles. It seems that in the first year, NO_2-N partially replaced nitrogen from the soil. Though the tendency of increasing needle dry weight was not significant, further growth effects may be encountered in the following years (Gebauer *et al.*, 1994), and total biomass production should be considered in the long term.

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