Incorporation of atmospheric $^{15}NO_2$-nitrogen into free amino acids by Norway spruce *Picea abies* (L.) Karst.

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Abstract. During spring and autumn 1991, potted 6-year-old spruce trees (*Picea abies* (L.) Karst.) were fumigated with $60 \ nl \cdot l^{-1} \ ^{15}NO_2$ for 4 days under controlled conditions in constant light. Current and previous flush needles, the bark and the fine roots were analysed for total ^{15}N content and incorporation of ^{15}N into the α-amino nitrogen of free amino acids. In addition, *in vitro* nitrate reductase activity and stomatal conductance of the needles were measured. Nitrate reductase activity was significantly higher in the needles of fumigated trees compared to control trees exposed to filtered air. With an average of $9.1\% \ ^{15}N$, free glutamate was the pool with the most label. Taking into account the time-course of the labelling of this pool, this figure can be taken as an estimate of the minimum contribution of NO_2 to the N nutrition of the needles. ^{15}N-labelled amino acids were also detected in the bark and the roots, indicating export from the needles.

Key words: Amino acids $-^{15}N$ incorporation – Nitrate reductase activity – Nitrogen dioxide – *Picea abies*

Effects of atmospheric nitrogen oxides on plants have been studied extensively (for review see Rajagopal and Saxe 1988; Wellburn 1990). Besides their phytotoxic properties (Sabaratnam and Gupta 1988; Srivastava et al. 1975; Saxe 1986b), their function as a source of nitrogen has been recognized (Faller 1972; Rogers et al. 1979a; Kaji et al. 1980; Okano et al. 1984; Latus et al. 1990). In herbaceous species an increase in nitrate reductase activity (NRA) has been detected. In some cases this response to NO_2 was modulated by the nitrate supply to the roots (Zeevaart 1974; Srivastava and Ormrod 1984; Murray and Wellburn 1985, 1987; Rowland et al. 1987). Conifer seedlings [*Pinus sylvestris* (L.), *Picea rubens* (Sarg.)] also had higher NRA in needles *in vivo* when fumigated with NO_2 (Wingsle et al. 1987; Norby et al. 1989). Aseptically grown *Picea abies* [(L.) Karst.] seedlings in hydroponic culture had a high *in*

vivo NRA in the needles but low NRA activity in the roots when exposed to $500 \ nl \cdot l^{-1} \ NO_2$ for 11 weeks (Tischner et al. 1988). *P. abies* trees 4–8 years old showed an increase in needle NRA when fumigated with ecologically relevant concentrations of NO_2 (Egger et al. 1988; Thoene et al. 1991).

The finding that, if atmospheric resistance is ignored, NO_2 absorption by leaves is mainly controlled by stomatal aperture (Rogers et al. 1979b; Fuhrer and Erismann 1980; Kaji et al. 1980; Saxe 1986a; Okano et al. 1988; Hanson et al. 1989; Rowland-Bamford et al. 1989; Thoene et al. 1991) suggests that NO_2 is taken up by the plant via the stomata. It is dissolved in the cytosol at least to some extent as nitrate (Norby et al. 1989). The nitrogen can then be incorporated into amino acids through the assimilatory nitrate reduction pathway common to all higher plants (Beevers and Hageman 1980; Runge 1983). Using $^{15}NO_2$ it was shown that amino acids were indeed labelled with the tracer in several herbaceous species (Durmishidze and Nutsubitze 1976; Yoneyama and Sasakawa 1979; Kaji et al. 1980). Based on model calculations for a healthy spruce stand, Schulze (1989) estimated that 1.7% of the total nitrogen supply of *P. abies* growing on acid soil could be provided by gaseous aerial uptake at ambient average NO_x concentrations of $14–20 \ nl \cdot l^{-1}$. This nitrogen input adds to the load reaching the plant via wet and occult deposition (Unsworth and Wilshaw 1989). Airborne N inputs could lead to nutrient imbalances in forests (Schulze 1989; Weikert et al. 1989).

The aim of this study was to analyse and quantify the incorporation of NO_2-N into amino acids in *P. abies* under controlled conditions.

Materials and methods

Plant material

Four-year-old *P. abies* trees grown in the nursery from seeds from the same site were potted in polyethylene pots (20 l, diameter 340 mm, height 260 mm) containing 40% bark compost, 40% peat and 20% sand. At the end of the experiments the soil parameters listed in

Table 1. pH and nutrient content of the soil at the end of the experiments

Parameter		Mean	Range
pH (H$_2$O)		7.2	7.1 – 7.2
N-Kjeldahl	(mg·g^{-1} DW)	5.47	4.46– 6.52
C$_{tot}$	(mg·g^{-1} DW)	118	99 –160
NH$_4^+$–N	(mg·g^{-1} DW)	0.15	0.14– 0.17
NO$_3^-$–N	(µg·g^{-1} DW)	26.9	16.5 – 43.9
P	(µg·g^{-1} DW)	5.4	4.6 – 6.3
K	(µg·g^{-1} DW)	25.6	21.3 – 32.6
Mg	(µg·g^{-1} DW)	134	86 –184

Mean values and range of six samples, three each from spring and autumn, are presented. Nutrients were extracted as follows: NH$_4^+$/NO$_3^-$: 1M KCL; P/K: CO$_2$-saturated water; Mg: 0.0125M CaCl$_2$. There were no significant differences between spring and autumn

Table 2. Mean value ± SD of continuously monitored parameters in the control chambers

Parameter	Spring	Autumn
NO$_2$ (nl·l^{-1})	7±5	2±2*
NO (nl·l^{-1})	6±3	2±2*
O$_3$ (nl·l^{-1})	2±2*	2±2*
CO$_2$ (µl·l^{-1})	400±15	380±30
Temperature (°C)	21± 0.5	21± 0.5
Relative humidity (%)	61± 0.5	61± 1
Light (µE·m^{-2}·s^{-1} PAR)	320±15	260±10
Soil temperature (°C)	23± 0.5	21± 1
Soil water tension (hPa)	20–30	20–30

Values marked with an asterisk are within the detection limit of the respective monitor

Table 1 were determined. The pots were placed in the shade of a forest clearing near Bern (Switzerland) with a light intensity of 100 µE·m^{-2}·s^{-1} photosynthetically active radiation (PAR) on sunny summer days. Total annual rainfall amounted to 1122 mm with an average volume-weighted pH of 4.89. The pots were watered regularly with deionized water. NO$_2$ concentrations monitored by passive sampling devices (Passam, Switzerland) were 10 nl·l^{-1} in August and 20 nl·l^{-1} in January and February. Bulk deposition measured in an open field nearby was 6.02 and 4.65 kg·ha^{-1}·year^{-1} for NH$_4^+$–N and NO$_3^-$–N, respectively. At the time of fumigation the plants were 6 years old.

Fumigation equipment

Two chambers, for the control treatment and the fumigation respectively, (1200 × 700 × 1000 mm, wind tunnel type), with stainless steel frames and UV-transparent acrylic glass (Sanalux, DESAG, Germany), were placed in a climate-controlled growth room equipped with metal-halogenide lamps (MRF 250 BU, EYE Corp., Japan). Preconditioned air from this room was passed through a filter unit with the following elements in sequence: particle filter (Macropur F, Delbag GmbH, Germany), permanganate oxidizing absorption filter (Purafil Inc., USA), and two types of activated charcoal (Acolit A$_1$ and Acolit C$_1$, Delbag GmbH, Germany). Air flow was equivalent to 4.5 air changes per minute in the chambers. The pressurized gas mixture for fumigation containing 0.1% NO$_2$ (99% ^{15}N) in synthetic air (Carbagas, Switzerland) was added to the air stream between the filter unit and the fumigation chamber. The gas flow was controlled by a thermic mass flow controller (5850TR, Brooks Instruments BV, The Netherlands).

Three trees were used in each treatment. Four ventilators assured turbulent mixture in the chamber, resulting in a wind speed in the crown ranging from 0.5 to 1.0 m·s^{-1}. Between the trees a LI-190SA quantum sensor (Li-Cor, USA), a combined temperature/humidity sensor (MP-100, Rotronic, Switzerland) as well as two PTFE tubes for continuous sampling of the trace gas composition were installed. The following monitors were used: Siemens Ultramat 5 E (Siemens, Germany) for CO$_2$, Tecan CLD 502 chemiluminescence monitor (Tecan, Switzerland) for NO$_x$ and Daisibi 1003 PC (Daisibi, USA) for O$_3$.

Soil water tension in each pot was measured with tensiometers (Tensior 2, Umweltanalytische Messgeräte, Germany) and maintained at 20–30 hPa. Stomatal conductance of the current and the previous flush needles were measured daily with a steady state porometer (LI-6200, Li-Cor, USA), using a quarter-litre chamber. Boundary layer conductance was estimated by the heat transfer system described by Jones (1983) at chamber conditions (20° C, wind speed 0.7–0.8 m·s^{-1}) using an aluminium model twig.

Fumigation experiments ran from 15 March to 25 March just before bud break (spring fumigation); and 23 September to 4 October (autumn fumigation) in 1991.

Experimental conditions

The plants were sequentially exposed to the following three periods of controlled constant conditions:

A. Adaptation to temperature, humidity and light conditions in a climate-controlled growth room of the type used for the fumigation experiments (4 days). NO$_2$ concentration was 13–19 nl·l^{-1}, all other parameters corresponded to those listed in Table 2.

B. Adaptation to filtered air and wind speed in the chambers used for fumigation (2 days). For environmental conditions see Table 2.

C. Fumigation experiment (4 days). All parameters listed in Table 2 remained unchanged. NO$_2$ concentration in the fumigation chamber was increased to 59±4 nl NO$_2$·l^{-1} in spring and to 60±2 nl NO$_2$·l^{-1} in autumn without any detectable effect on NO and O$_3$ concentrations.

Harvest of plant material

Immediately after the end of the fumigation shoots were separated from roots and both parts harvested in parallel.

A. Shoots: twigs were separated into different age classes. The current and the previous flush were immediately immersed in liquid nitrogen, and the needles were taken off and stored at −80° C until further use. The projected area of the needles of the twigs used for porometer measurements was determined with a LI-3000 area meter (Li-Cor, USA) and total surface computed by multiplying with a factor of 2.65 (Oren et al. 1986). This value was used to calculate area based stomatal conductance. The entire bark of the flush of 1987 was peeled from the wood. Both bark and wood were stored at −80° C until further use.

B. Roots: the soil was removed from the roots by rinsing with tap water followed by deionized water. Roots < 1 mm in diameter were cleaned of mycorrhizal rhizomorphs, blotted dry with paper, weighed and then stored at −80° C until further use.

Extraction of free amino acids

Using a Polytron (Kinematica, Switzerland) 0.5–1 g plant material (needles, bark, roots) was homogenized in 20 ml 80% methanol containing known amounts of internal standard (α-aminobutyric acid for the neutral and amide fraction, α-aminoadipic acid for the acidic fraction). After boiling under reflux for 10 min at 70° C, the suspension was centrifuged for 10 min at 17000 g (Sorvall RC5,

Du Pont Instruments, USA). The pellet was resuspended in 10 ml 20% methanol and the boiling and centrifugation procedure repeated. The combined supernatants were rotary-evaporated to dryness under reduced pressure at 45°C using a Rotavap (Büchi, Switzerland). The residue was redissolved sequentially by addition of 2 ml 80% methanol, 4 ml chloroform and 5 ml H_2O (all HPLC grade). Phase separation of the combined washing fluids was completed by centrifugation for 30 min at 3000 g. The upper (methanol/water) phase was used for the isolation of free amino acids.

Isolation and purification of glutamate and aspartate

Glutamate and aspartate were isolated from the neutral and acidic fraction by Dowex-1-acetate ion-exchange chromatography (Sims and Ferguson 1974; Rhodes et al. 1981). The methanol/water phase from the extraction was applied to a 3×1 cm column of the resin (mesh 200–400) equilibrated with H_2O. Neutral amino acids and amides were collected by washing with 6 ml H_2O and glutamate and aspartate were jointly eluted with 6 ml 2 M acetic acid. The latter fraction was rotary-evaporated to dryness, dissolved in 2 ml H_2O and applied to a 3×1 cm Dowex-50W H^+ column (mesh 100–200) equilibrated with H_2O, together with another 2 ml wash of the flask. The column was washed with 6 ml H_2O and glutamate and aspartate jointly eluted with 6 ml 25% NH_4OH. The sample was rotary-evaporated to dryness, redissolved in 1 ml 60% methanol and stored at $-80°C$ until further use.

Purification of neutral amino acids and amides

The neutral amino acid/amide fraction from Dowex-1-acetate chromatography was applied to a Dowex-50W H^+ column (3×1 cm, mesh 100–200) equilibrated with H_2O. The column was washed with 6 ml H_2O and neutral amino acids and amides jointly eluted with 6 ml NH_4OH. The sample was rotary-evaporated to dryness, redissolved in 2 ml H_2O and the flask washed with another 2 ml of water. Dowex-50W H^+ chromatography was repeated as described in the previous paragraph and the dissolved amino acids and amides, together with another 2 ml water wash of the flask, applied to a Dowex-1-chloride column (3×1 cm, mesh 200–400) equilibrated with H_2O. The column was washed with 4 ml water and the washing fluid rotary-evaporated to dryness. The sample was redissolved in 1 ml 60% methanol and stored at $-80°C$ until further use.

Derivatization of amino acids

The derivatization procedures for free amino acids followed those described by Rhodes et al. (1981) based on MacKenzie and Tenaschuk (1974, 1979a, b). At the end the samples were dissolved in 50–250 µl ethyl acetate:acetic anhydride (1:1, v/v), stored at 4°C and analysed within 2 days.

GLC and gas-chromatography-mass-spectrometry (GC-MS) of amino acids

Quantitation by GLC: Samples were co-derivatized with a standard amino acid mixture (Standard H, Pierce) supplemented with known amounts of γ-aminobutyric acid (gaba) as well as the internal standards α-aminobutyric acid and α-aminoadipic acid. For quantitation a Carlo Erba (Italy) Fractovap GLC with flame ionization detection was used equipped with a 30 m x 0.32 mm (internal diameter) DB-1 fused silica capillary column (0.25 µm film). The carrier gas employed was H_2 at 1.47 hPa. The temperatures of both injector and detector were maintained at 250°C. Aliquots of 1 µl were injected. The oven temperature program was 100°C (4 min) to 260°C at 6°C per min. A Beckman 427 Integrator (Beckman, USA) was used for peak area integration.

Determination of ^{15}N-enrichment of the amino acids by GC-MS. Aliquots of 0.2–1 µl were analysed on a Varian MAT44S quadrupole GC-MS system in the electron ionization mode, equipped with an SS 200 data acquisition system. The column type and oven temperature program were the same as for GLC. The carrier gas was He at 1.88 hPa. The source temperature, injector temperature and interface temperature were all 250°C, and electron energy was 70 eV. Complete scans over the mass range 200–320 for the neutral and amide fraction and 240–320 for the acidic fraction were performed throughout the chromatogram every second.

The following selected ion pair ratios of each amino acid, containing the α-amino nitrogen, were used for calculation of ^{15}N enrichment (in elution sequence): ala: 240/241; gly: 226/227; val: 268/269; thr: 253/254; ser: 239/240; leu: 282/283; ile: 282/283; gaba: 282/283; pro: 266/267; met: 253/254; asx: 284/285; phe: 316/317; glx: 298/299; lys: 280/281; arg: 266/267; his: 306/307. Ratios were determined from area counts of the respective ion current profile peaks and enrichments calculated according to Rhodes et al. (1981). Standard deviation (s) of each amino acid from zero enrichment was determined from 10 unlabelled spruce needle samples and an enrichment detection limit of 2s was applied (Lodge 1989).

In order to account for $^{14}NO_2$ in the chamber, calculated enrichments were corrected by dividing by the respective $^{15}NO_2$ fraction. Throughout this paper the corrected values are used for enrichments in percent or mol ^{15}N.

Determination of ^{15}N enrichment in total nitrogen

The plant material was ground to a fine powder according to von Arb and Brunold (1990). Drying at 80°C yielded fresh weight:dry weight ratio. Total nitrogen and carbon content was determined with a C/N analyzer (Carlo Erba, Italy) by WSL, Birmensdorf, Switzerland. ^{15}N enrichment of total nitrogen was measured by on-line combustion gas-isotope ratio mass spectrometry by ECN, Petten, The Netherlands. Air was used as standard and the values corrected as described above for the amino acids.

In vitro nitrate reductase assay

Measurement of nitrate reductase (E.C. 1.6.6.1./1.6.6.2) activity followed the procedures described by Neyra and Hageman (1975). Using a Polytron, 1 part needles were homogenized in 10 parts (w/v) phosphate buffer (0.1 M, pH 7.7) containing 1% (v/v) Tween 80, 1% (w/v) polyvinylpyrrolidone K30, 5 mM EDTA, 10 mM 1,4-dithioerythritol, 10 mM L-cysteine and 20 µM FAD (all chemicals supplied by Fluka, Switzerland). Homogenates were made cell-free by passing through two layers of viscose-fleece (Milette, Migros, Switzerland). In a final volume of 1 ml the assay mixture contained 25 mM phosphate buffer pH 7.5, 3.5 mM KNO_3, 0.15 mM each of a NADH/NADPH mixture and crude extract with up to 1.6 mg protein. A blank, where NADH/NADPH was replaced by phosphate buffer, was run in parallel. The mixture was placed in a water bath and heated to 30°C. After 20 min the reaction was stopped by adding 400 µl 0.125 M zinc-acetate. The protein content of the extracts was determined according to Bradford (1976) using BSA as a standard.

Statistical analysis

For statistical analysis of differences between mean values the Wilcoxon-Mann-Whitney U-test (Sachs 1984) was used.

Results

The total free amino acid contents of control trees, in filtered air, of both spring and autumn experiments were comparable in all samples (Fig. 1). In autumn the free amino acid content in needles of fumigated trees was roughly double that of control trees (statistically different at $P \leq 0.05$). In bark and roots no difference was found between the two treatments. No visible injury of needles caused by NO_2 was detected. Stomatal conductance for water vapor during the experiments was in the range $0.4–2.3 \, mm \cdot s^{-1}$ with no detectable diurnal variation (data not shown). Estimated boundary layer conductance was $74 \pm 3 \, mm \cdot s^{-1}$ $(n = 6)$.

Total N content of the needles at the end of the experiments was $8.4 \pm 1.5 \, mg \cdot g^{-1}$ dry weight (DW) in spring. In autumn current-flush needles contained significantly more N than those from the previous flush $(12.25 \pm 0.60$ and $9.82 \pm 1.13 \, mg \cdot g^{-1} DW$, respectively; $P \leq 0.005$). The N content of bark, wood and roots was lower in spring compared to autumn. There was no difference in total N content between controls and fumigated trees.

At the end of the experiments NRA was significantly higher in fumigated trees than in controls, in both current- and previous-flush needles $(P \leq 0.01$ and 0.005 for the current and previous flush, respectively; Fig. 2). This effect was more pronounced in autumn.

Combining spring and autumn experiments, $23 \pm 10\%$ $(n = 6)$ and $15 \pm 6\%$ $(n = 6)$ of the total label in needles was detected in free amino acids in current and previous flush, respectively, after fumigation for 4 days. At this time 13 out of 18 detectable amino acids of the needles were labelled in the α-amino N with ^{15}N from $^{15}NO_2$ (Fig. 3). The
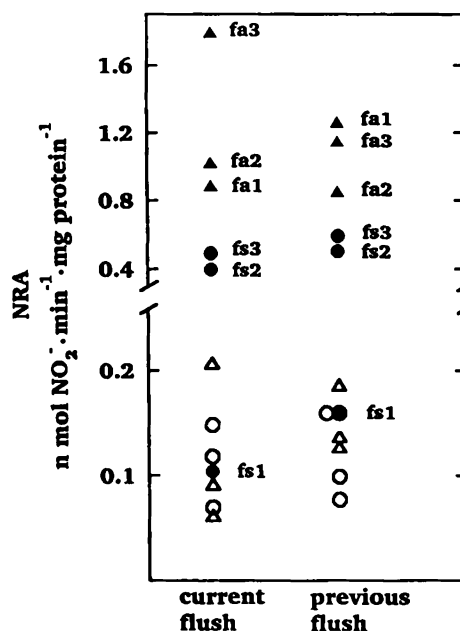
Fig. 2. Nitrate reductase activity of current and previous flush needles of individual 6-year-old *P. abies* trees exposed to $60 \, nl \cdot l^{-1}$ NO_2 for 4 days in spring (●, *fs*) and autumn (▲, *fa*). Controls were kept in filtered air in spring (○) or autumn (△)

glutamate pool was the most heavily labelled and contained $9.1 \pm 2.4\%$ ^{15}N $(n = 12)$. In samples of the spring experiment analysed after 1 and 2 days the α-amino N of glutamate contained 5.3 $(n = 1)$ and $5.3 \pm 1.3\%$ $(n = 4)$ ^{15}N, respectively (data not shown). Gly, gaba and asn were labelled in spring only. The amount and the label present in glu, asp and ser is shown in Fig. 4.

Figure 5 shows the amount of ^{15}N-label in free amino acids in the bark from both fumigation periods. Glutamine and glutamic acid contained most of the label. In the roots (Fig. 6) ala was labelled in all but one sample, while glu and gln were only labelled in autumn. Total ^{15}N content was $43–156, 9.0–15.4$ and $5.3–12.8 \, \mu g \cdot g^{-1}$ DW in needle, bark and root tissue, respectively. With the aim of obtaining an estimate of possible uptake of ^{15}N via the soil, the top 2 cm were analysed with respect to ^{15}N enrichment. The resulting $\delta^{15}N$ was $30–40\%$₀ relative to air.

Discussion

The NO_2 concentration used in this study is found in urban environments and along highways (BUWAL 1991).

Boundary layer conductance was not a major determining factor for deposition rates in our study, because it was up to two orders of magnitude higher than stomatal conductance. Thoene et al. (1991) demonstrated that NO_2 absorption by twigs of *P. abies* is mainly controlled by stomatal conductance. Absorption of NO_2 by the cuticle was shown before (Kisser-Priesack et al. 1987, 1990; Kisser-Priesack and Gebauer 1991). Thoene et al. (1991) calculated its contribution to total deposition as a maximum of 5%. Taking into account the NO_2 concentration and the duration of the experiment entry of the gas into the cells by diffusion through the cuticle is highly unlikely (K. Lendzian, personal communication). Using $^{15}NO_2$
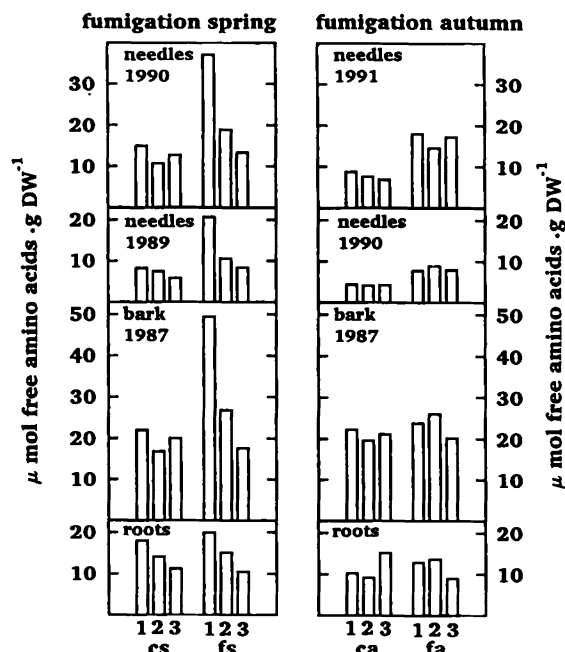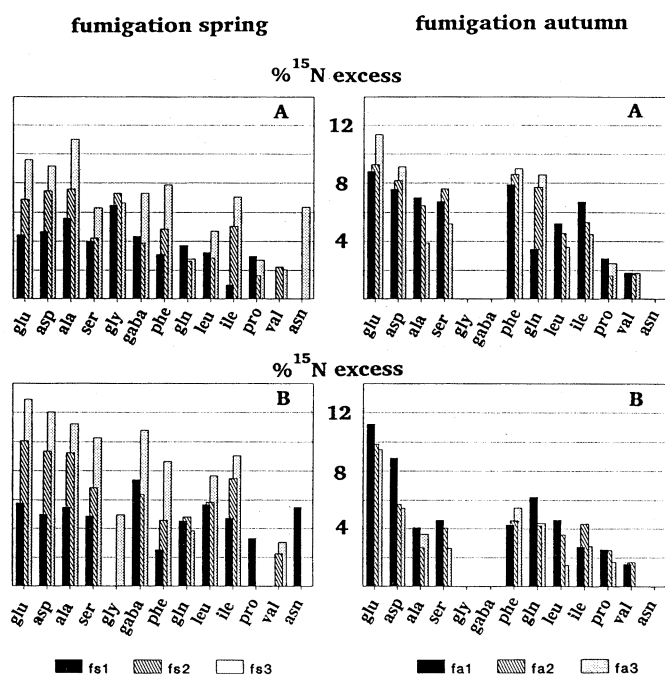

Fig. 1. Total amount of free amino acids in current and previous flush needles, entire bark of the flush of 1987 and roots of less than 1 mm in diameter of 6-year-old *Picea abies* trees exposed to $60 \, nl \cdot l^{-1}$ NO_2 for 4 days in spring (*fs*) or autumn (*fa*). Controls were in filtered air (*cs*, spring; *ca*, autumn). *Numbers* indicate individual trees

Fig. 3A, B. Percent ^{15}N excess in the α-amino nitrogen of the free amino acids in **A** current and **B** previous flush needles of 6-year-old *P. abies* trees fumigated with $60 \ nl \cdot l^{-1}$ [15]NO$_2$ in spring (*fs*) and autumn (*fa*). *Numbers* and *shadings* indicate individual trees: fs1, 2, 3 and fa1, 2, 3 are *solid, shaded and stippled columns*, respectively

fumigation we show here that the nitrogen from this gas is incorporated into amino acids of needles, bark and roots of *P. abies*.

Like other authors (Egger et al. 1988; Thoene et al. 1991) we found significantly higher nitrate reductase activities in needles of *P. abies* trees exposed to NO$_2$ than in controls in filtered air (Fig. 2), indicating an increase in the metabolic pool of nitrate (Beevers and Hageman 1980). This is probably due to solution of at least part of the NO$_2$ in this form in the cytosol. Kinetic evidence from other studies (Rogers et al. 1979a; Norby et al. 1989; Thoene et al. 1991) suggests that NO$_2$ uptake by plants cannot be explained by disproportionation to NO$_2^-$ and NO$_3^-$ alone as observed in pure water (Lee and Schwartz 1981) but is catalysed by compounds present in the atmosphere–cell interface (Ramge et al. 1992). The assimilatory nitrate reduction pathway common to all higher plants (Beevers and Hageman 1980; Runge 1983) has been shown to operate in *P. abies* needles (Tischner et al. 1988; Thoene et al. 1991). This is confirmed by the labelling of free amino acids during [15]NO$_2$ fumigation. Glutamate, the first free amino acid in this pathway to be labelled in the α-amino nitrogen, usually contained the highest label. The percentage of this pool that was labelled increased from day 1 to day 4 of the fumigation and reached an average of 9% at the end. This figure can therefore be taken as an estimate of the minimal contribution of NO$_2$ to the N nutrition of the needles under the chosen conditions. As calculated by Schulze (1989), the respective contribution of gaseous NO$_x$ to the N nutrition of a healthy spruce stand exposed to an average of 14–20 nl NO$_x \cdot l^{-1}$ amounts to 1.7%. Taking into account the linear relationship between NO$_2$ concentration and its absorption
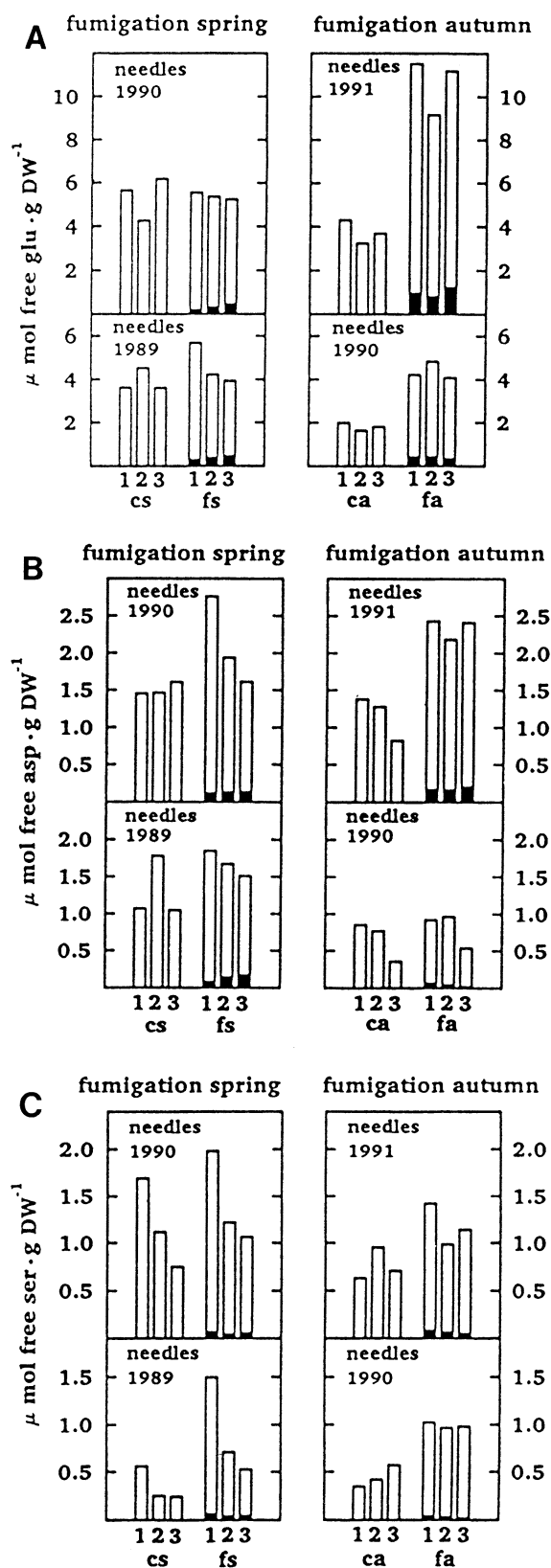

Fig. 4A–C. Content (*total column*) and ^{15}N label in α-amino nitrogen (*black column*) in **A** free glutamic acid **B** aspartic acid and **C** serine, of current and previous flush needles of three *P. abies* trees exposed to $60 \ nl \cdot l^{-1}$ [15]NO$_2$ for 4 days in spring (*fs*) or autumn (*fa*). Controls were in filtered air (*cs*, spring; *ca*, autumn). *Numbers* indicate individual trees
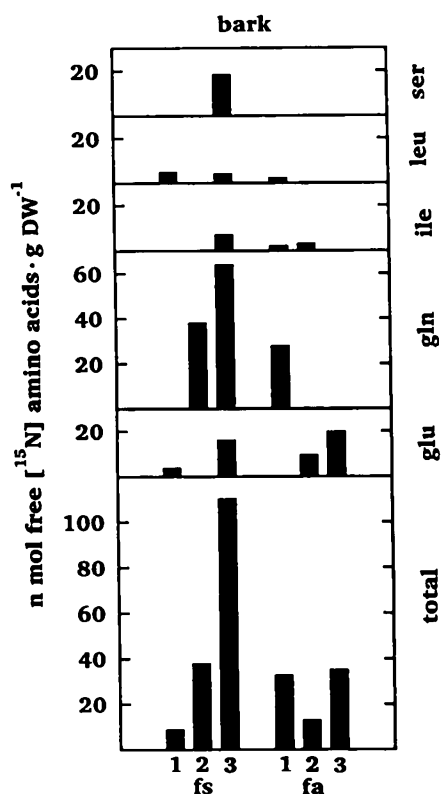
Fig. 5. ^{15}N label in total free amino acids and in leu, ile, gln and glu of the entire bark of the flush 1987 of 6-year-old *P. abies* trees fumigated for 4 days with 60 nl·l^{-1} $^{15}NO_2$ in spring (*fs*) and autumn (*fa*). Numbers indicate individual trees
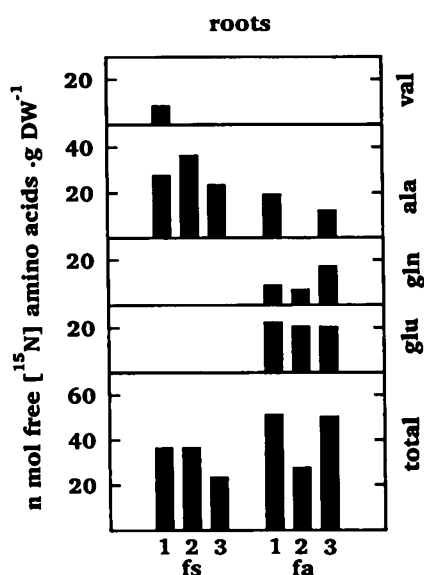

Fig. 6. ^{15}N label in total free amino acids and in ala, gln and glu of the fine roots of 6-year-old *P. abies* trees fumigated for 4 days with 60 nl·l^{-1} $^{15}NO_2$ in spring (*fs*) and autumn (*fa*). Numbers indicate individual trees

by *P. abies* twigs in this range (Theone et al. 1991), and assuming that the proportion of NO_2 incorporated into organic compounds remains constant as shown for sunflower (Latus et al. 1990), our estimated contribution from

NO_2-N is compatible with the value calculated by Schulze.

^{15}N enrichments in free amino acids were also detected in bark and roots. Since both organs contain appreciable nitrate reductase activity (Vogel 1992), import of amino acids from the needles as well as direct uptake and assimilation *in situ* are both possible explanations for the presence of the label. In the bark neither of the two possible ways of labelling can be excluded. In roots exclusive or almost exclusive import of the tracer from the shoot is the more likely explanation for the label in the amino acids, since the bulk of the mass of the roots found in the bottom half of the pots and the total ^{15}N enrichment of the fine roots was distinctly higher than that of the top soil layer.

In the spring the free amino acid pools did not change when exposed to NO_2 fumigation (Fig. 1). In autumn the total amino acid pool in the needles of fumigated trees roughly doubled compared to control trees, mostly due to an increase in the glutamate pool (Fig. 4). This effect cannot be attributed to additional synthesis due to the increased nitrogen supply from the air alone, since the labelled fraction did not make up for the difference. Other processes such as increased protein breakdown or decreased efflux to other pools could account for the increased amino acid content.

Our results show that NO_2 at moderate concentrations can act as a nitrogen fertilizer for *P. abies*. Whether this input is relevant with respect to possible adverse effects of increased nitrogen deposition postulated by Schulze (1989) remains to be elucidated.

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