Trees (2003) 17:144–152
DOI 10.1007/s00468-002-0215-1

ORIGINAL ARTICLE

M. Genenger · M. Jaeggi · R. Siegwolf · M. Chalot
E. Frossard · I. Brunner

Rapid ^{15}N uptake and metabolism in fine roots of Norway spruce

Received: 4 March 2002 / Accepted: 14 August 2002 / Published online: 21 September 2002

Abstract Short-term N uptake by Norway spruce roots was investigated after tracer application both in the field and in the laboratory. In the forest the influence of wood ash or liquid fertiliser treatments on the uptake was studied. A rapid uptake of the $^{15}NH_4{}^{15}NO_3$ tracer into the fine roots was observed. Within 1 day of application about 50% of the maximum $\delta^{15}N$ value observed was measured and within 1 week as much as 70–90%. The ^{15}N enrichment in fine roots was not affected by wood ash and liquid fertiliser treatments applied in the previous year. $\delta^{15}N$ increased continuously until 2 months after ^{15}N application and decreased to 60% of its maximum value within 1 year. Nine months after the tracer application, an analysis of $\delta^{15}N$ distribution throughout the root system was conducted. The $\delta^{15}N$ values were highest in roots of the topsoil compared to roots at deeper soil layers, and higher in fine roots than in larger roots. The ^{15}N was not translocated within the root system. The laboratory experiment, using Norway spruce seedlings, aimed at describing short-term $^{15}NO_3$ uptake and assimilation into free amino acids with 2 mM or 20 mM nitrate application. The ^{15}N was detected within 4 h to 1 day in the amino acids Glu, Gln, Asp of roots, while in Asn, Ala and Ser and in the shoots, the ^{15}N was retrieved at the earliest after 1 day. The enrichment factor in the amino acids increased to a maximum within 3–7 days, depending on the nitrate concentration applied.

M. Genenger · I. Brunner (✉)
WSL, Swiss Federal Institute of Forest,
Snow and Landscape Research, Zürcherstrasse 111,
8903 Birmensdorf, Switzerland
e-mail: brunner@wsl.ch
Tel.: +41-1-7392284, Fax: +41-1-7392215

M. Jaeggi · R. Siegwolf
Paul Scherrer Institut, 5232 Villigen PSI, Switzerland

M. Chalot
UMR INRA/UHO Interactions arbres-microorganisms,
UHP Nancy I, Faculté des Science et Techniques,
54506 Vandoeuvre, France

E. Frossard
Institute of Plant Science, Research Station Eschikon,
ETH Zürich, 8315 Lindau, Switzerland

Keywords *Picea abies* · Wood ash · Fertilisation · Amino acids · Tracer

Introduction

The consequences of high atmospheric N deposition from anthropogenic activities (Galloway 1995) on N limited ecosystems such as forests are still not fully understood (Rennenberg and Gessler 1999). In studying parts of the N cycle and its possible changes, the stable N isotope (^{15}N) provides an elegant tool for tracing source to sink relations. The natural abundance of $\delta^{15}N$ in plant tissue generally lies within the narrower range of –5‰ to +8‰ (Fry 1991). Therefore, when small amounts of highly enriched ^{15}N are added to the soil, the signal can easily be followed into different N pools (Nadelhoffer and Fry 1994). The $\delta^{15}N$ in the plant tissue reflects the $^{15}N/^{14}N$ isotope ratio of the available N, and the fine roots of trees in particular might provide reliable information on the $\delta^{15}N$ of available N in the forest soil (Högberg 1997).

In the laboratory the short-term uptake kinetics of ammonium and nitrate uptake in tree species have been studied in detail using the ^{13}N isotope (e.g. Kronzucker et al. 1995b, 1995c; Min et al. 1998). The short-term uptake of N into trees under natural conditions in the field, however, has usually been measured by depletion from an artificial solution (e.g. Marschner et al. 1991; George and Marschner 1996). Few field experiments on the short-term uptake of trees using ^{15}N have been conducted (e.g. Buchmann et al. 1995; Gessler et al. 1998). A pool of amino acids and other amino compounds such as gamma amino butyric acid (GABA) circulating between shoot and root is assumed to modulate the N uptake according to the N demand of the whole plant (Muller et al. 1996; Gessler et al. 1998). The amino acid pool is subject to a complex control by the enzymes of the amino acid biosynthesis and in plants is closely connected to their C metabolism (Lea and Ireland 1999). The main N assimilation pathways can be followed using the ^{15}N iso-

tope in combination with inhibitors. The free amino acids are central within the N metabolism of a plant as reviewed by Lea and Ireland (1999).

The ^{15}N tracer experiment was conducted within the scope of a forest management project, investigating the influences of wood ash versus optimal fertilisation on a Norway spruce forest (Genenger et al. 2001). We investigated how fast the ^{15}N signal appeared in the fine roots after the $\delta^{15}N$ of plant available N in the soil had been experimentally changed. Furthermore, the influence of the soil depth, root size and root tissue on the $\delta^{15}N$ within the root system of a single tree was analysed. The field results were supported by a laboratory study on the metabolism of Norway spruce seedlings, where the effects of different ^{15}N-nitrate regimes on free amino acids in roots and shoots over a period of 10 days were investigated.

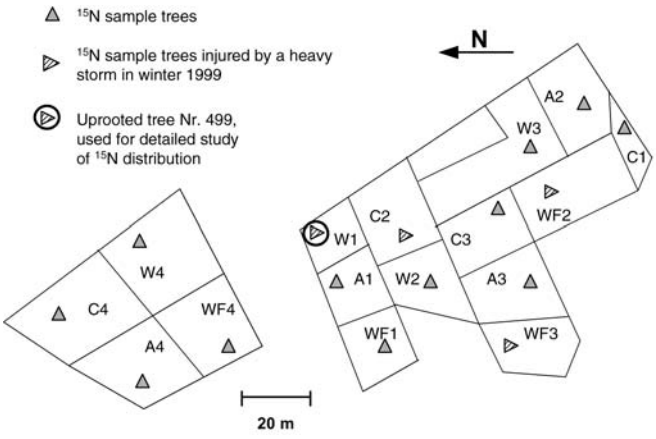

Fig. 1 Illustration of the field experimental site. *C* Control, *W* water, *WF* liquid fertiliser, *A* ash plots, location of spruce trees fed with ^{15}N (*triangles*), trees affected by the storm (*horizontal triangles*) and fallen tree investigated for ^{15}N distribution (*encircled*)

Materials and methods

Field experiment

The experiment was conducted in a 70-year-old spruce [*Picea abies* (L.) Karst.] forest, called 'Schladwald' (464 m a.s.l.), which is located about 25 km northwest of Zurich, Switzerland. The stand is dominated by Norway spruce, and the vegetation is characterised as a *Galio odorati – Fagetum luzuletosum* (Ellenberg and Klötzli 1972). The soil is an acidic brown forest soil. More detailed information is given by Bundt et al. (2001b). The average annual precipitation is 1,076 mm and the air temperature is 9.6°C (average of 15 years, SMA 1998). The experimental treatments were: a control without any treatment (C), water irrigated plots (W), water and fertiliser plots (WF) and plots supplied with wood ash (A). The WF (basically NPK full nutrient fertiliser containing 100 kg N ha^{-1} $year^{-1}$) and W treatments were irrigated daily during the 1998, 1999 and 2000 seasons (Genenger et al. 2001). The A treatment was applied manually in May 1998 and July 1999 with 4 t ha^{-1} of dry wood ash each year.

Sixteen mature spruce trees (aged between 43 and 62 years) were selected for sampling in this experiment as illustrated in Fig. 1. The severe storm 'Lothar' in December 1999 caused considerable damage to the forest site and reduced the number of sample trees to 12 individuals.

^{15}N labelling and sample analysis

In April 1999, the 16 spruce trees were supplied with 58.4 mg ^{15}N m^{-2} as double-labelled $^{15}NH_4{}^{15}NO_3$ (98% enriched, Cambridge Isotope Laboratories, USA) dissolved in water. The tracer amount was estimated to be large enough to ensure a visible ^{15}N signal in the roots (and in the needles) of mature spruce trees, yet small enough to avoid any unwanted physiological effects. The tracer was evenly distributed on a circular area of 30 m^2. Fine roots (diameter ≤2 mm) were sampled before the tracer application in April 1999, then 1, 3, 7, 28 days, and 2, 6 and 12 months later. Three root samples per tree were collected at a distance of 1 m from the trunk and maximum 5 cm below ground by hand, rinsed with demineralised water, frozen in liquid N and then stored at –80°C before the fine roots were freeze-dried for 48 h.

Single tree study

In January 2000, root samples belonging to different size and depth classes were taken from one of the labelled spruce trees, which had been uprooted 2 weeks earlier (Fig. 1, tree 499). Size classes were fine roots (FR 0–2 mm), very small roots (VSR 2–5 mm), small roots (SR 5–10 mm) and coarse roots (CR 10–20 mm). Depth classes were 0–10 cm (surface), 30–40 cm and 60–70 cm soil depth. The roots were lyophilised for 48 h. FR and VSR have been analysed in total. The bark of the SR was separated with a knife from the woody tissue and analysed separately. The bark of the CR was also separated and, in addition, the centre of the woody tissue was removed from the remaining wood with a small corer (diameter 4 mm) and measured separately. For each depth class and tissue three samples were analysed.

Mass spectrometry, ^{15}N analysis

The different root samples were ground with a mill (Retsch MM2000, Germany); 4.5–5 mg dry material of the fine root sampled several times was used for the analyses of total N and $\delta^{15}N$. The same amount was taken to analyse the FR and VSR from the uprooted tree. Furthermore, 7.5–8 mg of the bark of SR and CR, 15–16 mg of the SR wood, 35–36 mg of the central wood of CR and 30–31 mg of the remaining wood of CR was used.

The total N concentration and the isotopic signature of each sample were measured by combustion in an Elemental Analyser (EA-1110, Carlo Erba, Italy), which was connected to a continuous flow mass spectrometer (DELTA-S Finnigan MAT, Germany). The isotopic signatures, i.e. the relative amounts of the isotopes ^{15}N and ^{14}N compared to the international standard (N_2 in air), are expressed in the δ (in ‰) notation:

$$\delta^{15}\mathrm{N} = \left[\left(R_{\mathrm{sample}}/R_{\mathrm{standard}}\right) - 1\right] \times 1{,}000. \quad (1)$$

To avoid memory effects due to high enrichment and sample amounts, blank tin capsules were measured after every wood sample.

The ^{15}N recovery rate (in % of the applied ^{15}N) of the fine roots in the topsoil was calculated according to Buchmann et al. (1996). Therefore, samples were taken with a soil corer on a 5-m grid on each plot in April 1999 and April 2000. At each sampling point three soil cores (diameter 8.5 cm) were collected. The spruce fine roots were washed out of the A horizon (around 3 cm depth), dried and weighed.

Laboratory experiment

Ten boxes (34×95×8.5 cm) were sealed at the bottom with plastic foil (sterilised with ethanol), with several holes for drainage.

Vermiculite was sieved (>2 mm) and autoclaved twice for 60 min and then added to the prepared boxes. 600–700 *Picea abies* seeds (origin: WSL-No. 846) were surface sterilised with 30% H_2O_2 for 35 min, rinsed with sterile water and sown in each box (containing 3.5–4 l vermiculite). The boxes were covered with transparent film to ensure moist conditions for germination and placed in a growth chamber at 20°C and 70% humidity (16 h light per day, 100 µE m^{-2} s^{-1}). Once the seeds had germinated, the cover was removed.

Three weeks after sowing, the seedlings were irrigated regularly (1–2 times a week) with demineralised water. In the 5th week a 1:5 diluted modified Melin Norkrans (MMN) solution (Marx and Bryan 1975) without vitamins, glucose or malt was added (500 ml per box) instead of the demineralised water. Eight weeks after sowing, the plants were treated with either 2 mM or 20 mM nitrate, added as a potassium nitrate solution (98% ^{15}N enriched, Sigma-Aldrich, USA). Each nitrate solution was applied once to two boxes (500 ml per box). As controls two boxes were irrigated with the same amount of water. Only the substrate was watered in order to avoid ^{15}N contamination of the shoots. Samples of about 50–70 plants were taken 4 h, 1, 3, 7 and 10 days after nitrate fertilisation.

Sampling and amino acid extraction

At each harvest the plants were carefully taken out of the substrate. Seedlings were divided into root and shoot, washed with demineralised water and immediately frozen in liquid N. All the samples were stored at –80°C until extraction. At each harvest the roots were examined under a dissecting microscope and no mycorrhizal or contaminating fungi were evident on the root surface.

Extraction of amino compounds from the roots was based on the method described by Stoermer et al. (1997). The root material was freeze-dried for 48 h and ground 3 times for 1 min in liquid N with a mill (Retsch MM2000, Germany). Fifteen milligrams of the material was homogenised in 200 µl of a 0.02 M HEPES buffer containing 5 mM EGTA and 10 mM NaF. Then 50 µl of 10 mM norleucin (Fluka) were added to each sample as a standard. 1.6 ml methanol: chloroform (3.5:1.5, v/v) were added and incubated for 20 min on ice. The water-soluble compounds were extracted twice with 2.4 ml distilled water. The aqueous phases were combined and freeze dried in a centrifugal evaporator (RC10, Jouan, USA) overnight. The dried extracts were dissolved in 400 µl bidistilled water (and if necessary centrifuged). The extracts were purified according to Chalot et al. (1994) on a Dowex 50 W 8X-200 (Sigma-Aldrich, USA) column, and eluded with 5 ml of 4.5 M ammonia.

Derivatisation and measurements

The purified samples were freeze dried in a centrifugal evaporator and then redissolved in 10 µl DMF and 50 µl MTBSTFA (Pierce, USA). Samples were mixed and heated in a water-bath at 80°C for 25 min, and then allowed to cool down for about 90 min at room temperature before injection. The GC-MS was a Hewlett Packard (USA) 5989A MS engine interfaced to a model 5890 GC and a model 7673 auto-sampler. The capillary column (30×0.25 mm, HP5-MS, Hewlett Packard) was initially held for 3 min at 110°C; then the temperature was increased by 5°C per min to 260°C. The injector temperature and the maximum detector temperature was 260°C. The MS peak $[M\text{-}57]^+$ was monitored and used for calculation of ^{15}N enrichment of all amino acids (Mawhinney et al. 1986). The amino acids Ile and Leu could not be separated, and the applied procedures were not suitable for measuring Arg properly (double peak, Arg partly overlaid with His peak, Mawhinney et al. 1986). The enrichment factor (EF) of each amino acid is expressed in atom % excess compared to the control samples and was calculated according to Campbell (1974):

$$\mathrm{EF\,(at\%excess)} = \left[\left(R_{\mathrm{sample}} - R_{\mathrm{control}}\right) \times 100\right] / \left(R_{sample} - R_{control} + 1\right), \quad (2)$$

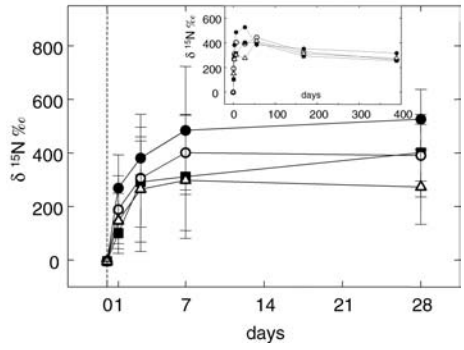

Fig. 2 Changes in $\delta^{15}N$ (‰) in Norway spruce fine roots over time (days), split by different treatments: *square C* control, *filled circle W* water, *clear circle WF* liquid fertiliser, *triangle A* ash plots, means (n=12) ± SE; *inset* long-term changes in $\delta^{15}N$

where R_{control} is the average of eight to ten control samples. Data are presented as the mean of two extractions and measurements.

In a similar study of Persson and Näsholms (2001) the relative standard deviation (RSD) of the GC-MS method was 5.8% concerning the amino acid concentrations and the RSD<2.1% concerning the $\delta^{15}N$ values.

Statistics

Statistics were performed as provided by Statview 5.0 (SAS, USA) for Macintosh. The differences in the $\delta^{15}N$ or %N distribution in the root system was analysed by a one-way ANOVA with Fisher's PLSD test on depth or tissue effects at a 5% probability level.

Results

Field experiment

A rapid increase of the $\delta^{15}N$ in the fine roots of Norway spruce was observed (Fig. 2). Within 1 day of application about 50% of the maximum $\delta^{15}N$ value observed was measured in the roots. Within the 1st week after tracer application 70–90% of the maximum $\delta^{15}N$ measured was detected (Fig. 2). The highest $\delta^{15}N$ values were reached within 1–2 months. One year after the tracer application the enrichment in the fine roots of Norway spruce was about 60% of the maximum $\delta^{15}N$ values measured (Fig. 2, inset). Beside the tendency towards a faster $\delta^{15}N$ increase in the irrigated plots (WF and W) within the 1st week, no significant effects of the treatments applied in the year prior to the ^{15}N experiment on the $\delta^{15}N$ change were observed.

The ^{15}N recovery rate of the fine roots has increased to around 1% within 1–3 days after tracer application and to maximum of 3% after 1 year (Table 1).

^{15}N distribution within the root system

The $\delta^{15}N$ enrichment of the root system of an uprooted tree was analysed 9 months after tracer application

Table 1 Mean ^{15}N recovery (%) ±SE in the fine roots as measured 0–389 days after tracer application (n=4) and fine root biomass (g/m^2) of Norway spruce in the uppermost soil horizon (3 cm depth) used for calculation

Day	Year	C	A	WF	W
^{15}N recovery (%)					
0	1999	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
1	1999	–0.31±0.46	0.59±0.54	1.08±0.63	0.69±0.48
3	1999	0.66±0.07	1.10±0.77	1.22±0.42	1.14±0.37
7	1999	0.43±0.32	0.52±0.62	1.71±0.53	1.14±0.24
28	1999	0.81±0.43	0.31±0.45	1.56±0.46	1.37±0.33
56	1999	0.96±0.35	1.46±0.88	2.25±1.43	0.90±0.41
168	1999	0.74±0.38	1.16±0.93	1.84±0.33	0.95±0.11
389	2000	0.36±0.57	3.02±0.77	3.29±0.32	1.37±0.86
Fine root biomass (mg/m^2)					
	1999	38.3±3.0	44.5±10.6	44.1±6.4	29.2±4.0
	2000	42.7±5.0	65.6±18.4	51.6±6.0	37.0±8.4

Table 2 δ^{15}N values (‰) and N content (%N) in root samples of an uprooted tree of different root sizes or tissues and from three different soil depths, 9 months after ^{15}N application (n=3). Different letters indicate significant differences between the soil depths at a 5% probability level (one-way ANOVA)

Soil depth	Fine 0–2 mm Total	Very small 2–5 mm Total	Small 5–10 mm		Coarse 10–20 mm Ø root size		
			Bark	Wood	Bark	Wood	Inner wood
δ^{15}N							
0–10 cm	227.1a	163.8a	51.6a	58.1a	36.8a	47.8a	31.0a
30–40 cm	33.9b	37.3b	28.6ab	31.7ab	9.8b	12.0b	8.7b
60–70 cm	33.6b	–4.0b	–2.7b	–2.6b	–4.2c	–6.8c	–4.9b
% N							
0–10 cm	1.32a	0.87a	0.69a	0.39a	0.66a	0.33a	0.28a
30–40 cm	0.73b	0.71b	0.68a	0.25b	0.70a	0.15a	0.17b
60–70 cm	0.82b	0.75ab	0.72a	0.21b	0.69a	0.15a	0.13b

(Table 2). The root values differed significantly depending on the depth at which they had been growing in the soil (Table 2), and on the root size and tissue. The δ^{15}N was significantly higher in the fine and very small roots of the samples from the topsoil (0–10 cm depth) compared to all the other root sizes and tissues measured in the same horizon (analysis not shown). A continuous trend towards lower δ^{15}N values was observed with increasing root size. Further, the more woody material in the sample, the lower was the δ^{15}N. The same trend was found in the root samples from 30–40 cm soil depth. However, the differences between samples of various root sizes from this depth were not significant at P<0.05 (statistics not shown). The root samples at a soil depth of 60–70 cm did not show any significant differences between the root sizes or tissues either (statistics not shown).

Comparing the δ^{15}N values of root tissue of the three depth classes, all samples from the upper soil were significantly more enriched in ^{15}N after 9 months than the samples from the 60–70 cm soil depth (Table 2). Furthermore, all tissues of the topsoil root samples had higher δ^{15}N values than the root samples of the 30–40 cm depth class, except for bark and wood of the small roots (Table 2). The bark and wood of roots more than 1 cm in diameter at 30–40 cm depth were significantly more enriched with the tracer than the bark and wood of the root samples at 60–70 cm depth. There was no significant difference in the δ^{15}N between these two depth classes in any of the other tissues (Table 2, upper part).

The %N was higher in the FR than in all the other tissues of roots over 0.5 cm in diameter. The %N of VSR was intermediate between FR and SR. The %N in FR, VSR, in the wood of SR, and in the inner wood of CR was significantly higher in the root samples from 0 to 10 cm than in roots deriving from the deeper soil layers (Table 1, lower part).

Laboratory experiment

After nitrate fertilisation, changes in the free amino acids in roots and shoots of Norway spruce seedlings were measured. Within 4 h after the nitrate application no differences in the amino acid concentrations were observed (Fig. 3). After 1 and 3 days an increase of the amino acid concentrations in the shoots of the spruce seedlings was observed that was most pronounced in Glu, Gln, and Asp, while in Asn, Ala, and Ser the effects were faint. After 7 days, the amino acid concentrations decreased again and 10 days after the nitrate application the concentrations measured were in the range of the control (Fig. 3).

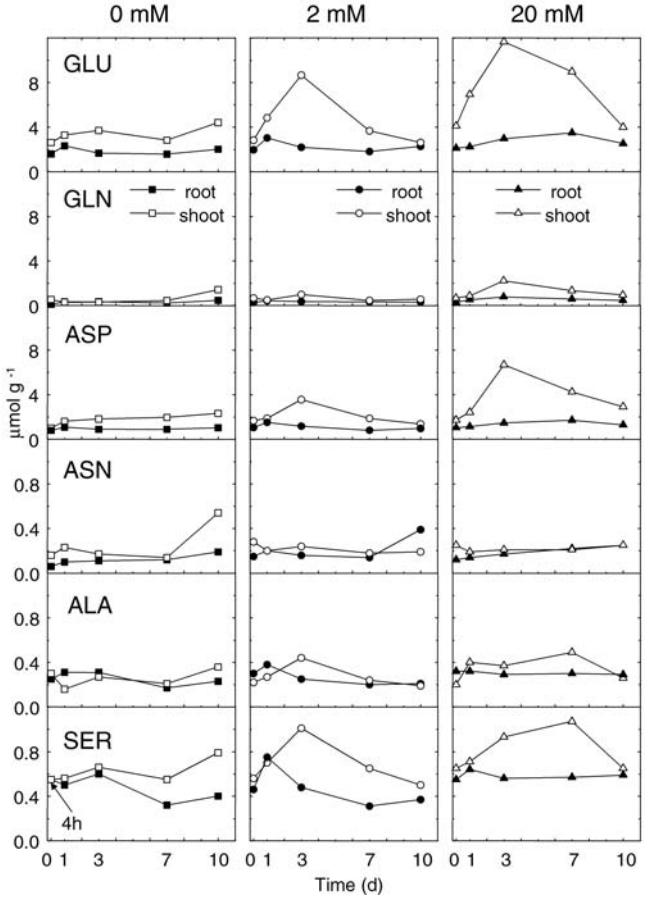

Fig. 3 Concentrations (µmol g $^{-1}$) of free amino acids of Norway spruce roots and shoots as affected by different nitrate regimes with time (days): *square* control, *circle* 2 mM nitrate, *triangle* 20 mM nitrate

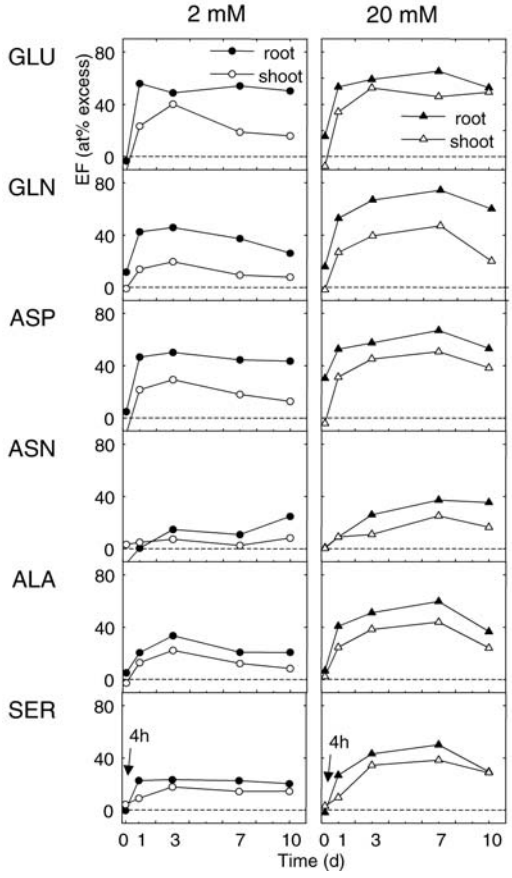

Fig. 4 Enrichment factor (*EF*) of ^{15}N (at% excess ^{15}N) in free amino acids of Norway spruce roots and shoots as affected by different nitrate regimes with time (days): *circle* 2 mM nitrate, *triangle* 20 mM nitrate

The natural ^{15}N abundance of the amino acids in spruce roots was comparable to that in shoots, and ranged between –10 and +10 at%. After tracer application the enrichment of the amino acids in the shoots was generally lower than in the roots with both tracer concentrations at all harvests (Fig. 4). Within 4 h after the tracer application, the EF (at% excess) of ^{15}N was enhanced in Glu, Gln and Asp of the roots compared to the shoots after the 20 mM nitrate treatment. The EF of ^{15}N in Asn, Ala and Ser was enhanced 1 day after application in roots and shoots. The EF increased in all amino acids up to the 3rd day after the 2 mM, and up to the 7th day after the 20 mM nitrate application. Thereafter the EF of ^{15}N in the amino acids decreased (Fig. 4). In general the application of 20 mM nitrate resulted in higher at% ^{15}N excess values and amino acid concentrations than the 2 mM nitrate treatment (Figs. 3, 4).

Discussion

^{15}N uptake in the field

The natural abundance of stable ^{15}N isotope in field grown roots was within the range observed in other European forests (Emmett et al. 1998). After the ^{15}N application in the forest, δ^{15}N values in the fine roots increased rapidly, irrespective of the treatment applied. Within the 1st week δ^{15}N increased slightly more rapidly in the roots of the WF and W treatments than in the roots of the A and C treatments. These treatments had increased the N input (WF added 100 kg N ha^{-1} year^{-1}; W about 5–8 kg N ha^{-1} year^{-1}) to the forest and thus the uptake capacity in the year before the tracer experiment was improved. However, the relatively high amount of N in the WF treatment was expected to lead to lower uptake rates in the long run as biological N sinks became more saturated. Yet such an effect on the N uptake was not observed, which agrees with the findings of Nadelhoffer et al. (1995), who investigated the influence of low and high nitrate input rates on the N recovery in various tree species in the United States.

Emmerton et al. (2001) reported a strong influence of the ectomycorrhiza (EM) on the $\delta^{15}N$ in the plant tissue. In addition, fertilisation with N altered the EM species composition in a spruce forest (Peter et al. 2001). Thus, beside a change in N uptake, the N isotope fractionation pattern may be changed with the changing mycorrhizal partners in the field. The wood ash treatment is an 'N-free' fertilisation, which first leads to enhanced microbial activity and mineralisation and thus an increased plant availability (but also loss) of N, and can reduce the total N content of the soil in the long run, since the N mobilised can be washed out (Demeyer et al. 2001). In the present experiment these changes of the soil N, however, had no effect on the ^{15}N uptake.

The inorganic ^{15}N added in the field is assumed to be immobilised very quickly, mainly in the upper organic soil (Buchmann et al. 1996), while the mineralisation, i. e. the release of new plant available N compounds from the organic N pool, in coniferous forests on acid soil is slow compared to that in broadleaf forests (Gebauer et al. 2000). The N immobilisation in the soil was estimated to occur within less than 2 weeks (Gebauer et al. 2000), which is about the time period in which we observed a rapid $\delta^{15}N$ increase in the fine roots in the field experiment. Subsequently organic N sources, such as simple amino acids, may also be taken up by Norway spruce, as has been found in laboratory (Johnsson et al. 1999) as well as in field experiments (Näsholm et al. 1998). This ability is attributed mainly to the mycorrhizas (Blaudez et al. 2001).

Bundt et al. (2001a) observed maximum $\delta^{15}N$ enrichment in total fine roots of the topsoil after 1 month (the first sampling time) at the same experimental site with applying similar amounts of ^{15}N, which agrees with the findings in the present study for the fine roots of Norway spruce. The $\delta^{15}N$ in the soil of the uppermost 9 cm was determined to be around 0‰ before tracer application, rising to 1–2‰ within 1 month and remaining at this level after 12 months. The maximal enrichment in the roots measured in the present study was higher than in an experiment by Buchmann et al. (1996), even though similar amounts of ^{15}N tracer were applied. The discrepancies between the two studies might be due to differences in root size because of different sampling strategies. In Buchmann's study, root material up to 10 mm diameter was analysed, whereas in the present investigation only the finest roots up to 2 mm in diameter were used. This material consists mostly of mycorrhizas and the EM generally have a higher $\delta^{15}N$ than non-mycorrhizal root tissue (Högberg et al. 1996), which contributes to the higher $\delta^{15}N$ of the fine roots compared to bigger roots.

A recovery rate for ^{15}N was estimated with fine root biomass results from soil cores according to Buchmann et al. (1996), who applied similar amounts of ^{15}N m^{-2}. The ^{15}N recovery in the finest roots of the topsoil was in the range of about 1 mg ^{15}N m^{-2}, i.e. up to 3% recovery, after 12 months. These results are comparable to those of Buchmann et al. (1996), who described ^{15}N recovery from ammonium as accounting for about 0.6 mg ^{15}N m^{-2} (1.0%) and from nitrate for about 2 mg ^{15}N m^{-2} (3.5%). However, the majority of ^{15}N is assumed to be immobilised in the soil and litter (Buchmann et al. 1996; Schleppi et al. 1999).

^{15}N distribution within the root system

The data on the ^{15}N distribution in a root system presented here were only observations from a single tree. Thus a generalisation is impossible, because the data measured were dependent on the individual tree, soil type and small-scale factors, for example nutrition patches or flow-paths. Bearing this in mind, the observations will be discussed in the context of other studies.

In an undisturbed forest the $\delta^{15}N$ values of the roots increase with increasing soil depth (Mariotti et al. 1980; Gebauer and Schulze 1991; Högberg 1997; Bundt et al. 2001a) while the N concentrations decrease. Nine months after applying a highly ^{15}N enriched tracer on the forest floor, the $\delta^{15}N$ values in the present study were highest in the roots of the topsoil and decreased with depth. At 60–70 cm depth, the average $\delta^{15}N$ values of roots were in the range of the natural ^{15}N abundance. Bundt et al. (2001a) observed a close correlation between changes in soil $\delta^{15}N$ and in the fine root $\delta^{15}N$ of a spruce forest in a study concerning the importance of preferential flow paths in the soil. This study was conducted in the same forest less than 200 m from the plots, so that a similar correlation between the soil $\delta^{15}N$ for the present study could be assumed. Consequently only a small part of the ^{15}N had already infiltrated into the deeper soil layers, similar to the observations made by Buchmann et al. (1996).

The $\delta^{15}N$ values in the roots of Norway spruce were in a similar range from about 50‰ (in roots of 5–10 mm diameter) to more than 200‰ in the finest roots as reported by Buchmann et al. (1996), whose values were between –4.4‰ and about 55‰ with labelled ammonium and about 137‰ with labelled nitrate. The bulk of N is taken up from the organic soil layers as reported by Gebauer and Schulze (1991). However, in the present experiment we found most of the ^{15}N in the root tissue, which is responsible for the N uptake. The tracer was not distributed in the root system to a noticeable extent. Yet the translocation of N to the shoot happened rather rapidly. One month after the tracer application, the ^{15}N was highly enriched in the needles of the 42- to 63-year-old sample trees within the same experiment (Jaeggi, unpublished data). In a comparable experiment, Buchmann et al. (1995) detected a significant increase of $\delta^{15}N$ in the current-year foliage in 15-year-old spruce trees only 11 days (first sampling) after tracer application. In the laboratory experiment presented here, the major amino acids were enriched in the shoots after only 4 h to 1 day.

The amount of N applied as tracer in the present experiment was estimated to account for about 2% of the annual N deposition, and thus was minute compared to the N deposition and N pool in the soil. Coarse roots

serve as N storage, as Dyckmans and Flessa (2001) concluded from an enhanced translocation of N to coarse roots in N-deprived trees. In the present experiment, in small and coarse roots differences in %N due to the soil depth were observed only in woody tissue. This finding could be due to a N storage function of the wood of roots, in contrast to the root bark. However, Millard (1996) reported that the main N storage tissue in whole evergreen trees is in the needles.

Amino acid metabolism

In the present study, rather low concentrations of Gln and Asp were measured in the roots, in contrast to Glu, while other studies have described Gln as one of the main amino acid in the roots of Norway spruce seedlings (Chalot et al. 1991; Aarnes et al. 1995; Gessler et al. 1998). The low Gln concentrations could be due to N depletion in our laboratory study, as no N was applied before the tracer application. Comparable values in pine needles were observed at sites where the N supply was generally low (Raitio and Sarjala 2000).

The changes observed in the amino acid concentration after N application agree well with the results from laboratory experiments obtained with *Picea glauca* Moench (Kronzucker et al. 1995a) which described the induction of nitrate uptake and reduction. The maximum influx rates were observed 3 days after exposure to elevated nitrate and declined again afterwards (Kronzucker et al. 1995a).

Previous studies of short-term ammonium ^{15}N labelling of mycorrhizal eucalypt (Turnbull et al. 1995) or spruce (Chalot et al. 1991; Aarnes et al. 1995) seedlings reported the greatest flow of ^{15}N via the amide group of Gln, with a label also in the amino group of Gln, Glu, Ala and GABA. While the latter was not measured in the present study, the high enrichment patterns of Gln, Glu and Ala were confirmed in non-mycorrhizal spruce seedlings after 4 h to 1 day. Compared to the incorporation of ammonium into amino acids of *Daucus* and *P. glauca* (Lea and Ireland 1999) the uptake and incorporation of nitrate was slower. While the former investigation reported times of minutes to hours for detection of the ^{15}N signal in the amino acids, in the present study times were in the range of days. Spruce is known to prefer ammonium to nitrate (Kronzucker et al. 1997), which, with a low uptake of nitrate, might have slowed down enrichment of the amino acids. Yet the slower uptake metabolism of Norway spruce could also be due to species-dependent differences in the N uptake and metabolism kinetics in general, such as have been observed between trembling aspen and lodgepole pine (Min et al. 1998).

The differences between the at%^{15}N enrichment values in roots and shoots demonstrated a dilution effect already described by Aarnes et al. (1995) in white spruce. The amino acid concentrations increased after the nitrate fertilisation mainly in the shoot of the Norway spruce seedlings, while concentrations in the roots remained unchanged. Thus the surplus of N had been transported to the main pools of storage in the needles (Millard 1996). Nevertheless, the changes in the concentrations of the free amino acid pool must be interpreted as the initial physiological signal of a nutritional improvement rather than being quantitatively relevant for N storage (Bauer et al. 2000), because this pool contributes less than 1% of the total N in needles (Bauer 1997).

Flaig and Mohr (1992) reported that a distinct accumulation of free amino acids did not occur with intensive nitrate fertilisation in a laboratory experiment with *Pinus*. Similarly, in the present study the free amino acid pool in the roots remained almost unaffected. In the shoots a transient increase (depending on the fertilisation amount) in Glu and Asp was observed, which lasted for about a week and returned to the control level afterwards, as has been observed before in *Pinus* (Flaig and Mohr 1992). These observations lead to the conclusion that a surplus of N is metabolised in a steady state to storage pools as, for example, Arg or storage proteins.

In field samples an enrichment of ^{15}N in amino acids was not detectable (data not shown). This might have been due to the dilution of applied ^{15}N in the N pool of the soil or to the relevant time period after tracer application being missed between the measurements.

Conclusions

The results of this study showed a fast uptake of N into the fine roots in the laboratory as well as in the field. In the field the potential influence of the soil immobilisation properties on the kinetics of uptake of N deposited on the forest floor was observed. The N taken up is not distributed inside the root system to any noteworthy extent within several months. However, a rapid N transport to the shoot was observed in laboratory as well as in the field experiment. No significant effects of optimal (high NPK) fertilisation or ash treatment on the N uptake were observed in the field. Therefore, the uptake capacity of trees might not be a suitable short-term indicator of N saturation in the forest soils.

Acknowledgements We would like to thank S. Brodbeck, R. Belleville and K. Bleidissel for skilful laboratory work. Furthermore we would like to thank Prof. B. Botton and his team at the University of Nancy for hospitality in their laboratory. We are grateful to M.J. Sieber for help with the English and to two reviewers for critical comments on earlier versions of the manuscript. The project was funded by the Swiss Federal Office for Education and Science (BBW) and the Swiss Agency for the Environment, Forests and Landscape (BUWAL) as part of the COST E6 Eurosilva Project: Forest tree physiology research. The Cooperation between the WSL and the Laboratoire de Biologie Forestière, Nancy was supported by the CRICEPF program and the EU/COST short-term scientific mission support.

References

Aarnes H, Eriksen AB, Southon TE (1995) Metabolism of nitrate and ammonium in seedlings of Norway spruce (*Picea abies*) measured by *in vivo* ^{14}N and ^{15}N NMR spectroscopy. Physiol Plant 94:384–390

Bauer G (1997) Stickstoffhaushalt und Wachstum von Fichten- und Buchenwäldern entlang eines europäischen Nord-Südtransektes unter besonderer Berücksichtigung der Nitratnutzung. Dissertation, University of Bayreuth

Bauer GA, Persson H, Persson T, Mund M, Hein M, Kummetz E, Matteucci H, Oene H van, Scarascia-Mugnozza G, Schulze ED (2000) Linking plant nutrition and ecosystem processes. In: Schulze ED (ed) Carbon and nitrogen cycling in European forest ecosystems, vol 142. Springer, Berlin Heidelberg New York, pp 63–98

Blaudez D, Botton B, Dizengremel P, Chalot M (2001) The fate of [C-14]glutamate and [C-14]malate in birch roots is strongly modified under inoculation with *Paxillus involutus*. Plant Cell Environ 24:449–457

Buchmann N, Schulze ED, Gebauer G (1995) ^{15}N-ammonium and ^{15}N-nitrate uptake of a 15-year-old *Picea abies* plantation. Oecologia 102:361–370

Buchmann N, Gebauer G, Schulze ED (1996) Partitioning of N-^{15}-labelled ammonium and nitrate among soil, litter, below- and above-ground biomass of trees and understory in a 15-year-old *Picea abies* plantation. Biogeochemistry 33:1–23

Bundt M, Jaeggi M, Blaser P, Siegwolf R, Hagedorn F (2001a) Carbon and nitrogen dynamics in preferential flow paths and matrix of a forest soil. Soil Sci Soc Am J 65:1529–1538

Bundt M, Widmer F, Pesaro M, Zeyer J, Blaser P (2001b) Preferential flow paths: biological 'hot spots' in soils. Soil Biol Biochem 33:729–738

Campbell IM (1974) Incorporation and dilution values – their calculation in mass spectrally assayed stable isotope labeling experiments. Bioorg Chem 3:386–397

Chalot M, Stewart GR, Brun A, Martin F, Botton B (1991) Ammonium assimilation by spruce-*Hebeloma* sp. ectomycorrhizas. New Phytol 119:541–550

Chalot M, Brun A, Finlay RD, Söderström B (1994) Respiration of [^{14}C] alanine by the ectomycorrhizal fungus *Paxillus involutus*. FEMS Microbiol Lett 121:87–92

Demeyer A, Nkana JCV, Verloo MG (2001) Characteristics of wood ash and influence on soil properties and nutrient uptake: an overview. Bioresour Technol 77:287–295

Dyckmans J, Flessa H (2001) Influence of tree internal N status on uptake and translocation of C and N in beech: a dual C-13 and N-15 labeling approach. Tree Physiol 21:395–401

Ellenberg H, Klötzli F (1972) Waldgesellschaften und Waldstandorte der Schweiz. Mitt EAFV 42:587–930

Emmerton KS, Callaghan TV, Jones HE, Leake JR, Michelsen A, Read DJ (2001) Assimilation and isotopic fractionation of nitrogen by mycorrhizal fungi. New Phytol 151:503–511

Emmett BA, Kjonaas OJ, Gundersen P, Koopmans C, Tietema A, Sleep D (1998) Natural abundance of N-15 in forests across a nitrogen deposition gradient. For Ecol Manage 101:9–18

Flaig H, Mohr H (1992) Assimilation of nitrate and ammonium by Scots pine (*Pinus sylvestris*) seedlings under conditions of high nitrogen supply. Physiol Plant 84:568–576

Fry B (1991) Stable isotope diagrams of freshwater food webs. Ecology 72:2293–2297

Galloway JN (1995) Acid deposition: perspectives in time and space. Water Air Soil Pollut 85:15–24

Gebauer G, Schulze ED (1991) Carbon and nitrogen isotope ratios in different compartments of a healthy and a declining *Picea abies* forest in the Fichtelgebirge, NE Bavaria. Oecologia 87:198–207

Gebauer G, Zeller B, Schmidt G, May C, Buchmann N, Colin-Belgrand M, Dambrine E, Martin F, Schulze ED, Bottner P (2000) The fate of ^{15}N labelled nitrogen inputs to coniferous and broadleaf trees. In: Schulze ED (ed) Carbon and nitrogen cycling in European forest ecosystems, vol 142. Springer, Berlin Heidelberg New York, pp 144–170

Genenger M, Frossard E, Zimmermann S, Brunner I (2001) Nitrate reductase activity in fine roots of Norway spruce – a biochemical marker for nitrogen conditions in the soil? In: Horst WJ, Schenk MK, Bürkert A, Claassen N, Flessa H, Frommer WB, Goldbach H, Olfs H-W, Römheld V, Sattelmacher B, Schmidthalter U, Schubert S, Wirén N v, Wittenmayer L (eds) Plant nutrition – food security and sustainability of agro-ecosystems. Kluwer Academic, Dordrecht, pp 918–919

George E, Marschner H (1996) Nutrient and water uptake by roots of forest trees. Z Pflanzenernaehr Bodenkd 159:11–21

Gessler A, Schneider S, Sengbusch D, Weber P, Hanemann U, Huber C, Rothe A, Kreutzer K, Rennenberg H (1998) Field and laboratory experiments on net uptake of spruce (*Picea abies*) and beech (*Fagus sylvatica*) trees. New Phytol 138: 275–285

Högberg P (1997) N-15 natural abundance in soil-plant systems. New Phytol 137:179–203

Högberg P, Högbom L, Schinkel H, Högberg M, Johannisson C, Wallmark H (1996) N^{15} abundance of surface soils, roots and mycorrhizas in profiles of European forest soils. Oecologia 108:207–214

Johnsson L, Berggren D, Karen O (1999) Content and bioavailability of organic forms of nitrogen in the O horizon of a podzol. Eur J Soil Sci 50:591–600

Kronzucker HJ, Glass ADM, Siddiqi MY (1995a) Nitrate induction in spruce – an approach using compartmental analysis. Planta 196:683–690

Kronzucker HJ, Siddiqi MY, Glass ADM (1995b) Compartmentation and flux characteristics of ammonium in spruce. Planta 196:691–698

Kronzucker HJ, Siddiqi MY, Glass ADM (1995c) Kinetics of NO_3– influx in spruce. Plant Physiol 109:319–326

Kronzucker HJ, Siddiqi MY, Glass ADM (1997) Conifer root discrimination against soil nitrate and the ecology of forest succession. Nature 385:59–61

Lea PJ, Ireland RJ (1999) Nitrogen metabolism in higher plants. In: Singh BJ (ed) Plant amino acids: biochemistry and biotechnology. Dekker, New York, pp 1–47

Mariotti A, Pierre D, Vedy JC, Bruckert S, Guillemot J (1980) The abundance of natural nitrogen 15 in the organic matter of soils along an altitudinal gradient. Catena 7:293–300

Marschner H, Häussling M, George E (1991) Ammonium and nitrate uptake rates and rhizosphere pH in non-mycorrhizal roots of Norway spruce [*Picea abies* (L.) Karst.]. Trees 5:14–21

Marx DH, Bryan WC (1975) Growth and ectomycorrhizal development of Loblolly pine seedlings in fumigated soil infested with the fungal symbiont *Pisolithus tinctorius*. For Sci 21:245–254

Mawhinney TP, Robinett RSR, Atalay A, Madson MA (1986) Analysis of amino acids as their tert.-butyldimethylsilylderivates by gas-liquid chromatography and mass spectrometry. J Chromatogr 358:231–242

Millard P (1996) Ecophysiology of the internal cycling of nitrogen for tree growth. Z Pflanzenernaehr Bodenkd 159:1–10

Min X, Siddiqi MY, Guy R, Glass ADM, Kronzucker HJ (1998) Induction of nitrate uptake and nitrate reductase activity in trembling aspen and lodgepole pine. Plant Cell Environ 21:1039–1046

Muller B, Touraine B, Rennenberg H (1996) Interaction between atmospheric and pedospheric nitrogen nutrition in spruce (*Picea abies* (L.) Karst.) seedlings. Plant Cell Environ 19:345–355

Nadelhoffer KJ, Fry B (1994) Nitrogen isotope studies in forest ecosystems. In: Lajtha K, Michener R (eds) Stable isotopes in ecology and environmental science. Blackwell, Oxford, pp 22–44

Nadelhoffer KJ, Downs MR, Fry B, Aber JD, Magill AH, Melillo JM (1995) The fate of ^{15}N-labelled nitrate additions to a northern hardwood forest in eastern Maine, USA. Oecologia 103:292–301

Näsholm T, Ekblad A, Nordin A, Giesler R, Högberg M, Högberg P (1998) Boreal forest plants take up organic nitrogen. Nature 392:914–916

Persson J, Näsholm T (2001) A GC-MS method for determination of amino acid uptake by plants. Physiol Plant 113:352–358

Peter M, Ayer F, Egli S (2001) Nitrogen addition in a Norway spruce stand altered macromycete sporocarp production and

below-ground ectomycorrhizal species composition. New Phytol 149:311–325

Raitio H, Sarjala T (2000) Effect of provenance on free amino acid and chemical composition of Scots pine needles. Plant Soil 221:231–238

Rennenberg H, Gessler A (1999) Consequences of N deposition to forest ecosystems – recent results and future research needs. Water Air Soil Pollut 116:47–64

Schleppi P, Bucher Wallin L, Siegwolf R, Saurer M, Muller N, Bucher JB (1999) Simulation of increased nitrogen deposition to a montane forest ecosystem: partitioning of the added N-15. Water Air Soil Pollut 116:129–134

SMA (1998) Annalen der Schweizerischen Meteorologischen Anstalt SMA 135. MeteoSchweiz, Zürich

Stoermer H, Seith B, Hanemann U, George E, Rennenberg H (1997) Nitrogen distribution in young Norway spruce (*Picea abies*) trees as affected by pedospheric nitrogen supply. Physiol Plant 101:764–769

Turnbull MH, Goodall R, Stewart GR (1995) The impact of mycorrhizal colonization upon nitrogen source utilization and metabolism in seedlings of *Eucalyptus grandis* Hill ex Maiden and *Eucalyptus maculata* Hook. Plant Cell Environ 18: 1386–1394