Plant and Soil **168–169:** 135–150, 1995.

Monitoring uptake and contents of Mg, Ca and K in Norway spruce as influenced by pH and Al, using microprobe analysis and stable isotope labelling

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Key words: Al effect, microprobe analysis, nutrient uptake, pH effect, *Picea abies*, stable isotope labelling

Abstract

In a model system using intact spruce trees (*Picea abies* [L.] Karst.) we followed the path of magnesium, calcium and potassium during uptake into the root and during long-range transport into the shoot, by multiple stable isotope labelling. The roots of two- and three-year-old spruce trees originating from soil culture were removed from the soil and, in part or in toto, exposed to labelling solutions containing the stable isotopes ^{25}Mg or ^{26}Mg, ^{41}K and ^{42}Ca or ^{44}Ca. Optical-emission-spectroscopy (ICP-OES) of plant fractions and labelling solutions was combined with the quantitative analysis of stable isotope ratios in sections of shock frozen, cryosubstituted material using the laser-microprobe-mass-analyser (LAMMA). This combination allowed us to distinguish, both in bulk samples and on the cellular level between (i) the fraction of elements originally present in the plant before the start of the labelling, (ii) the material taken up from the labelling solution into the plant and (iii) any material released by the plant into the labelling solution.

In single-root labelling experiments, roots of three-year-old spruce trees, grown in nursery soil, were exposed to various pH conditions. The exchange of Mg and Ca with the labelling solution was nearly 100% in the cell walls of the mycorrhized finest roots. This exchange was only slightly affected by a step down to pH 3.5. The absolute Mg and Ca content in the cell walls was moderately reduced by incubation at pH 3.5 and strongly reduced in the presence of Al at this pH. After a pH 3.5 and 2 m*M* Al treatment we found Al in the xylem cell walls and the cortex cell lumina at elevated concentrations. To analyse the combined effect of high Al and high proton concentrations on the long-range transport, we used a "split-root system". The root mass of an intact two-year-old spruce tree, grown in mineral soil, was divided into even parts and both halves incubated in solutions with two sets of different stable isotopes of Mg and Ca (side A: no Al, ^{25}Mg and ^{42}Ca; side B: +Al, ^{26}Mg and ^{44}Ca) and ^{41}K on both sides. We observed a large uptake of Mg, Ca and K into the plant and a pronounced release. The net uptake of all three elements was lower from the Al-doted solution. In cross-sections of the apical shoot we found after a seven-day labelling period about 60–70% of the Mg and Ca and 30% of the K content in the xylem cell walls originating from both labelling solutions. The clear majority of the Mg and Ca label originated from the Al-doted side.

Introduction

The uptake of the macro-nutrients under conditions simulating possible causes of the "new type forest decline" was investigated in Norway spruce (*Picea abies*). This species is seriously subjected to the "new type forest decline" and covers 3.7 million hectares in german forests (36% of the total forest area, BML, 1992). The decline was originally reported in Germany for *Abies alba* as early as 1977 (Schütt, 1977) and later for other conifers - including Norway spruce - and broad leaved trees as well. The available evidence points to two generally favoured types of possible causes: (i) direct effects of air pollutants on leaves or needles and (ii) indirect effects of air pollutants via changes in the chemical and ecological condition of the soil. Direct effects of air pollutants on leaves, respectively needles, as the primary factor responsible for the observed damages may be restricted to some few heavily stressed forest stands (BMFT, 1990). Whenev-

er air pollutants have been shown to provoke various stress reactions, i.e. in needles of spruce, these effects are modulated by the water and mineral uptake of the affected trees. In the majority of forest decline stands indirect effects via the soil seem to be major factors (e.g. Hüttl, 1991; Kreutzer and Göttlein,1991; Schulze, 1989; Ulrich, 1981; Zech and Popp,1983; Zöttl and Hüttl,1985). Therefore, for both lines of thought, a detailed knowledge of the influence of the chemical condition of the soil on the uptake of water and mineral nutrients in trees is of vital importance.

Both, the early reported proton input, in turn leading to an increasing acidification of the soil frequently associated with an increased concentration of Al^{3+} and Al-complexes (Ulrich, 1981), and the high nitrogen input into the soil (Mohr, 1994), are reported to have considerable impact. Before the advent of acid rain research toxic effects by low pH and Al on root structure, plant growth and nutrient uptake have often been reported, especially in the field of agricultural science (e.g. Clarkson and Sanderson, 1971; Foy, 1984; Foy et al., 1978). Adverse effects of low pH and Al were shown for trees, particularly those grown in hydroponic cultures (e.g. Evers, 1983; Hecht-Buchholz et al., 1987; Junga, 1984; Rost-Siebert, 1983, 1985; Schaedle et al., 1989; Stienen and Bauch, 1988; Tischner et al., 1983). In many acid soils throughout the world aluminium is the most important growth impeding factor (Rengel,1992). A number of different possible mechanisms of pH- and Al-toxicity are under discussion (e.g. Foy, 1984; Huang and Bachelard, 1993; Jorns, 1988; Marschner, 1991; Rengel, 1992; Ryan et al., 1993; Schimansky, 1991; Taylor, 1991; Wagatsuma et al., 1987). There are rare studies where, opposed to the reported adverse effects, Al stimulation of growth or nutrient uptake of plants have been reported (Asp et al., 1991; Cumming et al., 1985; Foy, 1984; Huang and Bachelard, 1993; Mulette, 1975).

The displacement of bivalent cations like Ca^{2+} and Mg^{2+} by Al from adsorption sites, e.g. cell walls and membranes – generally discussed as Al-antagonism – may be of crucial importance for the influence of Al on the nutrient supply of plants. Good evidence exists that the long-range transport into the shoot is influenced by adsorption processes, antagonistic effects, the formation of complexes, and the physiological state of the plant (e.g.: Bradfield, 1976; Dambrine et al., 1993; Ferguson, 1980; Gülpen et al., 1993; Isermann, 1969, 1978; Thomas, 1967, 1969; Türk et al., 1993; Wieneke, 1969, 1974; Wieneke and Führ, 1973). Evidence for displacement of bivalent cations from the cell walls in roots has been reported in several studies (e.g. Bauch and Schröder, 1982; Godbold et al., 1988b; Jentschke et al., 1991; Murach, 1984; Rademacher, 1986; Schröder et al., 1988; Ulrich,1981; Wilkinson and Duncan, 1993). The mechanisms of Mg and Ca uptake into the root and the influence of low pH and Al-ions have not been sufficiently clarified yet. It seems obvious that these mechanisms and the long-range transport can only be understood if the study includes analytical methods allowing access to the cellular level.

This study presents data on the uptake of the macronutrients of Mg, Ca and K in mycorrhized finest roots. We first analysed the element distribution in cross-sections of finest roots using microprobe techniques in the starting material. We then compared these findings with the element distribution after a pH step down experiment and after a combined step up in Al and proton concentration. Similar conditions might occur on natural stands subjected to pollution. Later we follow the Mg, Ca and K label into the shoot after double labelling in a split-root system. The use of stable isotopes allows us to differentiate in these experiments between (i) the fraction of each element originating from the plant before the start of the experiment and (ii) the material released into the labelling solution and (iii) the material taken up from the labelling solution during the exposure period.

Materials and methods

Plants

Seeds of Norway spruce (*Picea abies* [L.] Karst.) originating from the German provenance Harzvorland / Westerhof (division: 840-03, 840-10 and 840-13) were germinated and seedlings were raised in sandculture for three months. Single seedlings were planted in 1 L pots and grown for one, respectively two years, in a greenhouse. The two types of soil were (i) a nursery soil with a high nutrient content and (ii) a mineral soil taken from a depth of 10–30 cm at the well described forest location "Postturm" (for a detailed analysis see: Michaelis and Bauch, 1992). The element composition of the soil solutions was determined according to Rademacher (1986) at the time of depotting. We found the following concentrations, given in m*M* in the nursery soil: 7.13 Ca, 1.67 Mg, 5.42 K, 1.10 Na, 0.06 Al, pH of the solution: 5.90, pH of the soil (in KCl): 6.36 and in the case of the mineral soil: 0.40 Ca, 0.25

Table 1. Natural isotope composition of the elements Ca, Mg and K (after Weast, 1989) and composition of the stable isotope label, according to the supplier. Composition in % of total number of atoms. Only the relevant isotopes are shown

Isotope	40	42	44
Ca–(natural)	96.941	0.647	2.086
42-Ca–label	11.21	87.70	0.90
44-Ca–label	2.90	0.06	97.00

Isotope	24	25	26
Mg–(natural)	78.99	10.00	11.01
25-Mg–label	2.29	96.90	0.81
26-Mg–label	1.82	1.08	97.10

Isotope	39	41
K–(natural)	93.258	6.730
41-K-label	4.2	95.8

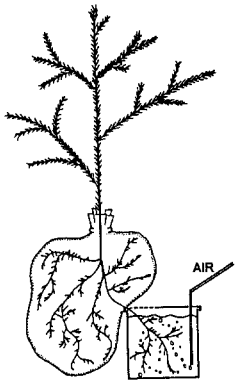

Fig. 1. Schematic diagram of the single-root labelling experiments. The composition of the labelling solution was: 1 m*M* each $^{25}MgCl_2$, ^{41}KCl, $^{44}CaCl_2$, NaCl. For the pH and the Al additions see the text below.

Mg, 0.73 K, 0.86 Na, 0.40 Al, pH of the solution: 3.49, pH of the soil (in KCl): 3.34. The reported experiments were carried out at the end of the natural growth period at the end of September.

Stable isotope labelling

The plants were transferred to a plant growth chamber with the following day/night cycle: day: 16 h, +23°C, 60 % rel. humidity, 280 μE light intensity; night: 8 h, +15°C, 90 % rel. humidity, complete darkness. Slow light / dark changes for dusk and dawn were within one

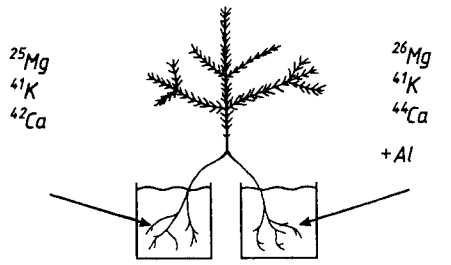

Fig. 2. Schematic diagram of the split-root labelling experiments. The composition of the labelling solutions were **∅Al**: 1 m*M* each $^{25}MgCl_2$, ^{41}KCl, $^{42}CaCl_2$, NaCl; **+Al**: 1 m*M* each $^{26}MgCl_2$, ^{41}KCl, $^{44}CaCl_2$, NaCl and 2 m*M* $AlCl_3$.

hour. After a 10 day period the labelling experiments were started. Plants were removed carefully from the soil. Any visible adhering soil was removed using a fine brush, if necessary under stereomicroscopic control. The last cleaning step was an approximate 10 s rinse in distilled water. Only plants where no wounding of the root system could be detected under stereomicroscopic observation were used. The presence of rhizomorphs or hyphae penetrating into the soil were rarely detected. The cleaned roots were then labelled according to two protocols, (i) "single-root labelling experiments" and (ii) "split-root" or "double labelling" experiments. In both cases highly enriched stable isotope preparations of magnesium, calcium and potassium, obtained from Medgenix, Düsseldorf, FRG, were used. Table 1 shows the detailed relative isotope composition of the enriched labels as compared to the natural isotope abundances. The relative isotope composition as specified by the supplier could be confirmed by LAMMA measurements within the accuracy of our procedure of 1 %. As this precision is lower than the precision obtained by the instrumentation of the supplier, we show the supplier's specification.

For the single-root labelling experiments (Fig. 1) roots from three-year-old spruce seedlings, grown in nursery soil, with a height of approximately 25 cm above ground, were prepared as described above. A 15 % to 20 % fraction of the root was subsequently incubated for 24 h and 48 h, respectively, in 20 mL labelling solution of 1 m*M* each $^{25}MgCl_2$, ^{41}KCl and $^{44}CaCl_2$ in the presence of 1 m*M* NaCl. The part of the roots that was not subjected to labelling was wrapped in a protective layer of wet cellulose. Where indicated, aluminum was added to 2m*M* as $AlCl_3$. The labelling solutions were aerated and in the case of the 48 h incubation exchanged once after 24 h.

Two-year-old Norway spruce, grown in mineral soil, with a height of approximately 10 cm above ground, were chosen for the double labelling or split-root experiments (Fig. 2). The younger, smaller plants were selected because of the high cost of the labelling in the larger volume and the use of the precious minor stable isotope ^{42}Ca. The root system was cleaned as described above and divided into halves named "OAl" and "+Al". Labelling was performed under the same conditions as above, except that both sides were incubated in a 20 mL solution with two sets of different stable isotopes of Mg and Ca ("OAl":1 m*M* each $^{25}MgCl_2$, ^{41}KCl, $^{42}CaCl_2$, NaCl; "+ Al": 2 m*M* $AlCl_3$ added to 1 m*M* each $^{26}MgCl_2$, ^{41}KCl and $^{44}CaCl_2$, NaCl). Potassium double labelling was not possible, since besides ^{39}K only one major stable isotope, ^{41}K, is available. Labelling was for seven days, and all solutions were exchanged every 24 h. Since measurements of the O_2-partial pressure in the exchanged solutions demonstrated at least 50 % saturation, the labelling solutions were not aerated.

Shock-freezing and cryosubstitution

All the following manipulations were carried out rapidly. Under stereomicroscopic control we took samples of 1–2 cm length. These were blotted on filter paper and shock-frozen by immersion into liquid propane, that was near its freezing point at a temperature of ≈-190°C. The propane (≈30 mL) was kept frozen in a brass container that was cooled by liquid nitrogen (≈-196°C). It was liquified prior to the sample immersion by dipping a somewhat warmer metal rod into the cryogen. Shortly after the rod was removed the liquid propane reached its freezing point (fp.:≈-190°C) again, indicated by a freezing front at the edges of the container. This procedure assured the lowest possible freezing temperature of the quenching liquid. After at least 30 s the shock-frozen samples were taken out of the propane, the major part of the adhering propane was removed, and the samples were transferred within the cold nitrogen gas phase into 20 mL polypropylene scintillation vials (Packard, No. 6008117) containing 10 mL water-free acetone with 0.5 % OsO_4 and kept for 3 days at -80°C. The samples were slowly warmed up to -20°C within some 24 h and to room temperature (+20°C) within 16 h.

Embedding and ultramicrotomy

The cryo-substituted samples were embedded after thoroughly removing residual OsO_4 by a fivefold rinsing step in water-free acetone in a low viscosity medium, modified after Spurr (1969) and Mascorro et al. (1976): viscosity 20 centipoise, 5 g ERL 4206 (vinylcyclohexene dioxide), 10 g HXSA (hexenyl succinic anhydride), 0.4 g Araldite RD2 (DY026), 0.15 g DMAE (dimethyl aminoethanol) (all obtained from TAAB). The samples were infiltrated in a graded series of embedding medium to acetone ratios (1/3; 1/1; 3/1; 1/0; 1/0) for 8 hours each step. Polymerization took place under exclusion of oxygen in "easy molds"; LKB #2208-156/180 with 5.6 or 8.0 mm diameter. After polymerization the blocks were trimmed to the

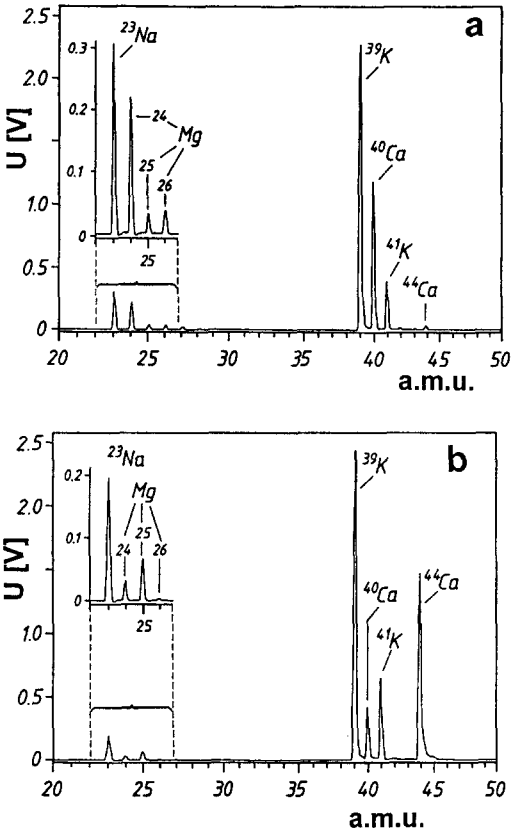

Fig. 3. LAMMA spectra of positive ions obtained from cortex cell walls in 0.5 μm thin sections of shock-frozen and cryo-substituted Norway spruce mycorrhizae. The major isotopes of Mg, Ca and K are clearly visible (see Table 1). Na is monotopic at mass 23. The upper spectrum (**a**) originates from an unlabelled control sample. The lower spectrum (**b**) was obtained from a spruce root sample after incubation in a labelling solution containing the stable isotopes ^{25}Mg, ^{44}Ca and ^{41}K, at 1 m*M* concentration. Note the change in the isotope ratios for the labelled elements in the lower spectrum. The insert shows part of the same spectrum at expanded scale. The dynamic range of the spectra is large enough to quantitatively determine the smaller peaks of the less frequent isotopes e.g. ^{44}Ca just visible here or ^{42}Ca that cannot be resolved in this graph. U = voltage at detector; a.m.u. = atomic mass unit.

required size, just slightly larger than the samples to be analysed. To facilitate sectioning for optimal orientation of the samples, these blocks were re-embedded in the following embedding medium: viscosity 200 centipoise; 54.5% (w/w) DDSA (dodecenyl succinic acid anhydride); 25% Epon 812; 20% Araldit CY212; 0.5% 2,4,6-tri- (dimethylaminomethyl)-Phenol (all obtained from SERVA). The re-embedded blocks were trimmed to final dimensions, using a diamond milling cutter. Sections, 0.5 μm thick, were cut on a "Ultracut", Reichert-Jung ultramicrotome, using a glass knife without floating liquid, since floating the sections on liquid would result in material loss or redistribution (Harvey et al., 1976).

LAMMA analysis

Sections were analysed using the laser-microprobe-mass-analyser LAMMA 500 (Leybold, Köln, FRG). In brief, the LAMMA 500 is a laser light miroscope with a high energy pulse laser fitted to a time-of-flight mass spectrometer. A small area in a 0.5 μm thin section, mounted on a 3 mm copper grid, is selected for analysis under light microscopic control and then evaporated by a flash of UV light (256 nm). In our samples the lateral resolution was 1–2 μm.

The evaporated material is analysed in the attached time of flight mass spectrometer. (For a detailed description see: Heinen et al., 1980; Hillenkamp et al., 1975). The resulting voltage signal at the detector is processed, and the area under the peak is expressed in relative units. Quantitative analysis giving absolute amounts of material is nearly impossible. Relative measurements, comparing LAMMA signals at different locations within one section, or comparing different samples, can be performed with high accuracy and were employed here. Using LAMMA we analysed both the relative element concentration and the isotope ratios on thin sections. The isotope ratios from a single spot analysis for each element, taken from a labelled specimen, do not necessarily represent the true ratio of material present before the onset of the labelling and the true percentage of label taken up from the labelling solution. As shown in Table 1, the "old", originally present material (e.g. Mg consisting of the natural mixture of the isotopes 24, 25, 26) and our labels (e.g. ^{25}Mg containing a small fraction of ^{24}Mg and ^{26}Mg) consist of a mixture of isotopes. Within the spectra measured by LAMMA (Fig. 3b), each peak of a given atomic mass unit is composed of a mixture of isotopes of different origin. These distinct fractions can be deconvoluted in a matrix correction algorithm. Using this procedure we computed in all spectra the fractions of the material present before the labelling and the material originating from each label. The correction procedure is described in detail in Fain and Schröder (1987). For each point of analysis (e.g. cell type) defined below and for each experimental condition (e.g. pH value) we analysed at least two different root samples of each plant. From each of these root samples we analysed 30–50 LAMMA spectra from 3–4 sections, resulting in 60 to 100 spectra per plant and per point of analysis. In the split-root experiments we analysed three samples from different areas of the root halves. In this case we averaged all spectra taken from one root half. In the single-root labelling experiments we used two or three plants per condition. In these cases we show the averages of all spectra from these plants. The data for the pH 6.7 to pH 3.5 conditions were pooled. In this case we show the mean of the averages from the spectra of each plant. In all cases we show twice the standard-deviation of the mean according to Peter's formula: $SD(M) = 1.25 \times ({}^{N}\Sigma_i\ |X_i - X_M|) / [N \times (N-1)^{1/2}]$ (Squires, 1971). X_M is the mean value of a number N of single spectra X_i. Assuming that the distribution of the data is Gaussian, the twofold standard-deviation of the mean includes the true value with a 95% probability.

Quantitative multi-element analysis of bulk samples

The tissue samples were first ashed at high pressure and then resuspended according to Rademacher (1986). The liquid samples were analysed directly in diluted form. All bulk samples were analysed by ICP-OES (inductively coupled plasma optical emission spectroscopy; comp. Michaelis et al., 1985) using an ARL model. The isotope ratios of these samples were determined with LAMMA. Since LAMMA can only analyse small areas of a few μm^2, 50 μL aliquots from all samples were sprayed onto nitrocellulose support films on copper grids, and the resulting microdroplets were analysed by LAMMA analysis after drying. 50 spectra were averaged to determine the isotope ratios of the samples.

Results

When finest roots of spruce trees were shock frozen, cryo-substituted and embedded under our conditions, we observed good structural preservation within the

Fig. 4. Cross-section of spruce mycorrhiza. The arrows indicate the points of LAMMA analysis. **1.C:** cell wall in direct contact to the soil or the labelling solution; **2.C** and **4.C:** cortex cell walls; **Lu:** cortex cell lumen; **Xyl:** cell wall of primary xylem cells. The calibration *bar* represents 50μm.

limits of the light microscopic resolution and the typical appearance of ectomycorrhizae (Fig. 4). All of the finest roots were monotypically mycorrhized except for very rare unmycorrhized cases that were discarded. A single-layered Hartig net extends to the endodermis layer, while the surrounding hyphae mantle is typically small.

To get a first impression of the relative element distribution for a number of elements, we processed roots without any further treatment for microprobe analysis. One example of the LAMMA spectra obtained is shown above (Fig. 3a). Within the samples of the finest roots, we selected those areas where the primary xylem just started to appear developed, showing 10–20 lignified xylem cells within a complete cross-section. Points of analysis (see Fig. 4) were the cell walls that seerned most representative for the radial distribution of the analysed elements.

In addition, we analysed the lumina (Lu) of the cortex cells from the second to innermost cell layer. We found no obvious difference between the data for the lumen measurements in different cortex locations. Therefore we pooled the data from the lumen measurements. The element content of Mg, Ca, Al, K and Na, as measured by LAMMA, is shown in Figure 5 as control values for the following experiments. With LAMMA we cannot determine the absolute amount of material present, therefore the relative element distribution is shown. The analysed areas in the cell walls of the cortex do include fungal hyphae as can be seen in Figure 4. The 1 μm lateral resolution of the LAMMA is not sufficient to resolve these structures in the analysis (see discussion). We found a characteristic distribution of high levels of Ca in the cell walls with a gradient along the cross-section. Al is present primarily in the outer cortex cell walls. Mg is more evenly distributed at low levels in the cell walls. The isotope ratios of the elements Mg, Ca and K were determined from these spectra and were found to agree well with the tabulated and expected values within 1% of the total sum of all isotopes of each element.

A step up experiment in proton concentration

The roots of three-year-old Norway spruce grown in nursery soil were removed from the soil and labelled for two days as described above (see Fig. 1) by incubation in various labelling solutions with pH values of

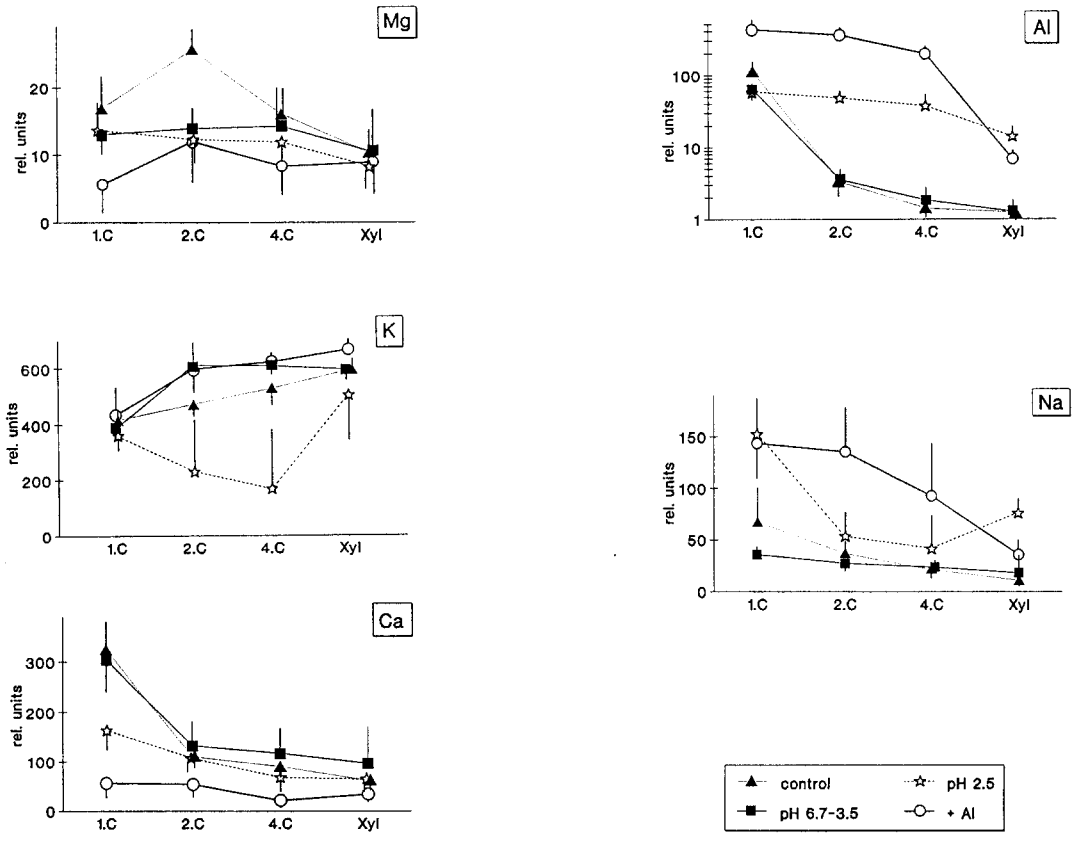

Fig. 5. Radial element distribution in the apoplasm of mycorrhizae from three-year-old Norway spruce grown in nursery soil. The roots were labelled by a 48h incubation of the roots in solutions with pH values ranging from pH 6.7 to pH 2.5 (two plants each per condition) and in one case for 24 h at pH 3.5 and 2m*M* $AlCl_3$ (three plants), containing the stable isotopes ^{25}Mg, ^{41}K and ^{44}Ca (each 1 m*M*). Points of analysis were as shown in Figure 4: cell walls in direct contact with the solution of the first, outermost cortex cell layer (1.C), of the 2nd cortex cell row (2.C), the innermost cortex cell row adjacent to the endodermis layer, typically the 4th cortex cell row (4.C), and the cell walls of the primary xylem cells (Xyl). Since no significant difference between the data from pH 6.7 to pH 3.5 was found, the data from these experiments were pooled. The mean values and twice the standard-deviation of the mean are plotted. Control root samples were taken from three plants without any other handling. Each element analysed is shown separately as indicated in the figures

pH 6.7, pH 5.6, pH 4.5, pH 3.5 and pH 2.5. As the pH of the soil solution was near pH 6 this resulted in a step up in proton concentration, except for the pH 6.7 condition. The plants were allowed to modulate the pH in the labelling solutions, as the solutions were not buffered. As expected, the pH changed during the experiment. The pH of the solutions with a starting value of pH 6.7 to pH 3.5 changed within the incubation time of 48 h uniformly to 6.7–7.2. In the case of the incubation in the labelling solution at pH 2.5 the observed pH at the end of the labelling period was pH 2.9–pH 3.0.

Relative element distribution within the apoplasm

To obtain the radial relative element distribution within the apoplasm, we took LAMMA spectra from cell walls in cross-sections from this pH-step experiment and compared the results in Figure 5 with the data of the starting material. We could not detect any significant difference between the datasets obtained from the different experiments with pH values from pH 6.7 to pH 3.5. Therefore we cumulated these data into one single line in this graph for clarity. As compared to the starting material, we found in the outer cortex cell row a noticeable, and in the second cortex cell row a pronounced reduction in the Mg levels in the cell walls at pH 6.7 to pH 3.5. The Ca levels were changed slightly

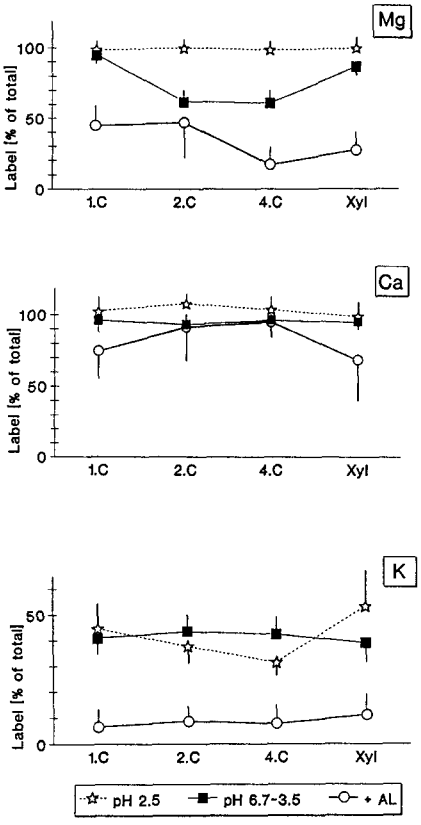

Fig. 6. Fractions of label in Mg, Ca and K, expressed as percent of total element. The data from the same spectra and the same locations as shown in Figure 5 were used to compute the fraction of material originating from the labelling solution to which the roots were exposed.

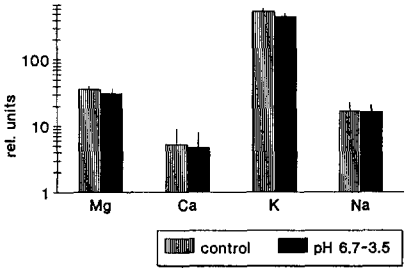

Fig. 7. Relative element content in the lumina of mycorrhizae before and after a pH 6.7 to pH 3.5 treatment during stable isotope labelling. The conditions of incubation and of analysis were identical to the ones in Figure 5.

under these conditions. No change was detectable for the Al distribution under these conditions. However, at pH 2.5, the extreme end of the range of the low pH treatment, rather large differences to the starting material and the material from the pH 6.7 to pH 3.5

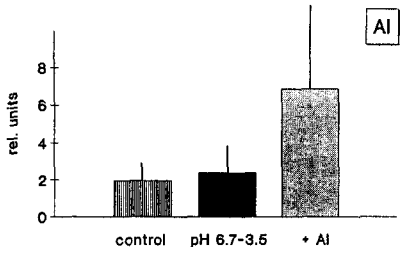

Fig. 8. Relative aluminum concentration in the lumina of mycorrhizae before and after a pH 6.7 to pH 3.5 and a pH 3.5 + 2m*M* $AlCl_3$ treatment during stable isotope labelling. The conditions of incubation and of analysis were identical to the ones in Figure 5.

were observed (Fig. 5). Incubation at pH 2.5 caused a major increase in the concentration of Al well into the cortex and into more centrally located cell walls of the mycorrhizae. Al rises in the xylem about 10–20 fold relative to the original content. In addition, the K content decreased and the Na content increased in cell walls of the cortex and the primary xylem in these samples after the pH 2.5 treatment (for restrictions in the K and Na measurements see discussion).

Fraction of Mg, Ca and K in the apoplasm, originating from the label

We now used the same spectra to determine the fraction of label within the total amount of Mg, Ca and K in the cell walls of mycorrhizae (Fig. 6). In the case of Ca we found under all conditions a surprisingly high 100% value for the exchanged fraction, as shown in Figure 6, indicating a fully quantitative equilibration of the Ca originally present in the cell walls with the Ca in the labelling solution. This is in contrast to the exchange of potassium, where only approximately 40% of the total element is the label. This low percentage of exchange is consistent within all pH values used here. The percent-values for the labelled fraction of Mg vary with the position of the analysis and with the treatment. (As will be discussed below, this may be due to an interference of an Mg signal from other structures.)

Analysis of the cortex cell lumen

Together with the analysis in the apoplasm shown above we took measurements in the lumen of the cortex cells on the indicated locations 2.C and 4.C in Figure 4 on the same sections. The Ca and the Mg values appear to be the reverse of the content in the apoplasm. The Ca concentration is low and the Mg concentration is high (Fig. 7). The incubation in pH 6.7 to pH 3.5 results in almost no change in the concentration of the analysed elements, including Al (Fig. 8). Again, since no differ-

ences among the data for the different pH conditions were detectable, the data from these experiments were pooled. The exchange between the labelling solutions and the lumen of the cortex cells is also high in the case of Ca, where we determined a 85% ± 15% fraction of Ca from the label, while the exchange of Mg is small (< 10%). The labelled fraction in K was always similar to the values found in the apoplasm. In contrast, the concentrations for Mg, Ca, K and Na in cross-sections from mycorrhizae incubated in pH 2.5 were reduced to less than 1% of the control values. Therefore no isotope ratios were determined. The fine structure within the cytoplasm in the cortex cells of the roots - that was visible in the starting material and in the material after the more moderate pH treatment - could not be detected in these samples, which is compatible with the assumption that the cell structures in the lumen were destroyed.

The effect of 2 mM Al and pH 3.4 in the labelling solution

We analysed the effect of 2 m*M* Al added to a labelling solution of pH 3.4. We observed in preliminary experiments, that the plant induced pH change in the labelling solutions, noticed above, was abolished under these conditions. The pH of the labelling solutions changed during the incubation period merely from pH 3.4 to pH 3.5. However, as the effects on the Mg and Ca content in the apoplasm of mycorrhizae seemed quite strong already after one day, we decided to reduce the time of exposure in this experiment to 24 h to avoid prolonged stress due to the long exposure to the low pH. The element distribution (Fig. 5) in mycorrhizae of this treatment shows the expected dramatic increase in Al content in the cortex cell walls as well as in the corresponding lumina (Fig. 8). The second effect that can be measured is a pronounced loss of Mg and Ca from the apoplasm of the mycorrhizae. The content of Mg and Ca was much less than that found after incubation in solutions of pH 6.7–pH 3.5 without added Al, even after the shorter exposure time. We also found a large increase in Na in the 2.C and 4.C position in the cortex cell walls. The exchanged fractions (Fig. 6) of Mg and K are strongly decreased in the mycorrhizae from the Al experiments but not significantly reduced in the case of Ca. However, as the exposure time was shorter compared to the pH 3.5 experiment without Al, this effect may at least partially be due to the shorter incubation time.

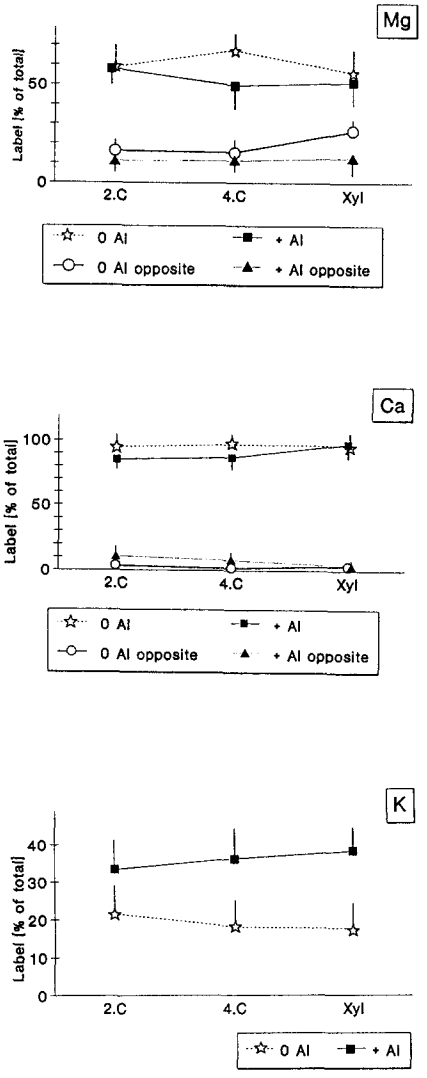

Fig. 9. Fractions of label in Mg, Ca and K in mycorrhizae from a split-root double labelling experiment. The roots were removed from the soil, split into halves and labelled as shown in Figure 2 with two sets of labelling solutions, one control (∅Al, labelled with ^{25}Mg, ^{41}K, ^{42}Ca) and one containing 2m*M* $AlCl_3$ (+Al, labelled with ^{26}Mg, ^{41}K, ^{44}Ca). LAMMA point measurements were taken as shown above. In the case of Mg and Ca both sides were labelled with different isotopes. Analog to the single-root labelling experiments shown in Figure 6 we show here for both roots the fraction of the label originating from the incubation solution to which each root was exposed (the upper traces, marked as ∅Al and +Al). In addition we show the fraction of the second label, to which the roots were not exposed, originating from the opposite labelling solution (the lower traces, marked as ∅Al-opposite and +Al-opposite). Since in the case of potassium only one label is available, we cannot distinguish the origin of the label and therefore show two curves only. The fractions of label are expressed as percent of total element, consisting of (i) label one, (ii) label two and (iii) the original element fraction, present before the labelling.

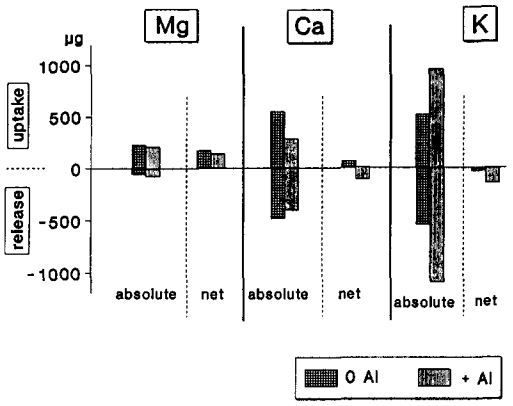

Fig. 10. Uptake and release from both labelling solutions (∅Al and +Al) during the 7 day lasting split-root experiment. The absolute element content and the isotope fractions of Mg, Ca and K in the labelling solutions were determined using ICP-OES and LAMMA. Changes in the fraction of the label and in total element content during the experiment were used to calculate the absolute uptake and release from and into the labelling solution, expressed in µg. The "net" values shown are the difference between uptake and release.

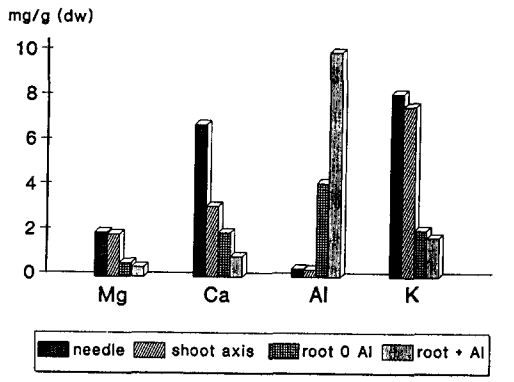

Fig. 11. Total element content in the dry weight of plant fractions, taken at the end of the 7-day split-root experiment. For the root we show the two halves incubated in the control solution (∅Al) and the Al-doted one (+Al) separately. The concentrations were determined by ICP-OES.

The effect of Al on the exchanged fraction of label in the lumen of the cortex cells was small. Compared to the pH 6.7–pH 3.5 conditions we found a similar large fraction of Ca exchanged (68%) and a similar small exchanged fraction of Mg (6%). The fraction of exchanged K was smaller than without the addition of Al, again similar to the values found in the apoplasm. However, this may also be dependent on the shorter exposure time in this experiment.

A split-root experiment to study the influence of Al on ion uptake and long-range transport

In order to analyse the effect of Al on element uptake separate from other accompanying effects, we developed a split-root system optimized for the direct comparison of conditions. In order to reduce unspecific stress due to changes of conditions we selected plants from a soil with a low pH similar to our previous conditions and a lower nutrient content. The closest condition we could find that was similar to a natural stand were two-year-old Norway spruce grown in mineral soil from a forest stand with a pH 3.5 in the soil solution. The design of the experiment is shown above in Figure 2. Here we show in a single case study the results obtained from the analysis of one single tree. In this split-root double labelling experiment 2m*M* Al was added to the labelling solution only on one side. The labelling solutions differed in the stable isotopes of Mg and Ca in both incubation solutions. In these experiments we observed no root-induced pH change of the labelling solutions.

Fraction of label in mycorrhizae

It was reassuring, though not surprising, that the same results as seen in the single-root labelling experiments were obtained when we analysed the relative element distribution of Mg, Ca and K in cross-sections of mycorrhizae from this experiment. We obtained the same distribution patterns and similar values for the analysed elements. However, additional information on the level of the mycorrhizae was obtained by the analysis of the fraction of label in root cross-sections. Figure 9 describes LAMMA microprobe analysis of thin sections from mycorrhizae similar to the data presented above, but now from two labelling sides. The data show the labelled element fractions in the cell walls of the inner cortex cells and of the primary xylem which originated from either the Al doted solution or the undoted solution. The analysis shows that after 7 days only a minor effect of Al on the exchanged fraction of Ca and Mg in the cell walls of mycorrhizae occurred, whereas the labelled fraction of K is higher on the Al doted side.

It was rather unexpected, that we could detect in cross-sections of mycorrhizae of one side a quite noticeable fraction of those Mg and Ca isotopes, which clearly originated from the opposite side of labelling (lower traces in Fig. 9). This effect is quite large for Mg. Up to 26% of the total Mg found in the primary xylem of mycorrhizae incubated in the control solution without any aluminum added originates from the labelling solution on the opposite side. The effect seems to be

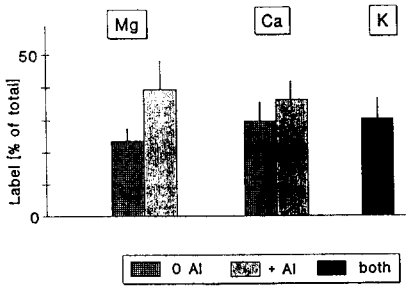

Fig. 12. Fraction of label in the one year old part of the shoot, originating from the control root (ØAl) and the Al-doted root (+Al) after the split-root double labelling experiment. Averages and one standard deviation of 80 LAMMA spectra from cross-sections in the cell walls of earlywood and latewood.

under the influence of Al both in the case of Mg and in the case of Ca. The Ca amount is smaller but still highly significant, since we can detect the isotopes with high precision and the standard deviations are very small. Unfortunately, in this experiment we cannot determine the origin of the K label, as only one stable isotope was available for labelling.

Uptake and release as determined from the labelling solutions

We determined by ICP-OES and LAMMA the absolute element concentrations and the isotope ratios of all solutions before and after use. Both the unidirectional fluxes of uptake and release and the resulting difference, the net flux of the labelled elements were computed from the consumption of the solution (10.3 mL from the undoted side and 9.8 mL from the Al doted side) and the changes in concentration and in the isotope composition. The absolute amounts taken up or released during the 7–day labelling period, are shown in Figure 10. For all three elements shown the unidirectional fluxes are large, compared to the net flux. The presence of Al largely increased both unidirectional fluxes of K, decreased both for Ca and slightly decreased the Mg uptake and increased Mg release. These changes resulted in a decrease of the Mg net uptake and inverted the direction of the net flux in the case of Ca and K from a net uptake into a net release.

Element concentrations in plant fractions

In parallel to the analysis of the labelling solutions, we analysed the absolute concentrations of Mg, Ca, Al and K in fractions of the needles, the shoot axis and the two root halves from the two labelling sides by ICP-OES (Fig. 11). In the root fractions a clear difference in the element content of Ca and a minor difference in Mg and K can be detected. The root half, that was not exposed to the Al doted solution, apparently did contain a high amount of Al, typical for plants from this stand and soil. Exposure to the Al doted solution did increase this value substantially, as expected. The Ca and K concentrations are highest in the needles while the Mg concentration is generally lower and about even in the needles and in the shoot axis.

The stable isotope label in the shoot

A quick check of the isotope ratios in the plant fractions used for the bulk analysis shown above (data not shown) indicated a strong labelling. This enabled us to detect the label by microprobe analysis in cross-sections taken from the cell walls of earlywood and latewood in the youngest shoot area. After the 7–day labelling period approximately 60% of the total Mg content, 65% of the Ca and 30% of the K in the xylem cell walls of the apical shoot were derived from both labelling solutions. Figure 12 shows the measured fractions of the label and distinguishes the origin of the label (not possible for K), whether it originated from the Al doted or the undoted labelling solution. For both Mg and Ca the fraction of label originating from the Al doted solution is significantly higher.

Discussion

One advantage of stable isotope labelling is, that we can work as close as possible to natural conditions. This includes that no misleading element substitutes are necessary (e.g. Rb instead of K; see Marschner and Schimansky, 1968). We are not restricted to radioactive isotope laboratories and experiments at natural sites are possible. Here we used intact plants in growth chambers to obtain the highest possible chance for reproduction of the experiments. The plants were grown in soil and the roots were fully mycorrhized. Plants without mycorrhization did not seem suitable, since under natural conditions Norway spruce -like most European forest tree species- are mycorrhized (Allen, 1991, 1992; Kottke and Oberwinkler,1986). The mycorrhization with a single selected fungal species would be advantageous for this type of study. This is possible in the culture of smaller seedlings under sterile conditions, where the inoculated fungus can be maintained in the mycorrhizae (see: Jentschke et al., 1991; Kottke et al., 1987; Kuhn et al., 1988). Such attempts failed for forest trees, if they were grown for longer periods of

time in soil culture. In these cases (e.g. Feil, 1989) and in the one presented here spontaneous monotypic mycorrhizae developed.

On the cellular scale we demonstrated good structural preservation after shock freezing and cryosubstitution (Fig. 4). Ice crystal damage is below the limits of the lateral resolution of the microprobe analysis. However, this does not necessarily imply the absence of redistribution or loss of diffusible material. Loss or redistribution can only be minimized if cryosections of shock frozen material are analysed (see: Steinbrecht and Zierold,1987). Plant samples of the large volumes required in this study cannot be obtained with these techniques. We therefore used a cryosubstitution protocol that was shown previously in another system to keep Ca in place (Fain and Schröder,1985). The solubility of Mg and Ca in dry acetone is very low. Isotope labelling offers one inherent control for the absence of redistribution. If roots are only shortly immersed in Mg and Ca label we measure the natural isotopic abundance throughout the sample. In contrast, we find only the labelling isotopes in the extracellular material of the first cell layer, in direct contact with the labelling solution. We take this as strong evidence that no relocation of the labelled elements occurs. Moreover, we could show using the radioactive tracer ^{45}Ca, that loss of Ca during our specimen preparation protocol is low, if present at all (Kuhn, 1993). Since the solubility of Al in the solvents involved is lower than that of Mg and Ca, we assume minimal loss of this element. In the case of the monovalent ions Na and K we may expect some loss. Therefore these data should be taken with caution. Despite this, the data for Na and K from cryosubstituted samples are shown, since the high fraction of "old", unlabelled K corresponds well with the large release of K into the labelling solutions. This large fraction of "old" K could at least in part originate from a back-transport of K from the shoot. The pronounced pH and Al dependent Na effect seemed relevant enough to show, even after taking into account that the quantification is vague in view of the possible loss of material. This effect should be taken as a qualitative change.

We were surprised by the strong labelling well into the shoot from the only 1 m*M* concentrations in the labelling solutions. These are still within the range of values that can be found in soil solutions of various forests: Na $\approx$ 0.96 m*M*[0.17–2.9], Mg $\approx$ 0.25 m*M*[0.08–1.17], K $\approx$ 0.36 m*M* [0.10–1.56], Ca $\approx$ 0.6 m*M*[0.07–2.0], pH $\approx$ 4.3[3.5–5.9] (A_h-layer; mean value and range in brackets; according to Scheffer and Schachtschabel, 1976). If we incubate the roots from the nursery soil (with even higher Mg, Ca and K concentrations) in the labelling solutions we create a step down in the nutrient concentration, even though most likely not all of the Mg and Ca in the soil solution will be in free ionic form. The cost to use the same high concentrations for the labelling solutions would be prohibitive. Nevertheless, incubation of the roots in labelling solutions at the same pH (6.7) or at pH values down to pH 3.5 results in only a slight change of the Ca and K concentration in the cell walls of the cortex and the xylem. The Mg concentration is only affected in the cell walls of the outer cell layers.

At pH 6.7–pH 3.5 we find a quantitative exchange of Ca in the cell walls of both the cortex and the xylem. The exchange of Mg in these locations is very high as well. The exchange of Mg is complete in the outer cell layer and in the xylem. In the second to fourth cortex cell layer the measurements show an approximately 30% unexchanged Mg fraction. Possibly there are at least three pools in these analysed areas. One pool is the actual cell wall material, the second one is in the fungal hyphae within the cell walls and the third pool is contained in a small amount of cytoplasmic material, possibly adhering to the cell walls or in plasmodesmata. We are not able to physically resolve these in our LAMMA measurements and therefore these pools are all analysed together within the cell walls. The material from the lumen may be concentrated along the inner edge of the cell walls. Since we know that the Mg exchange in the lumen is smaller than 10 %, this would reduce the observed changed fraction. Any Mg in the "old" Mg fraction may originate from these sources, or it may originate from other parts of the plant. This would also be true for K. The exchanged fraction of K was much smaller than that of Ca and Mg under all conditions.

The step down experiment to pH 2.5 did lead to a destruction of the cortex cells. This finding is relevant, since the pH localized in the rhizosphere may be up to two pH values lower than the pH measured in the soil (Marschner and Römheld, 1983). In various forest stands pH values lower than 3.0 are reported for soil solutions (e.g. Rademacher, 1986). Dramatic shifts in soil pH occur especially after drought periods (Murach, 1984). As one might expect after the destruction of the cytoplasm, the exchanged fraction of the Mg label went up to 100%. Under the assumption that the pH 2.5 treatment did not change the cell wall structure, this result can be taken as supportive evidence for an unrestricted movement of Mg in the cell walls within the cortex. The pH 2.5 step results in a relocation of

Al. We find a reduction of Al, originally present in peripheral cortex cell walls and a pronounced increase of Al in the inner cortex and the primary xylem where it would be available for the long-range transport. At this position it may be more relevant for phytotoxic effects than if restrained to peripheral cortex areas (see Puhe et al., 1986; Radermacher, 1986).

Loss of both Mg and Ca from cell walls exposed to a combination of low (soil) pH and high Al concentration (Bengtsson, 1992; Godbold et al., 1988a; Jentschke et al., 1991; Schröder et al., 1988) has been reported previously. Our data are in agreement with these findings. We also demonstrate that Al present in the incubation solutions can be found in the lumina of the cortex cells and in the primary xylem. The size of the exchanged fraction of Ca however is not affected by the reduction in the total concentration. The larger fraction of unlabelled, "old" Mg after Al treatment shown in Figure 6 may in part be explained by Mg originating from the shoot or from unlabelled other parts of the root system. This is supported by the split-root experiment where we found a higher fraction of Mg from the corresponding root labelled with a different isotope. Cycling of water from root to root (van Bavel and Baker, 1985) and cycling of amino-nitrogen (Cooper and Clarkson, 1989) have been reported before. However, Mg cycling was not accessible to analysis until now because of the lack of a suitable tracer.

The data shown from the split-root experiment are obtained from one single tree. The reported case is the first complete dataset combining microprobe and bulk analysis obtained from a series of experiments with a range of various Al and nutrient concentrations. Additional data from 6 trees, originating from experiments with comparable nutrient and Al concentration, were similar for (i) the observed release, (ii) uptake and (iii) the labelled fractions in the shoot and in the needles (data not shown).

The analysis of the labelling solutions illustrates in all experiments both a large uptake for Mg, Ca and K and a large release for Ca and K. A large release of "old" material would be expected from accessible binding sites within the apoplasm of the root. Analysis of the incubation solutions, collected after the first and second day of incubation, shows the expected large fraction of ^{24}Mg, ^{40}Ca and ^{39}K, the unlabelled, originally present material, released by the roots (data not shown). This is in agreement with the data shown for the 100% exchange of Mg and Ca in the apoplasm of the mycorrhizae (Fig. 6) within the same period of time. However in the solutions from the third to the seventh day of incubation we still find a large fraction of "old" Mg, Ca and K. This can only in part be accounted for as coming from secondary roots since the total amount of Ca in roots of control trees was smaller than the total amount of "old" Mg and Ca released into the labelling solutions. This may indicate cycling from the shoot.

The large uptake as determined from the analysis of the incubation solutions is matched by the high fraction of label in the shoot (Fig. 12). We found a higher uptake from the Al doted solution for both Mg and Ca. This is in contrast to the observed lower net uptake from the labelling solutions and reported adverse effects of Al on the uptake of these nutrients in long-time experiments (see: Foy, 1984; Schaedle et al., 1989). We could reconcile these opposing findings if we assume that Al blocks negative binding sites in the apoplasm in the root and the shoot. Facilitated apoplasmic movement of bivalent ions in cell walls by Al^{3+} or other competing ions has been previously reported (Clarkson, 1984; Huang and Bachelard, 1993; Isermann, 1969; Läuchli, 1976; Lüttge and Higinbotham, 1979). In this way each individual ion would travel faster along the long axis of a vessel as it is not retarded by constant binding and release from binding sites. The label and the originally present material as it is now released from its binding sites would appear faster in the shoot than the label and original material from the undoted side. This could result in a transient "flooding" of the shoot with Mg and Ca. This effect would be independent from any other mechanism involved in the long-range transport e.g. transport inhibiting Al effects. We still might observe this effect under conditions where the long term total transport rate of Mg and Ca were increased, unchanged or decreased, depending on the time regime.

The combination of isotope labelling and microprobe analysis with bulk element analysis was very powerful in tracing the element uptake and transport. In contrast to previous studies where the unidirectional fluxes between root and environment could not be distinguished, we can separately measure uptake, release and cycling simultaneously for several elements in one physiological experiment.

Acknowledgements

This reseach was supported by grants #03392218B and #0339328A given to J B and A J K by the German Federal Ministry of Research and Technology. This work would not have been possible with-

out the excellent technical assistance of A Einerhand H Erkens (Jülich), R Endeward, T Schwarz and O Dünisch (Hamburg). Furthermore we thank the GKSS-Research Centre Geesthacht, Prof W Michaelis and coworkers, for the use of their ICP-OES instrument. We thank Prof F Führ, Institut für Radioagronomie, Forschungszentrum Jülich for the possibility to use the growth chamber and L Molday for critically reading the manuscript.

References

Allen M F 1991 The Ecology of Mycorrhizae. Cambridge Studies in Ecology, Cambridge University Press. 184p.

Allen M F 1992 Mycorrhizal functioning: an integrative plant-fungal process. Routledge, Chapman and Hall, Inc., New York, 534p.

Asp H, Bengtsson B and Jensén P 1991 Influence of aluminium on phosphorus and calcium localization in roots of beech (*Fagus sylvatica*). Physiol. Plant. 83, 41–46.

Bauch J and Schröder W H 1982 Zellulärer Nachweis einiger Elemente in den Feinwurzeln gesunder und erkrankter Tannen (*Abies alba* Mill.) und Fichten (*Picea abies*[L.] Karst.).Forstwiss. Centralbl. 101, 285–294.

Bavel van C H M and Baker J M 1985 Water transfer by plant roots from wet to dry soil. Naturwissenschaften 72, 606–607.

Bengtsson B 1992 Influence of aluminium and nitrogen on uptake and distribution of minerals in beech roots (*Fagus sylvatica*). Vegetatio 101, 35–41.

Bradfield E G 1976 Calcium complexes in the xylem sap of apple shoots. Plant and Soil 44, 495–499.

BMFT 1990 Programmreport: Waldschadensforschung. Bundesministerium für Forschung und Technologie, Referat Öffentlichkeitsarbeit / Bonn. ISBN 3-88135-219-8. 39p.

BML 1992 Waldzustandsbericht der Bundesregierung 1992. Schriftenreihe des Bundesministers für Ernährung, Landwirtschaft und Forsten, Heft 415. ISBN 3-7843-0415-X. 78p.

Clarkson D T 1984 Calcium transport between tissues and its distribution in the plant. Plant Cell Environ. 7, 449–456.

Clarkson D T and Sanderson J 1971 Inhibition of the uptake and long-distance transport of calcium by aluminium and other polyvalent cations. J. Exp. Bot. 22, 837–851.

Cooper H D and Clarkson D T 1989 Cycling of amino-nitrogen and other nutrients between shoot and roots in cereals - a possible mechanism integrating shoot and root in the regulation of nutrient uptake. J. Exp. Bot. 40, 753–762.

Cumming J R, Eckert R T and Evans L S 1985 Effect of aluminium on potassium uptake by red spruce seedlings. Can. J. Bot. 63, 1099–1103.

Dambrine E, Carisey N, Pollier B and Granier A 1993 Effects of drought on the yellowing status and the dynamics of mineral elements in the xylem sap of declining spruce (*Picea abies* L.). Plant and Soil 150, 303–306.

Evers F H 1983 Ein Versuch zur Aluminium-Toxizität bei Fichte. Ergebnisse eines Gefäßkulturversuchs mit bewurzelten Fichtenstecklingen. Forst. Holzwirt. 38 (12), 305–307.

Fain G L and Schröder W H 1985 Calcium content and calcium exchange in dark-adapted toad rods. J. Physiol. 368, 641–665.

Fain G L and Schröder W H 1987 Calcium in dark-adapted toad rods: evidence for pooling and cyclic-guanosine-3′-5′-monophospate-dependent release. J. Physiol. 389, 361–384.

Ferguson I B 1980 The uptake and transport of calcium in the fruit tree. Acta Hortic. 92, 183–191.

Feil W 1989 Morphologische und anatomische Untersuchungen zur Reaktion der Mykorrhizen von *Picea abies*[L.] Karst. auf natürlichen und experimentellen Trockenstreß. Dissertation, Universität Tübingen.

Foy C D 1984 Physiological effects of hydrogen, aluminum, and manganese toxicities in acid soil. *In* Soil Acidity and Liming. Ed. F Adams. pp 57–97. Agronomy, American Society of Agronomy, Madison, Wisconsin, USA.

Foy C D, Chaney R L and White M C 1978 The physiology of metal toxicity in plants. Ann. Rev. Plant Physiol. 29, 511–566.

Godbold D L, Dictus K and Hüttermann A 1988a Influence of aluminum and nitrate on root growth and mineral nutrition of Norway spruce (*Picea abies*) seedlings. Can. J. For. Res. 18, 1167–1171.

Godbold D L, Fritz E and Hüttermann A 1988b Aluminium toxicity and forest decline. Proc. Natl. Acad. Sci. USA 85 (Ecology). 3888–3892.

Gülpen M, Türk S and Fink S 1993 Der Effekt von Kalkung und Magnesiumdüngung auf die Aufnahme, den Transport und die chemische Bindungsform von Calcium und Magnesium in Koniferen. KfK-PEF (Kernforschungszentrum Karlsruhe) 104, 195–207.

Harvey D M R, Hall J L and Flowers T J 1976 The use of freeze-substitution in the preparation of plant tissue for ion localization studies. J. Microsc. 107, 189–198.

Hecht-Buchholz Ch, Jorns C A and Keil P 1987 Effect of excess aluminium and manganese on Norway spruce seedlings as related to magnesium nutrition during Al stress. J. Plant Nutr. 10, 1103–1110.

Heinen H J, Hillenkamp F, Kaufmann R, Schröder W H and Wechsung R 1980 LAMMA: A new laser microprobe mass analyser for biomedicine and biological materials analysis. *In* Recent Developements in Mass Spectrometry in Biochemistry and Medicine. Eds. A Frigerio and M McCamish. pp 435–451. Elsevier Scientific Publishing Company, Amsterdam.

Hillenkamp F, Unsöld E, Kaufmann R and Nitsche R 1975 A high-sensitivity laser microprobe mass analyzer. Appl. Phys. 8, 341–348.

Huang J and Bachelard E P 1993 Effects of aluminium on growth and cation uptake in seedlings of *Eucalyptus mannifera* and *Pinus radiata*. Plant and Soil 149, 121–127.

Hüttl R F 1991 Die Nährelementversorgung geschädigter Wälder in Europa und Nordamerika. Frei. Bodenkd. Abh. 28. 440.

Isermann K 1969 Aufnahme und Verlagerung von Calcium bei der höheren Pflanze. Dissertation, Universität Hohenheim. 132p.

Isermann K 1978 Einfluß von Chelatoren auf die Calcium-Verlagerung im Sproß höherer Pflanzen. Z. Pflanzenernähr. Bodenkd. 141, 285–298.

Jentschke G, Schlegel H and Godbold D L 1991 The effect of aluminium on uptake and distribution of magnesium and calcium in roots of mycorrhizal Norway spruce seedlings. Physiol. Plant. 82, 266–270.

Jorns A 1988 Aluminiumtoxizität bei Sämlingen der Fichte [*Picea abies*(L.) Karst.] in Nährlösungskultur. Ber. Forschungsz. Waldökosysteme / Waldsterben (Göttingen), Reihe A, Bd. 42, 86p.

Junga U 1984 Sterilkulturen als Modellsystem zur Untersuchung des Mechanismus der Aluminium-Toxizität bei Fichtenkeimlingen (*Picea abies* Karst.). Dissertation, Universität Göttingen.

Kottke I and Oberwinkler F 1986 Mycorrhiza of forest trees - structure and function. Trees 1, 1–24.

Kottke I, Guttenberger M, Hampp R and Oberwinkler F 1987 An in vitro method for establishing mycorrhizae on coniferous tree seedlings. Trees 1, 191–194.

Kreutzer K and Göttlein A 1991 Ökosystemforschung Höglwald. Verlag Paul Parey, Hamburg, Berlin. 261p.

Kuhn A J, Schröder W H, Bauch J, Haug I, Kottke I and Oberwinkler F 1988 Mikrosonden-Untersuchungen zum Ionenaustausch und zur Ionenaufnahme in sterilen und mykorrhizierten Feinstwurzeln. *In* Das Forschungsprogramm Waldschäden am Standort "Postturm", Forstamt Farchau / Ratzeburg. Eds. J Bauch and W Michaelis. pp 255–286. GKSS-Forschungszentrum Geesthacht, GKSS 88 / E / 55.

Kuhn A J 1993 Mikrosonden-Analysen zur Ionenaufnahme in Fichten (*Picea abies*[L.] Karst.). Berichte des Forschungszentrum Jülich, Jül-2744, ISSN 0366-0885. Dissertation Universität Hamburg, Fachbereich Biologie (1992). 204p.

Läuchli A 1976 Apoplasmic transport in tissues. Enc. Plant. Physiol. 2B, 3–34.

Lüttge U and Higinbotham N 1979 Transport in Plants. Springer-Verlag, New York-Berlin-Heidelberg. 468p.

Marschner H and Schimansky Chr 1968 Unterschiedliche Aufnahme von Kalium und Rubidium durch Gerste. Naturwissenschaften 55 (10), 499.

Marschner H and Römheld V 1983 In vivo measurement of root-induced pH changes at the soil-root interface: Effect of plant species and nitrogen source. Z. Pflanzenphysiol. 111, 241–251.

Marschner H 1991 Mechanisms of adaptation of plants to acid soils. Plant and Soil 134, 1–20.

Mascorro J A, Ladd M W and Yates R D 1976 Rapid infiltration of biological tissues utilising n-hexenyl succinic anhydride (HXSA) / vinyl cyclohexene dioxide (VCD), an ultra-low viscosity embedding medium. 34th. Ann. Proc. E.M.S.A. pp 346–347.

Michaelis W and Bauch J 1992 Luftverunreinigungen und Waldschäden am Standort "Postturm", Forstamt Farchau / Ratzeburg, GKSS-Forschungszentrum Geesthacht GmbH., GKSS 92 / E / 100. 455 p.

Michaelis W, Fanger H U, Niedergesäß R and Schwenke H 1985 Symp. Instrumental Multielement Analysis, April 2–5, Jülich. *In* Instrumentelle Multielement-Analyse. Ed. B Sansoni. p 693. VCH Verlagsgesellschaft, Weinheim.

Mohr H 1994 Stickstoffeintrag als Ursache neuartiger Waldschäden. Spektrum der Wissenschaft 1, 48–53.

Mullette K J 1975 Stimulation of growth in *Eucalyptus* due to aluminium. Plant and Soil 42, 619–636.

Murach D 1984 Die Reaktion der Feinwurzeln von Fichten (*Picea abies* Karst.) auf zunehmende Bodenversauerung. Göttinger Bodenkd. Ber. 77, 1–126.

Puhe J, Persson H and Börjesson I 1986 Wurzelwachstum und Wurzelschäden in skandinavischen Nadelwäldern. Allg. Forstz. 20, 488–492.

Rademacher P 1986 Morphologische und physiologische Eigenschaften von Fichten (*Picea abies* [L.] Karst.), Tannen (*Abies alba* Mill.), Kiefern (*Pinus sylvestris* L.) und Buchen (*Fagus sylvatica* L.) gesunder und erkrankter Waldstandorte. GKSS-Forschungszentrum Geesthacht, GKSS 86 / E / 10. 274p.

Rengel Z 1992 Role of calcium in aluminium toxicity. New Phytol. 121, 499–513.

Rost-Siebert K 1983 Aluminium – Toxizität und Toleranz an Keimpflanzen von Fichte (*Picea abies* Karst.) und Buche (*Fagus silvatica* L.). Allg. Forstz. 38 (26/27), 686–689.

Rost-Siebert K 1985 Untersuchungen zur H- und Al-Ionen-Toxizität an Keimpflanzen von Fichte (*Picea abies* Karst.) und Buche (*Fagus sylvatica* L.) in Lösungskultur. Ber. Forschungsz. Waldökosysteme / Waldsterben (Göttingen) 12, 1–219.

Ryan P R, Ditomaso J M and Kochian L V 1993 Aluminium toxicity in roots: an investigation of spatial sensitivity and the role of the root cap. J. Exp. Bot. Vol. 44, No. 259, 437–446.

Schaedle M, Thornton F C, Raynal D J and Trepper H B 1989 Response of tree seedlings to aluminum. Tree Physiol. 5, 337–356.

Scheffer F and Schachtschabel P 1976 Lehrbuch der Bodenkunde. Ferdinand Enke Verlag, Stuttgart. 394p.

Schimansky Chr 1991 Einfluß von Aluminium und Protonen auf die Aufnahme und den Transport von Magnesium (^{28}Mg) bei Gerste (*Hordeum vulgare* L.). Z. Pflanzenernähr. Bodenkd. 154, 1–4.

Schröder W H 1981 Quantitative LAMMA analysis of biological specimens. I. Standards. II. Isotope labelling. Fresenius Z. Anal. Chem. 308, 212–217.

Schröder W H, Bauch J and Endeward R 1988 Microbeam analysis of Ca exchange and uptake in the fine roots of spruce: influence of pH and aluminum. Trees 2, 96–103.

Schulze E D 1989 Air pollution and forest decline in a spruce (*Picea abies*) forest. Science 244, 776–783.

Schütt P 1977 Das Tannensterben. Der Stand unseres Wissens über eine aktuelle und gefährliche Komplex-Krankheit der Weißtanne (*Abies alba* Mill.). Forstwiss. Centralbl. 96, 177–186.

Spurr A R 1969 A low viscosity embedding medium for electron microscopy. J. Ultrastructure Res. 1, 239–246.

Squires G L 1971 Meßergebnisse und ihre Auswertung. Walter de Gruyter, Berlin-New York. 240p.

Steinbrecht R A and Zierold K 1987 Cryotechniques in Biological Electron Microscopy. Springer-Verlag, Berlin-Heidelberg, N Y, London, Paris, Tokyo. 297p.

Stienen H and Bauch J 1988 Element content in tissues of spruce seedlings from hydroponic cultures simulating acidification and deacidification. Plant and Soil 106, 232–238.

Taylor G J 1991 Current views of the aluminum stress response, the physiological basis of tolerance. Curr. Top. Plant Biochem. Physiol. 10, 57–93.

Tischner R, Kaiser U and Hüttermann A 1983 Untersuchungen zum Einfluß von Aluminium-Ionen auf das Wachstum von Fichtenkeimlingen in Abhängigkeit vom pH-Wert. Forstwiss Centralbl. 102, 329–336.

Thomas W A 1967 Dye and calcium ascent in dogwood trees. Plant Physiol. 42, 1800–1802.

Thomas W A 1969 Accumulation and cycling of calcium by dogwood trees. Ecol. Monogr. 39, 101–120.

Türk S, Gülpen M and Fink S 1993 Aufnahme, Transport und Verbleib von Calcium und Magnesium in Fichten (*Picea abies* [L.] Karst.) und Kiefern (*Pinus silvestris* L.) bei unterschiedlicher Ernährung und Schadstoffbelastung. Forstwiss. Centralbl. 112, 191–208.

Ulrich B 1981 Eine ökosystemare Hypothese über die Ursachen des Tannensterbens (*Abies alba* Mill.). Forstwiss. Centralbl. 100, 228–236.

Wagatsuma T, Kaneko M and Hayasaka Y 1987 Destruction process of plant root cells by aluminum in the roots. Soil Sci. Plant Nutr. 33, 161–175.

Weast R C 1989 Handbook of Chemistry and Physics. 70st edn. Chemical Rubber Co., Cleveland.

Wieneke J 1969 Calciumtranslokation und Ätiologie der Stippigkeit beim Apfel. Der Erwerbsobstbau 11. Jahrg., Heft 12, 225–231.

Wieneke J 1974 Untersuchungen zur Translokation von ^{45}Ca im Apfelbaum. II. Transport zur Frucht und Verteilung. Gartenbauwissenschaft 39, 57–67.

Wieneke J and Führ F 1973 Untersuchungen zur Translokation von ^{45}Ca im Apfelbaum. I. Transport und Verteilung in Abhängigkeit

vom Aufnahmezeitpunkt. Gartenbauwissenschaft 38 (20), 91–108.

Wilkinson R E and Duncan R R 1993 Calcium ($^{45}Ca^{2+}$) absorption inhibition by aluminum (Al^{3+}) in *Sorghum* roots. J. Plant Nutr. 16, 235–240.

Woods F W and Brock K 1964 Interspecific transfer of Ca-45 and P-32 by root systems. Ecology 45, 886–889.

Zech W and Popp E 1983 Magnesiummangel, einer der Gründe für das Fichten-und Tannensterben in NO-Bayern. Forstwiss. Centralbl. 102, 50–55.

Zöttl H W and Hüttl R F 1985 Schadsymptome und Ernährungszustand von Fichtenbeständen im südwestdeutschen Alpenvorland. Allg. Forstz. 9/10, 197–199.