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In-situ application of stable isotope tracers in the rhizosphere of an oak seedling

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Abstract In a controlled rhizotrone experiment, stable isotope tracers of Mg, Ca and K were applied directly to the rhizosphere of an oak seedling using a 2D-array of micro ceramic cups. Before starting isotope application the oak root induced a significant reduction of K^+ , Ca^{2+} , Mg^{2+} and NO_3^- in the soil solution of the rhizosphere, as well as an increase of Al^{3+} . The effect of adding stable isotopes in the soil (soil solution and exchangeable cations) was mainly restricted to a distance of about 1 cm from the point of application. All stable isotopes were taken up by the oak seedling, especially Ca which according to leaf analysis was in the range of insufficiency. As expected, Ca showed low mobility in the phloem, resulting in a low percentage of label in the root tip as compared to other root segments. Our experiment proved, that in-situ application is an easy to handle tool for carrying out tracer studies in real soil.

Introduction

Micro suction cups (Göttlein et al. 1996) in combination with micro analytical methods (Göttlein and Blasek 1996) have been used successfully for the collection and characterization of soil solution with a spatial resolution

of 1 cm and below. This resolution is high enough to observe root-induced changes in soil solution chemistry. In experiments with oak (Göttlein et al. 1999), spruce (Dieffenbach et al. 1997) and beech (Wang et al. 2001) a depletion of nutrient cations in the soil solution of the rhizosphere could be observed. The main effects were restricted to distances smaller than 5 mm to the growing root, as expected from findings in the literature made for root-induced changes in pH (Schaller and Fischer 1985), water extractable cations (Kirlow and Bouldin 1987), phosphate depletion (Claassen et al. 1981) and according to model calculations (Kuchenbuch et al. 1986; Nye and Marriot 1969).

To prove, whether ion depletion is caused by ion uptake of the root, tracer studies are a valuable tool. Experiments for metal cation uptake using stable isotopes, however, up to now only have been done using solution culture or by immersing roots to a solution containing the labeled cations (Kuhn et al. 1995, 2000). The in-situ application of stable isotope tracers to the rhizosphere soil would avoid the disadvantage of an unnatural environment. For this purpose, micro ceramic cups may serve as an 'on the point' application system. To test whether this is a promising approach, we conducted a rhizotrone experiment, the aim of which was to prove the practicability of the application system, to estimate the propagation of the added label in the soil and to verify the uptake of the label by the plant.

Material and methods

Seedlings of oak (*Quercus robur* L.) were obtained by germination of acorns on moist cellulose at 15°C. When their root reached a length of 1 cm, two acorns were planted in a dual chamber rhizotron filled with 660 ml of an acid forest soil (B-horizon of a Cambic Podsol derived from granitic bedrock; $pH_{H_2O} = 4.21$; $CEC_e = 75.2 \mu mol_c/g$; $BS = 6.6\%$; for details see Manderscheid and Göttlein 1995). The soil was dried, sieved (< 2 mm) and homogenized before filling the rhizotron. For irrigation,

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porous ceramic cups were embedded in the soil when filling the rhizotrones and set to a water potential of -60 hPa (for details see Göttelein et al. 1999). The irrigation solution was obtained by a batch soil water extract (soil water ratio 1/3.3), which in order to get a good equilibrium subsequently for one time was slowly percolated through the rhizotrone. The rhizotrone was set up at an angle of 45° , so that the roots were forced to grow along the transparent front plate. The front plate was covered by a black plastic sheet, which was removed only for root observation. The root showing the best development and contact to the front plate was chosen for the experiment. A dense grid of 30 micro suction cups having an outer diameter of 1 mm (Göttelein et al. 1996) was placed just in front of the growing root. The micro-suction cups were installed in such a way, that in its further development the root passed the nearest micro-suction cup at a maximum distance of 2.5 mm (see Fig. 1).

Micro-suction cups were continuously sampled under a vacuum of -300 hPa and samples were taken at intervals of 3 days. Solutions were analyzed by capillary electrophoresis (Phoresis1000, Thermo Separation Produkts) using a pyromelic acid buffer for anions and a metol buffer for cations (for details see Göttelein and Blasek 1996). To simplify data presentation the individual measurements were grouped according to week number and distance class (see Table 1). After 7 weeks (51 days after installing the suction cups) four micro-suction cups near the main root were taken for the application of stable isotopes. We used a solution containing 0.86 mg/l Mg [$^{25}\text{MgSO}_4$], 3.17 mg/l Ca [$^{44}\text{Ca}(\text{NO}_3)_2$] and 3.08 mg/l K [^{41}KCl], which was about double the concentration of Mg and threefold the concentration of Ca and K as compared to the solutions uninfluenced by root growth. About 0.5 ml of isotope solution per day was slowly injected using syringes. After 14 days the experiment was stopped and the root was cut into 1-cm segments according to Fig. 2. The root segments, as well as the acorn, the axis and the leaves were analyzed for stable isotopes by a laser-microprobe-mass-analyzer (LAMMA, compare Kuhn et al. 1995, 2000).

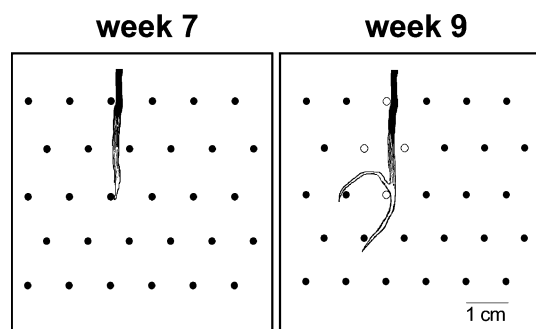


Fig. 1 Installation scheme of micro suction cups and root development. The open circles in week 9 indicate the micro cups taken for isotope application

Table 1 Mean soil solution concentrations (mg/l) of cations and anions before (week 7) and after (week 9) isotope application, grouped according to their distance to the application cups

Distance to isotope application (cm)	Before isotope application (week 7)				After isotope application (week 9)			
	0	1–2	2–3	≥ 3	0 ^a	1–2	2–3	≥ 3
K ⁺	0.34*	1.09	1.17	1.26	3.08	1.15	1.34	1.29
Ca ²⁺	0.46*	0.78	0.75	0.87	3.17	0.85	0.93	0.88
Mg ²⁺	0.27*	0.35	0.35	0.39	0.86	0.40	0.44	0.42
Al ³⁺	2.13*	0.17	0.16	0.08	–	0.32	0.15	0.17
SO ₄ ²⁻	14.27	11.56	13.96	16.28	3.18	16.74	14.64	17.44
NO ₃ ⁻	0.65*	1.11	0.98	1.19	8.93	3.29*	1.69	1.15
Cl ⁻	3.82	3.21	3.65	4.34	2.66	5.29	4.37	4.20

^aConcentration in isotope solution is given at distance 0; no statistics calculated for this distance class

*Significant difference between two neighboring distance classes ($P < 0.05$) as calculated by the LSD-procedure

Results and discussion

Soil solution chemistry

Figure 1 shows the installation scheme of micro-suction cups and the development of the oak root. Root growth was relatively slow and only during the last 2 weeks did the main root split into two root branches. No fine roots were formed.

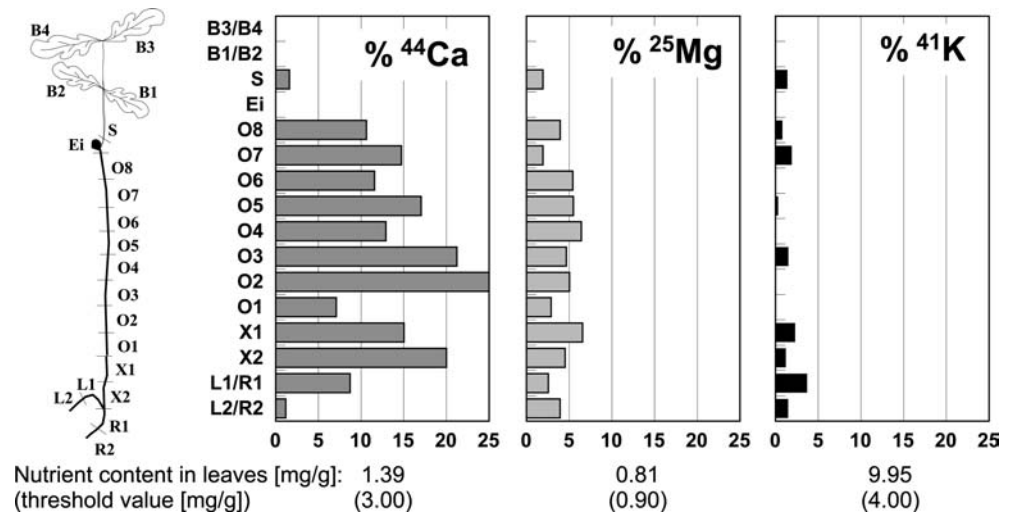
In week 7, just before starting isotope application a significant reduction of K⁺, Ca²⁺, Mg²⁺ and NO₃⁻ concentrations in soil solution could be observed in the immediate vicinity of the oak root (Table 1). In contrast to this, the Al³⁺ concentration showed a significant increase near the root. This effect could also be observed in another experiment with oak and is mainly attributed to the buffering of root exuded protons at the mineral phase (Göttelein et al. 1999). Comparable to previous studies (Göttelein et al. 1999; Dieffenbach et al. 1997; Wang et al. 2001) significant effects were restricted to distances smaller than 1 cm from the growing root. Application of the isotope solution raised the availability of K⁺, Ca²⁺, Mg²⁺ and NO₃⁻ in the immediate vicinity of the root. For K⁺, Ca²⁺ and Mg²⁺ this

Table 2 Mean percentage of stable isotopes in soil solutions at various distances from the point of application, in the root segments and estimation of an enrichment factor for the label in root tissue relative to soil solution

	Soil solution (% label)		Root segments (% label)	Enrichment factor
	d \leq 1 cm (n=3)	1 cm < d \leq 3 cm (n=2)		
K	3.1	b.d.	1.3	0.42
Ca	4.2	b.d.	12.6	3.02
Mg	24.7	2.8	4.3	0.17

n number of samples; b.d. below detection limit

Fig. 2 Proportion of stable isotopes at the total element content in different segments of the oak seedling. X_i are the segments nearest to the micro cups taken for the application of stable isotopes. L_i , R_i and O_i are the other root segments, B_i the leaves, E_i is the acorn and S the axis. Nutrient contents (mg/g) in leaves are given as well as threshold values indicating insufficient nutrient supply for mature oak (according to StMELF 1987)



effect was also restricted to distances lesser than 1 cm to isotope application. Only for NO_3^- , which was given in a nearly eightfold concentration as compared to the uninfluenced soil, a significant increase could be observed up to 2 cm distance to the point of isotope application.

Towards the end of the experiment some samples of soil solution were analyzed for their content of stable isotopes (Table 2). Up to 1-cm distance to the point of isotope application all stable isotopes could be found in the soil solution. At greater distances only ^{25}Mg was present, however, in a low percentage. The dispersion of the labeled cations in the soil should also depend on their adsorption to the solid phase. In general, cation adsorption in soils increases in the order $\text{K} < \text{Mg} < \text{Ca}$ (Schachtschabel et al. 1982). K, however, may not follow this ranking if K-fixing clay minerals are present. Comparing Ca and Mg in Table 2, it is obvious that Mg has a higher mobility in soil. The analysis of the soil solid phase (1 M NH_4Cl extract) at the end of the experiment showed that isotope application significantly raised the amount of exchangeable K, Ca, Mg and thus lowered Al-saturation in the immediate vicinity of the root. This effect also was restricted to distances smaller than 1 cm to the region of isotope application (K + Ca + Mg saturation: distance < 1 cm: $7.2\% \pm 0.7$, distance ≥ 1 cm: $3.1\% \pm 1.0$).

Uptake of stable isotopes

Experiments using stable isotopes in real soil are always tricky, because the added label is mixed with the naturally occurring ions and is also to some extent adsorbed to the exchange sites. So a low percentage of label in the plant is not necessarily an indication for low-ion uptake. In our experiment, however, we also did the laborious determination of stable isotopes for some samples of soil solution. Thus we have the possibility of estimating a relative accumulation factor, which is the ratio between percentage of label in the plant tissue and percentage of

label in close to root soil solution. For this calculation, however, we only used the below-ground compartments of the plant because there was no label detectable in the acorn and in the leaves. For the acorn, this is plausible because it is a nutrient and energy source for the germinating seedling, which loses its function and dies back as the plant gets older. However, for the leaves this finding is somewhat unexpected. It may be explained by the fact that we analyzed the leaves in total, which leads to a thinning of the label to values below detection limit. Obviously the time of 2 weeks of label application was not sufficient to bring enough label to all parts of the leaf. Also, to a minor extent, the acorn may still serve as a nutrient source for the above-ground compartments of the seedling. The highest percentage of label was found for Ca, the lowest for K. For K in some root segments, the values were below detection limit. The relative accumulation factor, as described above, indicates that Ca was preferably taken up by the oak seedling. Looking at the threshold values for sufficient nutrition, there is a clear nutritional deficit for this element. On the other hand, the low uptake of labeled K by the oak seedling may be due to the fact that the demand for this element was not very high because the plant showed very good K nutrition. In plant nutrition, it is a well-known principle that as the internal concentration of a nutrient is increasing its uptake rate is decreasing and vice versa, an effect which is well-documented for K, P, S and Fe (Marschner 1995). The threshold values given in Fig. 2, however, have to be used with caution, because they are available only for mature oak trees, not for oak seedlings. According to the data compilation given by van den Burg (1985, 1990) the nutrient demand (as expressed by nutrient concentrations in leaves) of juvenile oak, however, is comparable to that of mature oak trees.

The amount of label found in the root segments documents the different mobility of the cations within the plant (Larcher 1994). Ca is a cation that is nearly immobile in the phloem and thus is only translocated in acropetal direction. There are high amounts of ^{44}Ca in all root segments near the application cups and above.

In the root segments below the application cups, there is a marked decrease towards the root tips. Mg and especially K have a higher mobility in the phloem and thus can also be translocated in basipetal direction. This is consistent with the distribution of ^{25}Mg and ^{41}K in our experiment, reaching concentrations in the root tips comparable to other root segments. In-situ application offers the possibility of selectively feeding particular root zones, which are known to have different capabilities for ion uptake (Häussling et al. 1988). Especially, ion uptake by suberized and older roots could be easily studied by this method, a topic which is not very much considered in literature, but which is essential for the mineral nutrition of perennial plants (Chung and Kramer 1975; Comerford et al. 1994)

Conclusions

Micro-ceramic cups proved to be a suitable tool for in-situ application of tracer substances directly to selected zones of the rhizosphere of a growing root. The main effects of cation tracer application in soil and soil solution were restricted to a distance smaller than 1 cm from the point of application. Within the plant, the different mobility of Ca as compared to Mg and K in acropetal and basipetal direction confirm common literature knowledge. Obviously the oak seedling was able to selectively take up Ca, which according to leaf analysis was in the range of deficit.

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