Spatial and temporal variation in organic acid anion exudation and nutrient anion uptake in the rhizosphere of Lupinus albus L.

Plant Soil (2007) 301:123–134
DOI 10.1007/s11104-007-9427-x

REGULAR ARTICLE

Spatial and temporal variation in organic acid anion exudation and nutrient anion uptake in the rhizosphere of *Lupinus albus* L.

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Received: 12 December 2006 / Accepted: 16 September 2007 / Published online: 9 October 2007

Abstract We investigated *in situ* the temporal patterns and spatial extent of organic acid anion exudation into the rhizosphere solution of *Lupinus albus*, and its relation with the nutrient anions phosphate, nitrate and sulfate by means of a rhizobox micro suction cup method under P sufficient conditions. We compared the soil solution in the rhizosphere of cluster roots with that in the vicinity of normal roots, nodules and bulk soil. Compared to the other rhizosphere and soil compartments, concentrations of organic acid anions were higher in the vicinity of cluster roots during the exudative burst (citrate, oxalate) and nodules (acetate, malate), while concentrations of inorganic nutrient anions were highest in the bulk soil. Both active cluster roots and nodules were most efficient in taking up nitrate and phosphate. The intensity of citrate exudation by cluster roots was highly variable. The overall temporal patterns during the lifetime of cluster roots were overlaid by a diurnal pattern, i.e. in most cases, the exudation burst consisted of one or more peaks occurring in the afternoon. Multiple exudation peaks occurred daily or were separated by 1 or 2 days. Although citrate concentrations decreased with distance from the cluster root apex, they were still significantly higher at a distance of 6 to 10 mm than in the bulk soil. Phosphate concentrations were extremely variable in the proximity of cluster roots. While our results indicate that under P sufficient conditions cluster roots take up phosphate during their entire life time, the influence of citrate exudation on phosphate mobilization from soil could not be assessed conclusively because of the complex interactions between P uptake, organic acid anion exudation and P mobilization. However, we observed indications of P mobilization concurrent with the highest measured citrate concentrations. In conclusion, this study provides semiquantitative *in situ* data on the reactivity of different root segments of *L. albus* L. in terms of root exudation and nutrient uptake under nutrient sufficient conditions, in particular on the temporal variability during the lifetime of cluster roots.

Keywords Citrate · Cluster roots · *Lupinus albus* · Micro suction cups · Phosphate · Rhizobox

Responsible Editor: Philippe Hinsinger.

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Introduction

Several plant families have the ability to exude organic acid anions as a tolerance mechanism or as an adaptation to soils with low nutrient availability. *Lupinus albus*, a white-flowered Eurasian herb (Fabaceae-Leguminosae) that is widely cultivated for forage and also used for erosion control, is well known for its cluster roots that exude large amounts of citrate into the rhizosphere to cope with phosphate deficiency (Dinkelaker et al. 1995). Cluster roots are defined as a cluster of rootlets densely covered by root hairs that develop synchronously along a given length of a parent root (Dinkelaker et al. 1995; Marschner 1995; Skene et al. 1998). These specialized roots release citrate into the rhizosphere in a so-called exudative burst that is characterized by a sudden and marked release of high amounts of organic acid anions including citrate and malate as well as phosphatase and protons into the rhizosphere. This high exudative state lasts for 2–3 days before returning to a base level of exudation (senescence of the cluster root). This stage is followed by a decomposition of the cluster root after several weeks (Neumann and Martinoia 2002).

Citrate, one of the main compounds exuded by *L. albus*, is a short-chain molecule with three carboxylic acid groups allowing the complexation of metal cations in solution and the displacement of anions from the soil matrix. This property explains the important role of organic acid anions in several soil processes such as mobilization and uptake of nutrients by plants and microorganisms or detoxification (e.g. Al tolerance) (Gerke et al. 2000; Hue et al. 1986; Jones 1998; Jones and Darrah 1994; Marschner 1995). The plant availability of P is limited to a large extent by the rate of the reaction that replenishes the pool of soluble P. The benefits of having organic acid anions in the rhizosphere are twofold: they compete with phosphate groups for binding sites in the soil, and they form stronger complexes with Al, Fe and Ca than phosphate (Ryan et al. 2001). Thus, they may help to release phosphate from inorganic phases by ligand exchange or ligand-enhanced dissolution (Johnson and Loeppert 2006).

The exudation of organic acid anions by *L. albus* has been extensively investigated. Nevertheless, most of the studies have been performed using hydroponic systems (Neumann et al. 1999; Watt and Evans 1999b), by extraction of organic acid anions from rhizosphere soil separated from the roots (Bayon et al. 2006; Dinkelaker et al. 1989; Gardner and Parbery 1982a, b; Gerke et al. 1994; Hagström et al. 2001; Li et al. 1997) or by comparing the composition of leachates from pot or soil columns experiments (Egle et al. 2003; Gardner et al. 1983a; Johnson et al. 1996; Shen et al. 2004; Shu et al. 2005).

However, roots in hydroponic systems can behave differently compared to natural conditions in soils (Marschner 1995) and the growth medium appeared to affect the cluster root formation (Peek et al. 2003; Shu et al. 2005; Watt and Evans 1999b). Furthermore, soil extracts do not allow temporal studies and leachates from pot and soil columns give information on a whole rhizosphere system without any precise spatial information. Thus there is a need to get a better understanding on the temporal variability of organic acid anion exudation by *L. albus* cluster roots, and its spatial impact on nutrient availability in the cluster root rhizosphere solution as compared to the rhizosphere solution of other roots and bulk soil solutions.

The development of micro techniques for collection and analysis of soil solution has enabled microscale observation of soil solution chemistry (Göttlein et al. 1999; Göttlein et al. 1996; Vetterlein and Marschner 1993; Vetterlein et al. 1993). The use of micro suction cups in conjunction with rhizoboxes that allow to observe the development of root systems and sampling of the soil solution at defined distances from roots, has a large potential to study rhizosphere chemistry (Arocena et al. 2004; Dessureault-Rompré et al. 2006; Dieffenbach et al. 1997; Göttlein et al. 1999; Wang et al. 2004).

In our study we wanted to test the hypotheses (1) that different root segments of *L. albus* differ in root exudation and their effect on soil solution composition, (2) that exudation of organic acid anions by lupin cluster roots exhibits a diurnal variability, and (3) during intensive exudation of organic acid anions by cluster roots phosphate is strongly mobilised. To this end, we investigated *in situ* the temporal and spatial patterns of organic acid anions exuded by cluster roots of *L. albus* and their relation to soil solution chemistry, in particular to nutrients such as phosphate, nitrate and sulfate by means of a rhizobox micro suction cup method (Dessureault-Rompré et al. 2006). In addition, we compared cluster root soil solution with soil solution composition in the vicinity of "normal" roots, nodules and in the bulk soil. In

particular, no studies have been done so far according to our knowledge characterizing the soil solution composition surrounding nodules, which are of interest because of their symbiosis with rhizobia and their ability to fix atmospheric nitrogen.

Materials and methods

Rhizobox system

The rhizobox used in this study was described in detail by Dessureault-Rompré et al. (2006). It was adapted from the one introduced by Dieffenbach et al. (1997). We used a carbonate free soil (pH 6.4 (0.01 M $CaCl_2$), 15.1 g/kg C_{org}, 1.5 g/kg N_{tot}, 49 mg/kg $P_{available}$ (Kuo 1996), 862 mg/kg P_{org} (Kuo 1996), 36% sand, 49% silt, 15% clay). The soil was air dried, sieved (2 mm) and filled into the rhizoboxes at a bulk density of about 1.2 g/cm^3. First, the rhizoboxes were rinsed with synthetic rainwater using the irrigation system described below operating at a positive head for 6 weeks (1 l of leachate was collected from each rhizobox each week) in order to equilibrate the soil. Then the experimental conditions described below were established. Seeds of *L. albus* ("Weissblühende Tellerlupine" cultivar, Ufa AG, Switzerland) were pre-treated with 10% hydrogen peroxide (Liang and Li 2003) and then germinated in black garden soil for 1 week. The plants were not inoculated with rhizobia but nodules were visible during the experimental period. Healthy plants were gently washed with deionised water to remove the organic black soil and transplanted into the rhizoboxes 1 week after establishing experimental conditions. Three rhizoboxes planted each with a single plant were used in this study.

The rhizobox experiment was conducted under controlled conditions in a climate chamber (light 16 h with an intensity at canopy height of 150 µm m^{-2} s^{-1}, 80% humidity, temperature day/night: 20/16°C). The boxes were irrigated with synthetic rain water (ionic composition in µM: 70 NH_4, 70 NO_3, 3.2 PO_4, 17 Cl, 3.1 SO_4, 4.3 Na, 7.7 K, 5 Ca, 1.3 Mg, 0.15 Zn, pH= 5.5) using wicks that were made from a polymer tube (Rhizon irrigators, Rhizosphere research products, Netherlands) and installed at 5, 30 and 55 cm soil depth. A hanging water column of 40 cm was maintained between each wick and a corresponding reservoir. With these settings the following water fluxes occurred within the rhizobox system. In plant free rhizoboxes, covered to minimize evaporation, the gravitational water flow from the upper two irrigation wicks to the lowest irrigation wick and the bottom of the rhizobox was measured to be approximately 15 ml day^{-1}. Assuming a porosity of 50%, this amounts to a downward flux of 2 cm day^{-1}. Evaporation from the soil surface, measured in uncovered rhizoboxes, was about 10 ml day^{-1} or equal to an overall upward flux of 1.3 cm day^{-1} (corresponding to 1.7 mm day^{-1} of evaporation). In the planted rhizoboxes during the sampling periods, however, no outflow was observed. Total irrigation volume was about 80 ml day^{-1} with each irrigation wick delivering roughly the same amount of around 25 ml day^{-1}. This corresponded to about 20 ml of sampled solution plus an over-all evapotranspiration flux of about 60 ml day^{-1} or 10 mm day^{-1}.

After the experiment, the shoots and roots of the plants were separated from each other. The root system was characterized in terms of parental root length as reference for the number of cluster roots. Then the plant material was dried at 65°C and ground using a vibrating ball mill (Retsch MM 2000) equipped with an agate milling tool. The total chemical composition of shoots and roots were determined by ICP/OES analysis of acid micro wave digests.

Soil solution sampling

Samples were collected through the transparent front plate of the rhizoboxes as described by Dessureault-Rompré et al. (2006). The emergence of rootlets was the starting point for rhizosphere solution sampling around cluster roots. This happened between 4 and 7 weeks after sowing for the individual cluster roots, which were sampled for a period of 7 to 10 days, three times per day for 8 h (6–14 h, 14–22 h, 22–6 h). The positioning of the micro suction cups allowed a sampling-free time at each position of 16 h to reequilibrate the soil (Vetterlein and Jahn 2004). A total of nine micro suction cups were installed around a cluster root at three different distances to get spatial information (Fig. 1). Each layer of micro suction cups included the three daily sampling periods. The three layers of micro suction cups (<1, 1–5 and 6–10 mm from the rootlet apex) were operated alternatively for 8 h per day (6–14 h, 14–22 h, 22–6 h). The nine

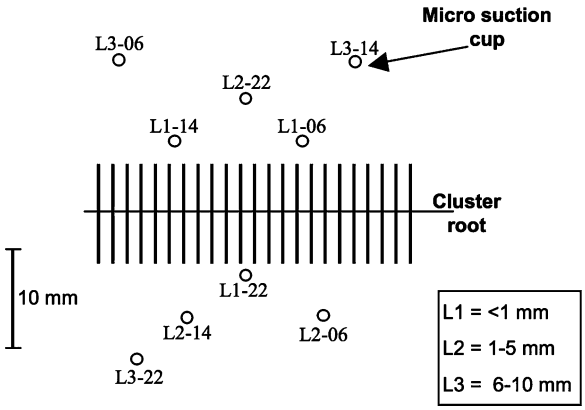

Fig. 1 Distribution of micro suction cups around a cluster root. For each position the layer (LX) and the beginning of the sampling period (06, 14, and 22 h) is indicated

micro suction cups were installed in such a way that (1) the interaction between the individual cups was minimized, and (2) their relative positions to the cluster roots were randomized. On 21 parental roots of about 60 cm length each, 11 cluster roots developed and were sampled (one cluster root per 115 cm parental root length; measured at the end of the experiment). However, only the results from seven cluster roots were included in the data analysis and interpretation. For the four remaining cluster roots, there were too many missing data due to insufficient material for analysis. During the 14–22 h time periods, we also sampled the soil solution along six "normal" roots (NR), near 10 apices of "normal" roots (NRA), near three nodules (NOD) and at 15 bulk soil locations (BS; >2 cm from the nearest root). For details on the sampling devices and sampling procedures we refer to Dessureault-Rompré et al. (2006).

Soil solution analysis

The volume was recorded for each soil solution sample. The samples were analyzed for low molecular weight organic acid anions (LMWOA) (acetate, citrate, formate, lactate, malate, oxalate, propionate) and inorganic anions (sulfate, nitrate, and phosphate) using ion chromatography (Dionex autosampler system, AS 11 column, eluent: potassium hydroxide (1 to 60 mM), flow: 1.5 ml min^{-1}) with 200 µl insert glass vials to reduce the sample volume needed. Statistical differences for spatial and temporal data were tested using ANOVA. If p values indicated significant differences at a level of <0.05, *post hoc* pairwise comparisons were carried out using Bonferroni/Dunn adjustment of probabilities. Analyses were carried out using SYSTAT 11.0.

Batch experiment to test phosphate mobilization from soil by citrate

Five grams of samples (oven-dried weight basis) of the same soil as used for the rhizobox experiment were shaken with 25 ml citrate solution (0, 500, 1,000, 2,500, 5,000 µM) in a 50 ml polypropylene centrifuge tube for 24 h at room temperature (~20°C). Formaldehyde solution was added at a concentration of 2% to the citrate solution to prevent microbial degradation of the organic acid anions during the experiment. The soil suspension was centrifuged at 1,060 g for 15 min and the supernatant filtered through Whatman Grade 602 $h^{1/2}$. Phosphate and citrate in the extracts were analyzed using ion chromatography as described above.

Results

Comparison between bulk and rhizosphere soil solutions

Table 1 shows the average concentrations of the measured anions in the soil solution of the different soil compartments sampled between 14 and 22 h. Shown are the means and standard errors of the average values measured at the individual sampling positions. The average concentrations of citrate and oxalate in layer 1 of the cluster root rhizospheres were higher than in all other compartments. Citrate concentrations in the NRA and NOD rhizospheres were lower than in the CR rhizospheres but larger than in the NR compartment and in the bulk soil. Malate concentrations were higher in all rhizosphere compartments compared to the bulk soil, but highest around nodules. Acetate showed a similar spatial distribution as malate. For propionate and lactate no significant differences were observed. However, there was a tendency for propionate to be higher near the nodules.

Phosphate and nitrate concentrations around cluster roots and nodules were lower than in all other compartments. When compared to the bulk soil, nitrate concentrations were also lower in the NRA and NR

Table 1 Organic acid and inorganic anion concentrations (in μM) sampled at 14–22 h in the rhizosphere solution of cluster roots (CR) in layer 1 (<1 mm), apices of normal roots (NRA), normal roots (NR), and nodules (NOD) of *Lupinus albus* as well as in bulk soil solution (BS)

	CR (n=7)	NRA (n=10)	NR (n=6)	NOD (n=3)	BS (n=15)
Citrate	1,231.3±570.1^{c}	31.9±12.4^{b}	10.9±12.4^{a}	37.7±20.3^{b}	6.3±10.3^{a}
Oxalate	16.7±12.0^{b}	4.2±4.1^{a}	3.0±6.4^{a}	5.4±3.9^{a}	3.2±0.9^{a}
Malate	3.0±0.4^{b}	1.7±2.3^{b}	1.7±1.5^{b}	7.1±2.8^{c}	0.2±0.9^{a}
Acetate	54.0±3.8^{b}	30.9±20.5^{ab}	19.4±10.0^{a}	153.2±63.2^{c}	25.3±19.4^{a}
Propionate	6.6±1.2^{a}	6.8±5.4^{a}	6.3±6.1^{a}	9.3±9.2^{a}	6.5±6.6^{a}
Lactate	29.1±1.9^{a}	35.2±12.1^{a}	33.4±9.2^{a}	27.5±12.3^{a}	33.6±11.6^{a}
Phosphate	70.9±16.2^{a}	152.6±8.3^{b}	163.2±15.1^{b}	109.4±25.3^{a}	184.2±73.3^{b}
Nitrate	33.1±13.2^{a}	154.8±19.9^{b}	101.6±23.8^{b}	29.7±9.3^{a}	301.6±124.5^{c}
Sulfate	26.2±4.6^{a}	26.0±6.8^{a}	19.8±5.4^{a}	15.7±7.5^{a}	45.8±17.2^{b}

Shown are the means and standard errors of the average concentrations at n individual sampling positions. In each row different letters indicate significant differences at $p<0.05$.

rhizospheres. Sulfate concentrations in the bulk soil were higher than in all rhizosphere compartments.

Temporal variability of organic acid anions in the cluster root rhizosphere

Figure 2 shows the rhizosphere concentrations of citrate during the lifetime of the seven cluster roots with a complete data set. The intensity and duration of the bursts of citrate differed considerably between individual cluster roots. Based on the data for layer 1, the behavior ranged from single exudation events (CR 2) to multiple exudation events separated by 1 (CR 1, 3, 4, 5), 2 (CR 7), or 3 days (CR 6). The concentration maxima occurred always in the time period from 14 to 22 h. The citrate concentrations in layer 3 were generally much smaller than in layer 1, but in most cases with an approximately similar temporal variation. The exception was CR 4, where during the second part of its life time high concentrations were observed in layer 3.

For most cluster roots, oxalate and malate concentrations in layer 1 varied irregularly at a low level (oxalate <10 μmol l^{-1}; malate <5 μmol l^{-1}; data not shown). Only for the two cluster roots characterized by particularly high citrate exudation (CR 1 and 2) a more specific behavior could be observed. Cluster root 2 was characterized by a pronounced maximum of malate of about 40 μmol l^{-1} during the same sampling period as the citrate maximum. Oxalate, however, varied irregularly. For CR 1, the multiple citrate maxima were exactly mirrored by oxalate maxima of 50 to 300 μmol l^{-1}, while only the second and fourth citrate maxima were coupled to maxima of malate of about 40 μmol l^{-1}. Furthermore, both oxalate and malate exhibited additional maxima of similar intensity during the second part of the lifetime of CR 1 with no correspondence in citrate.

Temporal variability of inorganic anions in the cluster root rhizosphere

Figure 2 also shows the rhizosphere concentrations of phosphate in layer 1 for all cluster roots with a complete data set. Generally, concentrations were lower than in the bulk soil solution (184 μM, dotted line). For most cluster roots (CR 2 to 7) phosphate exhibited a roughly similar behavior. Concentrations decreased somewhat or were low during the first part of the cluster root lifetime and increased slightly towards the end. The variability from sampling period to sampling period was small for CR 3, 5, and 7, while it was rather large for CR 2, 4, and 6. For CR 1, the very high citrate concentrations during the multiple exudation events were paralleled by elevated phosphate concentrations.

Figure 3 shows a plot of all citrate and phosphate data from layer 1 (<1 mm). No correlation between citrate and phosphate is visible. High P concentrations were observed both for low and high citrate concentrations and high citrate concentrations were often accompanied by low P concentrations. Moreover, two interesting observations can be made from the graph: concentrations of P higher than the bulk soil concentration are accompanied by intermediate citrate concentrations (10–1,000 μM) and at higher citrate

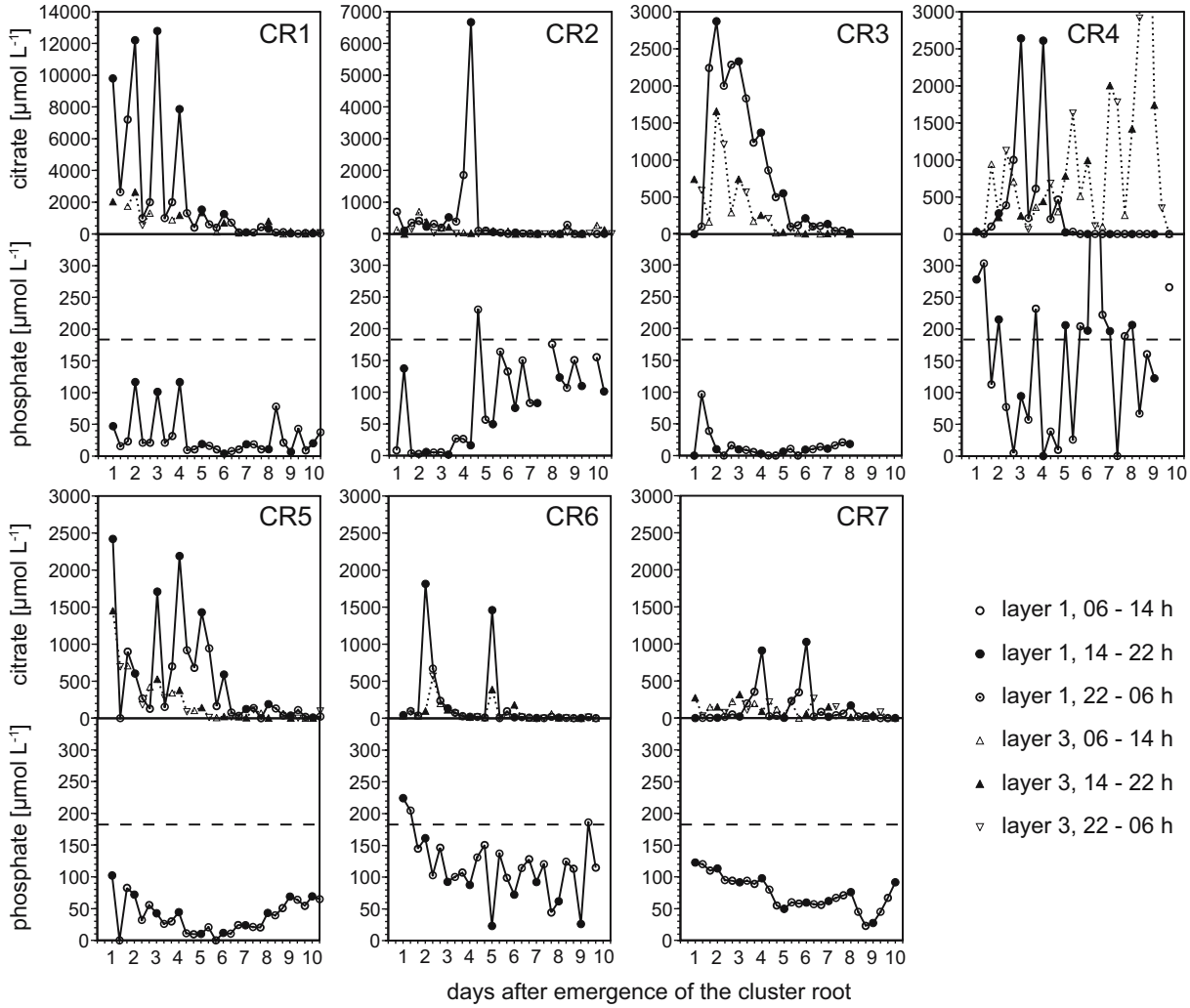

Fig. 2 Temporal variability of citrate (*upper part of panels*) and phosphate (*lower part of panels*) concentrations in the layers 1 and 3 of all seven *Lupinus albus* cluster roots with a complete data set. For easier reference in the text, the cluster roots are numbered from CR1 to CR7

concentrations the tendency for low phosphate concentrations is greater.

Nitrate and sulfate concentrations in layer 1 varied irregularly without obvious temporal patterns (data not shown).

Spatial distribution of organic acid and inorganic anions in the rhizosphere of cluster roots

Figure 4 shows that, for all daily sampling periods, the average concentrations of citrate and oxalate in soil solution during the time period of maximum exudation decreased with increasing distance from the rootlets. The differences were statistically significant, however, only for citrate during the 14–22 h sampling period, when concentrations even at a distance of 6–10 mm from the rootlets were larger than in the bulk soil.

While nitrate and phosphate concentrations were significantly higher in the bulk soil than in the cluster root rhizosphere, there were no significant differences between the concentrations of these anions at different distances within the rhizosphere.

Mobilization of phosphate by citrate in batch experiments

The batch experiment showed that citrate was able to mobilize phosphate from the used soil. The concen-

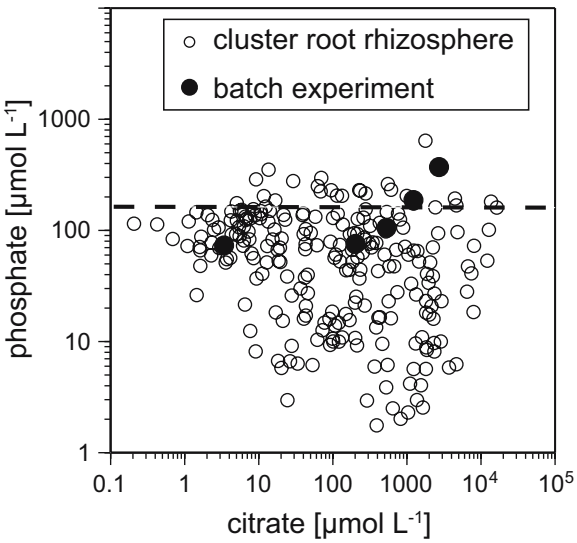

Fig. 3 Relationship between citrate and phosphate concentrations in layer 1 (<1 mm) of cluster roots (*open circles*) and in the batch experiment (*filled circles*). The *line* shows the phosphate concentration in the bulk soil solution

tration of mobilized phosphate was described by the following equation:

$$\mathrm{P}\left[\mu\mathrm{mol\,l^{-1}}\right] = 0.114 \times \mathrm{citrate(in\ equilibrium\ solution)} + 56.57\left(R^2 = 0.992\right)$$

The data are also presented in Fig. 3 (filled circles). Below 1.2 mM dissolved citrate there was little mobilization of phosphate but the phosphate concentration increased nearly five times at the highest input of citrate.

Discussion

Influence of sampling and system fluxes

Based on the observed water fluxes in the rhizoboxes one can conclude that during the sampling periods the major fluxes were the ones caused by transpiration and by sampling. Only at sampling positions in greater distance from roots, i.e. in the bulk soil or in layer 3 around cluster roots, some influence of gravitational flow can be expected. In the very vicinity of roots, it can be expected that, during the day, the matrix potential is smaller than applied at the irrigation wicks and gravitational flow is therefore smaller than measured in the plant free rhizobox. Together with the general considerations on the zone of influence for the sampling at a given position (Dessureault-Rompré et al. 2006), this has the following implications for the interpretation of the data: Measured concentrations should not be considered to quantitatively picture the situation at the place of sampling. They rather represent average conditions within a zone of influence that is given (1) by the sampling volume, (2) by the content of water filled pores emptying within the range of matrix potentials applied at the suction cup during sampling, and (3) by a directional preference governed by the transpirational and gravitational fluxes. Considering the dominant transpirational fluxes towards active root segments during the day, it can be expected that during this time all rhizosphere sampling positions should have a directional preference towards the bulk soil, i.e. the zone of influence is larger from the sampling position towards bulk soil than towards the root. As a consequence, the rhizosphere influence would actually be underestimated, and the measured differences between bulk and rhizosphere soil and the extent of spatial gradients is actually larger than measured. At night, when transpirational fluxes were low, the directional preference was probably smaller. An influence of gravitational flow during this time—dilution of rhizosphere influence at positions above and enhancement at positions below cluster roots—would have been averaged out by the even distribution of spatial orientations of cluster roots.

With respect to the temporal resolution of the sampling around cluster roots, it has to be noted that an overlap of the zones of influence of positions sampled during different time periods could not be avoided completely because of the small dimensions of the cluster roots. Thus, sampling at a given position captured also water from a not sampled position within its zone of influence.

Root exudation by cluster roots

Our results show that the intensity of citrate exudation by cluster roots is highly variable, although some influence by the exact distance of layer 1 from the rootlets cannot be excluded. The overall temporal patterns during the lifetime of a cluster roots are overlaid by a diurnal pattern, i.e. in most cases, the exudation burst actually consists of one or more peaks

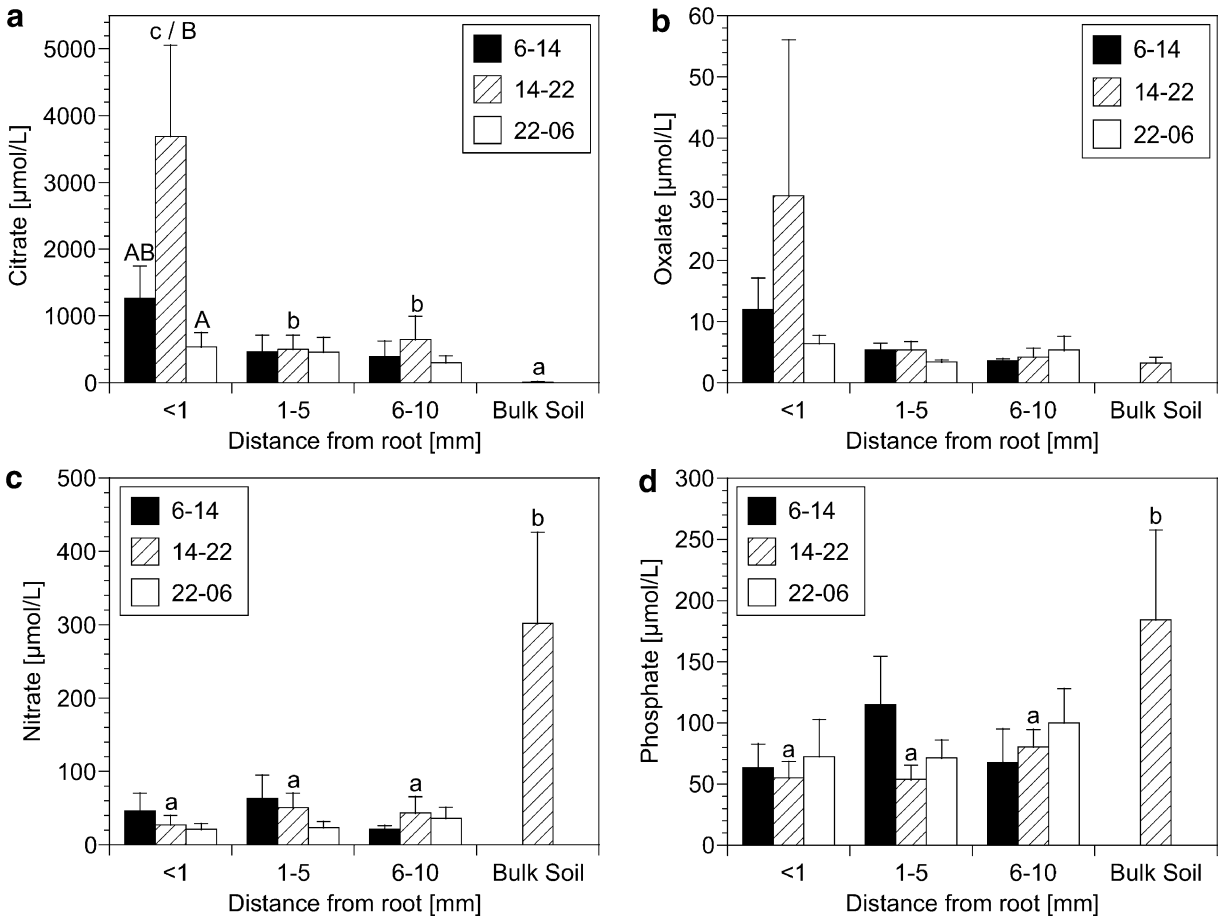

Fig. 4 Spatial and diurnal variation of citrate **a**, oxalate **b**, nitrate **c** and phosphate **d** concentrations in the rhizosphere of cluster roots of *Lupinus albus* during the time of maximum activity beginning on the 6–14 h sampling period before the first exudation peak and ending at the 22–6 h sampling period after the last exudation peak. Shown are the means and standard errors of the average concentrations for all seven cluster roots with a complete data set stratified for sampling period and distance. *Lower case lettering* denote significant differences between different distances at the same sampling period, *upper case lettering* significant differences for different sampling periods at the same distance (n=7 for CR, N=15 for BS, p<0.05)

occurring in the afternoon. Multiple exudation peaks can occur daily or be separated by one or 2 days.

The chance that hidden cluster roots are partly responsible for multiple peaks is small considering the following. (1) The size of the cluster roots was in the same range as the width of the rhizoboxes (1 cm) and the cluster roots formed dense rootlets with an average length of 5 mm. This spatial restriction reduced the possibilities of two cluster roots growing just one behind the other. (2) The rhizosphere around cluster roots was sampled to a distance of up to 1 cm. The gradient of decreasing concentrations of citrate with increasing distance from the root indicated that only one single cluster root was sampled, because a second hidden cluster root would have to be exactly at the same position and orientation relative to the suction cups to cause such a spatial concentration pattern. An exception is CR 4. Here, the data from layer 3 suggest the appearance of a second cluster root nearby during the second half of its lifetime. (3) The small spatial density of cluster roots, mentioned above, further reduced the chance of several cluster roots to be located very close to each other.

The mechanism by which exudation occurs is not well known (Watt and Evans 1999b), but it seems that carboxylate exudation may be sensitive to plant P status (Shane and Lambers 2005). Thus, if the exudation of citrate follows a diurnal pattern it should be in some way also linked to the P level of the plant. Major biochemical processes such as photosynthesis

and respiration are activated by inorganic phosphate or its organic derivatives (Raghothama and Karthikeyan 2005). We can hypothesize that when the plant is highly physiologically active in the second period of the day, plant P level may decrease to a certain threshold thus stimulating a signal for the exudative burst. On the other hand, the high production and release of organic anions can be directly linked to the high photosynthetic activity during this period of the day.

It is generally assumed that concentrations of exuded organic acid anions in soils rapidly decrease with time and with increasing distance from the roots due to microbial consumption, adsorption to soil surfaces and diffusion in the soil solution (Jones 1998). On the other hand, Weisskopf et al. (2006) revealed several mechanisms by which *L. albus* is able to reduce microbial activity during the most active period of a cluster root lifetime. The generally very strong diurnal variation during the exudation bursts suggests a rapid disappearance of the citrate exuded in the afternoon. The occurrence of significantly elevated citrate concentrations at a distance of 6–10 mm from the cluster roots at all daily sampling periods suggests, that the disappearance, apart from removal by sampling, microbial consumption, and adsorption to soil surfaces, can be attributed partly also to diffusive or convective transport away from the root.

Studies on spatial distribution of organic acid anions in soil are rare (Darrah 1991; Gardner and Boundy 1983; Gardner et al. 1983b; Hagström et al. 2001; Jones et al. 2003). Our results support the conclusions from early studies by Gardner et al. (1983, 1983a) on plants with cluster roots that there is a certain movement of the exuded organic acid anions in the soil. In an agar film experiment of Gardner et al. (1983b) citrate released by proteoid roots was effective over considerable distances of at least 5 mm from the nearest root. Gardner and Boundy (1983) found that plants with cluster roots also enhance nutrient acquisition of other plants rooting in their vicinity. On the other hand, Jones et al. (2003) suspected that, in general, due to the very low diffusion coefficients of most organic acid anions in soil, the size of hot spots of organic acid anions released from the tip of a root hair, fungal hyphae or bacterial cell, may be only a few μm in diameter. The relatively large zone around lupin cluster roots influenced by exudation may thus just be due to the high intensity of the exudative bursts and protective mechanisms inhibiting microbial consumption (Weisskopf et al. 2006).

Our results on organic acid anions other than citrate are not conclusive. To a large part this is probably due to the generally rather low concentrations and the respective analytical uncertainty. This is supported by the fact that more specific temporal patterns for oxalate and malate, however only partly mirroring citrate, were observed in cases where concentrations were rather high, i.e. for CR 1 and 2.

Root exudation and phosphate concentration in the rhizosphere soil solution of cluster roots

At the end of our experiment, the P level in the shoot of *L. albus* was sufficient (500 μmol g^{-1} dry weight) as was the level of other macro and micro nutrients (Marschner 1995), which is in good agreement with the high concentrations of available soil P and the high P concentrations in the bulk soil solution. The observed temporal patterns of citrate exudation are valid for these conditions, but might be different under P stress conditions. From the literature it is known that the citrate exudation is inversely related to plant P status (Keerthisinghe et al. 1998). The variability of exudation between different cluster roots of one single lupin plant, however, has not received much attention so far. Nevertheless, it is somewhat surprising to observe concentrations of up to 13 mM of citrate in cluster root rhizospheres under P-sufficient soil conditions, in particular when considering the postulated dilution effect by sampling. On the other hand, it is known that the frequency and extent of P-deficiency can determine the spacing and length of rootlets along the proteoid root axis. As the severity of phosphorus stress increases, the proportion of the root system covered with proteoid rootlets increases (Watt and Evans 1999a). When P is absent from the growth medium of lupin, cluster roots may constitute more than 50% of the root dry mass compared to 5% when P is sufficient (Gilbert et al. 2000). The low density of 1 cluster root in 115 cm of parental roots in our rhizoboxes are in good agreement with the observations by Watt and Evans (1999a) and Gilbert et al. (2000) for P-sufficient conditions. Our results may thus suggest that the plant's main adaptation to the P status of the soil is to

adjust the spatial density of cluster roots rather than the amount of organic acid anions exuded from individual cluster roots. However, this needs further similar studies under different P conditions.

It is commonly believed that citrate is exuded to mobilize phosphate from the soil. The reaction of organic acid anions is expected to be highly dependent on soil type and thus mobilization of P is highly a soil specific reaction (Jones and Darrah 1994). Nonetheless, soil extraction experiments and theoretical calculations indicate that at least a millimolar carboxylate concentration in soil solution is required to achieve efficient P mobilization (Neumann and Martinoia 2002; Neumann and Römheld 2000; Penaloza et al. 2002). This is confirmed by the results of our batch experiment, which in turn indicates that our soil behaved like a "normal" soil regarding phosphate mobilization. This can explain why a correlation between exudation and P mobilization could only be observed for the extremely high citrate peaks of CR 1. Otherwise our data only suggest that under P sufficient conditions cluster roots take up phosphate during their whole lifetime. The irregular temporal variability of phosphate concentrations during the lifetime of the cluster roots may be explained (1) by the fact that they were governed mainly by two competing processes, root uptake and mobilization from the soil matrix by organic acid anions, (2) by the influence of other root related processes such as the release of phosphatases and their impact on organic phosphate, and (3) by spatial variability of mobilizable soil P.

Exudation and nutrient uptake by normal roots and nodules

The higher citrate concentrations in the NRA rhizosphere compared to the NR rhizosphere and the BS are in good agreement with the root apex being the most active part of a root. However, compared to the cluster roots the concentrations of organic acid anions attributed to root exudation by normal roots were quite small. A key difference between exudation by cluster and normal roots is related to the fact that cluster root exudation occurs at the same location whereas the main region of exudation by normal roots moves through the soil as the root elongates (Skene 2003). It is the combination of the cluster root capacity to release carboxylates in an exudative burst and their morphological structure that allows the build-up of high concentrations in the rhizosphere (Lambers and Colmer 2005).

In addition, our study provides some first *in situ* characterization of the soil solution near root nodules. The presence of large amounts of acetate compared to other rhizosphere compartments is probably just indicative of the particularly high microbial activity in and around nodules. The elevated malate concentrations in the nodule rhizosphere may be related to the synthesis of malic acid serving as intermediate compound in the production of C substrates for the nitrogenase in the bacteroids (Marschner 1995). Considering the function of nodules to supply the plant with nitrogen by converting N_2 into ammonium, the intensive nitrate uptake by the nodules is probably related rather to microbial consumption than to plant uptake.

Conclusions

From our *in situ* soil solution study on citrate exudation by *L. albus* cluster roots under P sufficient conditions we conclude the following.

1. The overall temporal patterns during the lifetime of cluster roots are overlaid by a diurnal pattern, i.e. in most cases, the exudation burst actually consists of one or more peaks occurring in the afternoon. Multiple exudation peaks can occur daily or be separated by one or 2 days.
2. Our data are in favor of the notion that exuded citrate can diffuse in the soil solution to a distance of more than 5 mm from the cluster root apices.
3. Mobilization of phosphate by citrate could not be assessed conclusively, probably because of the complex interactions between P uptake, organic acid anion exudation and P mobilization. On the other hand, our results indicate P mobilization concurrent with the highest citrate concentrations encountered which supports earlier observations that only very high citrate concentrations lead to a significant P mobilization.

Furthermore, our study demonstrated again the potential of the rhizobox micro suction cup technique to study the influence of individual roots on soil

solution chemistry. In particular, this method seems to be well suited for a more detailed future study on rhizosphere effects around root nodules.

Acknowledgements This work was supported by project C02.0084, State Secretariat for Research and Education, Switzerland, within COST Action 631 (Understanding and Modelling Plant–Soil Interactions In the Rhizosphere Environment) and the Fond Québécois de la Recherche sur la Nature et les Technologies. The rhizoboxes were designed and produced by Arthur Kölliker, WSL. Lena Püschel helped with the set-up of the rhizoboxes and the intensive sampling of soil solutions.

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