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**Influence of root exudation of white lupine (*Lupinus albus* L.) on uranium
phytoavailability in a naturally uranium-rich soil**

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- 26 - Rhizotest study with contrasted P offer tested citrate effect on U phytoavailability
- 27 - Small (0.4% total U) but easily accessible U pool in the tested natural U-rich soil
- 28 - Accessible U pool in soil was not significantly affected by P or citrate concentration
- 29 - U translocation to shoots, but not global uptake, was related to lupine exudation rate
- 30 - Lupine plants extracted 25-40% of the estimated U available pool in 5 days

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Abstract

Mechanisms of uranium (U) transfer from soil to plants remain poorly understood. The kinetics of supply of U to the soil solution from solid phases could be a key point to understand its phytoavailability and implications for environmental risk assessment. Root activity, particularly the continuous release of organic acids in the rhizosphere, could have an effect on this supply. We tested the impact of citrate exudation by roots of *Lupinus albus*, either P-sufficient (P+) or P-deficient (P-), on the phytoavailability of U from a naturally contaminated soil (total content of 413 mg U kg⁻¹) using a rhizotest design. Combined effects of P (P-/P+ used to modulate plant physiology) and citrate (model exudate) on the solubilization of U contained in the soils were tested in closed reactors (batch). The batch experiment showed the existence of a low U available pool (0.4% total U) and high accessibility (k_d' around 20 L kg⁻¹) which was not significantly affected by P treatment or citrate concentrations. Analysis of U, Fe, Ca, P and citrate concentrations in the batches suggested a complex combination of mechanisms and factors including desorption, resorption, precipitation, co-sorption. On rhizotest, *L. albus* plants extracted 0.5 to 0.75% of the total U and between 25 and 40% of the estimated available U present in the rhizotest in 5 days. Uranium accumulation at the whole plant level (20 mg U kg⁻¹_{d.w.}, shoot to root ratio around 10⁻³) seemed to be dependent neither on the plant P nutrition status nor citrate exudation level, possibly in relation with the equivalent accessibility of U whatever the growth conditions. Yet differential translocation to shoots seemed to be positively correlated to citrate exudation.

Key-words

Lupinus albus L.

Natural U-rich soil

59	Rhizotest
60	Batch extraction
61	Exudation
62	Uptake
63	
64	

1. Introduction

Uranium is an ubiquitous radioactive metal with an average soil concentration of 3 mg kg⁻¹. Due to anthropogenic activities, U concentrations in soils can reach locally several hundreds of mg U kg⁻¹, which may result in contamination of soil and water resources (Ribera et al., 1996; Bourrelie and Berthelin, 1998; Ragnarsdottir and Charlet, 2000). The different U-mining operations (drilling, ore processing, on site-storage on tailings, post-mining operations) have resulted in areas where the environment may be contaminated by U (Pfeifer et al., 1994) and other metals (e.g. Ba, frequently used in exhaust water treatment). Phosphate rocks used as fertilizers in agriculture are also a source of U (among other metals) in cultivated soils: concentrations in phosphate fertilizer can reach several hundreds of mg U kg⁻¹ (Romero Guzman et al., 2002), with application of several hundred kg fertilizer per ha and per year.

Uranium has no physiological role in plant. Root uptake and translocation to shoots are very variable and result in Transfer Factors ($\text{Bq U kg}^{-1}_{\text{dry weight shoots}} / \text{Bq U kg}^{-1}_{\text{soil}}$) from 10⁻⁵ to 10⁻² (IAEA, 2010). In plant, U is mainly associated to roots, with concentrations as high as 10² mg U kg⁻¹_{d.w. roots} (Dushenkov et al., 1997; Tailliez et al., 2013). Phytotoxic effects have been recorded on growth and development (Sheppard et al., 1992; Straczek et al., 2009), chlorophyll content (Aery and Jain, 1997) and, oxidative stress (Vandenhove et al., 2006a, Vanhoudt et al., 2008). Uranium also affects the plants indirectly through interferences with phosphate (Misson et al., 2009) or iron (Viehweger and Geipel, 2010; Doustaly et al., 2014) homeostasis. Despite all these data, it is still difficult to clearly establish dose-response relationships between the concentration of U in soil (or soil solution), the concentration or distribution of U in plants, and their induced phytotoxicity (Sheppard et al., 2005). Indeed, depending on the study, toxic effects on plants have been recorded for total concentrations of U in soil that range from background levels (a few mg U kg⁻¹_{soil}) up to several hundred mg U

90 $\text{kg}^{-1}_{\text{soil}}$ (Sheppard et al., 1992), even thousands of $\text{mg U kg}^{-1}_{\text{soil}}$ (Meyer and McLendon, 1997;
91 Stojanovic et al., 2009). These discrepancies may hardly be related to parameters like plant
92 species or toxicity range, but may rather be related to the environmental bioavailability and
93 phytoavailability of U. Parameters responsible for U phytoavailability in soils are not well
94 understood despite the large literature available on U behaviour in soils (e.g. Ragnarsdottir
95 and Charlet, 2000). Indeed, way(s) by which U enters the root and moves in the plant are still
96 unidentified. In addition, studies in which speciation of U in solution or soil solution had been
97 explicitly considered have shown that several U species other than the free uranyl ion had to
98 be taken into account to correctly predict its transfer to plants (Ebbs et al., 1998; Vandenhove
99 et al., 2006b, Laroche, 2005; Mihalik et al., 2012). These studies allowed to hypothesize that
100 rhizospheric processes (processes at the soil/root interface, as defined by Hinsinger, 1998 or
101 Hinsinger et al. 2005), such as uptake and exudation, may drive the U phytoavailability.
102 Physico-chemical conditions in the rhizospheric soil may differ considerably from those of
103 the bulk soil because of root activities involving notably exudation processes. Variation of
104 rhizospheric pH and/or exudation of complexing agents (e.g. citrate), allow plants to stimulate
105 desorption of nutrients (e.g. Fe, P) from the soil solid phase, increases their solubility in soil
106 solution and subsequently their uptake and translocation (Duffner et al., 2012; Röhmeld,
107 1987; Vance et al., 2003; Briat, 2008). Citrate is continuously exuded by plant roots when
108 plants are experiencing Fe or P starvation (Kahm et al., 1999; Hinsinger, 2001). However,
109 organic acids are also good chelators for U, and they have been efficiently used in
110 amendment-assisted phytoremediation studies of U-contaminated soils (Huang et al. 1998;
111 Duquène et al., 2008; Mihalik et al., 2012), although it has been argued that its efficiency
112 might be limited because of the large amount needed and its quick biodegradability (Jones,
113 1998). It has been stated that citrate-U complexes may be available in the rhizosphere through
114 release of uranyl ion and/or uptake of complexes, which may also be the/one of the plant

circulation forms allowing for enhanced translocation (Laurette et al., 2012a, b). In soils, U is frequently associated with P and Fe carrying phases (Pfeifer et al., 1994; Payne et al., 1996; Fuller et al., 2002; Raicevic et al., 2006). Thus, during root exudation of protons or organic acids to increase P and Fe desorption from the solid phase (Hinsinger, 1998; McLaughlin et al., 1998; Kahm et al., 1999), U concentration may also increase in the rhizosphere. Finally, the phytoavailability of U may depend on the concomitant behaviour of the released elements, whether they are absorbed by roots, as free ion or complexes, or are subjected to precipitation, coprecipitation or resorption processes onto the soil solid phase.

The objective of this study was to evaluate if exudation of a model organic acid, namely citrate, may participate in maintaining a high U phytoavailability in soil solution. An experiment was performed with a modified RHIZOtest® (Bravin et al., 2010), used with naturally U-rich soils and white lupine plants (*Lupinus albus*). Plants were either P-starved or P-sufficient during the pre-culture period prior to soil exposure, to induce two different levels of citrate exudation. Uranium accumulated in roots and shoots were assessed with respect to citrate exudation level. We used white lupine because it is a model plant for P study, which induces proteoid roots exudating a high level of organic acids when P-starved (Keerthisinghe et al., 1998; Tailliez et al., 2013). To gain more insights on the dynamics of U at the soil/soil solution interface in relation with P and citrate levels, batch dynamic desorption experiments were also conducted.

2. Material and Methods

2.1. U-contaminated soils

The tested soil was sampled in the vicinity of one of the most U-concentrated pitchblende veins existing in Europe, on the site of La Creuzaz/Les Marécottes, 7 km West from Martigny, Switzerland. This site has been described by Pfeifer et al. (1994). It was characterized by an

on site radioactivity measurement (CoMo 170 analyser (Saphymo, France), 15 cm from the soil surface). The top soil (A horizon, 0-15 cm following removal of the OL horizon) was sampled, homogenized, dried at room temperature and sieved at 2-mm mesh size before use. Soil properties (accredited analysis; INRA, LAS, Arras, France) and soluble U (ICP-AES, see 2.4.3.3) analysis are displayed in Table 1. The soil is classified as Colluviosol (RP, 2008). It is characterized by an acidic pH and a high total U content of around 400 mg kg⁻¹. The available P content (P Olsen) is rather low as related to agricultural standards. During the study, 3 other soils were collected at different distances from the pechblende vein, in order to get a naturally-produced U gradient in the “same” soil, among which 2 were chosen. A second soil (soil 2) had similar properties but a higher U content (500 mg U kg⁻¹_{soil}) and was situated downwards soil 1 although the gradient was supposed to be related to distance from the vein. This could have signed a peculiar behavior regarding speciation, migration or (bio) availability. Thus, the complete experimental set up described for soil 1 was applied to soil 2. Results were equivalent to those of soil 1 are thus not detailed in the text but can be seen in supplementary S4.

2.2. Plant species

Seeds of white lupine (*Lupinus albus* L., cv. Amiga) were provided by S.A.S. Florimond-Desprez (Cappelle en Pévèle, France). The seed lot was treated by Wakil XL (Syngenta Agro S.A.S., Guyancourt, France) before use to prevent the post-germination development of diseases. Seeds were calibrated at 300±20 mg before use to guarantee a homogeneous initial development of the seedlings. Seeds were surface sterilized using a four-step protocol as described in Tailliez et al. (2013). They were re-moistened in ultrapure water for 24h at 24°C in the dark in order to homogenize and synchronize their germination.

2.3. Solutions

2.3.1. Nutrient solutions

The nutrient solution composition was identical to previous hydroponic studies (Tailliez et al., 2013). The basic composition was: 2 mM $\text{Ca}(\text{NO}_3)_2$, 0.7 mM K_2SO_4 , 0.5 mM $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.1 mM KCl , 20 μM $\text{Fe}(\text{III})\text{-EDTA}$, 10 μM H_3BO_3 , 0.5 μM $\text{MnSO}_4 \cdot \text{H}_2\text{O}$, 0.5 μM $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 0.2 μM $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, 0.01 μM $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$. Phosphorus was supplied as 100 μM (P-sufficient condition for plant physiology, labelled P+ in the document) or 1 μM (P-deficient condition labelled P- in the document) KH_2PO_4 . No pH regulation was used as it would have interfered with the lupine roots physiology regarding P status.

2.3.2. Solution for root exudate collection

For root exudate collection, a specifically-designed solution was used (Horst W., personal communication, 2011) to ensure the integrity of the biological membranes while not interfering with further organic acids analysis. Its composition was: 0.25 mM CaSO_4 , 10 μM H_3BO_3 , 0.5 μM $\text{MnSO}_4 \cdot \text{H}_2\text{O}$, 0.5 μM $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 0.2 μM $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ and 0.01 μM $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$.

2.4. Study of U phytoavailability in the rhizotest device

Lupine plants were grown and exposed to soil in a specifically designed device similar to the RHIZOtest[®] described in detail in Chaignon and Hinsinger (2003) and Bravin et al. (2010) (Figure 1). This device is composed of 2 parts, the upper one, closed at its basis by a 30- μm nylon mesh membrane, on which plant roots develop a root mat during the preculture step, and the bottom one, receiving the soil layer. Both are put into close contact during the exposure step. In this device, sampling of soils and roots is facilitated by their physical

separation by the membrane, which does not result in a chemical separation. Thus, uptake and exudation processes are preserved.

Some of the experimental parameters (duration of culture, solution composition) were chosen so as to match the conditions used in Tailliez et al. (2013). They aimed at obtaining lupine plants in the desired physiology state (P-sufficient vs P-deficient) as piloted by P-level in solution, and discriminated by their level of citrate exudation.

Figure 2 resumed the experimental set-up. Test of soil 2 only add to this set-up rhizotests devoted to soil exposure (5 upper parts with plants and 5 bottom parts with soil per P condition), the controls being common (supplementary material S4). These were conducted as those with soil 1.

2.4.1. Preculture step in hydroponics

Plants were grown on the upper part of the rhizotest from seeds. Preliminary experiments allowed optimizing rhizotest device parameters (number of seeds, duration of pre-culture step) to get an appropriate root mat for exposure to soil, a prerequisite for the use of this device. Six sterilized and re-imbibed seeds were sown on each rhizotest device. Thirty devices (each containing 6 plants) were prepared (10 devices dedicated to soil exposure, 10 devices for growth and hydroponic control and 10 extra devices to ensure a sufficient number of healthy and homogeneous devices at the end of pre-culture). Devices were disposed in two tanks containing nutrient solutions (15 devices in P- and 15 devices in P+) continuously aerated and renewed every week. The whole dispositive was kept in a growth box under controlled conditions: 16h/8h light/night cycle, 26/20±1°C day/night temperature, 60±5% relative air humidity and light intensity of 150 $\mu\text{mol m}^{-2} \text{s}^{-1}$. Seedlings were grown in hydroponics in P+ or P- nutrient solutions for 38 days, which was the delay to get a homogeneous root mat. Due to the constraint within the root mat, less proteoid roots were

observed in rhizotests in P– conditions than in free-roots experiment described in Tailliez et al. (2013), and some also appeared in P+ conditions. Yet analysis of organic acids exudation showed that conditions were adequate to obtained two different levels of exudation with that in P- being higher than in P+.

2.4.2. Exposure to soil

Twenty rhizotest devices were prepared. Each bottom part was filled with a 2-mm-thick soil layer, corresponding to 20 g of dry soil. Bottom parts were connected to 0.5 dm³ tanks containing 50 mL of nutrient solution, P– or P+, each with 10 replicates. First, the bottom parts were incubated in the dark for 1 week, until the soil was homogenously equilibrated with the solution. The resultant soil humidity was around 20 %, close to half-saturation. Half of the rhizotest devices were kept bare as non rhizospheric controls and half received their corresponding upper parts with lupine plants. Among the 20 pre-cultured upper parts per P condition, 5 were randomly chosen to be displayed on soil. Exposure was conducted for 5 days. During pre-incubation and exposure, the nutrient solution was renewed each day.

For each P condition, 5 of the 15 upper parts launched in the pre-culture steps (previously qualified as “growth and hydroponic control”) were also randomly chosen. They were kept in hydroponics for 5 more days, in the same conditions than the rhizotests with soil (individual pot).

2.4.3. Sampling and measures

2.4.3.1. Growth of lupine plants

The growth of plants during the pre-culture step was evaluated by weekly counting the number of developed leaves.

Biomass (fresh and dry –after drying in a ventilated hood at 60°C until constant weight) of aerial parts and roots were measured: at the end of the pre-culture step on the extra rhizotests that were as healthy as the others (n=5 per P condition) and at the end of exposure, both on soil rhizotests (n=5 per P condition) and on “hydroponic control” rhizotests (n=5 per P condition).

Biomasses were around 0.5 and 1.9 g_{d.w.} rhizotest⁻¹ respectively for roots and shoots, whatever the conditions (details in Figure S1, supplementary material). Only a slight increase of biomass was recorded during the exposure phase. Biomasses were similar for both P conditions, ensuring that differences between P+ and P- rhizotests will not be related to differences in biomasses, but to other physiological differences resulting from P availability.

Periodic photography of the root mat allowed determining the appropriate time to start the exposure that is when the mesh surface was covered with roots. The volume of the nutrient solutions underneath the rhizotests was followed by periodic weighing, and evaporation was compensated by addition of new solution.

2.4.3.2. Exudation of organic acids: collection and analysis

Quantification of root mat exudation was conducted at the end of the pre-culture period on all rhizotests (per P condition, the 5 exposed rhizotests before application on soil layer, the 5 extra rhizotests and the 5 hydroponic control rhizotests) and after exposure to soil or hydroponic solution as described in Tailliez et al. (2013), with specific adaptation to the root mats of the rhizotest. Roots were first rinsed in deionised water, then submerged for 3 h into 100 mL of the root exudate collection solution.

Aliquots of 10 mL root exudates solutions were filtered on a 0.2 µm sterile filter (polyethersulfone, VWR) and kept frozen at -20°C after addition of 10⁻⁴ M NaN₃ for preservation until analysis. Aliquots were then evaporated to dryness with freeze

drying/vacuum concentration (SpeedVac, Jouan, Paris, France). Residues were dissolved in 150 μL deionised water and analysed for citrate and other organic acids with Ionic Liquid Chromatography (ILC, Dionex autosampler ICS 3000, AS 11 HC column, eluent KOH (1-45 mM, flow rate: 1 mL min⁻¹), suppressor ASRS 4 mm, detection by conductivity, injection volume: 100 μL , quantification limit: 10 $\mu\text{g L}^{-1}$). Despite several optimizing operations, it was not possible to separate correctly malate and succinate, thus only citrate, oxalate, formate, lactate and acetate acids were quantified.

2.4.3.3. Analysis of root and shoot U content

Dry biomass of roots and shoots were digested (65% HNO₃ and 30% H₂O₂, 120°C), then evaporated to dryness and redissolved in 10 mL 2% v:v HNO₃ before analysis. Uranium and major cation contents were analysed by Inductively Coupled Plasma-Atomic Emission Spectrometry (ICP-AES, OPTIMA 4300 DV, Perkin Elmer, quantification limit = 10 $\mu\text{g L}^{-1}$ for each element) or Inductively Coupled Plasma-Mass Spectrometry (ICP-MS, Agilent 7500Cs, detection limit = 10 ng L⁻¹) depending on their concentration (as related to tissue type and exposure condition).

Despite their highest purity grade, some salts, phosphate salts mainly, contained small amounts of U as impurity (< q.l. in nutrient solution). Final U concentrations measured in plants were corrected for this background, subtracting U concentrations measured in the controls (38d + 5d without soil exposure).

2.5. Batch study of U behaviour in natural U-contaminated soils

2.5.1. Calculation of citrate concentrations

Batch studies were used so as to be the “chemical” equivalent of a rhizotest. The chosen citrate concentrations in the solution were representative of those that could be present at the

soil/root interface in the rhizotest for the different conditions used (P level). We used the maximal values of citric acid exudation rates reported in hydroponics (Tailliez et al., 2013), that is $400 \mu\text{mol}_{\text{Cit}} \text{kg}^{-1}_{\text{d.w. root}} \text{h}^{-1}$ in P-U- condition and $100 \mu\text{mol}_{\text{Cit}} \text{kg}^{-1}_{\text{d.w. root}} \text{h}^{-1}$ in P+U+ condition. These values were used to calculate the corresponding quantity of citrate exuded per rhizotest (root mass from 6 plants, in contact with 20 g soil, for 5 days), corrected for the different soil saturation state in the batch system (4 g soil, V/m ratio) compared to the rhizotest. The corresponding tested concentrations in the batch system solutions were 10.15 and 40.6 mg L^{-1} citric acid, respectively for the P-sufficient and the P-deficient conditions.

2.5.2. Set up of the experiment

Preliminary experiments with different soil/solution ratios and different shaking times allowed for the definition of adequate conditions to reach an apparent steady state between soil and solution. A 50 mL vial was filled with 4 g of dry soil sieved at 2 mm and 20 mL of solution to reach the solution/soil ratio of 5 V/m (OECD, 2000). Six conditions were tested in triplicates: P+ and P- nutrient solutions previously described, 0 (C_0), 10.15 (C_{10}) and 40.6 (C_{40}) mg L^{-1} citric acid.

Two kinds of batches were tested: in the "continuous batch" the same solution was kept in n contacts with the soil for 5 days; in the "serial batch" the solution was renewed every 24 h to mimic the rhizotest experiments, the change of solution being considered as a surrogate of the "root uptake" effect. Batches were agitated at 400 rpm in a controlled incubator (dark, $25 \pm 1^\circ\text{C}$). After 24 h or 120 h, vials were centrifuged (6500 g, 1 h - Centrifuge 5430R, Eppendorf and Biofuge Stratos, Heraeus Instruments). The supernatants were recovered, filtered at $0.45 \mu\text{m}$ (polyethersulfone, VWR) and kept at 4°C until analysis. Uranium and major cations (K, Ca, Mg, Na, Fe) contents of the supernatants were analysed by ICP-AES and anions including organic acids and phosphate by ILC, as described above. Organic and

inorganic anions were analysed in one of the 3 replicates for each condition (P/citrate/type of batch/step). Calculations were performed taking into account the remaining solution in the soil residue after each centrifugation step.

Soil 2 was subjected to the same protocol than soil 1 (supplementary material S4).

2.5.3. Theoretical considerations and calculations

In batch systems, cations (among which U), inorganic anions (among which P), and organic acids (among which citrate) dynamically interacted with the soil, whether they were introduced with the test solution and/or extracted from the soil. They could be either released (desorbed) from the solid phase or disappear from the solution (being either sorbed, or part of a precipitation process or degraded). Both processes can occur simultaneously and the resulting measured concentration in solution indicated the dominant process.

Calculations were done according to Teramage et al. (2017) and are detailed below for U. The underlying hypothesis is that a fraction of U in soil is available (named U_{avail}) and thus equilibrates with the solution, with a partition coefficient named $k_{d,U}$, and that a fraction ($\%U_{fixed}$) remains fixed on the soil solid phase and never participates in the equilibrium process. At each step of the batch experiment, the mass balance of U in the batch is conserved. The analysis of U only in the supernatants at the end of each step, allows, based on mass balance, to calculate the resulting U concentration in the solid phase. The concentration of U on the soil solid phase is expressed by the following equations:

$$C_{U_solid_total_initial} = C_{U_solid_available_initial} + C_{U_solid_fixed_initial} \quad (Eq1)$$

$$C_{U_solid_total_final} = C_{U_solid_available_final} + C_{U_solid_fixed_final} \quad (Eq2)$$

with $C_{U_solid_total_initial}$, $C_{U_solid_total_final}$, $C_{U_solid_available_initial}$ and $C_{U_solid_available_final}$ respectively the total and available U concentrations ($mg\ g^{-1}$) at the initial and final step. The

initial concentration of fixed element is supposed to be constant throughout the experiment as the hypothesis is that it does not participate in the equilibrium process.

The equilibrium between $C_{U_solid-available_final}$ and $C_{U_solid-total-final}$ is given by the following equation:

$$C_{U_solid_available_final} = k'_{d_U} \times C_{U_solution_final} \quad (Eq3)$$

where $C_{U_solution_final}$ is the U concentration (in mg L⁻¹) in the solution at the end of the batch step considered and k'_{d_U} (L. kg⁻¹) is the partition coefficient between the solid available fraction and the solution.

Merging equations (Eq2) and (Eq3) gives the following formula, which is verified at each step of the batch experiment:

$$C_{U_solid_total_final} = k'_{d_U} \times C_{U_solution_final} + C_{U_solid_fixed_initial} \quad (Eq4)$$

Finally, $C_{U_solid_total_final}$ is plotted against $C_{U_solution_final}$ for each step, which allows deducing k'_{d_U} which is the slope of the linear part of the curve and $C_{U_solid_fixed_initial}$ the y-intercept.

The % of U as available and fixed pools can then be calculated.

The dataset was not complete for the C₁₀ condition on soil 2. Thus, for soil 2 only results for C₀ and C₄₀ will be displayed.

2.7. Statistical analysis

All statistical analyses were performed with R software (R Development Core Team, 2011). Results were subjected to one-way and two-way analysis of variance (ANOVA) with Tukey posthoc tests. Normality of the distributions and homogeneity of variance were verified by the appropriate tests and graphically on residuals. Heteroscedasticity was corrected when necessary by variance modelling. Results of posthoc tests are displayed through use of

different letters. Displayed values are generally means of 5 rhizotests or 3 batches, with their corresponding standard error (\pm s.e.).

3. Results

This chapter display results obtained with soil 1. Equivalent results obtained with soil 2, which can thus be viewed as a kind of replicate study, may be seen in supplementary material S4.

3.1. Relationship between root exudation and U transfers to plants (Rhizotests)

3.1.1 U accumulation in lupine plants

Most of the U was recovered in the roots (Fig. 3a) and root-to-shoot translocation was low (Fig. 3b, 3d). Accumulation in roots (Fig 3a.) was slightly higher in P- condition ($19.6 \text{ mg U kg}^{-1}_{\text{d.w. roots}}$) than in P+ condition ($17.8 \text{ mg U kg}^{-1}_{\text{d.w. roots}}$). Uranium accumulation in shoots (Fig. 3b) and shoot to root ratio (Fig. 3d) was higher in P+ condition than in P- condition. Yet, shoot U content was 4 times higher in P+ condition than in P- condition, but, due to the higher prevalence of root U stock, at the whole plant level, U content was equivalent in both P condition.

3.1.2. Root exudation of organic acids

Exudation was measured for each rhizotest at the end of the pre-culture and after exposure to soil. The results are displayed on Figure 4a for citrate, Figure 4b for oxalate and Figure 4c for formate.

Exudation was variable from one rhizotest to another and the different organic acids had different patterns. Exposure to soil 1 as compared to the pre-culture results increased citrate exudation in both P conditions. The level of citrate exudation was especially high in P+

condition ($193 \mu\text{mol}_{\text{citrate}} \text{kg}^{-1}_{\text{d.w. roots}} \text{h}^{-1}$) and it was higher than in the P- condition ($93 \mu\text{mol}_{\text{citrate}} \text{kg}^{-1}_{\text{d.w. roots}} \text{h}^{-1}$), although the ANOVA did not find the results significantly different due to high standard errors. In addition, citrate exudation was higher after soil exposure than in the corresponding controls which were exposed 5 days to nutrient solution.

Exposure to soil 1 increased oxalate exudation in the same way as that recorded for citrate for both conditions, except that oxalate flux (around $150 \mu\text{mol}_{\text{oxalate}} \text{kg}^{-1}_{\text{d.w. roots}} \text{h}^{-1}$) was higher than citrate flux. In addition, the level of oxalate exudation recorded on controls, after exposure was also higher than at the end of pre-culture. Exposure to soil 1 increased only slightly formate exudation. Surprisingly, the level of formate exudation recorded on controls was higher than those recorded with soil 1. The other organic acids were either absent or under the detection limit (acetate, lactate) or not quantifiable by our current analytical protocol.

3.1.3. Role of organic acids in the U transfer to plants

In order to assess the potential role of organic acids, especially citrate, in the U transfer to plants, U accumulation in shoots (U_{transloc}) and total U uptake (total U in plant as related to dry matter of roots, U_{up}) is displayed in figure 5, as related to the variation in citrate exudation rate measured between beginning and end of exposure period.

The U measurements in plants illustrate that less than 0.3% of U absorbed by plant was translocated to shoots whatever the conditions. At the whole plant level (Fig 5a.) the U transfer did not correlate either with the P nutrition or the level of citrate exudation as similar values of U are recorded at different mean exudation rates. On the contrary, translocation in shoots seems to correlate with the corresponding level of citrate exudation for each soil (difference between P- and P+).

3.2. U behaviour in soils in the presence of citrate (Batch experiments)

The batch experiment aimed at assessing the behaviour of U in the U-contaminated soil exposed to a known citrate concentration, with no other organic acids added (as compared to exudates) and no interference of plant physiology. Results obtained for U are displayed on Figure 6.

Results are equivalent in P⁻ and P⁺ conditions. The total amount of U desorbed is higher in serial (2-5 times) than in continuous batch, due mainly to the high extraction rate recorded during the first step. The addition of citrate increases the % U desorbed at first step. During the second step there was an inverse relation between % U desorbed and citrate concentration and no effect of citrate during the last 3 steps although citrate was still added in the solution. No citrate effect on total U desorbed was recorded in the continuous batch.

The final U concentrations on the solid phase and U concentrations in solution at each batch step were plotted, as illustrated on Figure 7. Results of the serial batches were fitted with a linear regression model, considering only the steps 2 to 5, as results obtained for the first step showed systematically a very different behaviour from the other steps, an indication either of transitory equilibrium and/or effect of peculiar mechanisms. The linear regression allows, as detailed in the calculation section, generating the slopes and y-intercepts for Eq. 4, the latter corresponding to the % U fixed (non-available) that further gives the % “available” U (Figure 8a). This pool is further qualified as “extractable pool” as it may be confusing to call it “available” pool when citrate concentrations are applied to the soil. Results obtained in P⁺ and P⁻ conditions were not significantly different. With increasing citrate concentration, the value for 1st step of the serial batch diverged towards higher U concentration in solution, which illustrated that mechanisms during the 1st day were different from the other steps. The relative difference in the results for the further steps is related to the extent of U release during the first step, but, as revealed by the close slopes of the regression lines, the behaviour of U

then is independent of P and citrate. For the C0 condition, the value of continuous batch was in good agreement with the linear function defined within steps 2 to 5. With increasing citrate, values diverged on the left-hand side of the equilibrium line.

In the absence of citrate, the U extractable pool was estimated to be $0.4 \pm 0.1\%$ of total soil U (Fig 8a.). The size of the extractable pool was not changed with the low citrate concentration (C10), but increased up to 0.75% with the high citrate concentration (C40), up to 0.75%. The extractable pool was easily accessible as shown by the corresponding low k_d' values for all modalities (Fig 8b). Values are different from those of “soluble U” displayed in Table 1 as they were not acquired in the same medium (nutrient solution vs water) and time.

3.3. Fe, Ca, P and organic acids behavior in batch experiment

Cations, anions and organic acids were analysed in one replicate of each group of 3 batches, chosen randomly. Results obtained for Ca, Fe, citrate and oxalate are displayed respectively on Figure 9a, 9b, 9c and 9d. Phosphate concentration was undetectable in solution whatever the step and condition indicating that phosphate was immediately sorbed and both initial conditions (P- and P+) ended up with the same soil solution composition. Except for the first day in serial batches (in which 20% of initial citrate was recovered in P- and 34% in P+, whatever the citrate concentration), citrate was nearly totally consumed during all steps (Fig. 9c). Calcium, and Fe concentrations in the batch solutions decreased during most steps and a correlation between Fe and U behaviours was found in initial steps in particular (as revealed by Kendall and Spearman correlation coefficient of 0.95). For continuous batch, around 12% Fe (in P- and P+) initially present in solution disappeared, with no real difference between citrate conditions (Fig. 9b). Removal of Ca from solution was higher (35.7%) in P- condition than in P+ condition (13.1%) in the absence of citrate (Fig. 9a). With increasing citrate, the % of Ca removed from solution increased to 30% in P+. For serial batch steps 2 to 5, around

30% of Fe and 35-40% of Ca disappeared, with no clear difference between P and citrate conditions (with the exception of C40_P+_day 4 condition which value was 60 %). During the first step of the serial batch, there was a marked decrease in Fe concentration in the P+ condition (40%) compared to P- condition (13.1%). Increasing the citrate concentration resulted for the highest citrate concentration in a release of Fe, the latter being higher in P+ than in P- condition. On the second day, removal of Fe in P+ condition (22-25%) reached a value that was still recorded at ulterior steps. In P- condition, Fe was less consumed in C0 and C10 conditions, with a release in C40 condition. Ca was consumed at each step with values of % Ca removed between 15 and 30% at day 1 and 35-40% further on. A small quantity of oxalate (5-10 mg kg⁻¹_{dry soil}) was released especially during the first day in serial batches, with lower values afterwards and in the continuous batches (Fig. 9d).

4. Discussion

Citrate addition in soils as an amendment is known to increase U solubility in the soil solution and further U transfer to plants (Huang et al., 1998; Mihalik et al., 2012). Roots of P-deficient plants are known to exudate large levels of organic acids, among which citrate, in order to increase P availability. Many soils may have low available P contents whether their total P content is high or not. There is thus an interest in unravelling mechanisms involving root exudation of citrate, P acquisition, U release and ultimately plant uptake in the rhizosphere in particular of plants combining exudation of protons and chelators for P or Fe (so called “strategy I plants”) acquisition.

To answer the question (whether citrate exudation may modulate U release and uptake) , we used a modified version of the RHIZOtest®, which is a normalized biotest, two naturally U-contaminated soils (in which U is supposed to be at equilibrium with the solid phase) and lupine as model plant, which exudation was piloted by P-nutrition level. The rhizotest

experiment was combined with batch experiments, providing some insights into the dynamics of elements between soil and solution (principally U), and quantifying the U available pool. As exposed in the 'soil' issue of the "Material and Methods" chapter, a second soil (soil 2) of similar properties but a higher U content ($500 \text{ mg U kg}^{-1}_{\text{soil}}$) and possible different behavior regarding speciation, migration or (bio) availability of U was tested. Results were equivalent and thus validate all statements made in this document for soil 1.

U and other elements dynamics in the soils (batch experiments)

The batch experiment aimed at giving insights on U behaviour in the tested soil in the presence of two P levels and two levels of citrate taken as model of exudate, considered as representative of those that could occur in the rhizotest experiment. Apart from U, which results have been detailed in the results section (Fig. 6, Fig. 7, Fig. 8), the dynamics of other major cations, anions and organic acids was recorded, with a specific focus on those that could form soluble complexes with citrate (U, Fe, P) and/or degrade (citrate) and/or be involved in sorption/precipitation processes with/without U (Ca, PO_4 , citrate) (Fig. 9).

Phosphate concentration was undetectable in solution whatever the step and condition indicating that phosphate was immediately sorbed and both initial conditions (P- and P+) ended up with the same soil solution composition. As a result, the main differences were observed between the different citrate concentrations only. Except for the first day citrate was nearly totally consumed during all steps, and calcium or Fe concentrations in the batch solutions decreased during most steps. Contrary to citrate, oxalate was not introduced in the system. The batch experiment was not conducted in sterile conditions, thus organic acids may be produced by microbial activity. No other organic acid was released, including citrate in C0 condition.

509 These results gave some insights into complex interactions and exchanges between elements
510 in the batches. Phosphate and citrate removed from solution, together with Ca suggest
511 complex associations between these ions and the soil matrix. Fe releases at high citrate
512 concentration, and its correlation with U, suggest that citrate had an effect on a common
513 bearing phase. This result suggests that at least part of the “available pool” of U could be
514 related to a Fe-bearing phase, also susceptible to desorption in the presence of citrate. Yet,
515 previous studies on these soils had suggested that U may be associated with Fe-
516 oxi(hydr)oxides (Pfeifer et al., 1994). The importance of adsorption of U on Fe-
517 oxi(hydr)oxides and its consequences on U dynamics in soils are well known (Hsi and
518 Langmuir, 1985; Waite et al., 1994; Duff and Amrhein, 1996; Payne et al., 1996; Lenhart and
519 Honeyman, 1999). At the soil pH (5.26 for soil 1), these oxides are positively charged thus
520 citrate, oxalate and phosphate ions can interact with them (Hsu, 1964; Parfitt et al., 1975;
521 Goldberg and Sposito, 1984). This is coherent with the observed decrease in citrate
522 concentration over time in the solution during our experiments. In addition, studies by
523 Oburger et al. (2011a,b) have shown that equilibrium between citrate or phosphate and soil
524 may be rapid, especially on Fe-bearing phases.

525 Because the batches were not performed in sterile conditions, degradation of citrate and
526 oxalate by microorganisms, which is known to be rapid (half-life of only a few hours; Jones,
527 1998), may have occurred in the continuous batch, resulting in the destruction of the
528 complexes initially formed between citrate and U (Figure 6) and thus in the release of U (and
529 Fe, P) which in turn could have undergone precipitation or re-adsorption processes on the
530 solid phase (Hafsteinsdóttir et al., 2015). Citrate, positively charged Fe oxides and U may also
531 be involved in the formation of ternary complexes, leading to the same result (Fein, 2002).
532 Allard et al. (1999) have studied the products of U-weathering from U deposits in the Massif
533 Central (France). They have shown that oxidation of U may lead to the formation of

associations with Si and Al that could then be entrapped in hydrous ferric oxides during ion precipitation. As U originates from a pitchblende vein, the studied soil sampled downwards the vein may have accumulated U either through particulate transport/erosion or in dissolved form and subsequent immobilisation through secondary U associations. Thus some of the processes mentioned above may have already occurred in this soil prior to its use in the batch experiment. Additional results of DRX and μ -fluorescence analyses performed on some soil samples (supplementary material S3) are in agreement with those statements as they have shown a mix of homogeneous U contamination and hotspots, and possible U secondary associations as stated in Allard et al. (1999) that could result in different U “bearing-phases”, characterized by different reactivity with citrate leading to variable U lability in the soil.

In absence of citrate, the continuous batch results are in accordance with the equilibrium model fitted on steps 2-5 of the serial batch. With increasing citrate concentration, the ratio between U in solid and liquid phases moved away from the line defining equilibrium. Such disequilibrium is generally due to kinetic limitations, possibly involving rearrangements of U interactions during the process.

During the first step of the serial batch, there was a high release of U, independent of P level but correlated to citrate concentration. As the soil was introduced dry in the batches, we hypothesized that soil manipulation and imbibition at the moment of batch launching had triggered a “priming effect” that could have either released dissolved organic carbon in solution and/or boosted microorganisms that can either exudate organic acids (Jenkinson, 1966; Eschenbach et al., 1998) or degrade them (Jones, 1998). This peak of chelates release (see for example the oxalate release, Fig. 9d) was responsible for the high U desorption in the absence of citrate and was superimposed to the citrate effect for the 2 other conditions. This phenomenon has also certainly occurred in the continuous batch, but degradation of citrate or

oxalate may have participated to their further disappearance in solution, in addition to their sorption onto the solid phase or precipitation/sorption of elements including U.

U phytoavailability and influence of exudation (Rhizotest experiment)

U uptake and translocation

The mean U concentration ratio (CR, ratio of shoot U concentration to soil U concentration at the end of exposure) of lupine plants after 5-days exposure to soils in the rhizotest was $(1.1 \pm 0.1) \cdot 10^{-3}$. Despite a limited time of exposure, the experimental CR was in accordance with Transfer Factor (analog of CR) values reported in the literature (IAEA, 2010) for leguminous fodder and the closest soil category tabulated, i.e. “sand” (mean value $2.4 \cdot 10^{-3}$, GSD 3.7). In accordance with existing data (Dushenkov et al., 1997; Shahandeh and Hossner, 2002; Laroche, 2005; Misson et al., 2009; Straczek et al., 2010), most of the U was recovered in the roots and root-to-shoot translocation was low ($<0.3\%$ of total plant U). Uranium accumulation at the whole plant level is thus equivalent in both P conditions due to the prevalence of root concentration (Fig 3a. and Fig 3c.). Uranium content of shoots was higher in P+ than in P- condition for both soils (Fig 3b. and Fig 3d.).

The differences between P conditions were not related to differences in the water flux through the rhizotest, as they were equivalent in all experiments (Supplementary material S2).

Effects of citrate exudation on phytoavailability

According to our calculations, batch results in C0 condition were used to estimate the U available pool in the rhizotest experiment. Plotting U accumulation results as a function of citrate exudation has shown that U translocated to shoots (Fig 5b), but not U accumulated in the whole plants (Fig 5a), was correlated to citrate exudation. The level of citrate exudation of lupine on soil, either for P+ or P- conditions, may be related respectively to C40 and C10

citrate conditions used in the batch experiment. The batch results have shown that the size of extractable U pool (Fig. 8a) increased at the highest citrate concentration. The associated k_d values (Fig. 8b) were low whatever the conditions and even decreased with increasing citrate, which showed that the extractable pool was easily desorbed. Levels of U desorbed in continuous batch were also equivalent whatever P and citrate condition.

From our results, we may conclude that: i) a small but easily accessible available U pool exists in soil (even in the absence of complexing agents), and therefore ii) exudation of organic acids such as citrate do not affect significantly U availability. Our results shows also that U accumulated by lupine represented less than 50% of the U available pool: U accumulated by lupine plants corresponded to $27.8 \pm 4.1\%$ and $25.8 \pm 2.7\%$ of the U available pool respectively for the P- and P+ conditions. Buffering of the soil solution by the solid phase was supposed to be the limiting step for phytoavailability and not plant uptake. Indeed, the affinity of plant roots for U, even if not further translocated, is high (Dushenkov et al., 1997; Shahandeh and Hossner, 2002; Laroche, 2005; Misson et al., 2009; Straczek et al., 2010). Moreover, in our experimental conditions, a combination of factors may have limited the diffusion of U to roots and favoured the soil solution/root step limitation: e.g. the low duration of contact and the geometry of rhizotest (only the surface of roots in contact with basal membrane is efficient for uptake). However, it should be kept in mind that the phytoavailability, as measured in our rhizotest experiment conditions, is thus only representative of a short time window compared to the whole growth cycle of the plant.

Part of the U absorbed by roots may be translocated to shoots, through internal physiological mechanisms that are not fully dependant of the processes controlling U exchanges at the solution/root interface. In the rhizotest experiment, a differential translocation of U to shoots of lupine plants with P status was recorded, that may be linked to the citrate exudation level. It has been shown that citrate enhanced U translocation to shoots (Laurette et al., 2012a,b;

Mihalik et al., 2012), with two underlying possible processes: uptake of citrate-U complexes or buffering of the soil solution with uranyl ion through complex dissociation at the root interface. Both may explain the enhanced translocation recorded for soil in P+ condition, as it is affected by higher citrate exudation rate in that condition.

Effects of exudation of organic acids on soils

Citrate was not the only organic acid exuded by lupine roots. For example, oxalate was also exuded at a higher rate than citrate (Figure 4), as also already recorded for lupine by Mimmo et al. (2011). Generally, citrate and malate are the two most studied organic acids regarding lupine exudation, and citrate seems to be the most effective organic acid in solubilizing inorganic phosphorus (Pi) (Oburger et al., 2011a). Oburger et al. (2011a, b) have intensively studied the dynamics of Pi and citrate in soils in order to extrapolate to rhizospheric conditions. They concluded on a complex mechanism, not fully understood and depending on numerous parameters such as soil pH, quantity of Fe/Al oxy/hydroxides (as binding phase for Pi and citrate) and concentration of metals (e.g; Fe) or other competing cations (Ca) in the soil solution, and the respective effect of organic acids as chelates and the concomitant release of protons. All of these parameters have been shown to interfere with U behaviour as also observed in our soil/plant system. Yet, in many studies including that of Mimmo et al. (2011), the effect of organic acids other than citrate, as well as mix of organic acids, or more generally rhizosphere exudation, on soil dynamics is not always described while they may be produced in significant amounts, as shown in our experiments (Fig. 4). It may be cumulative, and a better assessment of total exudation could be a more effective determinant of U availability/phytoavailability than solely citrate exudation.

4. Conclusions and perspectives

This study aimed at testing if root exudation of a model organic acid, citrate, could influence U availability and phytoavailability for lupine plants. The hypothesis was that citrate would enhance U phytoavailability, through the same mechanisms as those shown by citrate-assisted phytoextraction studies. High exudation of citrate is known to occur in P-deficient plants, thus the level of P was used to modulate plant physiology and exudation level, which has introduced more complexity in the system. Batch experiment was conducted to assess the influence of citrate alone on U availability at the soil/solution interface. The results show that the U-available pool was of limited size, but was easily extractable. As a consequence, in only one-week-exposure of the soil to lupine plants (exudation and uptake), up to 25-40% of the U-available pool depending the conditions was removed. Due to the complexity of the system, and also potentially to the apparent insensitivity of U available pool to citrate, it was not possible to conclude on the effect of citrate exudation on the U phytoavailability in the tested conditions. However, we showed that U translocation was a function of the citrate exudation level. Thus, the question of whether exudates may participate in the phytoavailability of U is still as stake, for example in soils where U speciation may be more significantly affected by the effect of organic acids. In addition, the combined effects of plant strategies towards acquisition of both Fe and P (through pH modification and/or exudation of protons/organic acids/phytosiderophores) on U phytoavailability should be assessed in future studies, at the root interface as well as for the whole growth cycle of plants.

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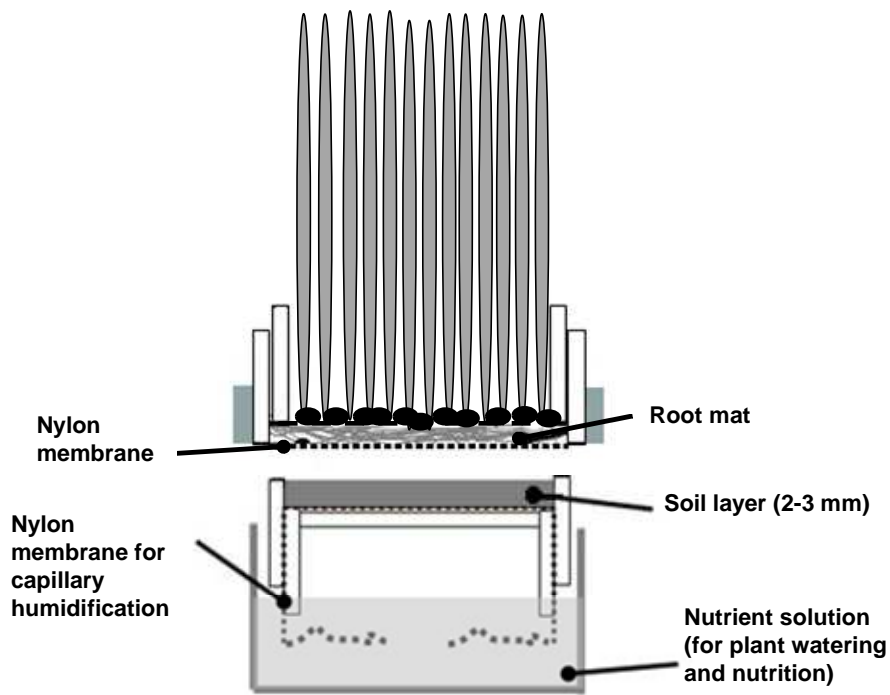


Figure 1: Rhizotest device.

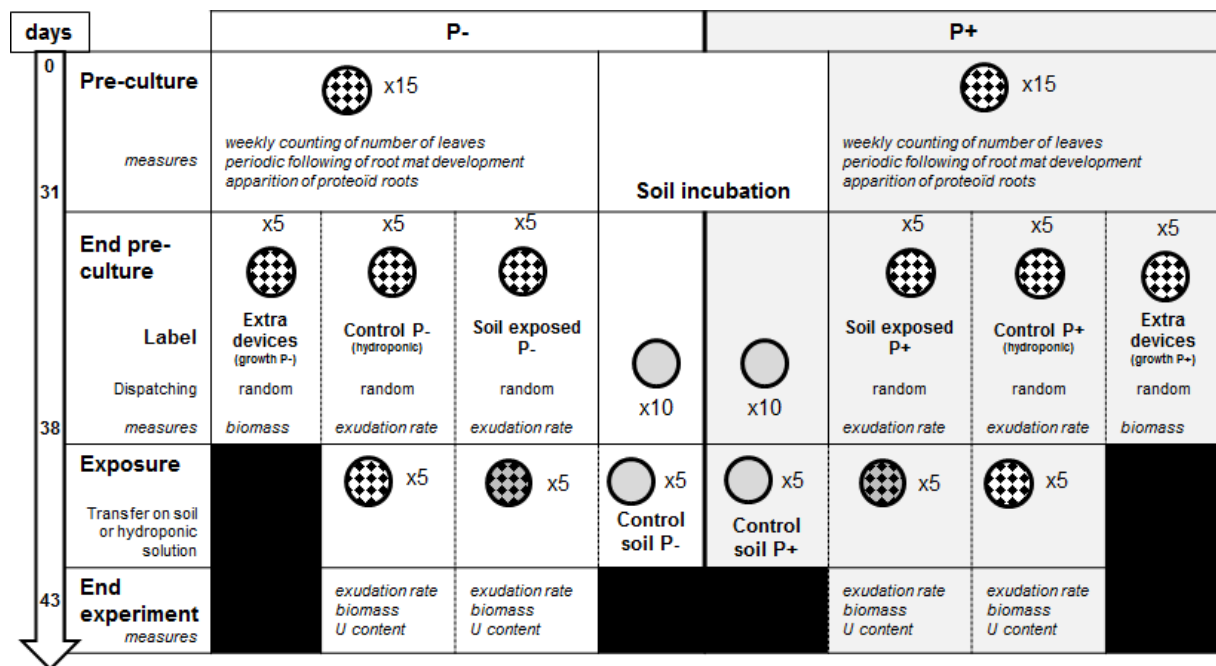
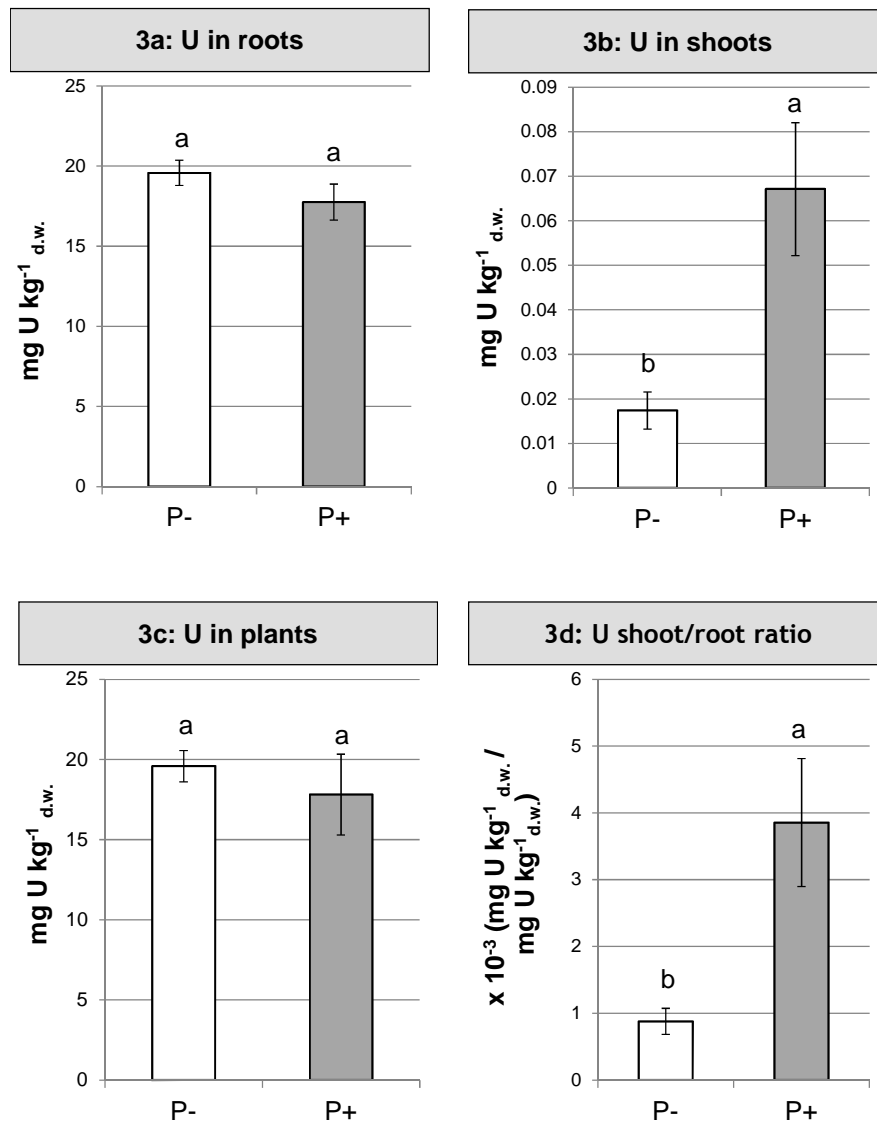


Figure 2: Experimental set-up for the rhizotest experiment including timeline and measures.



839

840 Figure 3: Accumulation of U in lupine plants after 5-day exposure to soil 1 in the rhizotest
 841 design. 3a: U in roots (in mg U per kg dry matter roots); 3b: U in shoots (in mg per kg dry
 842 matter shoots); 3c: U plants (in mg per kg dry matter shoots + roots); 3d: ratio of U
 843 accumulation in shoot vs root. Mean of 5 replicates \pm s.e. Letters: differences between P
 844 treatment, ANOVA, $p < 0.001$.

845

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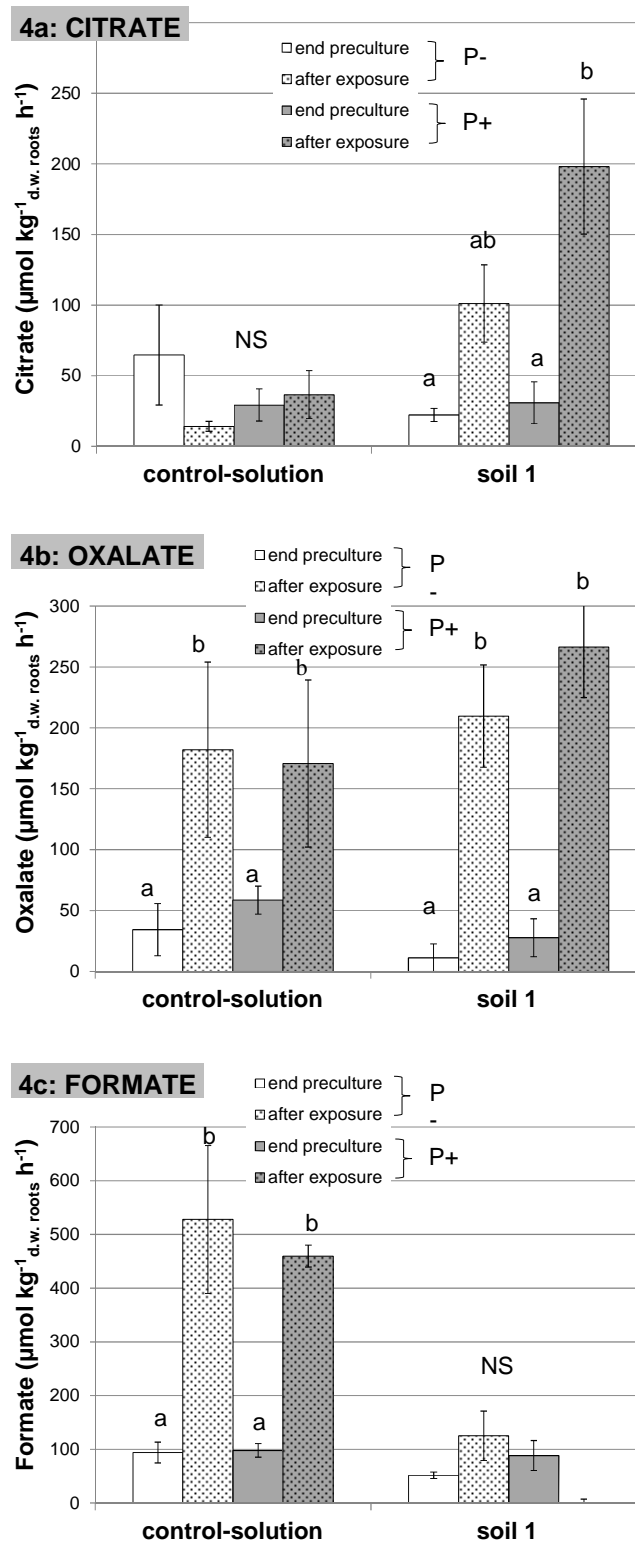


Figure 4: Root exudation of citrate (4a), oxalate (4b) and formate (4c) on the rhizotest design by lupine plants at the end of the pre-culture period and after 5 day-exposure to soil 1 or solution as control (mean of 5 replicates \pm standard error). Letters: results of 1-factor (condition) ANOVA for each kind of rhizotests (hydroponics/soil 1) ($P < 0.05$).

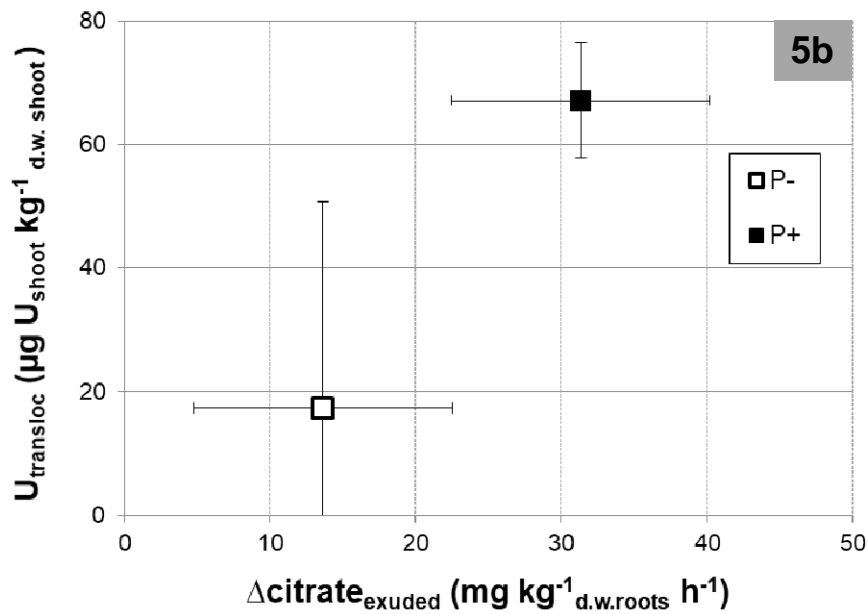
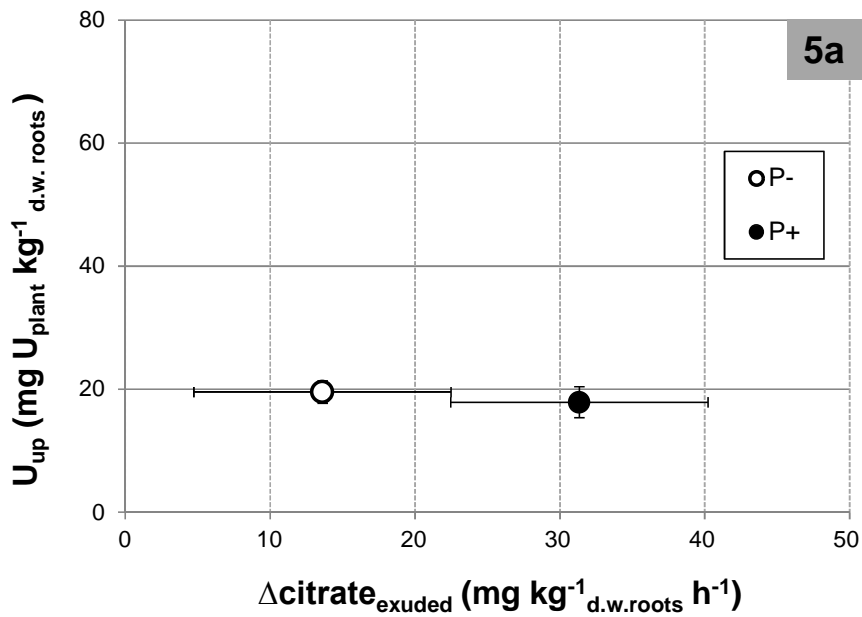
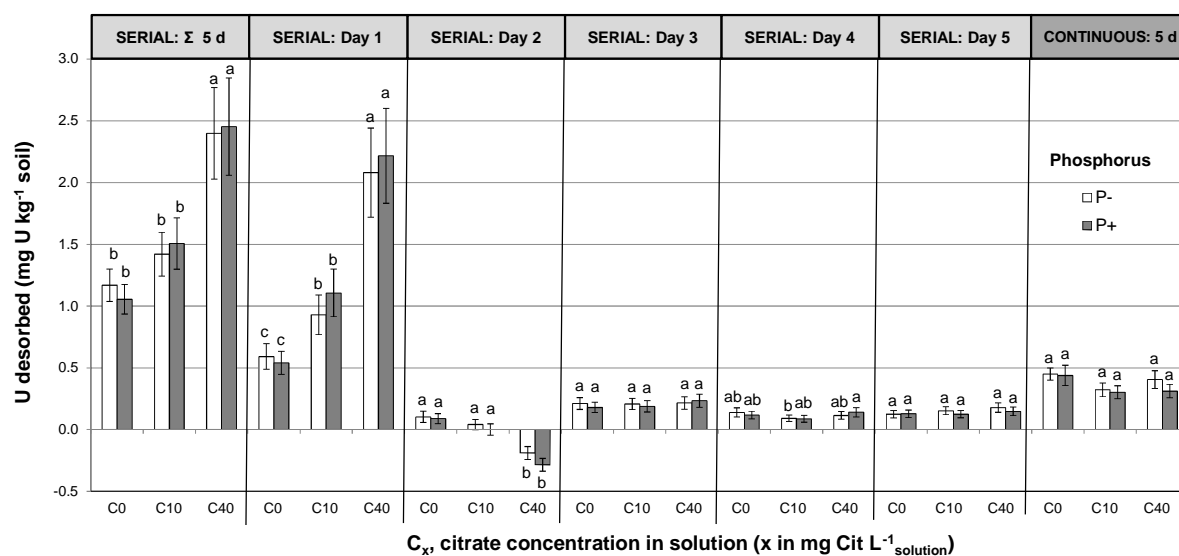


Figure 5: Results of U uptake (total U in plant as related to dry mass of roots, mg U_{plant} kg^{-1} roots d.w., 5a) and U translocated to shoots (μg U kg^{-1} shoots d.w., 5b) as a function of P level and variation in citrate exudation rate measured between beginning and end of exposure to soil 1 ($\Delta citrate_{exuded}$). Mean of 5 rhizotests \pm s.e.



870

871 Figure 6: U desorbed from soil 1 as a function of time either for a 5-day continuous extraction,
 872 or a 5-day serial extraction (with change of solution every day), P status of the solution and
 873 citrate concentration. Mean of 3 replicates \pm s.e. Letters: ANOVA for each day, $p < 0.01$.
 874 *Values may be positive (desorption) or negative (apparent sorption).*

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876

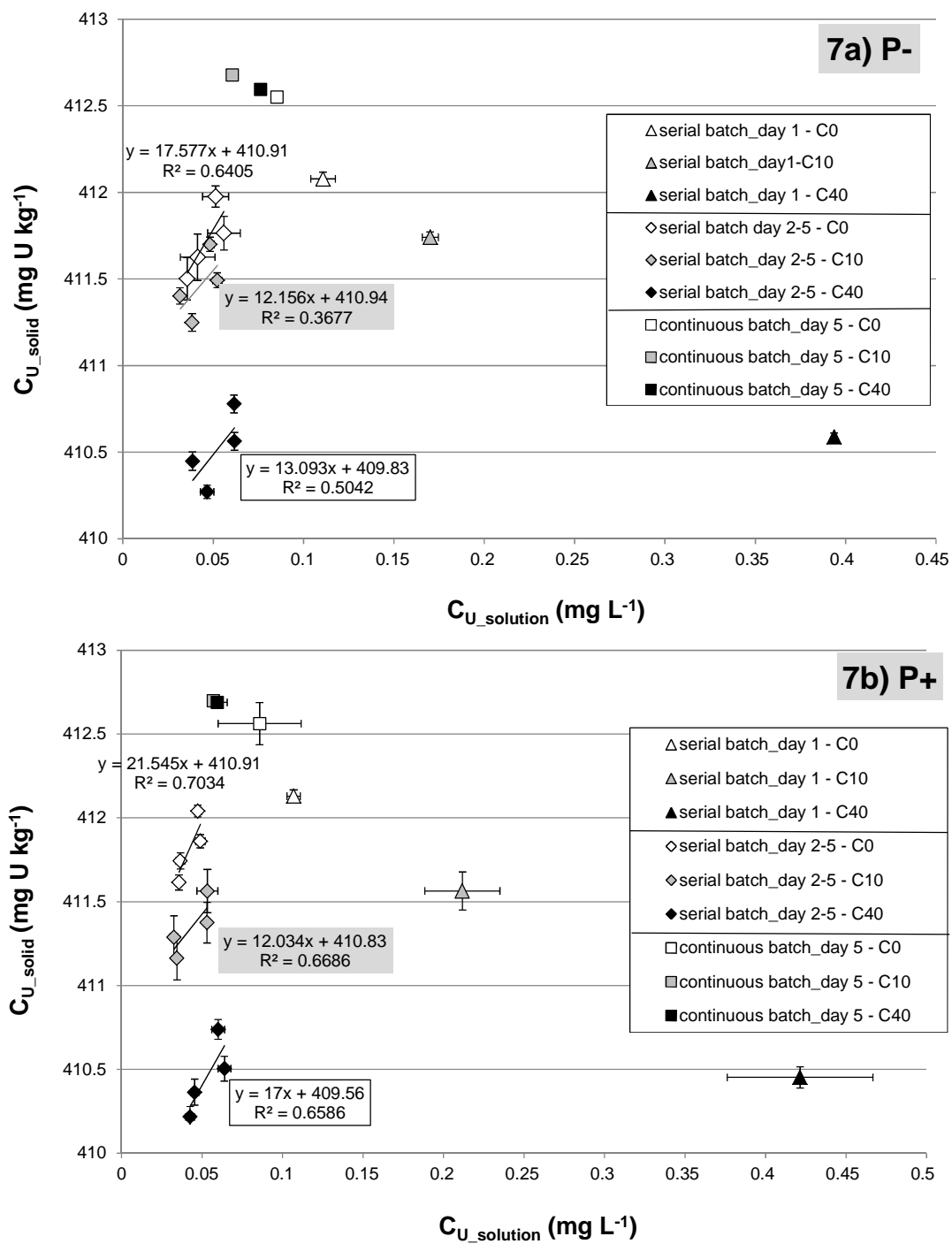


Figure 7: $C_{U_total_solid}$ as a function $C_{U_solution}$ at the end of each step of serial vs continuous batch for the a) P- and b) P+ conditions and all citrate concentrations. Mean of 3 replicates \pm s.e.

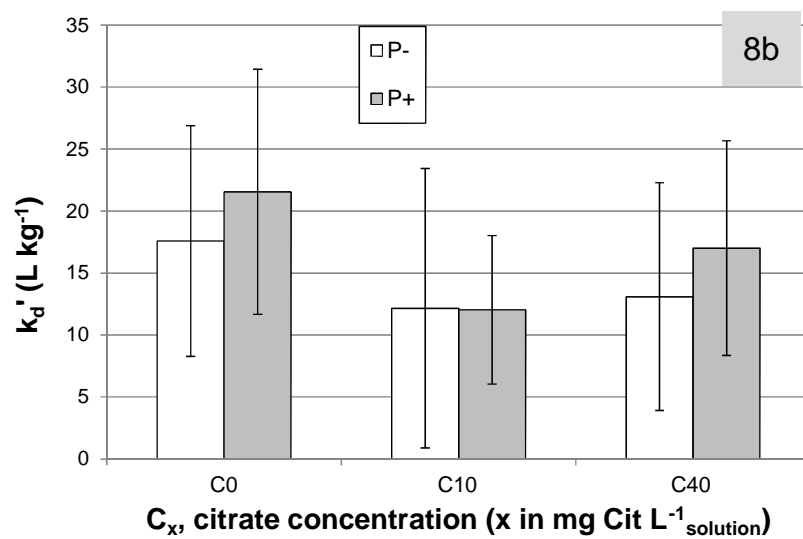
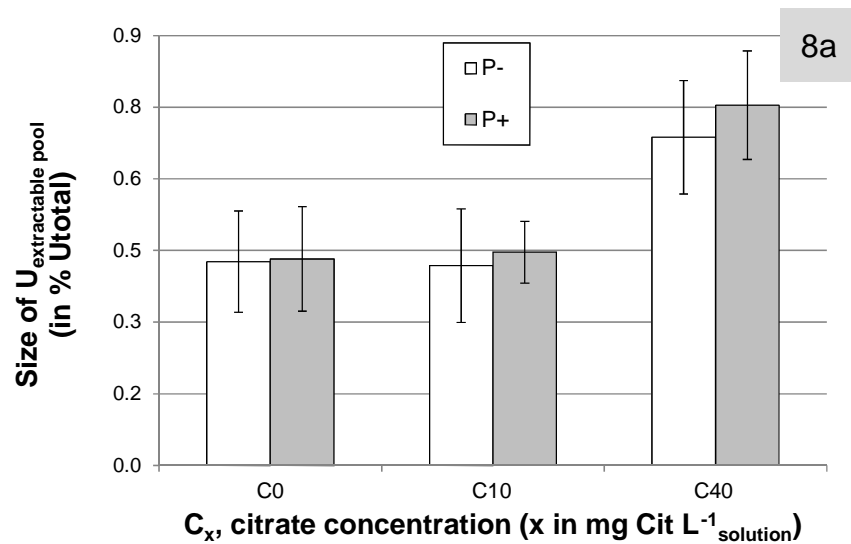
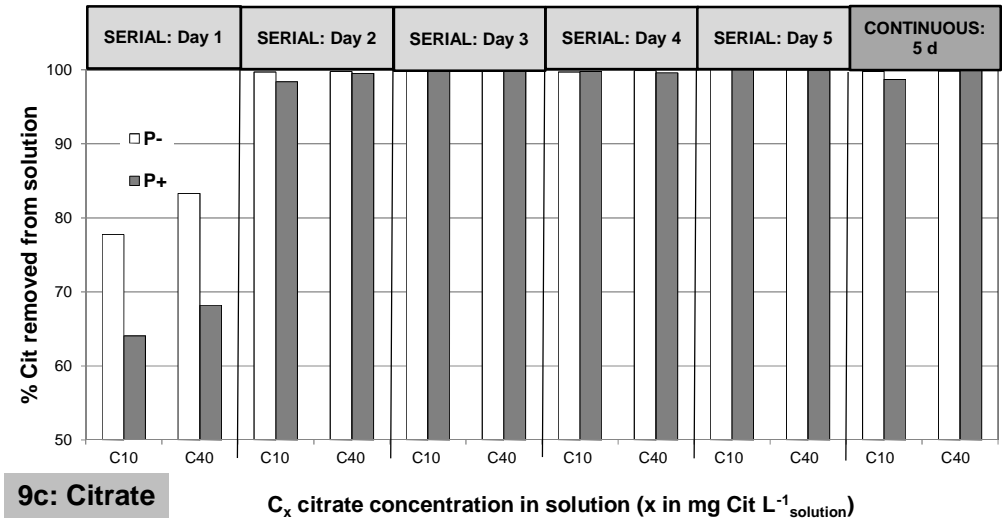
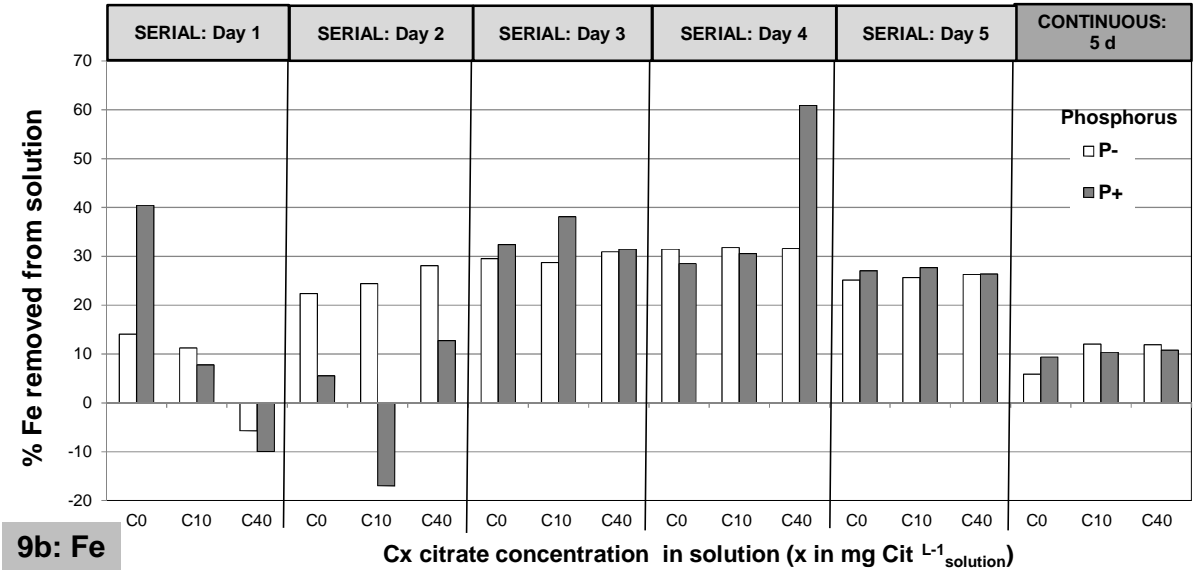
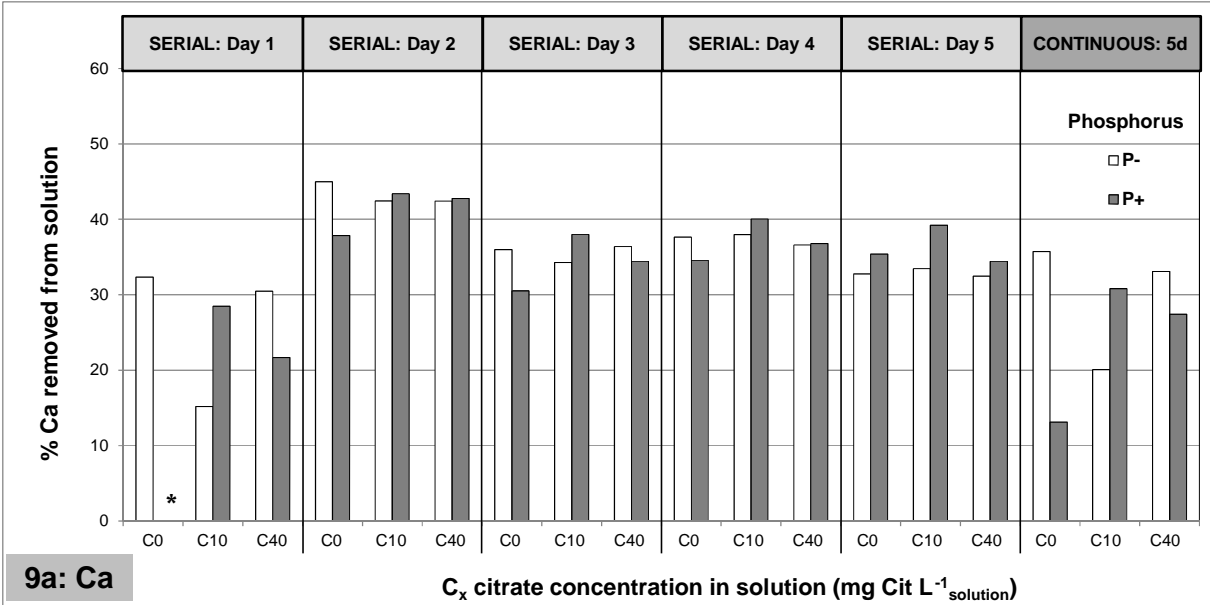
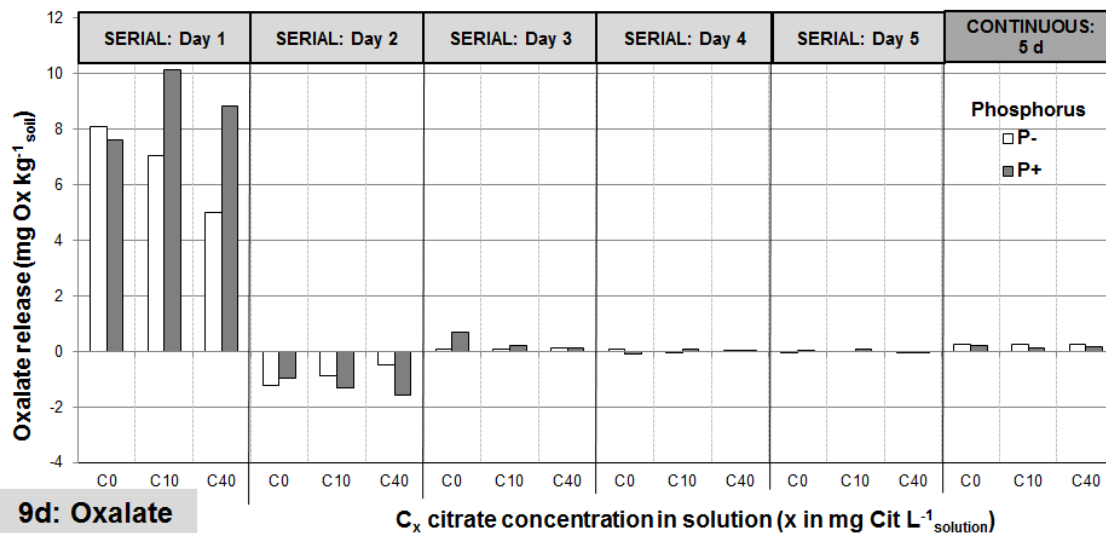


Figure 8: 8a-Size of U extractable pool (% of total soil U) and 8b- k_d' as a function of soil, P and citrate conditions (estimate \pm s.e.).





9d: Oxalate

C_x citrate concentration in solution (x in mg Cit L⁻¹ solution)

Figure 9: Calcium (9a), iron (9b) and citrate (9c) removal from solution and oxalate (9d) release in solution during each step as a function of citrate concentration in solution (C0/C10/C40 conditions), phosphorus status (P- or P+ for 1 and 100 μM P respectively) and type of batch (serial or continuous) *Contrary to U and oxalate, Ca, Fe and citrate are present in the initial solution, thus dynamics was calculated as the difference between final and initial solution concentration. Thus values may be positive (decrease compared to initial concentration) or negative (apparent release) depending which process was dominant during the corresponding period (24h or 5 days). * aberrant value.*

903 Table 1: Soil characteristics.

Characteristics	Units	Soil 1
Clay (< 2 μm)	g kg^{-1}	183
Loam (2-50 μm)	g kg^{-1}	358
Sand (50-2000 μm)	g kg^{-1}	459
Organic matter	g kg^{-1}	119.5
C/N		15.8
pH-H ₂ O		4.97
CEC Metson	cmol+ kg^{-1}	15.5
CEC cobaltihexamine	cmol+ kg^{-1}	5.73
Total U	mg kg^{-1}	413
Soluble U ^a	mg kg^{-1}	2.9
Total Fe	g kg^{-1}	43.4
Fe oxalate	%	24.3
Fe Mehra-Jackson	%	45.6
Total P	g kg^{-1}	2.2
Olsen P	g kg^{-1}	0.019
Exchangeable cations	K g kg^{-1}	0.22
	Ca g kg^{-1}	0.65
	Mg g kg^{-1}	0.08
Soluble elements ^b (in H ₂ O)	P g kg^{-1}	<0.002
	N mg kg^{-1}	48.94
	S mg kg^{-1}	9.99
	C _{org} mg kg^{-1}	670

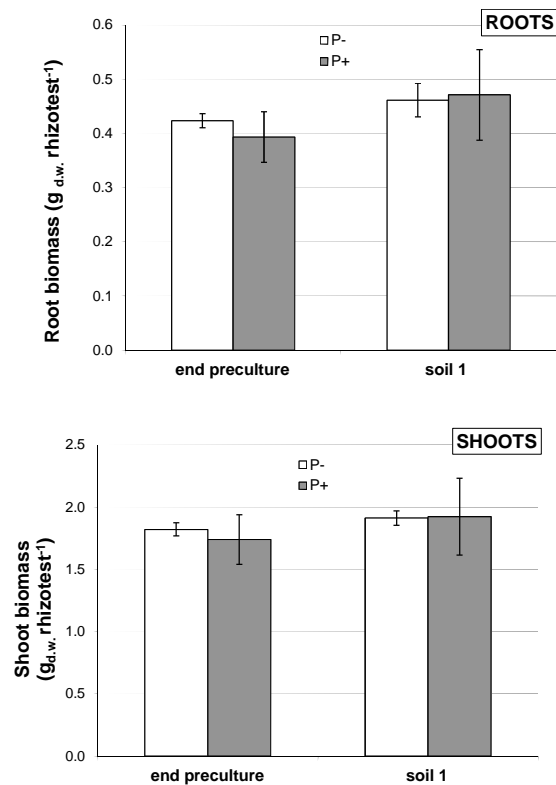
904 ^a Measured after 24h desorption in batch system, with 3g of soil and 30 mL water.

905 ^b INRA Method, water extraction, m/v 1/5, quantification in the extract by FAAS (Flame Atomic Absorption
906 Spectrometry) .

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908

909 **Supplementary material**

910 **S1: Biomass recorded during the rhizotest experiment**



912
913 **Figure S1 : Rhizotest: biomass of lupine plants**

914

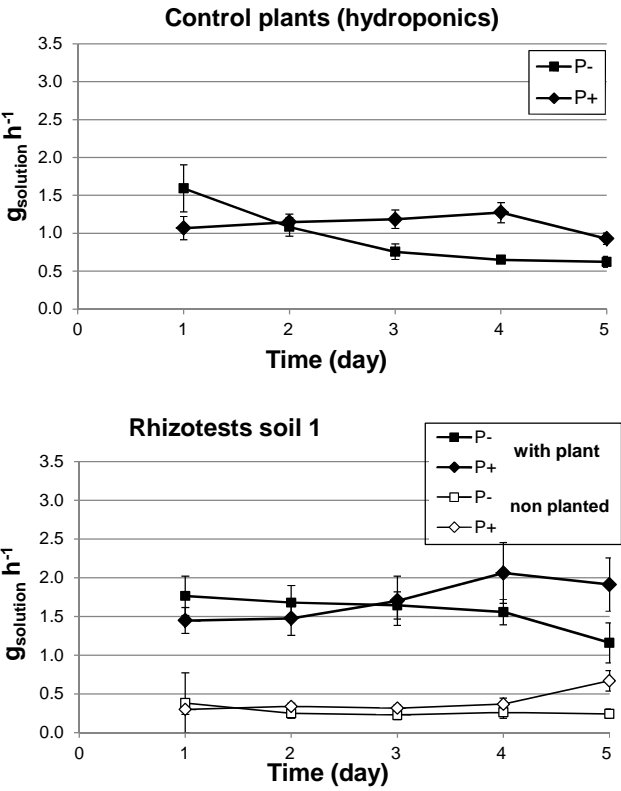
915 **Supplementary material**

916 **S2: Evapotranspiration on rhizotests – link with U uccumulation**

917 **S2-1: Evapotranspiration of lupine** on both soils was of the same order in both P condition

918 (175±26 ml in 5 days in –P and 169±10 ml in +P).

919

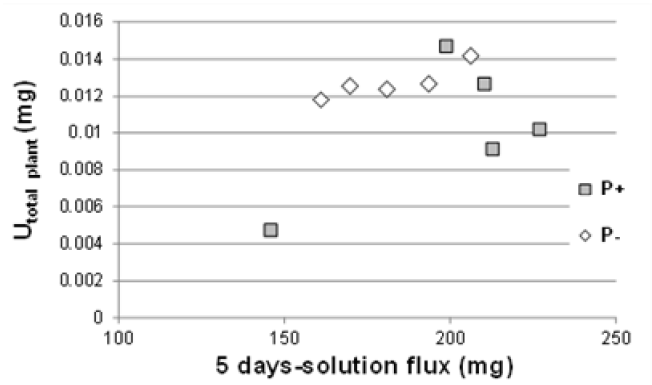


920

921 Figure S2-1: Solution fluxes in the rhizotest device (mean of 5 replicates \pm standard error).

922

923 S2-2: U accumulation as related to water flux through the rhizotest



924

925 Figure S2-2: Stock of U (mg) accumulated in lupine plant after 5-days exposure to soil 1 in

926 the rhizotest design as a function of the corresponding flux of solution across the rhizotest.

927

928

929 **Supplementary material**

930 **S3: Additional characterization of the soil samples**

931 To get insights in the possible different U forms (hotspots vs others) in soil which could either
932 highlight results detailed in this document and explain differences obtained with the similar
933 soil those results are displayed in the supplementary material, complementary analyses were
934 performed. While X-Ray diffraction did not detect any specific U-bearing minerals pointing
935 to a homogeneous U contamination, X-ray μ fluorescence analyses performed on X-Ray
936 Analytical microscope HORIBA Jobin Yvon XGT 7000 (data not shown) indicated a
937 background level of 0.3% U with some U-enriched zones with up to 1.5% U, which could be
938 responsible for the variability observed in the results. In addition, these U hotspots showed
939 concomitant lower Fe, Mn and S concentrations and higher K and Si concentrations, as
940 compared to background suggesting that U was preferentially associated with new minerals
941 containing K and Si in accordance with the observations made by Allard et al. (1999). These
942 analyses may again suggest that there are different U “bearing-phases”, characterized by
943 different reactivity with citrate leading to variable U lability in the soil(s). The U-available
944 bearing phase dissolved by citrate is different from the U-unavailable phase, quantitatively
945 more important and the only one detectable by X-ray fluorescence.

946

947 **Supplementary material**

948 **S4: Results obtained on soil 2**

949 During the study, 4 soils were collected at different distances from the pechblende vein, in
 950 order to get a naturally-produced U gradient in the “same” soil or at least soils with close
 951 properties. The experimental plan was too ambitious to be displayed on the 4 soils, thus only
 952 two were chosen. The second soil (soil 2) had similar properties (see table below) but a higher
 953 U content ($500 \text{ mg U kg}^{-1}_{\text{soil}}$) and was situated downwards soil 1 although the gradient was
 954 supposed to be related to distance from the vein. This could have signed a peculiar behavior
 955 regarding speciation, migration or (bio) availability. Thus, the complete experimental set up
 956 described for soil 1 was applied to soil 2. Results were equivalent to those of soil 1 are thus
 957 not detailed but displayed in supplementary material as they validate all statements made in
 958 this document.

959 **1. Soil 2 properties**

Characteristics	Units	Soil 2
Clay ($< 2 \mu\text{m}$)	g kg^{-1}	141
Loam ($2\text{-}50 \mu\text{m}$)	g kg^{-1}	285
Sand ($50\text{-}2000 \mu\text{m}$)	g kg^{-1}	574
Organic matter	g kg^{-1}	109.6
C/N		17.8
pH-H ₂ O		5.26
CEC Metson	cmol+ kg^{-1}	14.3
CEC cobaltihexamine	cmol+ kg^{-1}	7.2
Total U	mg kg^{-1}	525
Soluble U ^a	mg kg^{-1}	3.4
Total Fe	g kg^{-1}	46.8
Fe oxalate	%	17.7
Fe Mehra-Jackson	%	43.4
Total P	g kg^{-1}	2.7
Olsen P	g kg^{-1}	0.022
Exchangeable cations	K g kg^{-1}	0.24
	Ca g kg^{-1}	1.08
	Mg g kg^{-1}	0.14
Soluble elements ^b (in H ₂ O)	P g kg^{-1}	<0.002
	N mg kg^{-1}	47.33
	S mg kg^{-1}	15.37
	C _{org} mg kg^{-1}	1008

960 ^a Measured after 24h desorption in batch system, with 3g of soil and 30 mL water.

961 ^b INRA Method, water extraction, m/v 1/5, quantification in the extract by FAAS (Flame
 962 Atomic Absorption Spectrometry) .

2. Biomass recorded on rhizotests

Biomasses recorded on soil 2 are equivalent to those recorded on soil 1.

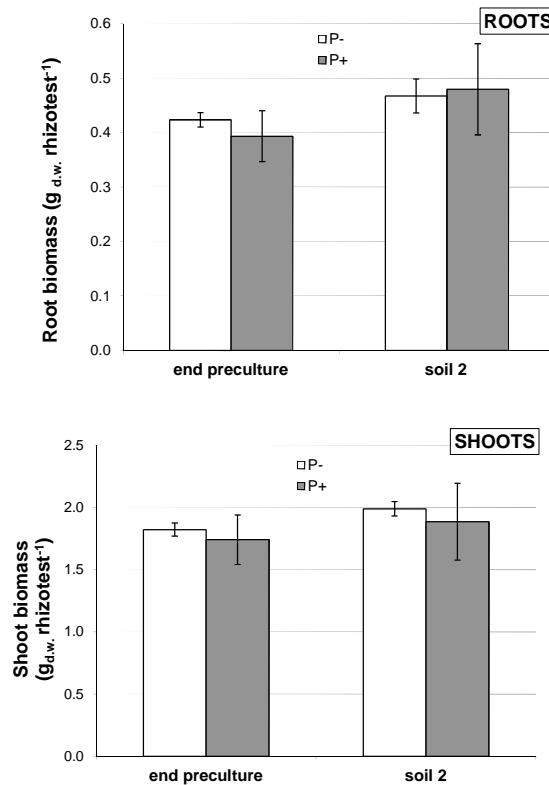


Figure S4-1: Biomass of lupine plants recorded for rhizotest with soil 2.

3. Evapotranspiration

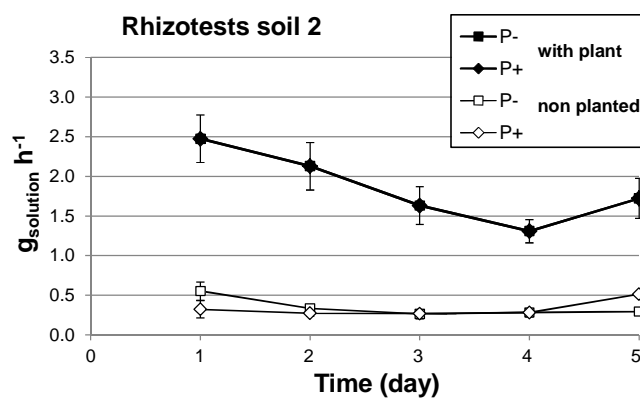


Figure S4-2: Evapotranspiration of rhizotests of soil 2 as a function of P treatment and type of rhizotest.

4. U accumulation

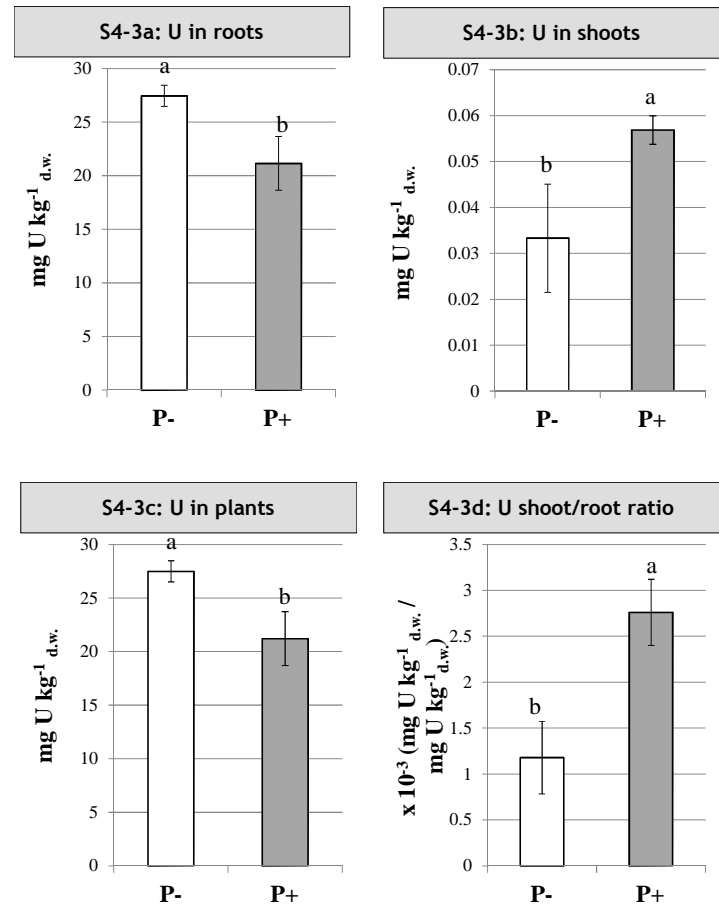


Figure S4-3: Accumulation of U in lupine plants after 5-day exposure to soil 2 in the rhizotest design. 3a: U in roots (in mg U per kg dry matter roots); 3b: U in shoots (in mg per kg dry matter shoots); 3c: U plants (in mg per kg dry matter shoots + roots); 3d: ratio of U accumulation in shoot vs root. Mean of 5 replicates \pm s.e. Letters: differences between P treatment, ANOVA, p<0.001.

Results (Fig. S4-3) are in adequation with those recorded on soil 1. The total U uptake is slightly lower than on soil 1 but the difference between P- and P+ for translocation to root is higher than on soil 1. Contrary to soil 1, there seems to be a small relation between U accumulated and water flux though the rhizotest (Figure S4-4).

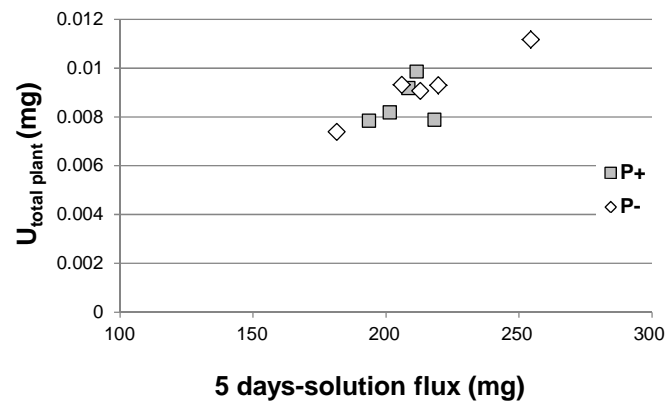
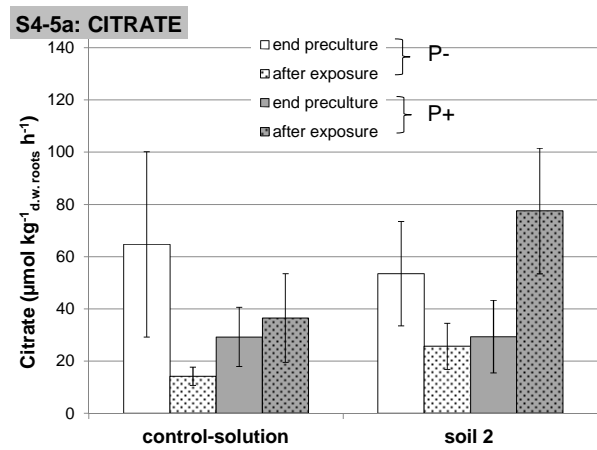


Figure S4-4: Stock of U (mg) accumulated in lupine plant after 5-days exposure to soil 2 in the rhizotest design as a function of the corresponding flux of solution across the rhizotest.

5. Exudation



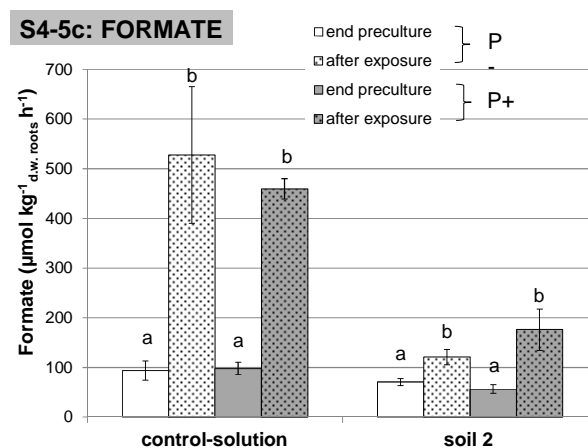
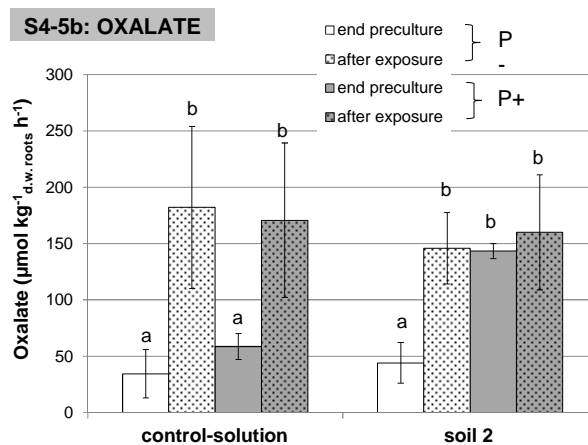


Figure S4-5: Root exudation of citrate (5a), oxalate (5b) and formate (5c) on the rhizotest design by lupine plants at the end of the pre-culture period (38 days) and after 5 day-exposure (43 days of growth in total) to soil or solution as control (mean of 5 replicates \pm standard error). Letters: statistical analysis ($P < 0.05$).

Main conclusions addressed for soil 1 are valid for soil 2 (Fig S4-5, Fig. S4-6) with the following differences: in P-, citrate exudation is not enhanced after soil exposure and for oxalate exudation there is no differences between P conditions (Fig. S4-5). The increase in U translocation in P+ condition compared to P- condition is recorded for lower citrate exudation rates in soil 2 than in soil 1 (Fig. S4-6).

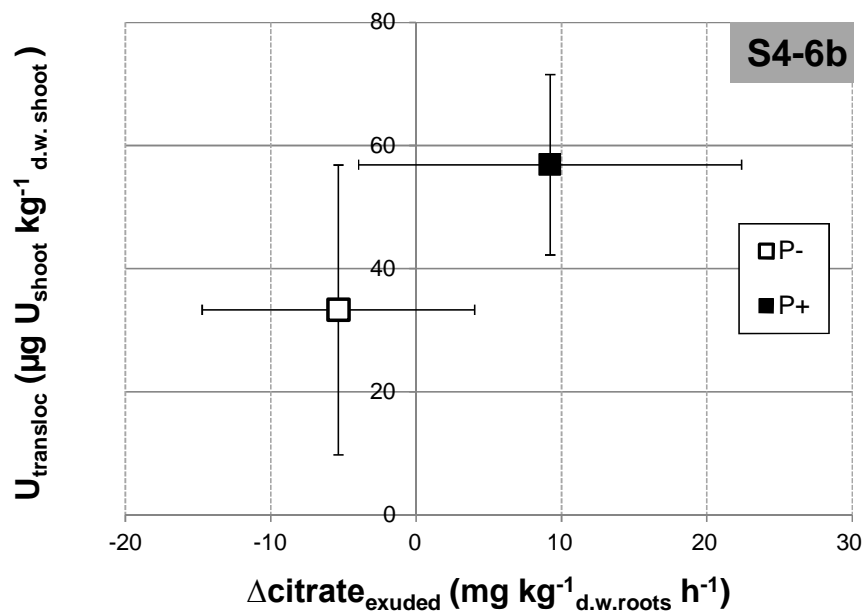
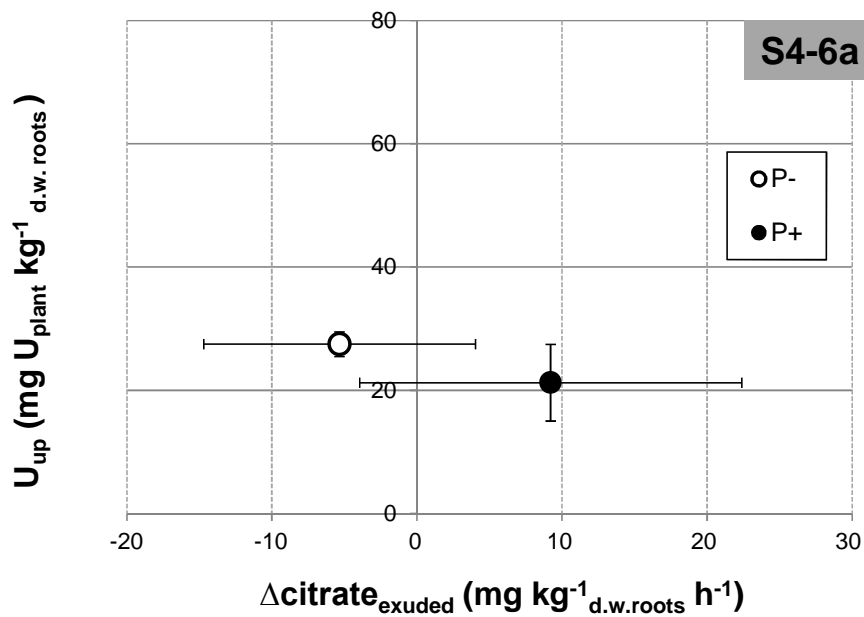
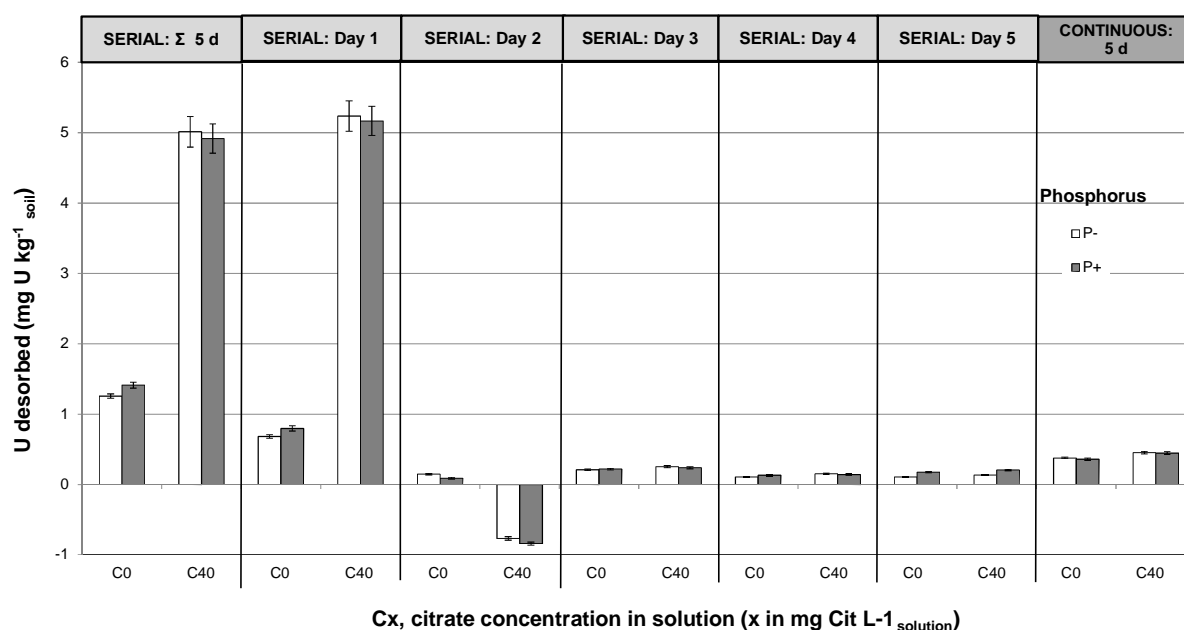


Figure S4-6: Results of U uptake (total U in plant as related to dry mass of roots, mg $U_{plant} \text{ kg}^{-1}$ roots d.w., 6a) and U translocated to shoots ($\mu\text{g } U \text{ kg}^{-1}$ shoots d.w., 6b) as a function of P level and variation in citrate exudation rate measured between beginning and end of exposure to soil2 ($\Delta \text{citrate}_{exuded}$). Mean of 5 rhizotests \pm s.e.

6. Batch results for soil 2

6.1. Uranium

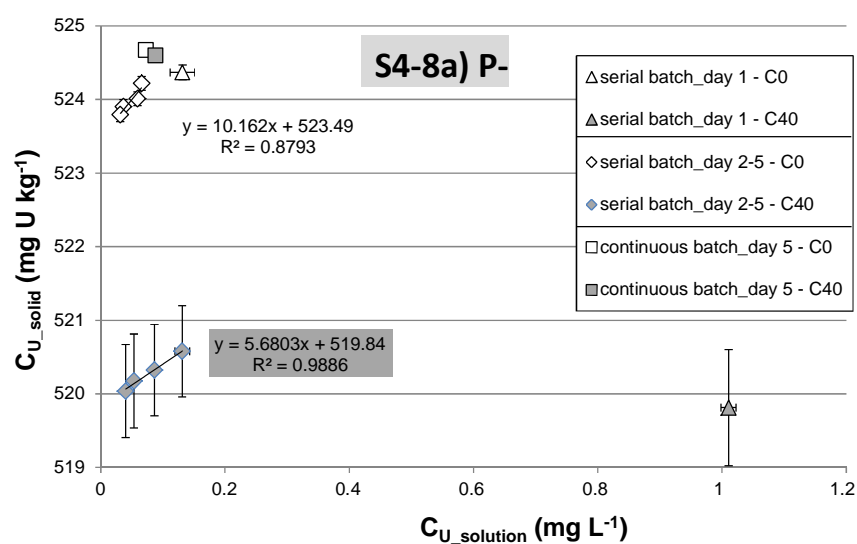
Results for soil 2, as detailed in the following figures are consistent with conclusions stated for soil 1 (Fig S4.7, S4-8).



1026

1027 Figure S4-7: U desorbed from soil 1 as a function of time either for a 5-day continuous
 1028 extraction, or a 5-day serial extraction (with change of solution every day), P status of the
 1029 solution and citrate concentration. Mean of 3 replicates \pm s.e. Letters: ANOVA for each day,
 1030 $p < 0.01$. Values may be positive (desorption) or negative (apparent sorption).

1031



1032

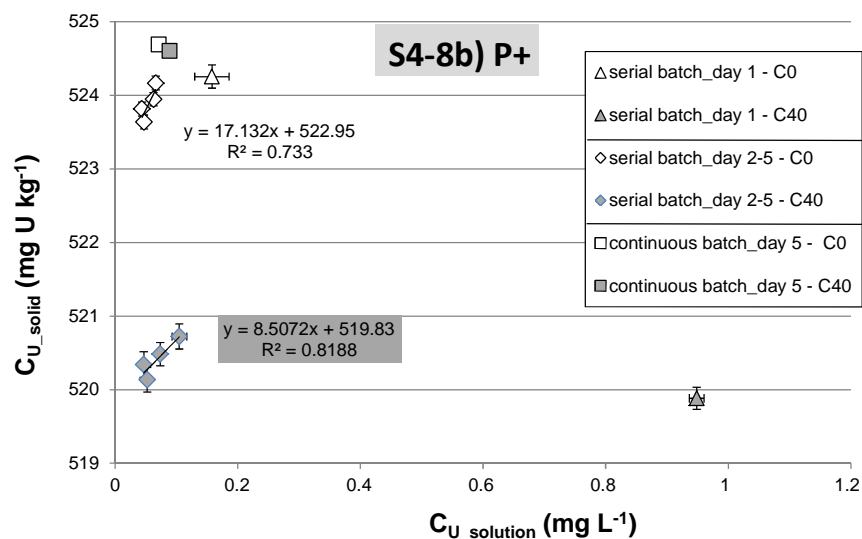
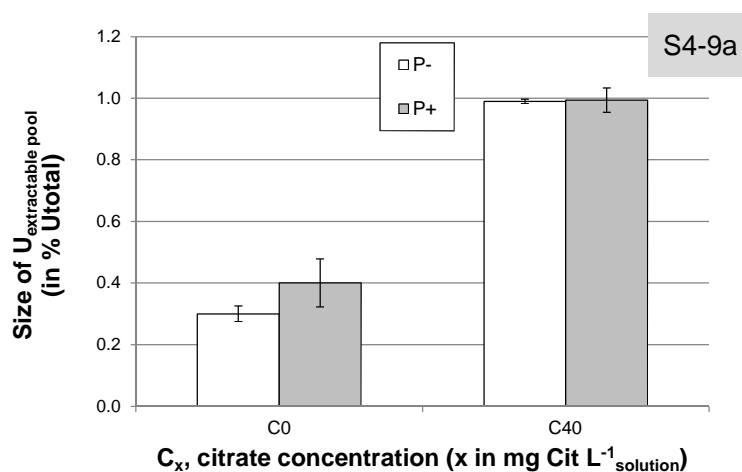
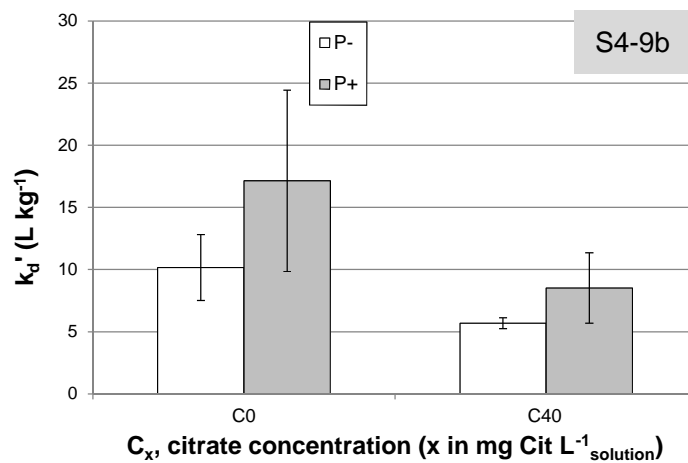


Figure S4-8: Soil 2- $C_{U_total_solid}$ as a function $C_{U_solution}$ at the end of each step of serial vs continuous batch for the a) P- and b) P+ conditions and all citrate concentrations. Mean of 3 replicates \pm s.e.

In the absence of citrate, the U extractable pool was estimated to be $0.4 \pm 0.1\%$ of total soil U as for soil 1, but the pool in P- condition ($0.3 \pm 0.1\%$) was slightly lower than in P+ condition (Fig S4-9a.). The size of the extractable pool increased with the high citrate concentration (C40) up to 0.99% for soil 2, a value higher than for soil 1. The extractable pool was easily accessible as shown by the corresponding low k_d' values for all modalities (Fig S4-9b). The availability (as estimated by the level of U extractability) in soil 2 tended to be lower than in soil 1, especially with citrate.



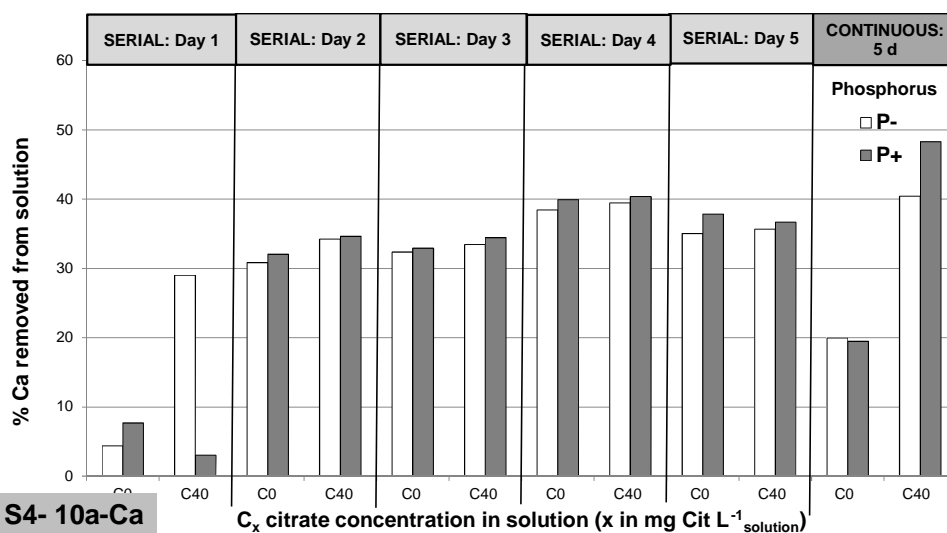


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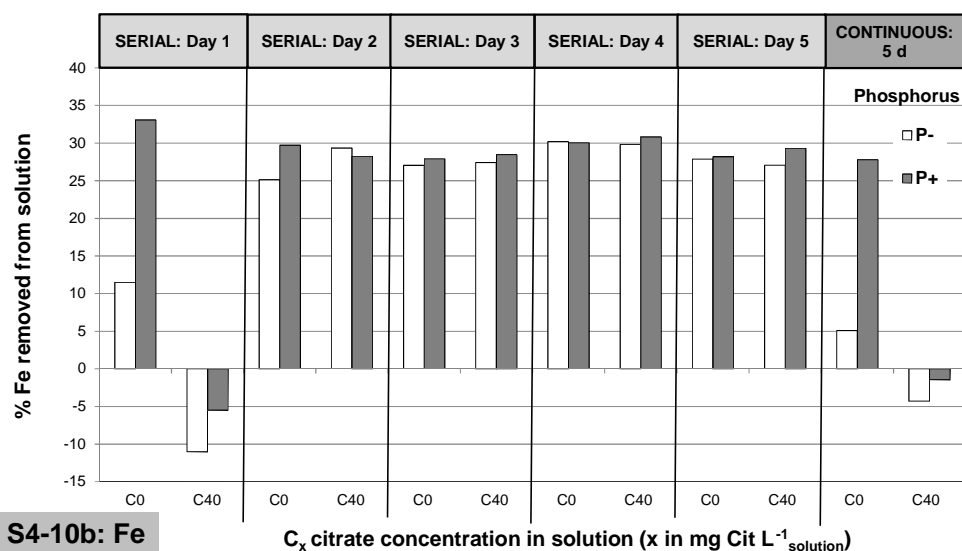
1047 Figure S4-9a-Size of U extractable pool (% of total soil U) and 9b- k_d' as a function of soil, P
 1048 and citrate conditions (estimate±s.e.).

1049

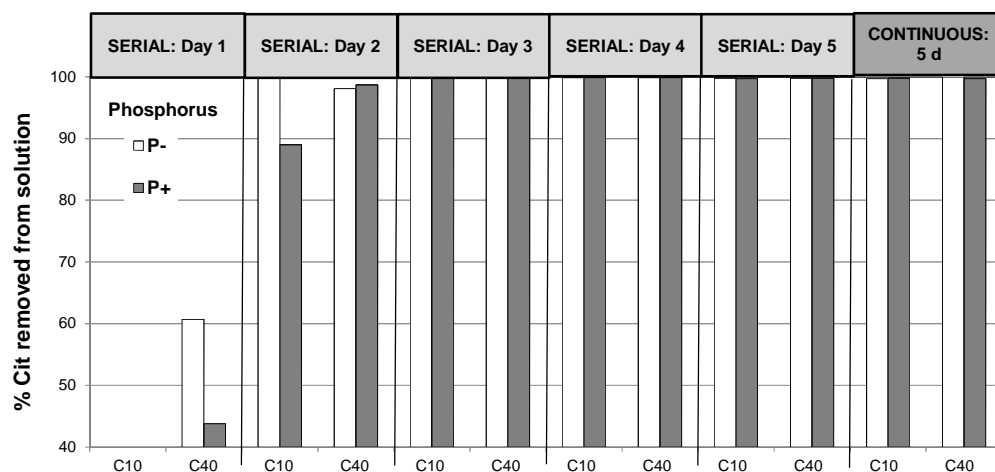
1050 6.2. Fe, Ca, P, citrate and oxalate



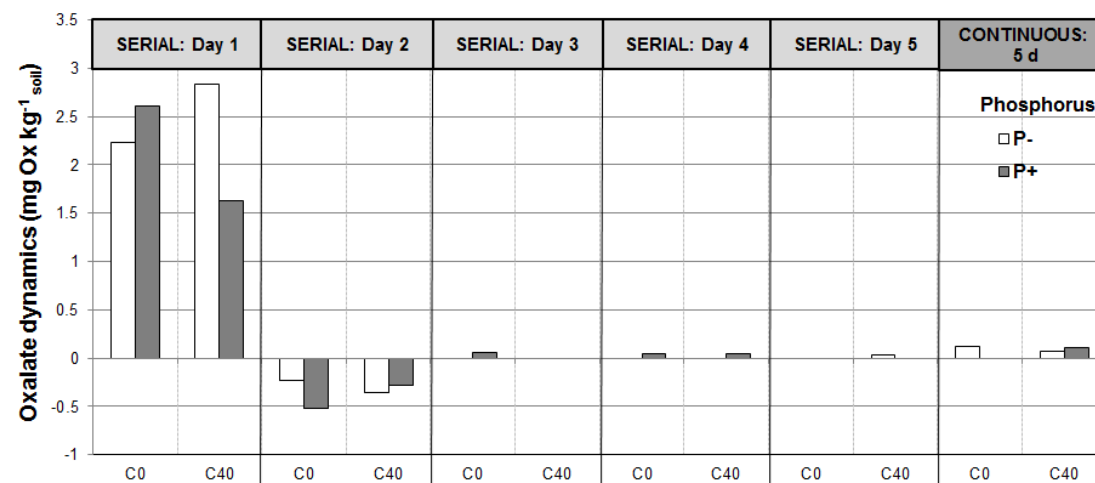
1051



S4-10b: Fe



S4-10c: Citrate



S4-10d: Oxalate

Figure S4-10: Calcium (10a), iron (10b) and citrate (10c) removal from solution and oxalate release (10d) during each step as a function of citrate concentration in solution (C0/C40 conditions*), phosphorus status (P- or P+ for 1 and 100 μM P respectively) and type of batch

1058 (serial or continuous). * *Contrary to U and Oxalate, Fe, Ca and citrate are present in the*
1059 *initial solution, thus dynamics was calculated as the difference between final and initial*
1060 *solution concentration. Values may be positive (decrease compared to initial concentration)*
1061 *or negative (apparent release) depending which process was dominant during the*
1062 *corresponding period (24h or 5 days).*

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