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

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Pascale Henner[1*], Félix Brédoire[1], Antoine Tailliez[1], Frédéric Coppin[1], Sylvie Pierrisnard[1], Virginie Camilleri[2], Catherine Keller[3]

[1]Institute for Radioprotection and Nuclear Safety (IRSN/PSE-ENV/SRTE), Laboratory of research on radionuclides transfer within terrestrial ecosystems (LR2T), Cadarache, Bat 183, BP 3, 13115 Saint Paul-lez-Durance, France

[2]Institute for Radioprotection and Nuclear Safety (IRSN/PSE-ENV/SRTE), Laboratory of research on radionuclides effects on ecosystems (LECO), Cadarache, Bat 183, BP 3, 13115 Saint Paul-lez-Durance, France

[3]Aix Marseille Univ, CNRS, IRD, INRA, Coll France, CEREGE, BP 80, 13545 Aix-en-Provence cedex 04, France

*Corresponding author

Tel. : +33(0)442199561

Fax : +33(0)442199151

E-mail address: pascale.henner@irsn.fr

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

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

Rhizotest

Batch extraction

Exudation

Uptake

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; Bourrelier 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 (Bq U $kg^{-1}_{\text{dry weight shoots}}$ / Bq U kg^{-1}_{soil}) from 10^{-5} to 10^{-2} (IAEA, 2010). In plant, U is mainly associated to roots, with concentrations as high as 10^{2} mg U $kg^{-1}_{\text{d.w. roots}}$ (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 kg^{-1}_{soil} (Sheppard et al., 1992), even thousands of mg U kg^{-1}_{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

115 circulation forms allowing for enhanced translocation (Laurette et al., 2012a, b). In soils, U is
116 frequently associated with P and Fe carrying phases (Pfeifer et al., 1994; Payne et al., 1996;
117 Fuller et al., 2002; Raicevic et al., 2006). Thus, during root exudation of protons or organic
118 acids to increase P and Fe desorption from the solid phase (Hinsinger, 1998; McLaughlin et
119 al., 1998; Kahm et al., 1999), U concentration may also increase in the rhizosphere. Finally,
120 the phytoavailability of U may depend on the concomitant behaviour of the released elements,
121 whether they are absorbed by roots, as free ion or complexes, or are subjected to precipitation,
122 coprecipitation or resorption processes onto the soil solid phase.
123 The objective of this study was to evaluate if exudation of a model organic acid, namely
124 citrate, may participate in maintaining a high U phytoavailability in soil solution. An
125 experiment was performed with a modified RHIZOtest® (Bravin et al., 2010), used with
126 naturally U-rich soils and white lupine plants (*Lupinus albus*). Plants were either P-starved or
127 P-sufficient during the pre-culture period prior to soil exposure, to induce two different levels
128 of citrate exudation. Uranium accumulated in roots and shoots were assessed with respect to
129 citrate exudation level. We used white lupine because it is a model plant for P study, which
130 induces proteoïd roots exudating a high level of organic acids when P-starved (Keerthisinghe
131 et al., 1998; Tailliez et al., 2013). To gain more insights on the dynamics of U at the soil/soil
132 solution interface in relation with P and citrate levels, batch dynamic desorption experiments
133 were also conducted.
134
135 2. Material and Methods
136 2.1. U-contaminated soils
137 The tested soil was sampled in the vicinity of one of the most U-concentrated pitchblende
138 veins existing in Europe, on the site of La Creuzaz/Les Marécottes, 7 km West from Martigny,
139 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.

165 2.3. Solutions

166 2.3.1. Nutrient solutions

167 The nutrient solution composition was identical to previous hydroponic studies (Tailliez et al.,
168 2013). The basic composition was: 2 mM $Ca(NO_3)_2$, 0.7 mM K_2SO_4, 0.5 mM $MgSO_4 . 7H_2O$,
169 0.1 mM KCl, 20 µM Fe(III)-EDTA, 10 µM H_3BO_3, 0.5 µM $MnSO_4 . H_2O$, 0.5 µM $ZnSO_4 .$
170 $7H_2O$, 0.2 µM $CuSO_4 . 5H_2O$, 0.01 µM $(NH_4)_6Mo_7O_{24} . 4H_2O$. Phosphorus was supplied as
171 100 µM (P-sufficient condition for plant physiology, labelled P+ in the document) or 1 µM
172 (P-deficient condition labelled P- in the document) KH_2PO_4. No pH regulation was used as it
173 would have interfered with the lupine roots physiology regarding P status.

174

175 2.3.2. Solution for root exudate collection

176 For root exudate collection, a specifically-designed solution was used (Horst W., personal
177 communication, 2011) to ensure the integrity of the biological membranes while not
178 interfering with further organic acids analysis. Its composition was: 0.25 mM $CaSO_4$, 10 µM
179 H_3BO_3, 0.5 µM $MnSO_4 . H_2O$, 0.5 µM $ZnSO_4 . 7H_2O$, 0.2 µM $CuSO_4 . 5H_2O$ and 0.01 µM
180 $(NH_4)_6Mo_7O_{24} . 4H_2O$.

181

182 2.4. Study of U phytoavailability in the rhizotest device

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

189 separation by the membrane, which does not result in a chemical separation. Thus, uptake and
190 exudation processes are preserved.
191 Some of the experimental parameters (duration of culture, solution composition) were chosen
192 so as to match the conditions used in Tailliez et al. (2013). They aimed at obtaining lupine
193 plants in the desired physiology state (P-sufficient vs P-deficient) as piloted by P-level in
194 solution, and discriminated by their level of citrate exudation.
195 Figure 2 resumed the experimental set-up. Test of soil 2 only add to this set-up rhizotests
196 devoted to soil exposure (5 upper parts with plants and 5 bottom parts with soil per P
197 condition), the controls being common (supplementary material S4). These were conducted as
198 those with soil 1.
199
200 2.4.1. Preculture step in hydroponics
201 Plants were grown on the upper part of the rhizotest from seeds. Preliminary experiments
202 allowed optimizing rhizotest device parameters (number of seeds, duration of pre-culture
203 step) to get an appropriate root mat for exposure to soil, a prerequisite for the use of this
204 device. Six sterilized and re-imbibed seeds were sown on each rhizotest device. Thirty
205 devices (each containing 6 plants) were prepared (10 devices dedicated to soil exposure, 10
206 devices for growth and hydroponic control and 10 extra devices to ensure a sufficient number
207 of healthy and homogeneous devices at the end of pre-culture). Devices were disposed in two
208 tanks containing nutrient solutions (15 devices in P- and 15 devices in P+) continuously
209 aerated and renewed every week. The whole dispositive was kept in a growth box under
210 controlled conditions: 16h/8h light/night cycle, 26/20±1°C day/night temperature, 60±5%
211 relative air humidity and light intensity of 150 µmol m^{-2} s^{-1}. Seedlings were grown in
212 hydroponics in P+ or P– nutrient solutions for 38 days, which was the delay to get a
213 homogeneous root mat. Due to the constraint within the root mat, less proteoïd 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 dm^3 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 rhizostests (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 NaN_3 for preservation until analysis. Aliquots were then evaporated to dryness with freeze

263 drying/vacuum concentration (SpeedVac, Jouan, Paris, France). Residues were dissolved in
264 150 µL deionised water and analysed for citrate and other organic acids with Ionic Liquid
265 Chromatography (ILC, Dionex autosampler ICS 3000, AS 11 HC column, eluent KOH (1-45
266 mM, flow rate: 1 mL min^{-1}), suppressor ASRS 4 mm, detection by conductivity, injection
267 volume: 100 µL, quantification quantification limit: 10 µg L^{-1}). Despite several optimizing
268 operations, it was not possible to separate correctly malate and succinate, thus only citrate,
269 oxalate, formate, lactate and acetate acids were quantified.
270
271 2.4.3.3. Analysis of root and shoot U content
272 Dry biomass of roots and shoots were digested (65% HNO_3 and 30% H_2O_2, 120°C), then
273 evaporated to dryness and redissolved in 10 mL 2% v:v HNO_3 before analysis. Uranium and
274 major cation contents were analysed by Inductively Coupled Plasma-Atomic Emission
275 Spectrometry (ICP-AES, OPTIMA 4300 DV, Perkin Elmer, quantification limit = 10 µg L^{-1}
276 for each element) or Inductively Coupled Plasma-Mass Spectrometry (ICP-MS, Agilent
277 7500Cs, detection limit = 10 ng L^{-1}) depending on their concentration (as related to tissue type
278 and exposure condition).
279 Despite their highest purity grade, some salts, phosphate salts mainly, contained small
280 amounts of U as impurity (< q.l. in nutrient solution). Final U concentrations measured in
281 plants were corrected for this background, subtracting U concentrations measured in the
282 controls (38d + 5d without soil exposure).
283
284 2.5. Batch study of U behaviour in natural U-contaminated soils
285 2.5.1. Calculation of citrate concentrations
286 Batch studies were used so as to be the “chemical” equivalent of a rhizotest. The chosen
287 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 $\mu mol_{Cit}\ kg^{-1}_{d.w.\ root}\ h^{-1}$ in P-U- condition and 100 $\mu mol_{Cit}\ kg^{-1}_{d.w.\ root}\ h^{-1}$ 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±1°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 µm (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

313 inorganic anions were analysed in one of the 3 replicates for each condition (P/citrate/type of
314 batch/step). Calculations were performed taking into account the remaining solution in the
315 soil residue after each centrifugation step.
316 Soil 2 was subjected to the same protocol than soil 1 (supplementary material S4).
317
318 2.5.3. Theoretical considerations and calculations
319 In batch systems, cations (among which U), inorganic anions (among which P), and organic
320 acids (among which citrate) dynamically interacted with the soil, whether they were
321 introduced with the test solution and/or extracted from the soil. They could be either released
322 (desorbed) from the solid phase or disappear from the solution (being either sorbed, or part of
323 a precipitation process or degraded). Both processes can occur simultaneously and the
324 resulting measured concentration in solution indicated the dominant process.
325 Calculations were done according to Teramage et al. (2017) and are detailed below for U. The
326 underlying hypothesis is that a fraction of U in soil is available (named U_{avail}) and thus
327 equilibrates with the solution, with a partition coefficient named k_{d_U}', and that a fraction
328 ($\%U_{fixed}$) remains fixed on the soil solid phase and never participates in the equilibrium
329 process. At each step of the batch experiment, the mass balance of U in the batch is conserved.
330 The analysis of U only in the supernatants at the end of each step, allows, based on mass
331 balance, to calculate the resulting U concentration in the solid phase. The concentration of U
332 on the soil solid phase is expressed by the following equations:

333 $$C_{U\_solid\_total\_initial} = C_{U\_solid\_available\_initial} + C_{U\_solid\_fixed\_initial} \quad \text{(Eq1)}$$

334 $$C_{U\_solid\_total\_final} = C_{U\_solid\_available\_final} + C_{U\_solid\_fixed\_final} \quad \text{(Eq2)}$$

335 with $C_{U\_solid\_total\_initial}$, $C_{U\_solid\_total\_final}$, $C_{U\_solid\_available\_initial}$ and $C_{U\_solid\_available\_final}$
336 respectively the total and available U concentrations (mg g^{-1}) at the initial and final step. The

337 initial concentration of fixed element is supposed to be constant throughout the experiment as
338 the hypothesis is that it does not participate in the equilibrium process.
339 The equilibrium between $C_{U\_solid-available\_final}$ and $C_{U\_solid-total-final}$ is given by the following
340 equation:
341 $$C_{U\_solid\_available\_final} = k'_{d\_U} \times C_{U\_solution\_final} \quad \text{(Eq3)}$$
342 where $C_{U\_solution\_final}$ is the U concentration (in mg L^{-1}) in the solution at the end of the batch
343 step considered and k'_{d_U} (L. kg^{-1}) is the partition coefficient between the solid available
344 fraction and the solution.
345 Merging equations (Eq2) and (Eq3) gives the following formula, which is verified at each
346 step of the batch experiment:
347 $$C_{U\_solid\_total\_final} = k'_{d\_U} \times C_{U\_solution\_final} + C_{U\_solid\_fixed\_initial} \quad \text{(Eq4)}$$
348 Finally, $C_{U\_solid\_total\_final}$ is plotted against $C_{U\_solution\_final}$ for each step, which allows deducing
349 k'_{d_U} which is the slope of the linear part of the curve and $C_{U\_solid\_fixed\_initial}$ the y-intercept.
350 The % of U as available and fixed pools can then be calculated.
351 The dataset was not complete for the C_{10} condition on soil 2. Thus, for soil 2 only results for
352 C_0 and C_{40} will be displayed.
353
354 2.7. Statistical analysis
355 All statistical analyses were performed with R software (R Development Core Team, 2011).
356 Results were subjected to one-way and two-way analysis of variance (ANOVA) with Tukey
357 posthoc tests. Normality of the distributions and homogeneity of variance were verified by the
358 appropriate tests and graphically on residuals. Heteroscedasticity was corrected when
359 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 (± 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 mg U $kg^{-1}_{d.w.\ roots}$) than in P+ condition (17.8 mg U $kg^{-1}_{d.w.\ roots}$). 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 $\mu mol_{citrate}\ kg^{-1}_{d.w.\ roots}\ h^{-1}$) and it was higher than in the P– condition (93 $\mu mol_{citrate}\ kg^{-1}_{d.w.roots}\ h^{-1}$), 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 $\mu mol_{oxalate}\ kg^{-1}_{d.w.\ roots}\ h^{-1}$) 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+).

410 3.2. U behaviour in soils in the presence of citrate (Batch experiments)

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

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

421 The final U concentrations on the solid phase and U concentrations in solution at each batch
422 step were plotted, as illustrated on Figure 7. Results of the serial batches were fitted with a
423 linear regression model, considering only the steps 2 to 5, as results obtained for the first step
424 showed systematically a very different behaviour from the other steps, an indication either of
425 transitory equilibrium and/or effect of peculiar mechanisms. The linear regression allows, as
426 detailed in the calculation section, generating the slopes and y-intercepts for Eq. 4, the latter
427 corresponding to the % U fixed (non-available) that further gives the % “available” U (Figure
428 8a). This pool is further qualified as “extractable pool” as it may be confusing to call it
429 “available” pool when citrate concentrations are applied to the soil. Results obtained in P+
430 and P- conditions were not significantly different. With increasing citrate concentration, the
431 value for 1st step of the serial batch diverged towards higher U concentration in solution,
432 which illustrated that mechanisms during the 1st day were different from the other steps. The
433 relative difference in the results for the further steps is related to the extent of U release during
434 the first step, but, as revealed by the close slopes of the regression lines, the behaviour of U

435 then is independent of P and citrate. For the C0 condition, the value of continuous batch was
436 in good agreement with the linear function defined within steps 2 to 5. With increasing citrate,
437 values diverged on the left-hand side of the equilibrium line.
438 In the absence of citrate, the U extractable pool was estimated to be 0.4±0.1% of total soil U
439 (Fig 8a.). The size of the extractable pool was not changed with the low citrate concentration
440 (C10), but increased up to 0.75%with the high citrate concentration (C40), up to 0.75%. The
441 extractable pool was easily accessible as shown by the corresponding low k_d' values for all
442 modalities (Fig 8b). Values are different from those of "soluble U" displayed in Table 1 as
443 they were not acquired in the same medium (nutrient solution vs water) and time.
444
445 3.3. Fe, Ca, P and organic acids behavior in batch experiment
446 Cations, anions and organic acids were analysed in one replicate of each group of 3 batches,
447 chosen randomly. Results obtained for Ca, Fe, citrate and oxalate are displayed respectively
448 on Figure 9a, 9b, 9c and 9d. Phosphate concentration was undetectable in solution whatever
449 the step and condition indicating that phosphate was immediately sorbed and both initial
450 conditions (P- and P+) ended up with the same soil solution composition. Except for the first
451 day in serial batches (in which 20% of initial citrate was recovered in P- and 34% in P+,
452 whatever the citrate concentration), citrate was nearly totally consumed during all steps (Fig.
453 9c). Calcium, and Fe concentrations in the batch solutions decreased during most steps and a
454 correlation between Fe and U behaviours was found in initial steps in particular (as revealed
455 by Kendall and **S**pearman correlation coefficient of 0.95). For continuous batch, around 12%
456 Fe (in P- and P+) initially present in solution disappeared, with no real difference between
457 citrate conditions (Fig. 9b). Removal of Ca from solution was higher (35.7%) in P- condition
458 than in P+ condition (13.1%) in the absence of citrate (Fig. 9a). With increasing citrate, the %
459 of Ca removed from solution increased to 30% in P+. For serial batch steps 2 to 5, around

460 30% of Fe and 35-40% of Ca disappeared, with no clear difference between P and citrate
461 conditions (with the exception of C40_P+_day 4 condition which value was 60 %). During
462 the first step of the serial batch, there was a marked decrease in Fe concentration in the P+
463 condition (40%) compared to P- condition (13.1%). Increasing the citrate concentration
464 resulted for the highest citrate concentration in a release of Fe, the latter being higher in P+
465 than in P- condition. On the second day, removal of Fe in P+ condition (22-25%) reached a
466 value that was still recorded at ulterior steps. In P- condition, Fe was less consumed in C0 and
467 C10 conditions, with a release in C40 condition. Ca was consumed at each step with values
468 of % Ca removed between 15 and 30% at day 1 and 35-40% further on. A small quantity of
469 oxalate (5-10 mg $kg^{-1}_{dry\ soil}$) was released especially during the first day in serial batches, with
470 lower values afterwards and in the continuous batches (Fig. 9d).
471
472 4. Discussion
473 Citrate addition in soils as an amendment is known to increase U solubility in the soil solution
474 and further U transfer to plants (Huang et al., 1998; Mihalik et al., 2012). Roots of P-deficient
475 plants are known to exudate large levels of organic acids, among which citrate, in order to
476 increase P availability. Many soils may have low available P contents whether their total P
477 content is high or not. There is thus an interest in unravelling mechanisms involving root
478 exudation of citrate, P acquisition, U release and ultimately plant uptake in the rhizosphere in
479 particular of plants combining exudation of protons and chelators for P or Fe (so called
480 “strategy I plants”) acquisition.
481 To answer the question (whether citrate exudation may modulate U release and uptake) , we
482 used a modified version of the RHIZOtest®, which is a normalized biotest, two naturally U-
483 contaminated soils (in which U is supposed to be at equilibrium with the solid phase) and
484 lupine as model plant, which exudation was piloted by P-nutrition level. The rhizotest

485 experiment was combined with batch experiments, providing some insights into the dynamics
486 of elements between soil and solution (principally U), and quantifying the U available pool.
487 As exposed ion the 'soil' issue of the "Material and Methods' chapter, a second soil (soil 2) of
488 similar properties but a higher U content (500 mg U kg^{-1}_{soil}) and possible different behavior
489 regarding speciation, migration or (bio) availability of U was tested. Results were equivalent
490 and thus validate all statements made in this document for soil 1.
491
492 U and other elements dynamics in the soils (batch experiments)
493 The batch experiment aimed at giving insights on U behaviour in the tested soil in the
494 presence of two P levels and two levels of citrate taken as model of exudate, considered as
495 representative of those that could occur in the rhizotest experiment. Apart from U, which
496 results have been detailed in the results section (Fig. 6, Fig. 7, Fig. 8), the dynamics of other
497 major cations, anions and organic acids was recorded, with a specific focus on those that
498 could form soluble complexes with citrate (U, Fe, P) and/or degrade (citrate) and/or be
499 involved in sorption/precipitation processes with/without U (Ca, PO_4, citrate) (Fig. 9).
500 Phosphate concentration was undetectable in solution whatever the step and condition
501 indicating that phosphate was immediately sorbed and both initial conditions (P- and P+)
502 ended up with the same soil solution composition. As a result, the main differences were
503 observed between the different citrate concentrations only. Except for the first day citrate was
504 nearly totally consumed during all steps, and calcium or Fe concentrations in the batch
505 solutions decreased during most steps. Contrary to citrate, oxalate was not introduced in the
506 system. The batch experiment was not conducted in sterile conditions, thus organic acids may
507 be produced by microbial activity. No other organic acid was released, including citrate in C0
508 condition.

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

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

558 oxalate may have participated to their further disappearance in solution, in addition to their
559 sorption onto the solid phase or precipitation/sorption of elements including U.
560
561 U phytoavailability and influence of exudation (Rhizotest experiment)
562 *U uptake and translocation*
563 The mean U concentration ratio (CR, ratio of shoot U concentration to soil U concentration at
564 the end of exposure) of lupine plants after 5-days exposure to soils in the rhizotest was
565 (1.1±0.1) 10^{-3}. Despite a limited time of exposure, the experimental CR was in accordance
566 with Transfer Factor (analog of CR) values reported in the literature (IAEA, 2010) for
567 leguminous fodder and the closest soil category tabulated, i.e. "sand" (mean value 2.4 10^{-3},
568 GSD 3.7). In accordance with existing data (Dushenkov et al., 1997; Shahandeh and Hossner,
569 2002; Laroche, 2005; Misson et al., 2009; Straczek et al., 2010), most of the U was recovered
570 in the roots and root-to-shoot translocation was low (<0.3% of total plant U). Uranium
571 accumulation at the whole plant level is thus equivalent in both P conditions due to the
572 prevalence of root concentration (Fig 3a. and Fig 3c.). Uranium content of shoots was higher
573 in P+ than in P- condition for both soils (Fig 3b. and Fig 3d.).
574 The differences between P conditions were not related to differences in the water flux through
575 the rhizotest, as they were equivalent in all experiments (Supplementary material S2).
576
577 *Effects of citrate exudation on phytoavailability*
578 According to our calculations, batch results in C0 condition were used to estimate the U
579 available pool in the rhizotest experiment. Plotting U accumulation results as a function of
580 citrate exudation has shown that U translocated to shoots (Fig 5b), but not U accumulated in
581 the whole plants (Fig 5a), was correlated to citrate exudation. The level of citrate exudation of
582 lupine on soil, either for P+ or P- conditions, may be related respectively to C40 and C10

583 citrate conditions used in the batch experiment. The batch results have shown that the size of
584 extractable U pool (Fig. 8a) increased at the highest citrate concentration. The associated k_d'
585 values (Fig. 8b) were low whatever the conditions and even decreased with increasing citrate,
586 which showed that the extractable pool was easily desorbed. Levels of U desorbed in
587 continuous batch were also equivalent whatever P and citrate condition.
588 From our results, we may conclude that: i) a small but easily accessible available U pool
589 exists in soil (even in the absence of complexing agents), and therefore ii) exudation of
590 organic acids such as citrate do not affect significantly U availability. Our results shows also
591 that U accumulated by lupine represented less than 50% of the U available pool: U
592 accumulated by lupine plants corresponded to 27.8±4.1% and 25.8±2.7% of the U available
593 pool respectively for the P- and P+ conditions. Buffering of the soil solution by the solid
594 phase was supposed to be the limiting step for phytoavailability and not plant uptake. Indeed,
595 the affinity of plant roots for U, even if not further translocated, is high (Dushenkov et al.,
596 1997; Shahandeh and Hossner, 2002; Laroche, 2005; Misson et al., 2009; Straczek et al.,
597 2010). Moreover, in our experimental conditions, a combination of factors may have limited
598 the diffusion of U to roots and favoured the soil solution/root step limitation: e.g. the low
599 duration of contact and the geometry of rhizotest (only the surface of roots in contact with
600 basal membrane is efficient for uptake). However, it should be kept in mind that the
601 phytoavailability, as measured in our rhizotest experiment conditions, is thus only
602 representative of a short time window compared to the whole growth cycle of the plant.
603 Part of the U absorbed by roots may be translocated to shoots, through internal physiological
604 mechanisms that are not fully dependant of the processes controlling U exchanges at the
605 solution/root interface. In the rhizotest experiment, a differential translocation of U to shoots
606 of lupine plants with P status was recorded, that may be linked to the citrate exudation level.
607 It has been shown that citrate enhanced U translocation to shoots (Laurette et al., 2012a,b;

608 Mihalik et al., 2012), with two underlying possible processes: uptake of citrate-U complexes
609 or buffering of the soil solution with uranyl ion through complex dissociation at the root
610 interface. Both may explain the enhanced translocation recorded for soil in P+ condition, as it
611 is affected by higher citrate exudation rate in that condition.
612
613 *Effects of exudation of organic acids on soils*
614 Citrate was not the only organic acid exuded by lupine roots. For example, oxalate was also
615 exuded at a higher rate than citrate (Figure 4), as also already recorded for lupine by Mimmo
616 et al. (2011). Generally, citrate and malate are the two most studied organic acids regarding
617 lupine exudation, and citrate seems to be the most effective organic acid in solubilizing
618 inorganic phosphorus (Pi) (Oburger et al., 2011a). Oburger et al. (2011a, b) have intensively
619 studied the dynamics of Pi and citrate in soils in order to extrapolate to rhizospheric
620 conditions. They concluded on a complex mechanism, not fully understood and depending on
621 numerous parameters such as soil pH, quantity of Fe/Al oxy/hydroxides (as binding phase for
622 Pi and citrate) and concentration of metals (e.g; Fe) or other competing cations (Ca) in the soil
623 solution, and the respective effect of organic acids as chelates and the concomitant release of
624 protons. All of these parameters have been shown to interfere with U behaviour as also
625 observed in our soil/plant system. Yet, in many studies including that of Mimmo et al. (2011),
626 the effect of organic acids other than citrate, as well as mix of organic acids, or more
627 generally rhizosphere exudation, on soil dynamics is not always described while they may be
628 produced in significant amounts, as shown in our experiments (Fig. 4). It may be cumulative,
629 and a better assessment of total exudation could be a more effective determinant of U
630 availability/phytoavailability than solely citrate exudation.
631
632 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.

Acknowledgments: Authors are very grateful to all reviewers for their valuable suggested improvements of the manuscript and to Daniel Borschneck (CEREGE) for the help in the X-ray fluorescence analysis.

5. References

Aery, N.C., Jain, G.S., 1997. Effect of uranyl nitrate on seed germination and early seedling growth on *Triticum aestivum*. Biologia (Bratislava). 52(1), 115-119.

Allard, T., Ildefonse, P., Beaucaire, C., Calas, G., 1999. Structural chemistry of uranium associated with Si, Al, Fe gels in a granitic uranium mine. Chemical Geol. 158, 81-103.

Bourrelier, P.-H., Berthelin, J., 1998. Contamination des sols par les éléments en traces : les risques et leur gestion. TEC & DOC Lavoisier, Paris, France.

Bravin, M.N., Michaud, A.M., Larabi, B., Hinsinger, P., 2010. RHIZOtest: a plant-based biotest to account for rhizosphere processes when assessing copper bioavailability. Environ. Pollut. 158, 3330-3337.

Briat, J.F., 2008. Iron dynamics in plants. Adv. Bot. Res. 46, 137-180.

Chaignon, V., Hinsinger, P., 2003. A biotest for evaluating copper bioavailability to plants in a contaminated soil. J. Environ. Qual. 32, 824-833.

Doustaly, F., Combes, F., Fiévet J.B., Berthet, S., Hugouvieux, V., Bastien, O., Aranjuelo, I., Leonhardt, N., Rivasseau, C., Carrière, M., Vavasseur, A., Renou, J.-P., Vandenbrouck, Y., Bourguignon, J., 2014. Uranium perturbs signalling and iron uptake response in *Arabidopsis thaliana* roots. Metallomics. 6, 809-821.

Duff, M.C., Amrhein, C., 1996. U(VI) sorption on goethite and soil in carbonate solutions. Soil Sci. Soc. Am. J. 60, 1393-1400.

Duffner, A., Hoffland, E., Temminghoff, E.J.M., 2012. Bioavailability of zinc and phosphorus in calcareous soils as affected by citrate exudation. Plant Soil, 361(1-2), 165-175.

Duquène, L., Tack, F., Meers, E., Baeten, J., Wanninjn, H., Vandenhove, H., 2008. Effect of biodegradable amendments on uranium solubility in contaminated soils. Sci. Tot. Environ. 391, 26-33.

682 Dushenkov, S., Vasudev, D., Kapulnik, Y., Gleba, D., Fleisher, D., Ting, K.C., Ensley, B.,
683 1997. Removal of uranium from water using terrestrial plants. Environ. Sci. Technol. 31,
684 3468-3474.
685 Ebbs, S.D., Brady, D.J., Kochian, L.V., 1998. Role of uranium speciation in the uptake and
686 translocation of uranium by plants. J. Exp. Bot. 49(324), 1183-1190.
687 Eschenbach, A., Wienberg, R., Mahro, B., 1998. Fate and stability of non-extractable residues
688 of ^{14}C PAH in contaminated soils under environmental stress conditions. Environ. Sci.
689 Technol. 32, 2585-2590.
690 Fein, J.B., 2002. The effects of ternary surface complexes on the adsorption of metal cations
691 and organic acids onto mineral surfaces. Water-Rock Interactions, Ore Deposits and
692 Environmental Geochemistry: A tribute to David A. Crerar. The Geochemical Society,
693 Special Publication n°7, Hellmann R. and Wood S.A. Eds.
694 Fuller, C.C., Bargar, J.R., Davis J.A., Piana, M.J., 2002. Mechanisms of uranium interactions
695 with hydroxyapatite: implications for groundwater remediation. Environ. Sci. Technol. 36,
696 158-165.
697 Goldberg, S., Sposito, G., 1984. A chemical model of phosphate adsorption by soils: I.
698 reference oxide minerals. Soil Sci. Soc. Am. J. 48(4), 772-778.
699 Hafsteinsdóttir, E.G., Camenzulli, D., Rocavert, A.L., Walworth, J., Gore, D.B., 2015.
700 Chemical immobilization of metals and metalloids by phosphates. Appl. Geochem. 59, 47-
701 62.
702 Hinsinger, P., 1998. How do plant roots acquire mineral nutrients? Adv. Agron. 64, 225-265.
703 Hinsinger, P., 2001. Bioavailability of soil inorganic P in the rhizosphere as affected by root-
704 induced chemical changes: a review. Plant Soil 237, 173-195.
705 Hinsinger, P.,Gobran, G., Wenzel , W.W., 2005. Rhizosphere geometry and heterogeneity
706 arising from root-mediated physical and chemical processes. New Phytol. 168, 293-303.

Horst; W., 2011. Composition of solution for exudate collection. Personal communication.

Hsi, C.K.D., Langmuir, D., 1985. Adsorption of uranyl onto ferric oxyhydroxides: application of the surface complexation site-binding model. Geochim. Cosmochim. Acta. 49, 1931-1941.

Hsu, P.H., 1964. Adsorption of phosphate by aluminium and iron in soils. Soil Sci. Soc. Am. J., 28(4), 474-478.

Huang, J.W., Blaylock, M.J., Kapulnik, Y., Ensley, B.D., 1998. Phytoremediation of uranium-contaminated soils: Role of organic acids in triggering uranium hyperaccumulation in plants. Environ. Sci. Technol. 32, 2004-2008.

IAEA-International Atomic Energy Agency, 2010. Handbook of parameter values for the prediction of radionuclide transfer in terrestrial and freshwater environments. Technical Reports Series n°472, 208p.

Jenkinson, D.S., 1966. The priming action. In “The use of isotope in soil organic matter studies”, Braunschweig, Pergamon Press, New York (United States), p 199-208. Report for the FAO/IAEA.

Jones, S.L., 1998. Organic acids in the rhizosphere- a critical review. Plant Soil. 205, 25-44.

Kahm, M., Horst, W.J., Amer, F., Mostafa, H., Maier, P., 1999. Mobilization of soil and fertilizer phosphate by cover crops. Plant Soil 211, 19-27.

Keerthisinghe, G., Hocking, P.J., Ryan, P.R., Delhaize, E., 1998. Effect of phosphorus supply on the formation and function of proteoïd roots of white lupin (*Lupinus albus* L.). Plant Cell Environ. 21(5), 103-122.

Laroche, L., 2005. Transfert racinaire de l’uranium (VI) en solution chez une plante supérieure : spéciation en solution hydroponique, prise en charge par la plante, microlocalisation et effets biologiques induits. Thèse doctorat en Sciences de

731 l'Environnement, spécialité Biosciences de l'environnement, chimie et santé, Université
732 d'Aix-Marseille 1, n° ISRN IRSN-2005/52-FR, 242p.
733 Laurette, J., Larue, C., Llorens, I., Jaillard, D., Jouneau, P.-H., Bourguignon, J., Carrière, M.,
734 2012a. Speciation of uranium in plants upon root accumulation and root-to-shoot
735 translocation: a XAS and TEM study. Env. Exp. Bot. 77, 87-95.
736 Laurette, J., Larue, C., Mariet, C., Brisset, F., Khodja, H., P.-H., Bourguignon, J., Carrière, M.,
737 2012b. Influence of uranium speciation on its accumulation and translocation in three plant
738 species: oilseed rape, sunflower and wheat. Env. Exp. Bot., 77, 96-107.
739 Lenhart, J.J., Honeyman, B.D., 1999. Uranium(VI) sorption to hematite in the presence of
740 humic acid. Geochim. Cosmochim. Acta 63, 2891-2901.
741 McLaughlin, M.J., Smolders, E., Merckx, E., 1998. Soil-root interface: Physico-chemical
742 processes. In Huang, P.M., Adriano, D.C., Logan, T.J., Checkai, R.YT. (Eds) Soil chemistry
743 and ecosystem health. Special Publication n°52, Soil Science Society of America, Madison,
744 pp233-277.
745 Meyer, M.C., McLendon, T., 1997. Phytotoxicity of depleted uranium on three grasses
746 characteristic of different successional stages. J. Environ. Qual. 26, 748-752.
747 Mihalik, J., Henner, P., Frelon, S., Camilleri V., Février, L., 2012. Citrate assisted
748 phytoextraction of uranium by sunflowers: study of fluxes in soils and plants and resulting
749 intra-planta distribution of Fe and U. Env. Exp. Bot. 77, 249-258.
750 Mimmo, T., Hann, S., Jaitz, L., Cesco, S., Gessa, C.E., Puschenreiter, M., 2011. Time and
751 substrate dependent exudation of carboxylates by *Lupinus albus* L. and *Brassica napus* L.
752 Plant Physiol. Biochem. 49, 1272-1278.
753 Misson, J., Henner, P., Morello, M., Floriani, M., Wu, T.-D., Guerquin-Kern, J.-L., Février,
754 L., 2009. Use of phosphate to avoid uranium toxicity in *Arabidopsis thaliana* leads to

755 alterations of morphological and physiological responses regulated by phosphate
756 availability. Environ. Exp. Bot. 67, 353-362.
757 Oburger, E., Jones, D.L., Wenzel, W.W., 2011a. Phosphorus saturation and pH differentially
758 regulate the efficiency of organic acid anion-mediated P solubilisation mechanisms in soil.
759 Plant Soil 341, 363-382.
760 Oburger, E., Leitner, D., Jones, D.L., Zygalakis, K.C., Schnepf, A., Roose, T., 2011b.
761 Adsorption and desorption dynamics of citric acid anions in soil. Eur. J. Soil Sci. 62, 733-
762 742.
763 OECD., 2000. Absorption-desorption using a batch equilibrium method. Test guidelines 106,
764 Organisation for Economic Co-operation and Development. Updated Guideline, January,
765 21st, 2000.
766 Parfitt, R.J., Atkinson, R.J., Smart, R.S.C., 1975. The mechanism of phosphate fixation by
767 iron oxides. Soil Sci. Soc. Am. J. 39(5), 837-841.
768 Payne, T.E., Davis, J.A., Waite, T.D., 1996. U sorption on ferrihydrite- effects of phosphate
769 and humic acid. Radiochim. Acta. 74, 239-243.
770 Pfeifer, H.-R., Vust, M., Meisser, N., Doppenberg, R., Croci Torti, R., Domergue, F.-L.,
771 Keller, K., Hunziker, J., 1994. Uranium-enrichment in soils and plants in the vicinity of a
772 pitchblende vein at La Creuzaz/Les Marécottes (W of Martigny, Valais, Switzerland).
773 Eclogae Geol. Helv., 87/2, 491-501.
774 R Development Core Team, 2011. The R project for statistical computing. URL http://www.r-
775 project.org.
776 Ragnarsdottir, K.V., Charlet, L., 2000. Uranium behaviour in natural environments. In:
777 Cotter-Howells, J.D., Campbell, L.S., Valsami-Jones, E., Batchelder, M. (Eds.)
778 Environmental Mineralogy: Microbial interactions, Anthropogenic Influences,
779 Contaminated Land and Waste Management. Vol. 9 of Mineralogical Society Series.

780 Mineralogical Society of Great Britainb and Ireland, Twickenham, United Kingdom, pp
781 245-289.
782 Raicevic, S., Wright, J.V., Velkojic, V., Conca, J.L., 2006. Theoretical stability assessment of
783 uranyl phosphates and apatites: selection of amendments for in situ remediation of uranium.
784 Sci. Tot. Environ. 355, 12-24.
785 Ribera, D., Labrot, F., Tisnerat, G., Narbonne, J.F., 1996. Uranium in the environment:
786 occurrence, transfer, and biological effects. Rev. Environ. Contam. Toxicol. 146, 53-89.
787 Romero Guzman, E.T., Esteller Alberich, M.V., Ordonez Regil, E., 2002. Uranium and
788 phosphate behavior in the vadose zone of a fertilized corn field. J. Radioanal. Nucl. Chem.
789 254(3), 509-517.
790 Römheld, V., 1987. Different strategies for iron acquisition in higher plants. Physiol.
791 Plantarum 70, 231-234.
792 RP, 2008. Référentiel pédologique 2008, Baize, D., Girard, M.-C. (Coord.à, AFES, Editions
793 Quae.
794 Shahandeh, H., Hossner, L.R., 2002. Enhancement of uranium phytoaccumulation from
795 contaminated soils. Soil Sci., 167(4), 269-280.
796 Sheppard, S.C., Evenden, W.G., Anderson, A.J., 1992. Multiple assays of uranium toxicity in
797 soil. Environ. Toxicol. Water Qual.: Int. J. 7, 275-294.
798 Sheppard, S.C., Sheppard, M.I., Gallerand, M.-O., Sanipelli, B., 2005. Derivation of
799 ecotoxicity thresholds for uranium. J. Environ. Radioactiv. 79, 55-83.
800 Stojanović, M.D., Stevanović, D.R., Milojković, J.V., Grubišić, M.S., Ileš D.A., 2009.
801 Phytotoxic effect of the uranium on the growing up and development of the plant of corn.
802 Water Air Soil Pollut. 209, 401-410.

803 Straczek, A., Wanninjn, J., Van Hees M., Thijs, H., Thiry, Y. 2009. Tolerance of hairy roots
804 of carrots to U chronic exposure in a standardized in vitro device. Environ. Exp. Bot. 65(1),
805 82-89.
806 Straczek, A., Duquene, L., Wegrzynek, D., Chinea-Cano, E., Wannijn, J., Navez, J.,
807 Vandenhove, H., 2010. Differences in U root to shoot translocation between plant species
808 explained by U distribution in roots. J. Environ. Radioactiv., 101(3), 258-266)
809 Tailliez, A., Pierrisnard, S., Camilleri, V., Keller, C., Henner, P., 2013. Do rhizospheric
810 processes linked to P nutrition participate in U absorption by *Lupinus albus* grown in
811 hydroponics? J. Environ. Radioactiv., 124, 255-265.
812 Teramage, M.T., Carasco, L., Orjollet, D., Coppin F., 2017. Radiocesium input forms and its
813 extractability in Fukushima forest soils. Under review by Soil Biol. Biochem.
814 Vance, C.P., Uhde-Stone, C., Allan, D.L., 2003. Phosphorus acquisition and use: critical
815 adaptations by plants for securing a nonrenewable resource. New Phytol. 157, 423-447.
816 Vandenhove, H., Cuypers, A., Van Hees, M., Koppen, G., Wannijn, J., 2006a. Oxidative
817 stress reactions induced in beans (*Phaseolus vulgaris*) following exposure to uranium. Plant
818 Physiol. Biochem. 44, 795-805.
819 Vandenhove, H., Van Hees, M., Wouters, K., Wannijn, J., Wang, L., 2006b. Can we predict
820 uranium bioavailability based on soil parameters? Part 2: Soil solution uranium concentration
821 is not a good bioavailability index. Environ. Pollut. 145(2), 577-586.
822 Vanhoudt, N., Vandenhove, H., Smeets, K., Remans, T., Van Hees, M., Wannijn, J.,
823 Vangronsveld, J., Cuypers, A., 2008. Effects of uranium and phosphate concentrations on
824 oxidative stress related responses induced in *Arabidopsis thaliana*. Plant Physiol. Biochem.
825 46, 987-996.
826 Viehweger, K., Geipel, G. 2010. Uranium accumulation and tolerance in *Arabidopsis halleri*
827 under native versus hydroponic conditions. Environ. Exp. Bot. 69, 39-46.

828 Waite, T.D., Davis, J.A., Payne, T.E., Waychunas, G.A., Xu, N., 1994. U(VI) sorption to
829 ferrihydrite: application of the surface complexation model. Geochim. Cosmochim. Acta. 58,
830 5465-5478.
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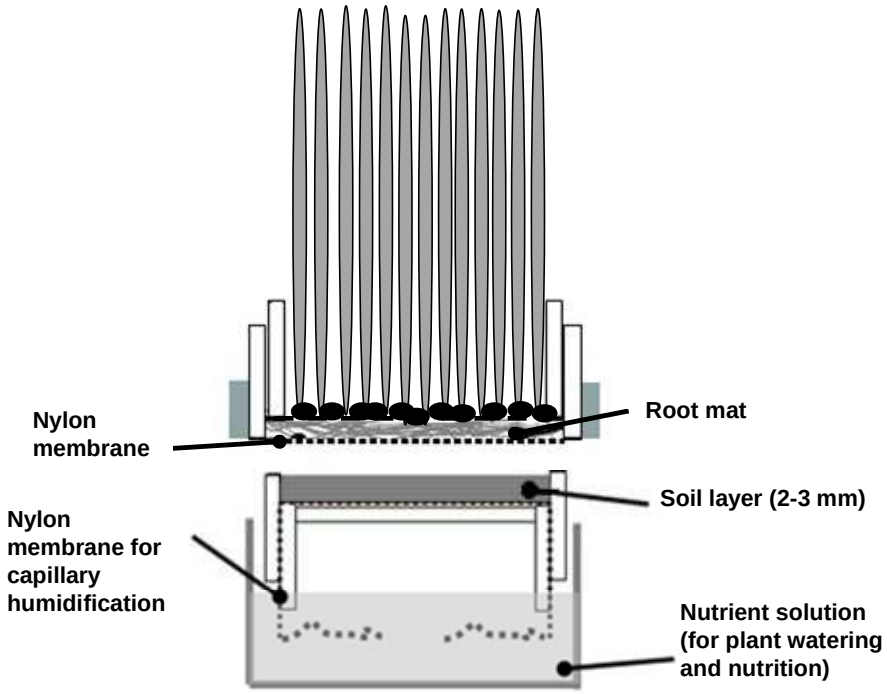

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833 Figure 1: Rhizotest device.

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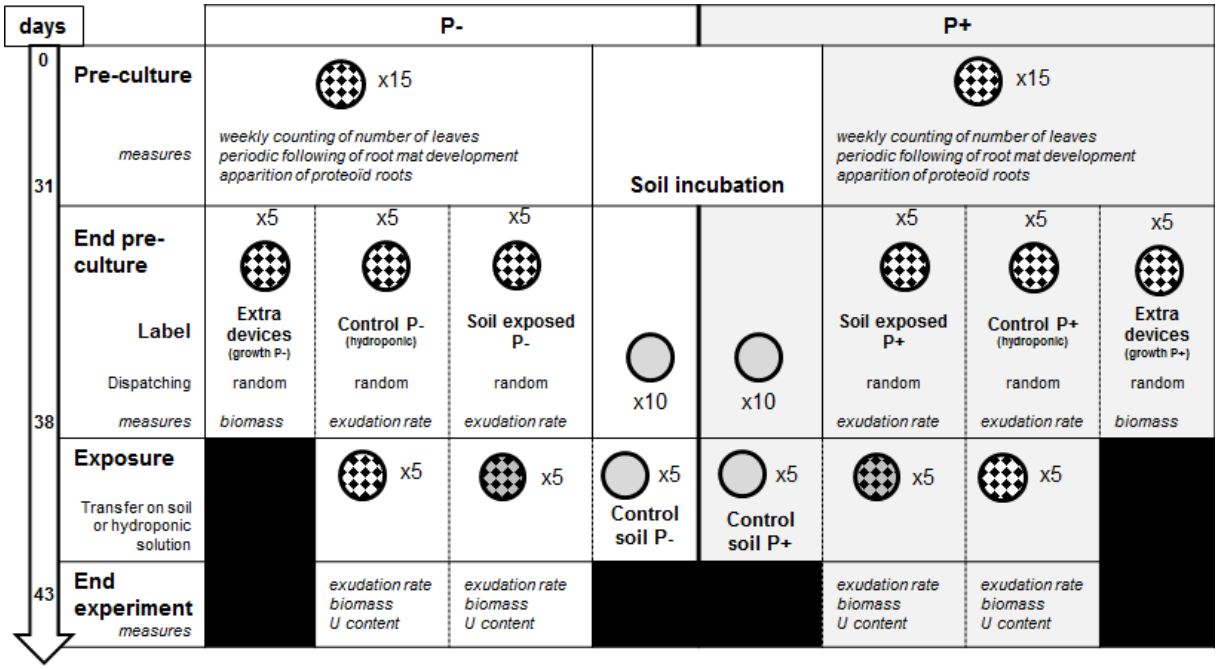


Figure 2: Experimental set-up for the rhizotest experiment including timeline and measures.

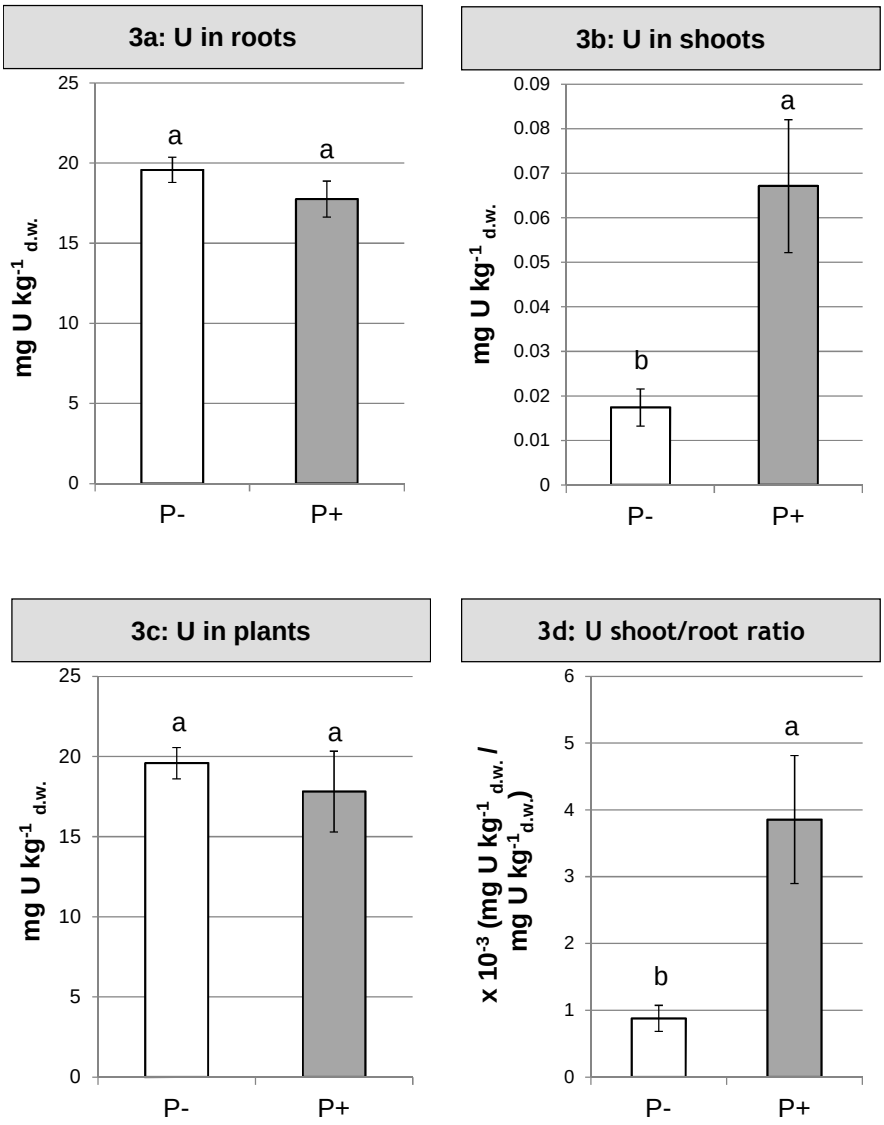
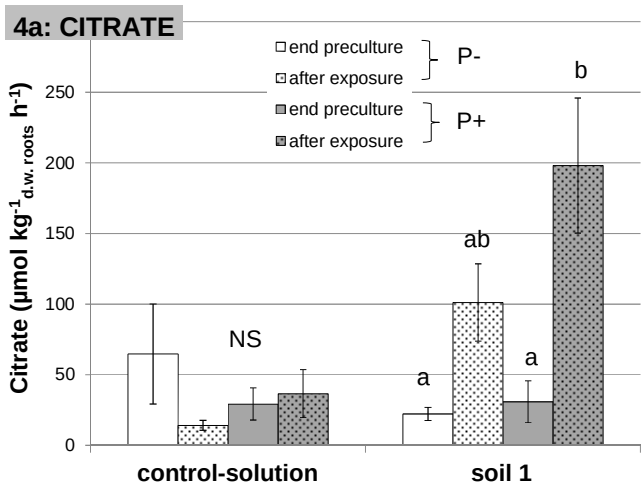


Figure 3**:** Accumulation of U in lupine plants after 5-day exposure to soil 1 in the rhizotest design. 3a: U in roots (in mg U per kg dry matter roots); 3b: U in shoots (in mg per kg dry matter shoots); 3c: U plants (in mg per kg dry matter shoots + roots); 3d: ratio of U accumulation in shoot vs root.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



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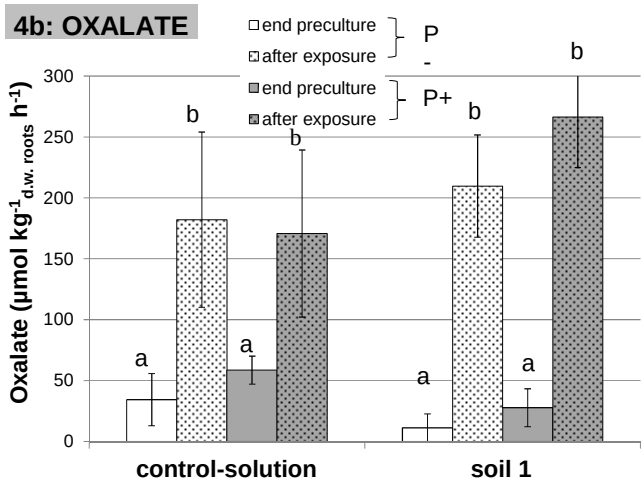

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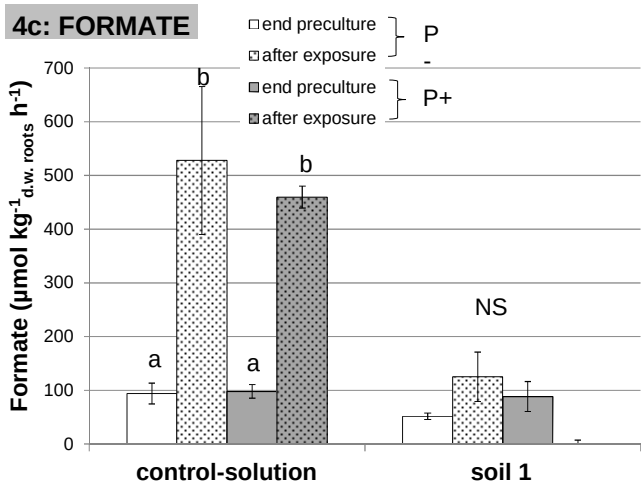

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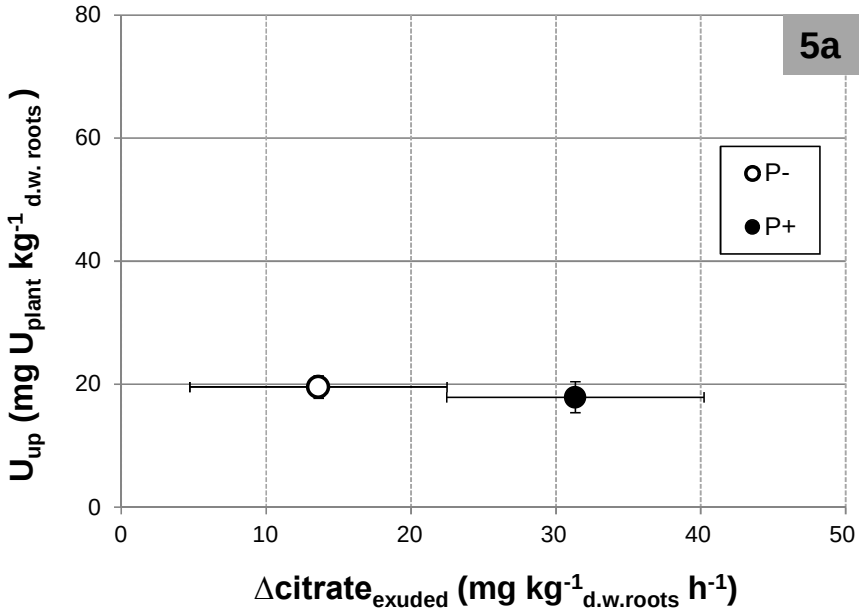
850 Figure 4: Root exudation of citrate (4a), oxalate (4b) and formate (4c) on the rhizotest design
851 by lupine plants at the end of the pre-culture period and after 5 day-exposure to soil 1 or
852 solution as control (mean of 5 replicates ± standard error). Letters: results of 1-factor
853 (condition)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



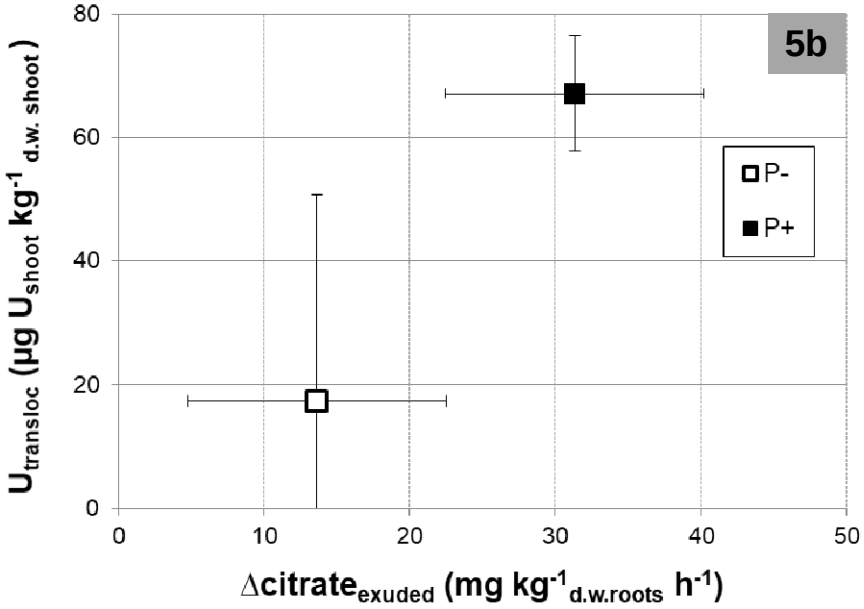
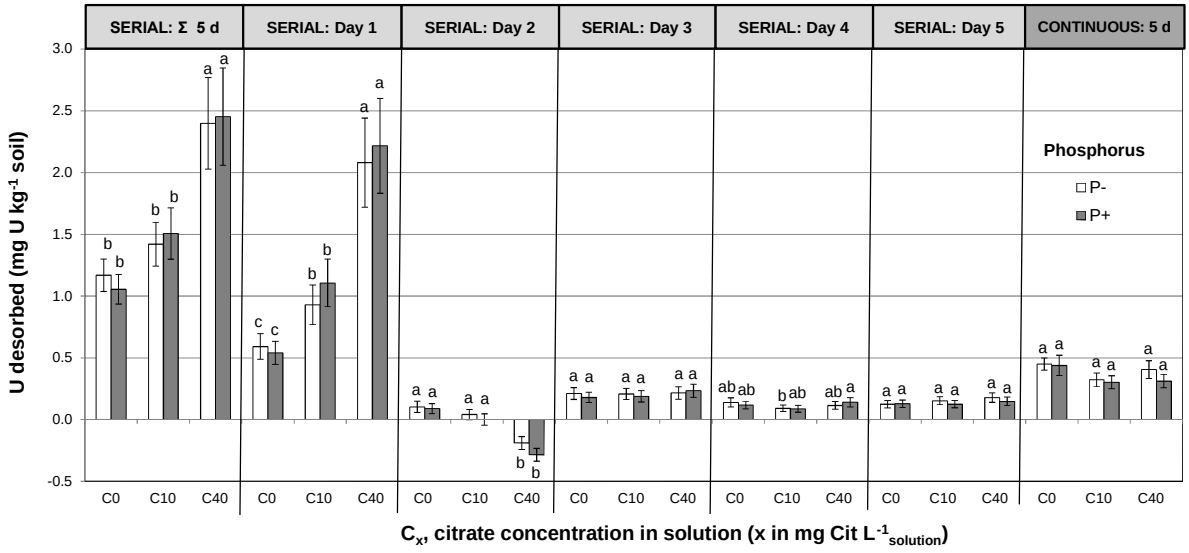


Figure 5: Results of U uptake (total U in plant as related to dry mass of roots, mg U_{plant} $kg^{-1}_{roots\ d.w.}$, 5a) and U translocated to shoots (µg U $kg^{-1}_{shoots\ d.w.}$, 5b) as a function of P level and variation in citrate exudation rate measured between beginning and end of exposure to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



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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 ± s.e. Letters: ANOVA for each day, $p<0.01$.

874 *Values may be positive (desorption) or negative (apparent sorption).*

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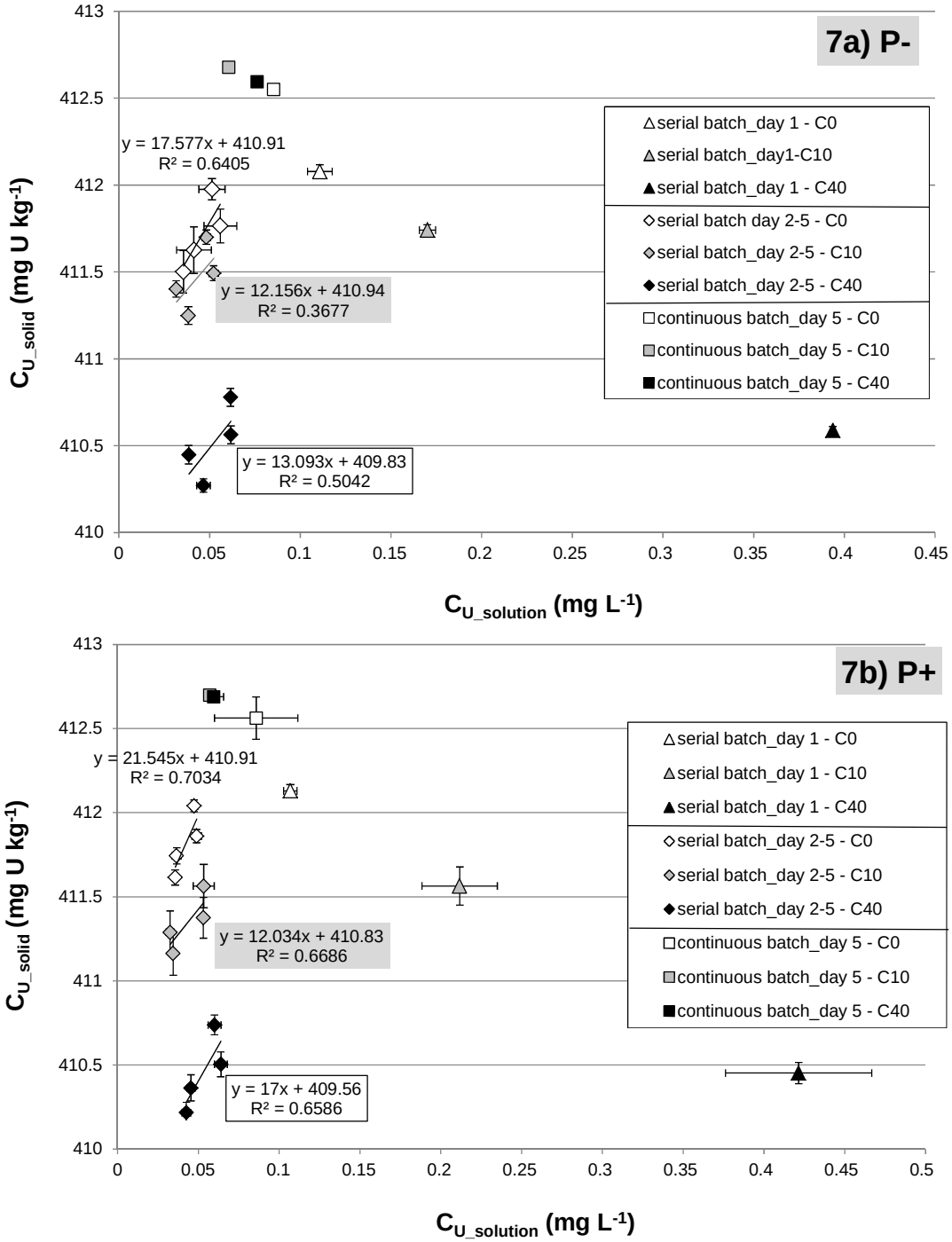

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878 Figure 7: $C_{U\_total\_solid}$ as a function $C_{U\_solution}$ at the end of each step of serial vs continuous

879 batch for the a) P- and b) P+ conditions and all citrate concentrations. Mean of 3 replicates ±

880 s.e.

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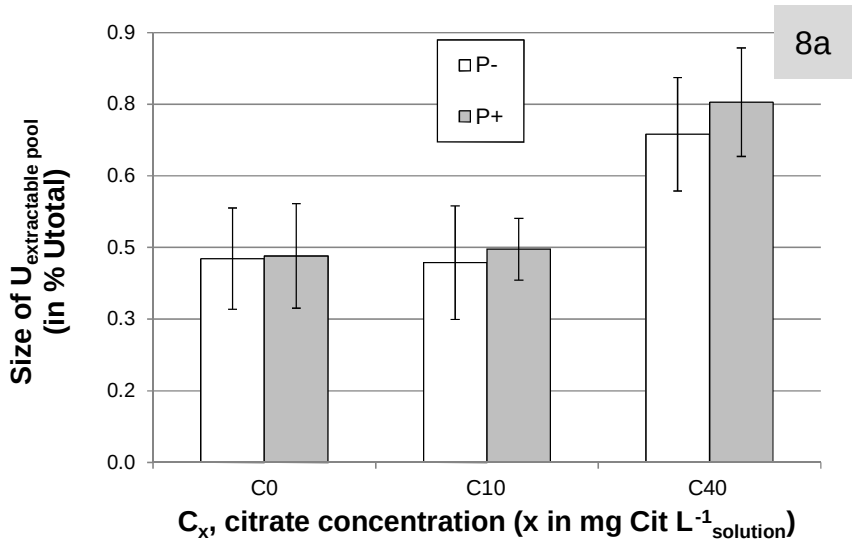

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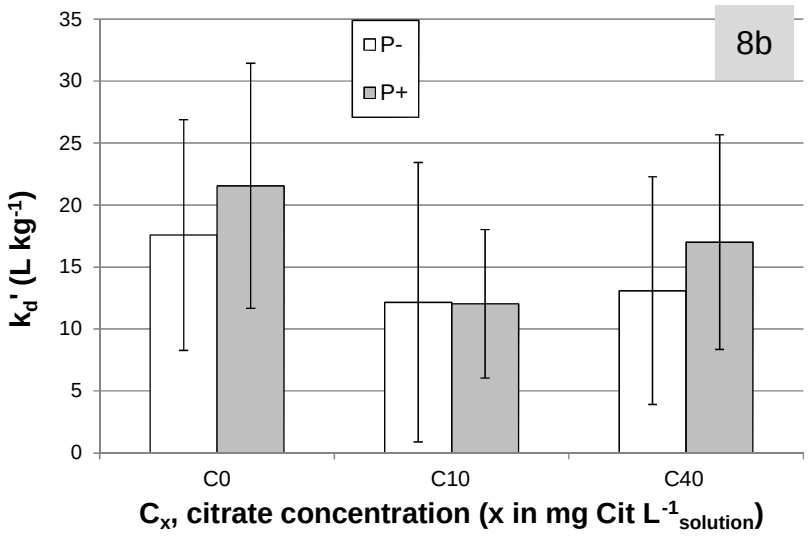

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885 Figure 8: 8a-Size of U extractable pool (% of total soil U) and 8b-k_d' as a function of soil, P

886 and citrate conditions (estimate±s.e.).

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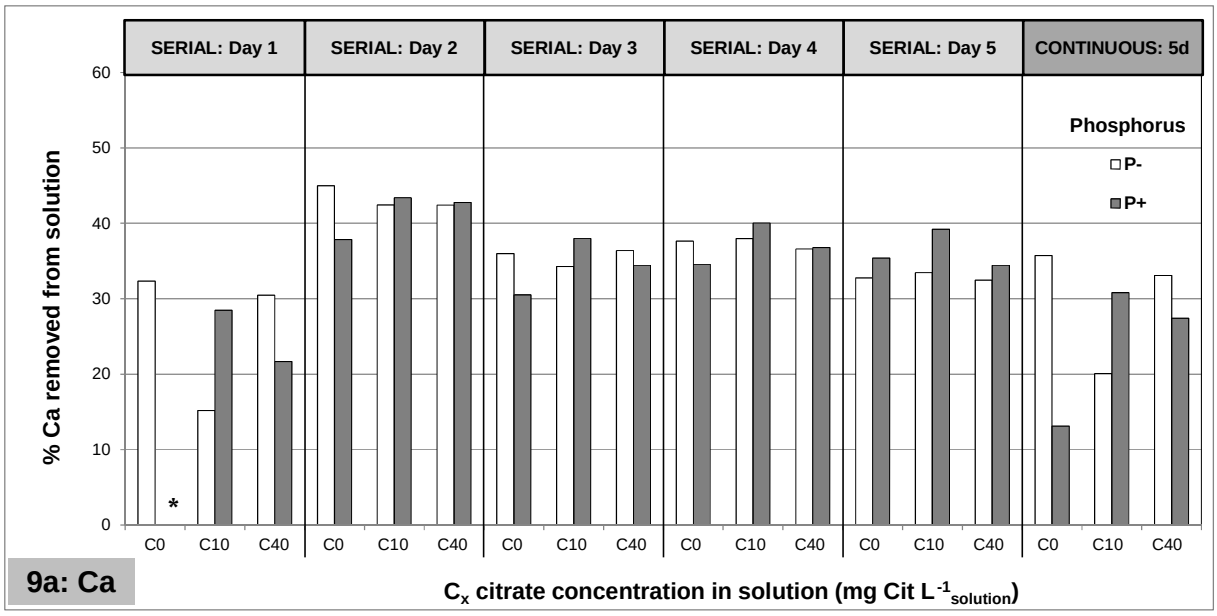
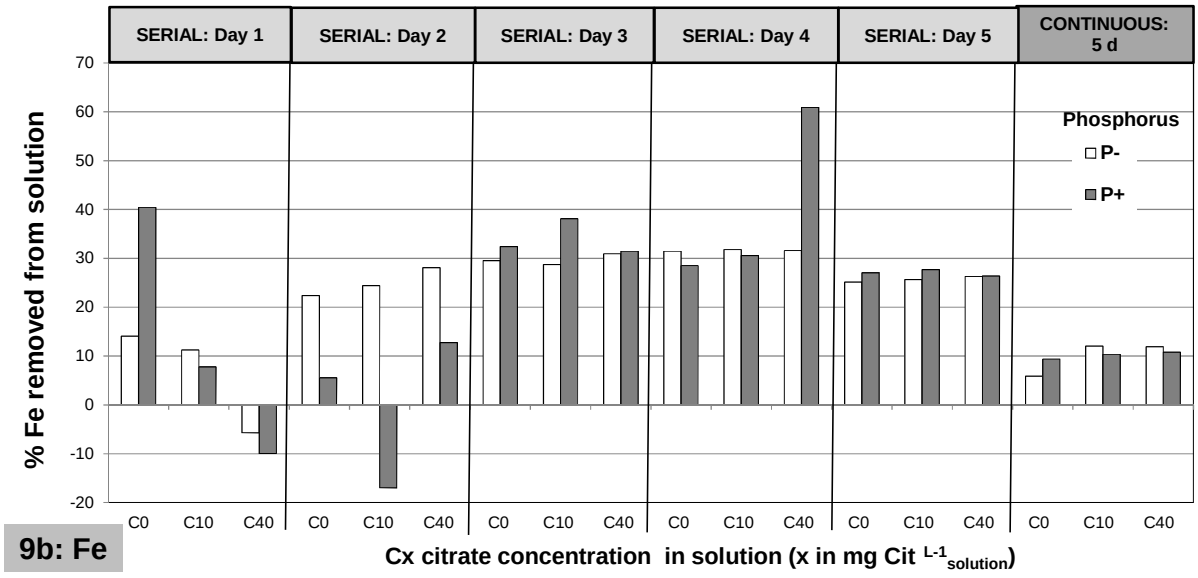
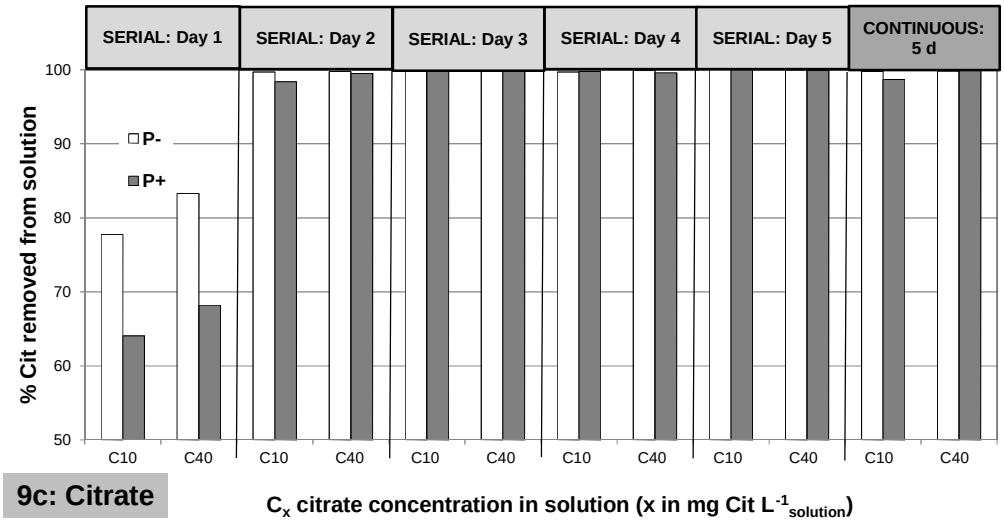


9a: Ca



9b: Fe



9c: Citrate

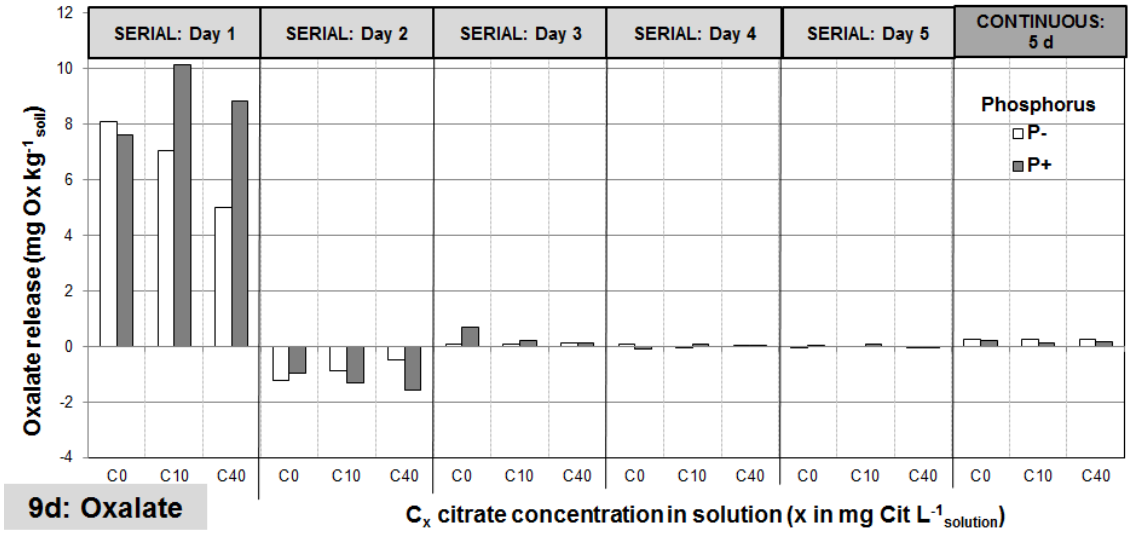


Figure 9: Calcium (9a), iron (9b) and citrate (9c) removal from solution and oxalate (9d) release in solution during each step as a function of citrate concentration in solution (C0/C10/C40 conditions), phosphorus status (P- or P+ for 1 and 100 µM P respectively) and type of batch (serial or continuous) *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_2O		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]	P g kg^{-1}	<0.002
	N mg kg^{-1}	48.94
(in H_2O)	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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Supplementary material

S1: Biomass recorded during the rhizotest experiment

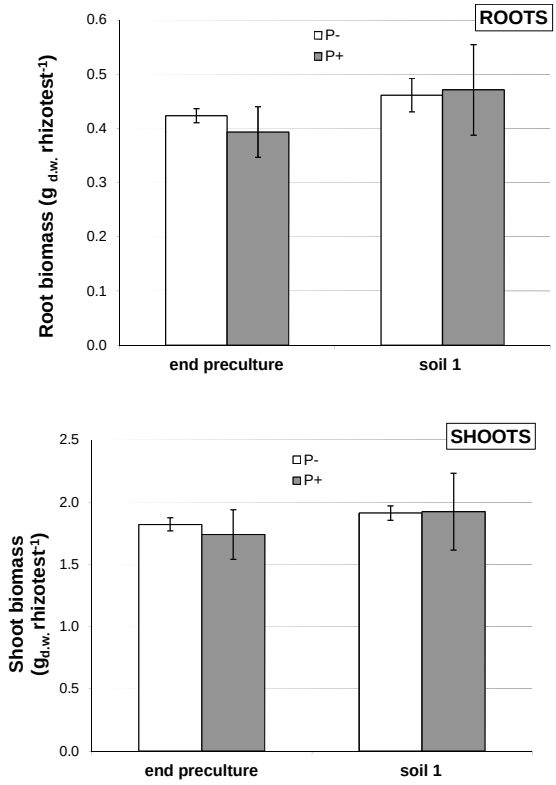

Figure S1 : Rhizotest: biomass of lupine plants

Supplementary material

S2: Evapotranspiration on rhizotests – link with U uccumulation

S2-1: **Evapotranspiration of lupine** on both soils was of the same order in both P condition (175±26 ml in 5 days in –P and 169±10 ml in +P).

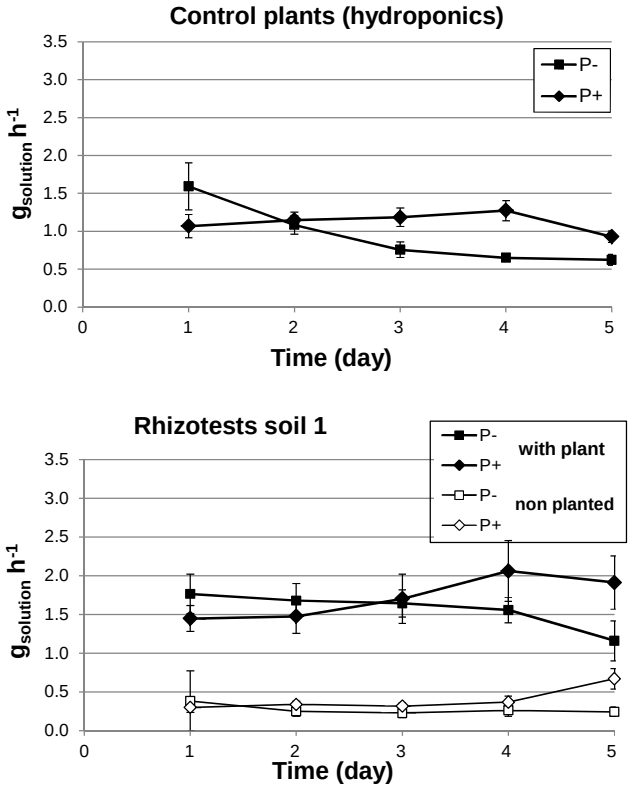

Figure S2-1: Solution fluxes in the rhizotest device (mean of 5 replicates ± standard error).

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

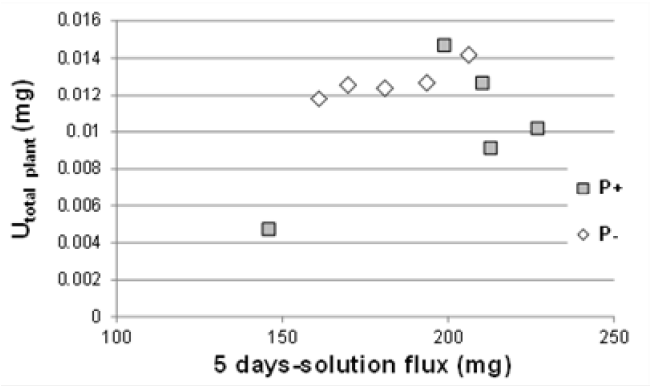

Figure S2-2: Stock of U (mg) accumulated in lupine plant after 5-days exposure to soil 1 in the rhizotest design as a function of the corresponding flux of solution across the rhizotest.

Supplementary material

S3: Additional characterization of the soil samples

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

Supplementary material

S4: Results obtained on soil 2

During the study, 4 soils were collected at different distances from the pechblende vein, in order to get a naturally-produced U gradient in the "same" soil or at least soils with close properties. The experimental plan was too ambitious to be displayed on the 4 soils, thus only two were chosen. The second soil (soil 2) had similar properties (see table below) 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 but displayed in supplementary material as they validate all statements made in this document.

1. Soil 2 properties

Characteristics	Units	Soil 2
Clay (< 2 µm)	g kg^{-1}	141
Loam (2-50 µm)	g kg^{-1}	285
Sand (50-2000 µm)	g kg^{-1}	574
Organic matter	g kg^{-1}	109.6
C/N		17.8
pH-H_2O		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_2O)	P g kg^{-1}	<0.002
	N mg kg^{-1}	47.33
	S mg kg^{-1}	15.37
	C_{org} mg kg^{-1}	1008

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

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

2. Biomass recorded on rhizotests

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

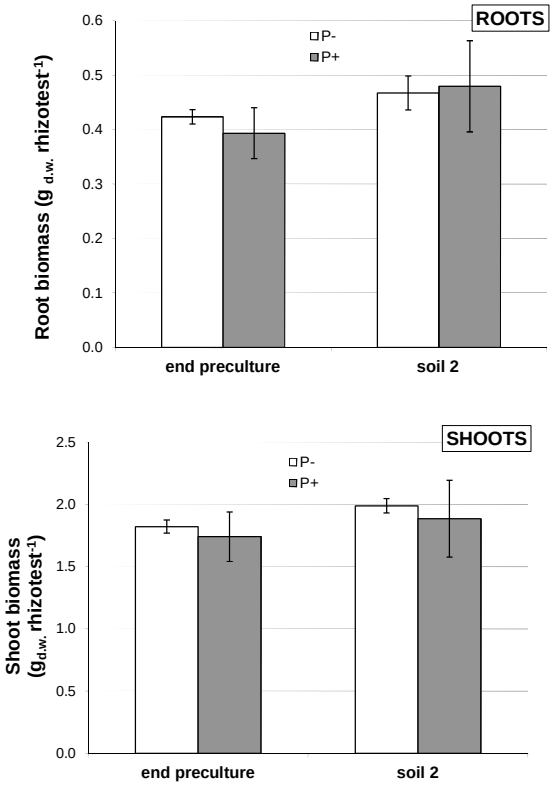

Figure S4-1: Biomass of lupine plants recorded for rhizotest with soil 2.

3. Evapotranspiration

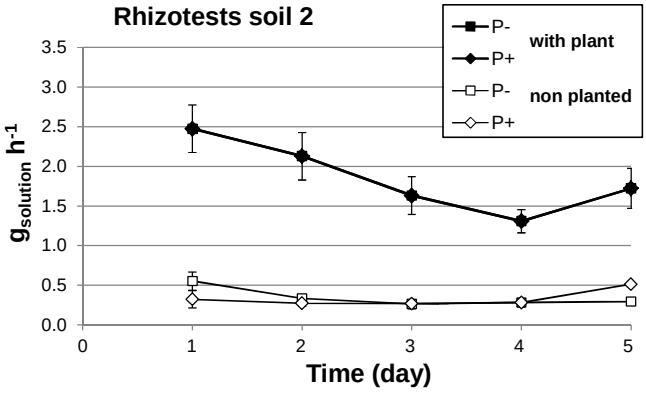

Figure S4-2: Evapotranspiration of rhizotests of soil 2 as a function of P treatment and type of rhizotest.

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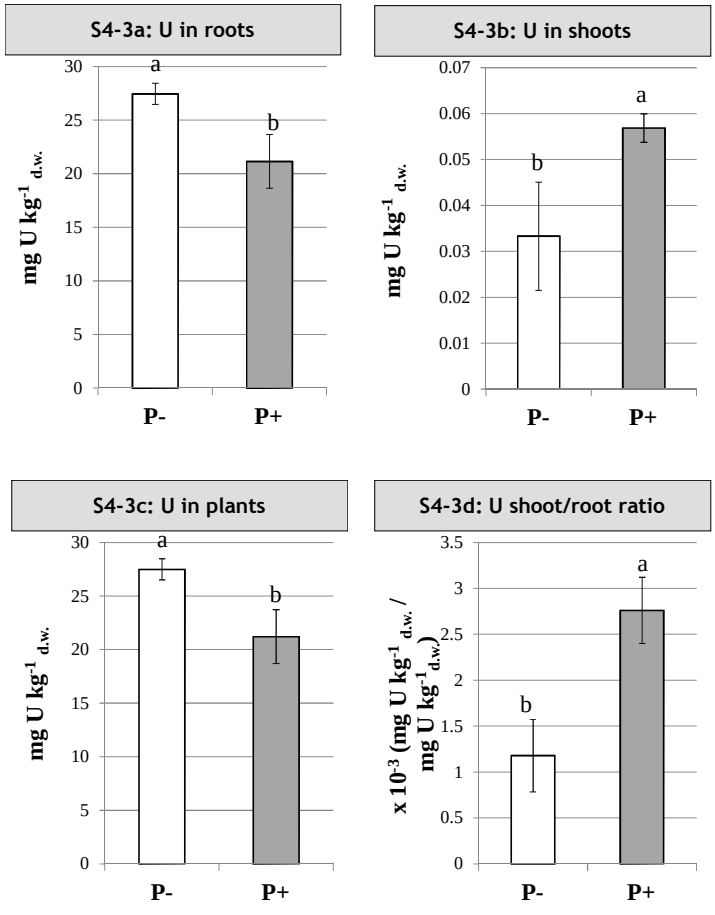
983 **4. U accumulation**



984

985 Figure S4-3**:** Accumulation of U in lupine plants after 5-day exposure to soil 2 in the rhizotest
986 design. 3a: U in roots (in mg U per kg dry matter roots); 3b: U in shoots (in mg per kg dry
987 matter shoots); 3c: U plants (in mg per kg dry matter shoots + roots); 3d: ratio of U
988 accumulation in shoot vs root. Mean of 5 replicates ± s.e. Letters: differences between P
989 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).

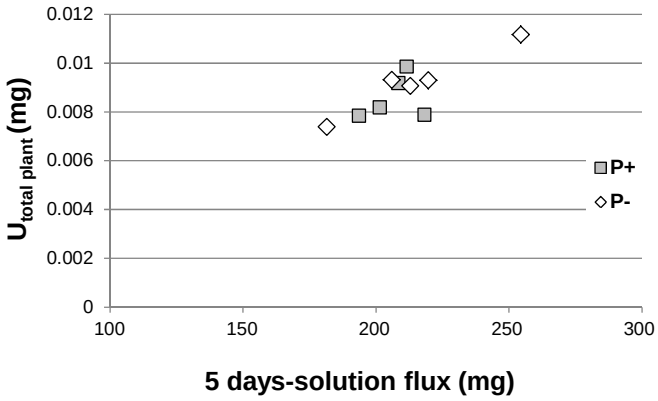
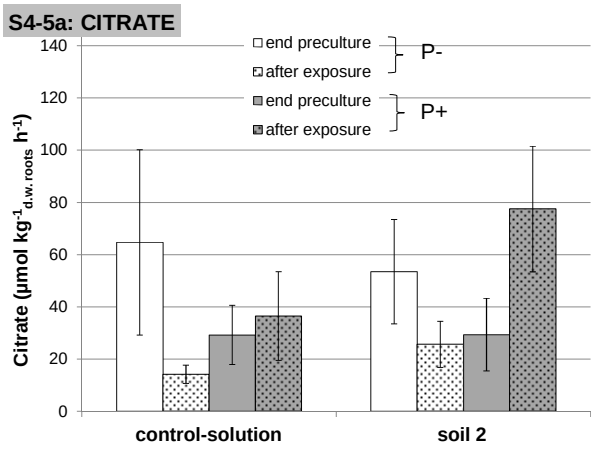
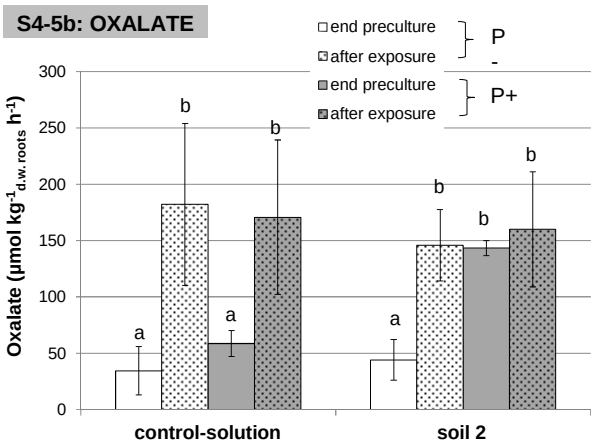


Figure S4-4: Stock of U (mg) accumulated in lupine plant after 5-days exposure to soil 2 in the rhizotest design as a function of the corresponding flux of solution across the rhizotest.

5. Exudation





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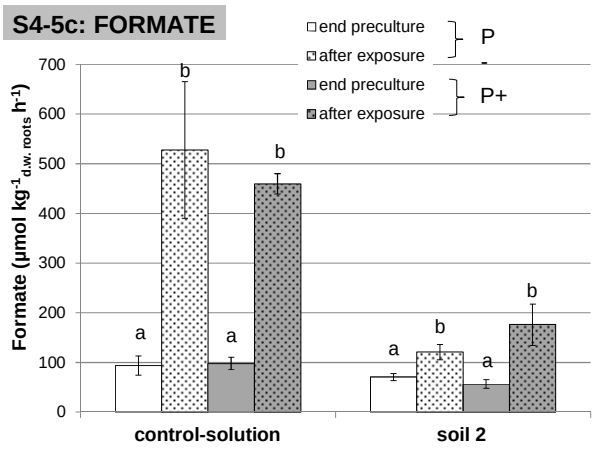
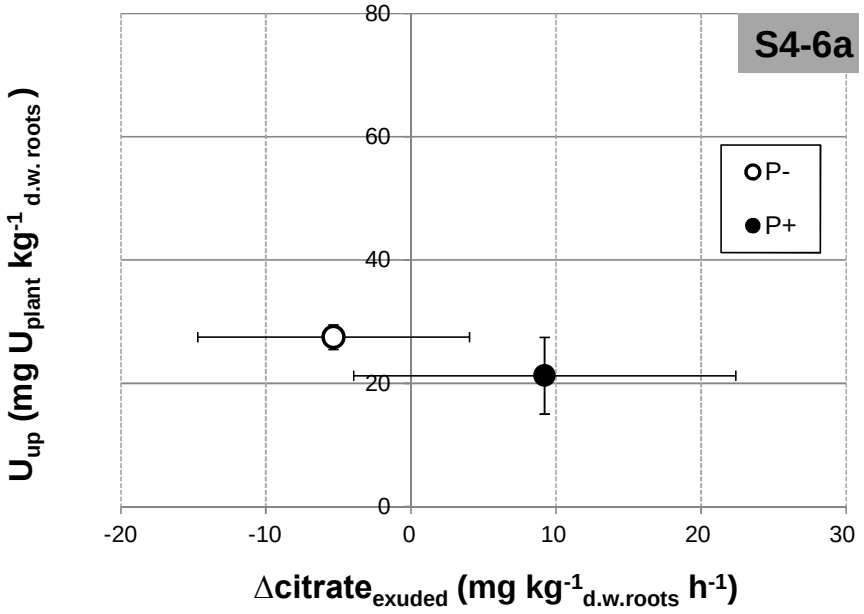


1004

1005 Figure S4-5: Root exudation of citrate (5a), oxalate (5b) and formate (5c) on the rhizotest
1006 design by lupine plants at the end of the pre-culture period (38 days) and after 5 day-exposure
1007 (43 days of growth in total) to soil or solution as control (mean of 5 replicates ± standard
1008 error). Letters: statistical analysis ($P<0.05$).

1009

1010 Main conclusions addressed for soil 1 are valid for soil 2 (Fig S4-5, Fig. S4-6) with the
1011 following differences: in P-, citrate exudation is not enhanced after soil exposure and for
1012 oxalate exudation there is no differences between P conditions (Fig. S4-5). The increase in U
1013 translocation in P+ condition compared to P- condition is recorded for lower citrate exudation
1014 rates in soil 2 than in soil 1 (Fig. S4-6).



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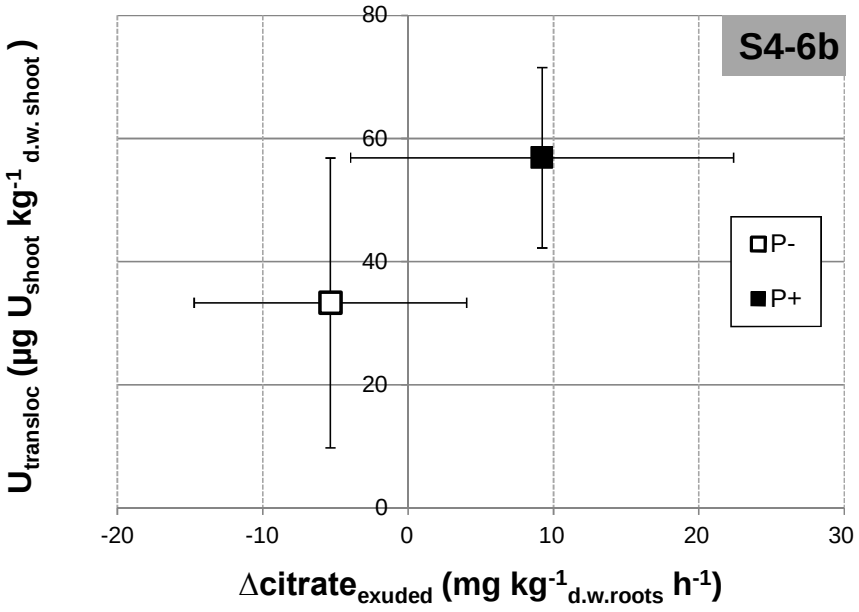

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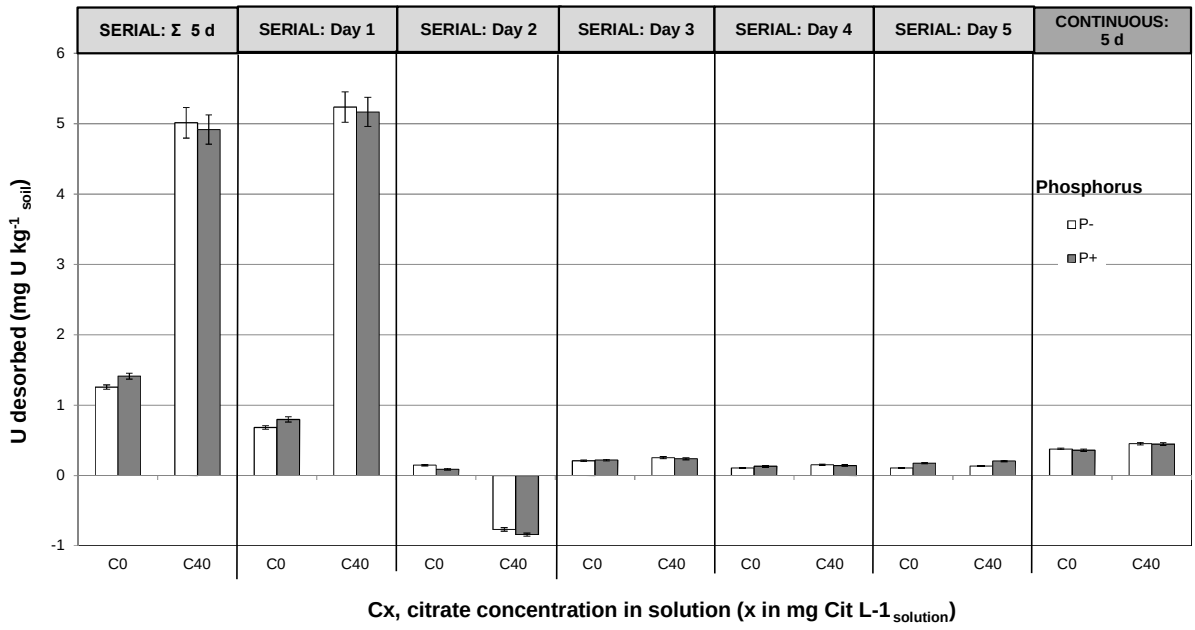
1017 Figure S4-6: Results of U uptake (total U in plant as related to dry mass of roots, mg U_{plant} kg^{-1}
1018 $_{roots\ d.w.}$, 6a) and U translocated to shoots (µg U kg^{-1} $_{shoots\ d.w.}$, 6b) as a function of P level and
1019 variation in citrate exudation rate measured between beginning and end of exposure to soil2
1020 ($\Delta citrate_{exuded}$). Mean of 5 rhizotests ±s.e.

1021

1022 **6. Batch results for soil 2**

1023 6.1. Uranium

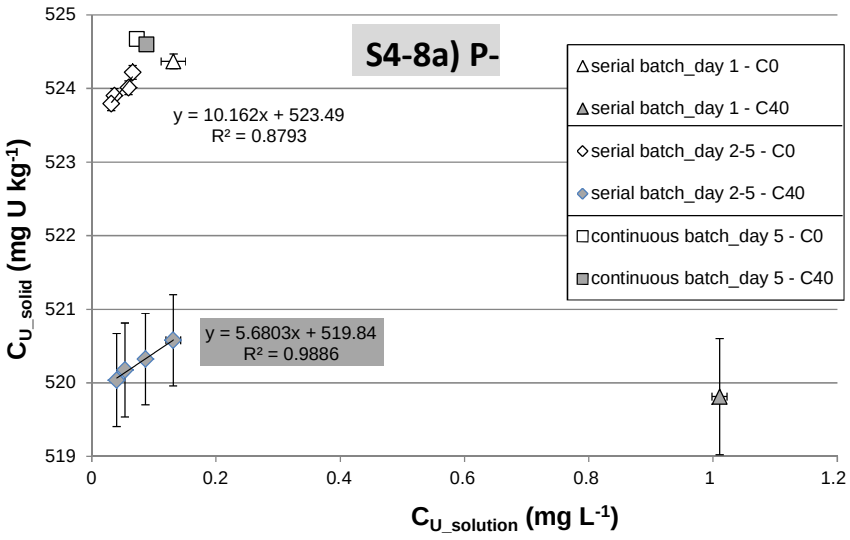
1024 Results for soil 2, as detailed in the following figures are consistent with conclusions stated
1025 for soil 1 (Fig S4.7, S4-8).



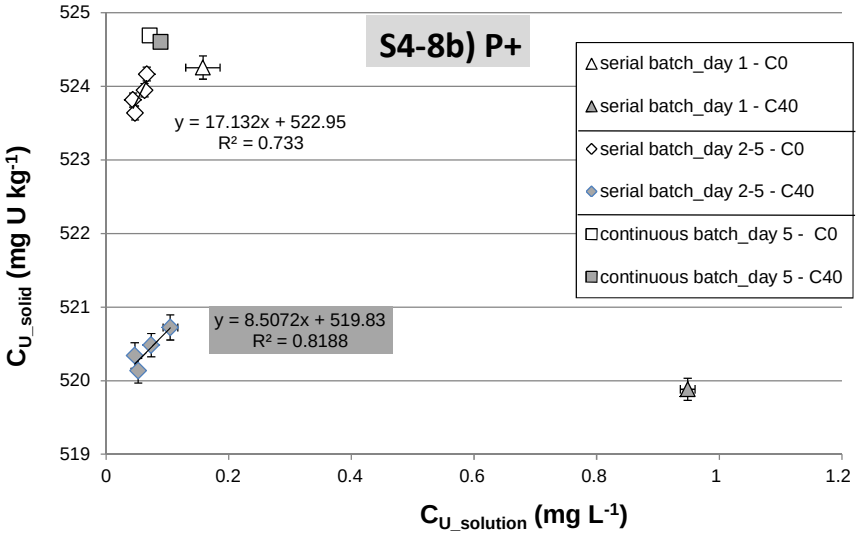
1026

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 ± s.e. Letters: ANOVA for each day,
1030 $p<0.01$. *Values may be positive (desorption) or negative (apparent sorption).*

1031



1032

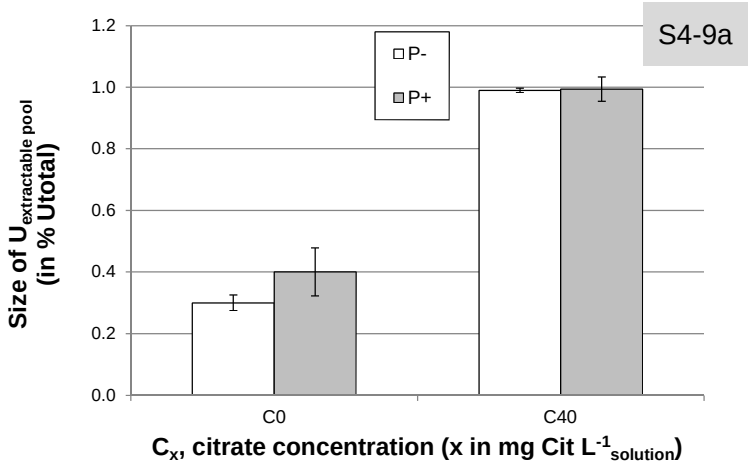


1033

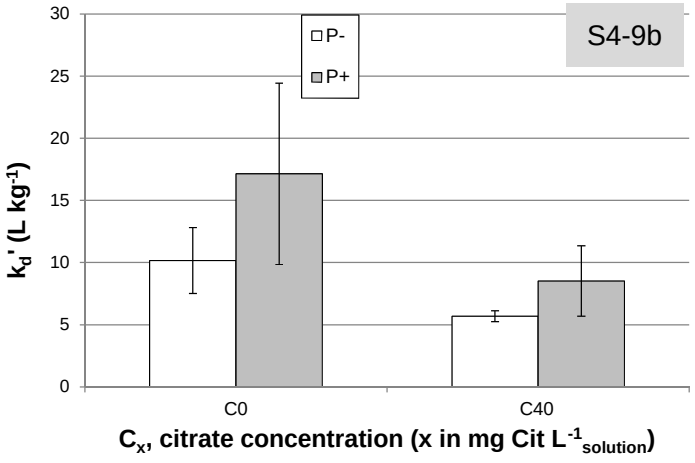
1034 Figure S4-8: Soil 2- $C_{U\_total\_solid}$ as a function $C_{U\_solution}$ at the end of each step of serial vs
1035 continuous batch for the a) P- and b) P+ conditions and all citrate concentrations. Mean of 3
1036 replicates ± s.e.

1037

1038 In the absence of citrate, the U extractable pool was estimated to be 0.4±0.1% of total soil U
1039 as for soil 1, but the pool in P- condition (0.3±0.1%) was slightly lower than in P+ condition
1040 (Fig S4-9a.). The size of the extractable pool increased with the high citrate concentration
1041 (C40) up to 0.99% for soil 2, a value higher than for soil 1. The extractable pool was easily
1042 accessible as shown by the corresponding low k_d' values for all modalities (Fig S4-9b). The
1043 availability (as estimated by the level of U extractability) in soil 2 tended to be lower than in
1044 soil 1, especially with citrate.



1045



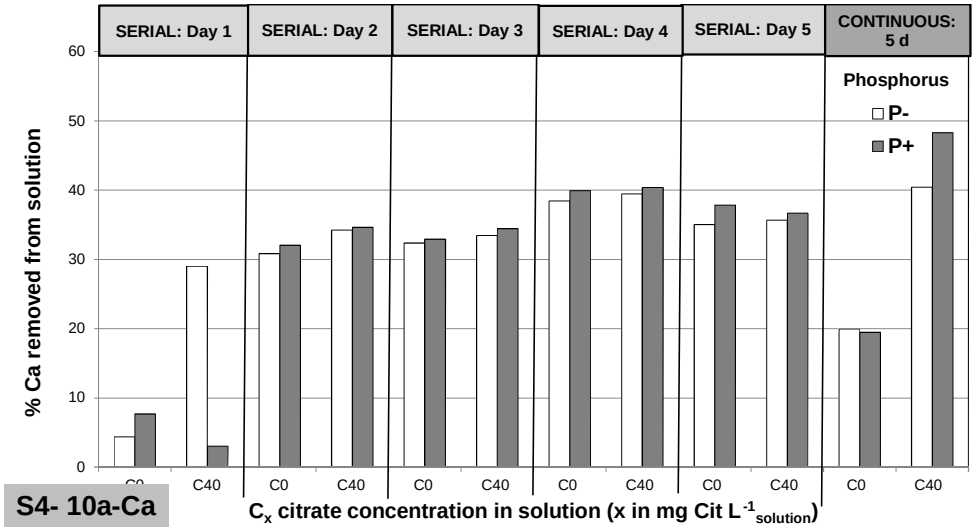
1046

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.).

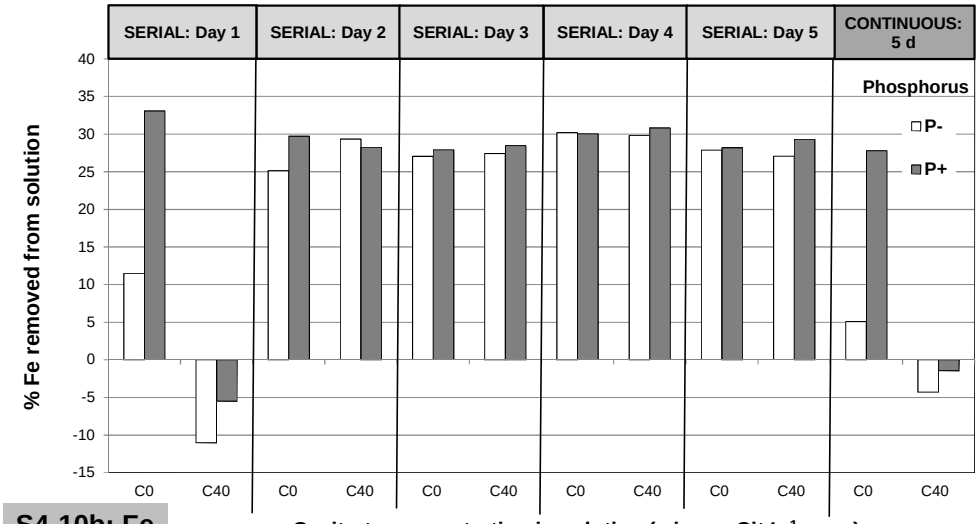
1049

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



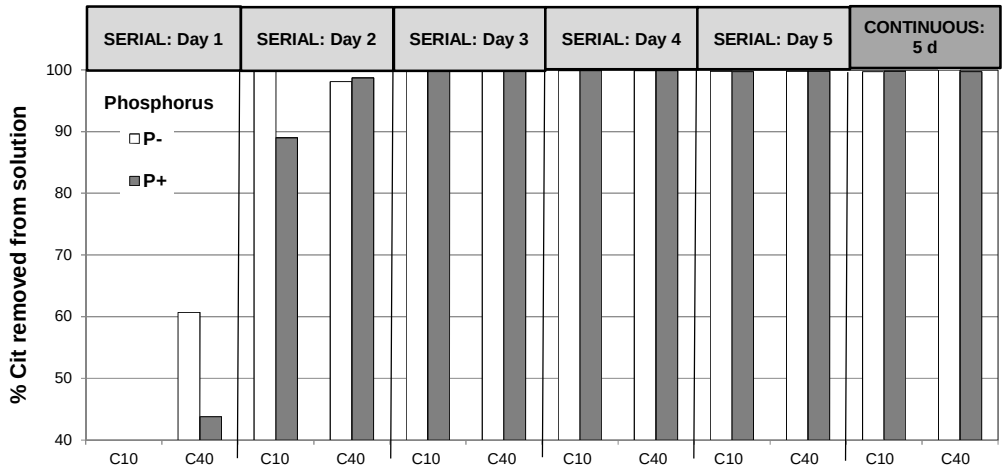
S4- 10a-Ca

1051



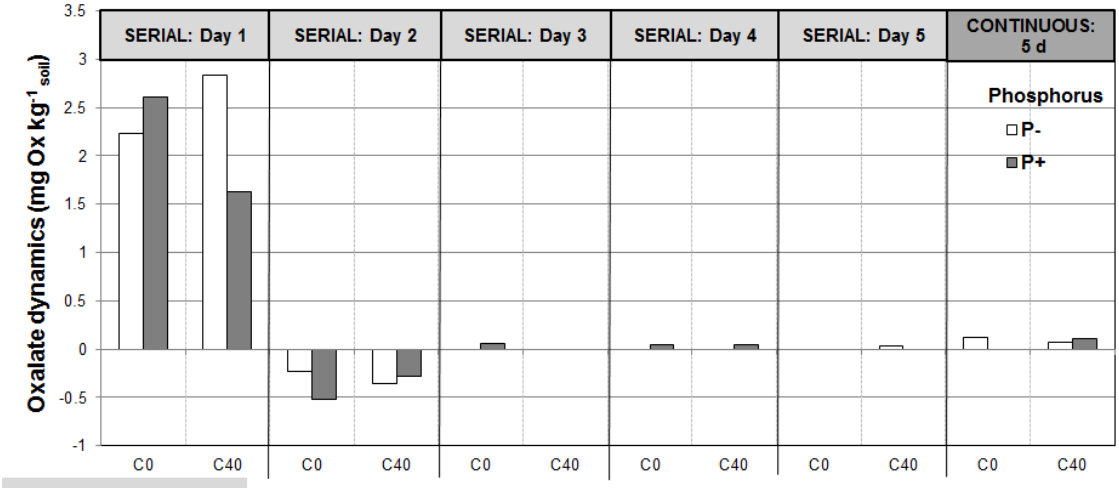
S4-10b: Fe

C_x citrate concentration in solution (x in mg Cit $L^{-1}_{solution}$)



S4-10-c: Citrate

C_x citrate concentration in solution (x in mg Cit $L^{-1}_{solution}$)



S4-10d: Oxalate

C_x citrate concentration in solution (x in mg Cit $L^{-1}_{solution}$)

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

(serial or continuous). *Contrary to U and Oxalate, Fe, Ca and citrate are present in the initial solution, thus dynamics was calculated as the difference between final and initial solution concentration. 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).*