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REGULAR ARTICLE

Fine scale measurement and mapping of uranium in soil solution in soil and plant-soil microcosms, with special reference to depleted uranium

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Abstract
Background and aims Residues from use of depleted uranium (DU) munitions pose a lasting environmental impact through persistent contamination of soils. Consequently, an understanding of the factors determining the fate of DU in soil is necessary. An understudied factor is the interaction of root exudates with DU. This study describes the use of 'Single-Cell-Sampling-and-Analysis' (SiCSA) for the first time in soil and investigates the effects of root exudates on DU dissolution.
Methods Soil solutions from soil and plant-soil microcosms containing DU fragments were sampled and analysed using SiCSA and capillary electrophoresis/ICP-MS for organic acids and uranium.
Results Nanolitre volumes of soil solution were sampled and analysed. Soils with DU fragments but no citrate addition showed low uranium concentrations in

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contrast to those with added citrate. Lupin root exudation gave concentrations up to 8 mM citrate and 4.4 mM malate in soil solution which solubilised DU fragments yielding transient solution concentrations of up to 30 mM.
Conclusions Root exudates solubilise DU giving high localised soil solution concentrations. This should be considered when assessing the environmental risk of DU munitions. The SiCSA method was used successfully in soil for the first time and enables investigations with high spatial and temporal resolution in the rhizosphere.

Keywords Organic acids · Small-volume-sampling-and-analysis · Plant exudates · Lupin · Rhizosphere · Proteiod roots

Introduction

The importance of the plant/soil interface as a distinct micro-site for physicochemical and biological processes is well recognised (Hinsinger et al. 2009). The many interconnecting processes occurring are complex, challenging to predict and need to be understood over a wide range of spatial and temporal scales. When the processes are "averaged" by large sample sizes, or are not time-resolved, important resolution is lost. This may lead to an incomplete understanding of interactions and processes essential for the prediction of behaviour at the larger scale. For example, the "average" concentration of a solute may not represent a value likely to have a biological impact, but this does not exclude the possibility that there are micro-sites where biologically significant concentrations do occur. At finer scales, corresponding to individual roots, quantitative descriptions and analyses of the dynamic heterogeneity of the rhizosphere are hampered by the physical opacity and impenetrability of soil. Oburger et al. (2011) recently drew attention to the lack of available techniques to measure organic acid anion gradients at sufficient spatial resolution in the rhizosphere. Luster et al. (2009) reviewed many ingenious approaches to sampling and analysing this medium. These include analysis of the soil solution that is the direct external medium for the activities of the cells of roots and microorganisms. These authors set four challenges that need to be overcome: 1. Gaining access to the soil solution in-situ. 2. Miniaturization of the sampling system to obtain (small enough) samples to suit the resolution required of the investigation. 3. Analytical techniques to match these sample sizes. 4. Achieving this in a native structured soil. Considerable progress has been achieved in the first three of these.

This paper describes the application of sampling and analysis techniques that have previously been used for the investigation of individual plant cells in-situ (Single Cell Sampling and Analysis—SiCSA) (Tomos and Leigh 1999). These techniques are combined with the soil microcosm approach that facilitates access to rhizosphere soils (Darwent et al. 2003; Dinkelaker and Marschner 1992). The SiCSA approach utilises glass micro-capillaries of micro-metre dimensions to sample fluid directly from the surface film of individual soil particles. It addresses two of the problems associated with soil solution analysis identified by Luster et al. (2009). These are the volume of sample, and hence spatial resolution, and the problem of contamination of these small samples. To address the latter, the technique uses a minimum number of transfer steps after sample capture and does not involve a concentration step. Some indication of the volume that samples are drawn from can be obtained from consideration of the sample volume and the percentage water of the soil. Samples of 30 μl (the smallest referred to by Luster et al. (2009)) would occupy some 176 μl of a 17 % w/w hydrated soil (even if air spaces were ignored). This would occupy a sphere of a radius of 3.48 mm. It can be assumed that many soils of interest would not be waterlogged. SiCSA-type analysis of biological samples has been performed for volumes below 1 pl (Webster et al. 1995). We have found, however, that volumes of 2–3 nl are more tractable for soil solutions where hydrostatic (matric) forces make extraction of small volumes difficult. A 2 nl sample would occupy 11.76 nl of soil which would occupy a sphere of radius 141 μm (for the same conditions as stated above), resulting in 25 times better spatial resolution than previously reported (Luster et al. 2009). Using micro-manipulation, such droplets can be obtained from accessible wet surfaces with negligible contamination. Moreover, by maintaining the micro-sample with no or minimal dilution the sample can be analysed rapidly by instrumental techniques without danger of further contamination. Anions or cations can be measured to micro-molar concentrations by X-ray microanalysis (EDX) (Tomos et al. 1994) and custom built capillary electrophoresis (Bazzanella et al. 1998). Another technique suitable for trace element analysis is ICP-MS, where a special nebulizer can allow the use of low volumes of sample (Luster et al. 2009; Takasaki et al. 2011). The merits of these analytical

approaches are listed by Luster et al. (2009), but there is now considerable potential to greatly increase the resolution of these techniques. Another feature of the SiCSA approach involves the use of constriction micro-pipettes to allow quantification via internal standardisation (Tomos et al. 1994; Tomos and Leigh 1999). The use of such pipettes greatly facilitates the sampling and analysis of soil solutions.

One application which is suited to the SiCSA approach is the investigation into the behaviour of Depleted Uranium (DU) in contaminated soils. DU-containing weapons have been used extensively in Iraq, Kosovo and Bosnia Herzegovina (UNEP 2003; 2005). On impact with hard objects, 10–35 % of the munitions are converted into dust or aerosols. Munitions that penetrate the soil corrode over time, a process that may contaminate local groundwater (Bleise et al. 2003). The subsequent main pathways into the human or animal body are though dust inhalation and ingestion of contaminated water (Choy et al. 2006). This raises health concerns, since DU is both weakly radioactive and chemically toxic (Bleise et al. 2003; Schott et al. 2006). The behaviour of DU is affected by abiotic and biotic factors in the soil. These can cause adsorption to or desorption from soil components, oxidation or reduction, dissolution or reprecipitation and complexation of the uranium (Crancon et al. 2010; Handley-Sidhu et al. 2010). All of these processes will affect the migration of DU in the soil and its leaching into the groundwater.

A substantial amount of work has recently been carried out on the fate of depleted uranium in the soil environment (Brittain et al. 2012; Crancon et al. 2010; Graham et al. 2011; Handley-Sidhu et al. 2010; Handley-Sidhu et al. 2009; Mellini and Riccobono 2005; Oliver et al. 2008; Oliver et al. 2007; Sajih et al. 2010; Schimmack et al. 2007; Torok et al. 2004). However, one process that has not been investigated fully is the effect of plant exudates on the solubility and movement of DU penetrator corrosion products. Using soil contaminated with uranium (U), Duquène et al. (2009) recently found that 1 month's growth of either Indian mustard or rye grass produced soil solution concentrations of U twenty fold higher in sandy soil and 2 fold higher in clay soil than in non-planted soil. This impact of plants may be mediated by root exudates that affect the solubility of U. The composition of root exudates is highly variable and dependent on plant species, plant age and physiochemical environment (Jones 1998). However, it is well known that organic acids are a universal component (Jones 1998; Jones and Darrah 1994). Their release has been suggested as a general mechanism for solubilizing metals from the soil's mineral phase, as an Al detoxification mechanism (Jones and Darrah 1994) and as a mechanism for solubilizing phosphorous by some plant species in P deficient soils (Dessureault-Rompre et al. 2007; Neumann and Romheld 1999). Of these organic acids, citric and malic acids are often released in the highest quantities (citrate ~0.072–569, malate ~0.288–1,217 $nmol^{-1} g^{-1} h^{-1}$; (Jones 1998; Jones and Darrah 1995).

In soil remediation trials, citric acid proved to be the most efficient amendment in solubilising U from soil (Duquène et al. 2008; Huang et al. 1998b) and in increasing its uptake by plants (Huang et al. 1998b). Malic acid proved to be next most efficient (Huang et al. 1998b; Sevostianova et al. 2010). Although there is a change of soil solution pH of between 0.5 – 1 units with the exudation of these acids (Huang et al. 1998b), pH is not the only parameter affecting U solubility. Sevostianova et al. (2010) recently found that citric acid and ammonium citrate had similar effects on plant uptake of U, implying that it is the citrate anion and not the change in pH that is most important in solubilising U. Citrate and malate both form complexes with uranium which both aids in the solubilisation of solid uranium minerals and in keeping uranium in solution (Huang et al. 1998b; Kirishima et al. 2008). At pH 6, uranium in oxidised solutions is mainly found in the form of uranyl hydroxide species while when citrate and malate are present at this pH or slightly lower, uranium is mainly complexed by them (Fox et al. 2006; Krestou and Panias 2004). These effects are of importance to the environment in general and human wellbeing in particular. It is therefore desirable to gain an improved understanding of the effect of root exudates on depleted uranium in soil.

In this investigation, our approach was to apply high resolution soil solution sampling and microanalysis in order to study root exudate effects on U in soil. Specifically, we investigated the impact of roots and root exudate compounds on the solubility of DU derived from munitions testing.

Materials and methods

Soil sampling and characterization

Sandy soil was sampled from the Eskmeals firing range in the NW of England in May 2006 (with

permission from QinetiQ, Eskmeals, Cumbria, UK). A history and description of the site is provided by Oliver et al. (2007). Two bulk soil samples (referred to as Soil 1 and Soil 2) were taken from the top 10 cm of a grassy dune area adjacent to the firing site, 50 m from the concrete firing apron. This had been intended as control material, but was found to contain depleted uranium (DU) at 86.9 and 592 $mg kg^{-1}$, respectively for Soil 1 and 2 (Table 1). Samples were sieved to 2 mm and stored at room temperature in sealed bags until required.

Fragments of yellow (corroded) DU shrapnel about 5 mm in diameter were collected from a known DU contaminated area adjacent to the concrete firing apron. Fragments, approximately 1 mm in diameter of this were used in the experiments described below.

Soil 1 was used for the mini-microcosms. Unlike the fresh soil, stored soil was found to be high in nitrate despite its storage at near dry conditions. In order to restore it to a more natural state, aliquots (500 g) of Soil 1 were planted with either wheat (*Triticum aestivum* var Hereward) or lupin seedlings (*Lupinus albus* v Dieta; Processors and Growers Research Organisation, Thornhaugh, Peterborough, UK). The seeds germinated and were allowed to grow for one month in a greenhouse without addition of light or heat (temperature 11–40 °C) and with daily watering. The soil was then harvested and the plant roots removed. This conditioned soil was subsequently stored moist at 4 °C with a small opening in the bag to allow for gas exchange. Prior to use it was made up to~17 % w/w moisture content (MC) using 18 MΩ water (Elga) and kept at room temperature for 3 days. The other two treatments were the original soil 1 brought up to 17 % MC using 18 MΩ water or adjusted to 17 % MC using 30 mM sodium citrate to simulate root exudates.

Table 1 Characteristics of soil samples from dunes approximately 50 m from test firing apron at Eskmeals firing range. Total U and initial soil solution U mean±SE (n=3)

	Soil 1	Soil 2
pH	5.7	6.0
Soil Organic Matter %	1.6	2.9
U $mg kg^{-1}$	86.9±9.0	592±49
Soil texture	Sand	Sand
Initial soil solution U mM	0.22±0.13	0.20±0.09

Soil pH was measured using a 1:1 ratio of soil to deionized water. After shaking, this was allowed to settle and was measured with an Orion 410A pH meter (Thermo Fisher Scientific Inc., Beverly, MA). Soil organic matter was estimated by loss-on-ignition (LOI, 500 °C, 6 h) on dried samples (105 °C) (West MC30, Vexstar Furnaces, Chesterfield, UK).

Soil mini-microcosms

In total, 8 mini-microcosms were prepared by backing a rectangle of ABS (acrylonitrile butadiene styrene, 8× 3.7 cm) with an oval window (6.9 cm long and 2 cm wide, 3 mm deep) with a glass microscope slide (Figure S1a). The microcosm lid was also a microscope slide, but during sampling this was replaced by an ABS lid with a 2 cm square window surrounded by moist filter paper (to reduce soil drying out under the microscope lights (fibre-optic cold lights, Intralux 5000, Volpi) Figure S1b). The microcosm was filled with sieved soil (~6.5 g moist soil, ~ 17 % MC, 5.4 g dry soil equivalent). At the start of the sampling period, a 1 mm fragment of DU was inserted into the soil such that it was still visible. Between sampling, the microcosm was held vertically (Figure S1c); during sampling, horizontally. Soil solution samples were taken from within 0.2 mm of the DU fragment and 3 mm below it (when vertical). Two replicates were performed for each treatment. Samples were analysed by capillary zone electrophoresis (CZE) for anions and inductively coupled plasma—mass spectrometry (ICP-MS) for uranium.

Plant-soil microcosms

To allow the growth of roots, larger microcosms were prepared on a Perspex back plate (30×15×0.5 cm, Figure S2). A Perspex lid of the same size was held in place with clips. Between the lid and the soil were 4 Perspex plates that could be removed to enable sampling of a small area with minimal disturbance to the rest of the microcosm. In total, 7 planted and 4 control microcosms were filled with Soil 2 made to 20 % MC with 18 MΩ water (268–280 g). *Lupinus albus* v Dieta (Processors and Growers Research Organisation, Peterborough) were inoculated with Rhizobium bacteria and planted at the top of the microcosm (where appropriate). Microcosms were maintained at ~20 % MC by periodically opening and spraying with deionised water. The microcosms were wrapped in black plastic (to avoid

phototropic effects and algal growth) and placed at a 30° angle, face down (to increase the number of roots accessible for sampling), in a growth chamber (Sanyo Fitotron SGC066.CPX) with a 16 h day (20 °C), 8 h night (16 °C) and 75 % humidity. Light intensity at leaf level was 480 μmol $m^{-2}s^{-1}$. The plants were allowed to grow and the roots examined regularly to identify when proteoid (cluster) roots were produced. Control microcosms were prepared and maintained in the same way as plant microcosms, but without the plants.

Under these conditions, it is thought that new proteoid roots elongate for 4 days and then exude large quantities of organic acids for 2–3 days (Watt and Evans 1999). When proteoid root initiation was identified, the roots were allowed to grow for 4 days. Then a sample was taken from next to the cluster root. A 1 mm fragment of DU was then placed in the same location. Sampling next to the DU fragment continued twice daily for 5 days. In parallel, a 1 mm fragment of DU was inserted into a control microcosm (without roots) and sampled in the same manner. Samples were analysed for anions by CZE and U by ICP-MS.

Small volume sampling

A modified version of the SiCSA approach was used to sample the soil solution (Tomos et al. 1994; Tomos and Leigh 1999). A schematic of the set up can be seen in Fig. 1. Small samples (~2 nl) were taken with a constriction micro-capillary pipette filled with water saturated silicon oil, mounted on a micromanipulator (Leitz) and observed under a stereomicroscope (Leitz Wild M8). The tip of the micro-capillary was placed in the solution and it was drawn into the capillary, up to the constriction, using a syringe with a 3 way valve (Tomos et al. 1994). The sampling constriction pipettes were pulled from borosilicate glass capillaries (O.D. 1 mm, I.D. 0.58 mm; Harvard Apparatus Ltd, Kent) using a commercial pipette puller (Model 700C, Harvard Instruments, Kent, UK.) and constrictions inserted with a microforge (de Fonbrune 5854, Ch Beaudouin, Paris, France). Samples for capillary zone electrophoresis (CZE) analysis were stored under paraffin oil (all chemicals were supplied by Sigma-Aldrich) saturated with 18 MΩ water on a microscopy slide with a numbered grid (Tomos et al. 1994). Samples for ICP-MS analysis (^{238}U measurement) were placed in 100 μl 10 % HNO_3 (Ultrapure) in the lid of a 2.5 ml Eppendorf vial. The vial was then closed and the sample centrifuged, mixed and centrifuged again to ensure complete mixing of sample and acid. In both cases, an internal standard (10 mM MoO_4^{2-} (as Na_2MoO_4) for CZE and 5 mM Cs (as CsCl) for ICP-MS) of an equal volume to the sample

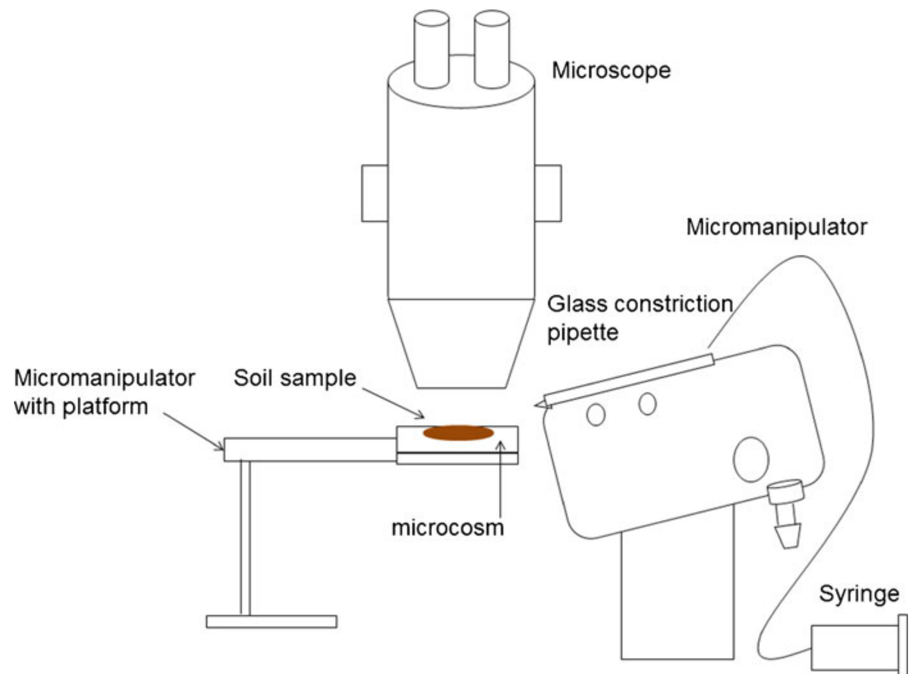

Fig. 1 Schematic of small volume sampling set up

(achieved by using the same, washed, constriction pipette) was added to the sample to permit quantification by internal standardisation.

Capillary zone electrophoresis

Organic anion analysis was performed, as described by Bazzanella et al. (1998), on a laboratory-built CZE system, equipped with a Lambda 1000 UV detector (Bischoff, Leonberg, Germany) and a high voltage power supply (HCN 6 M 30000, Omiran Ltd, Suffolk). Conditioned fused-silica capillaries, 60–80 cm long, of 50 μm i.d.; 365 μm o.d. (Composite Metal Services Ltd, Shipley, W. Yorks, UK.) were used. Sample injection was by manual suction using a syringe barrel while observing the sample and the end of the CZE capillary (under paraffin oil) with a dissecting microscope (Leitz Wild M8) before transfer to the cathode buffer. Anions, including citrate and malate, were analysed using an electrolyte containing 2.5 mM pyromellitic acid (PMA), 15 mM Tris (hydroxymethyl) aminomethane and 1 mM DoTAOH (Dodecyltrimethyl ammonium hydroxide). The last reagent was prepared from the bromide salt using an anion exchange column. Separation was achieved at 28–30 kV (4–500 V.cm^{-1}). Detection was by the indirect UV method (displacement of PMA) at 245 nm and the molybdate ion was used as internal standard. Concentrations were calculated using the conversion factor method (Beckers and Bocek 2004).

ICP-MS analysis

Uranium determination (^{238}U) was carried out using an ICP-MS (7500ce, Agilent Technologies, Wokingham, Berkshire, UK) fitted with an APEX Q high efficiency nebuliser system (Elemental Scientific Inc., Omaha, NE) which permitted the introduction of small samples (~ 45 μl). ICP-MS parameters were: Forward power 1,550 W; carrier gas 0.7–0.94 Lmin^{-1}; make up gas 0.22–0.3 Lmin^{-1}; data acquisition mode—Time resolved analysis; integration time 0.10 s/point for ^{238}U and ^{133}Cs. Apex Q parameters: heater 140 °C, chiller 2 °C, introduction of N_2 gas 3 bar, flowrate 10 ml min^{-1}. Instrument calibration was achieved using U and Cs standard solutions. 0.01, 0.1, 1 and 10 μgL^{-1} U and Cs standards were prepared in 10 % HNO_3 (Aristar, BDH, Poole, UK.) from serial dilution of the separate commercial 1,000 mgL^{-1} stock solutions (Claritas PPT, Spex CertiPrep Inc., Metuchan, NJ, USA). CsCl (5 mM) was added to collected soil solution samples as an internal standard.

Results

Mini-microcosms

The soluble uranium concentration in the control soil solution (17 % MC; Soil 1) prior to addition of the DU fragment was found to be 0.22±0.13 mM while the total concentration in soil was 0.365 mMoles kg^{-1} (86.9 mgkg^{-1}) (Table 1). These values indicate that the dune 50 m from the firing apron, from where the sample was taken, was significantly contaminated with the metal. The second sample (soil 2) had even higher levels.

When individual fragments of dry, corroded DU shrapnel (approximately 1 mm^3) were placed in the moist soil, occasionally a small transient increase in soluble U was noted close to the fragment (<1 mm) (Fig. 2a) but not 3 mm below (data not shown). This may have been brought about by the dry DU fragment surface becoming hydrated and dissolving a little as it was brought into contact with moist soil. This soluble DU was presumably subsequently (by 7 h) adsorbed to soil particles.

Soluble uranium did not exceed 0.7 mM next to the DU fragment or 0.5 mM, 3 mm below the fragment (3 mm data not shown) during the experiment. The conditioning process of growing wheat or lupin in the soil prior to its use had no measureable effect on its soluble U content and the transient increase was observed in all treatments. It did, however, significantly decrease the soil nitrate concentration as expected. Conditioned and unconditioned soil solution contained 0.05–0.19 mM and 14–20 mM soluble nitrate respectively, but this did not affect the soluble U concentrations. The control, lupin and wheat-conditioned soils had soluble citrate and malate concentrations below 0.5 mM .

From their colour and texture, the corroded shrapnel fragments appeared to be composed of metaschoepite ($UO_3.nH_2O$ n=< 2; ie. U^{VI}) (Handley-Sidhu et al. 2009). No metallic uranium (U^0) was observed in any of our samples.

The impact of citrate on solubilisation of uranium was determined by SiCSA. Soil was moistened with

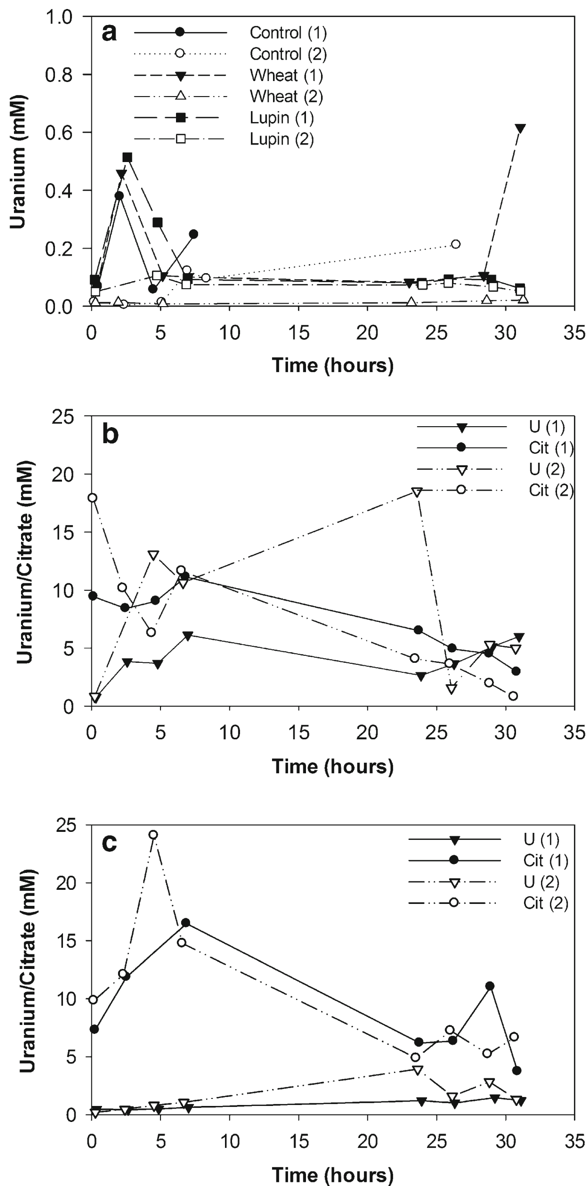

Fig. 2 Effect of pre-conditioning and of citrate on the time course of rhizosphere concentrations in mini-microcosms using soil 1 **a**) Uranium concentration in soil solution sampled next to a DU shrapnel fragment in control, wheat-or lupin-treated soil (no plant roots or added citrate; replicate experiments 1 and 2). **b** and **c**) Uranium and citrate concentrations in soil solutions of citrate-treated soil next to DU fragment (**b**) and 3 mm below fragment (**c**) (no plant roots; replicate experiments 1 and 2)

30 mM citrate solution, but at the start of sampling, 22 min after addition, the soluble citrate concentration was found to be only between 9.4 and 17.9 mM (Fig. 2b). Figure 2b and c demonstrate the effect the presence of citrate had on the dissolution of uranium from the shrapnel fragment. This was in contrast to the control and plant conditioned soils. Although the concentrations of soluble U varied, in one microcosm a concentration of 18 mM was recorded; which was higher than the apparent concentration of soluble citrate. Levels remained high for many hours although after 25 h it decreased in some cases. A lower concentration of uranium in solution was found 3 mm below the fragment (0.2–4 mM) compared to next to the fragment, up to 32 h after citrate addition (Fig. 2c). From these measurements it seems that diffusion is the mode of transport in these soil solutions.

Soil-plant microcosms

The soil of microcosms in which lupins were growing and of unplanted microcosms, were sampled over four experimental periods of 5 days each (Fig. 3a–f). This sampling was begun 4 days after the initiation of the growth of the proteoid roots. The first sample was taken close to the root surface and then a fragment of DU was placed in the same position and subsequent sampling was carried out next to the DU fragment. The initial samples prior to positioning of the DU fragment gave soil solution concentrations for U of 0.20±0.09 for unplanted and 0.51±0.34 mM in the planted microcosms. This shows that the presence of plant roots increased the concentration of U in soil solution prior to shrapnel being added. In several cases elevated levels of citrate or malate were not observed in the soil solution next to roots (presumably these were not functioning proteoid roots). Their concentrations remained in the same range as the control microcosms for citrate and malate (< 0.5 mM, data not shown) and resulted in low uranium concentrations in soil solution (<1 mM) which were similar to those found in control microcosms (Fig. 3a and b). In one control microcosm (Fig. 3a) a small transient increase of uranium was seen similar to that found in the mini-microcosms.

Four plant microcosms, however, generated appreciable concentrations of citrate and malate adjacent to evident proteoid roots over the expected 4 day trajectory (Fig. 3c–f) (Watt and Evans 1999). In three of the four (Fig. 3c–e), citrate concentrations reached between 5 and 8 mM. Malate levels were more modest and in the range 0.7–4.4 mM. In two microcosms (Fig. 3c and d) the uranium concentration was similar

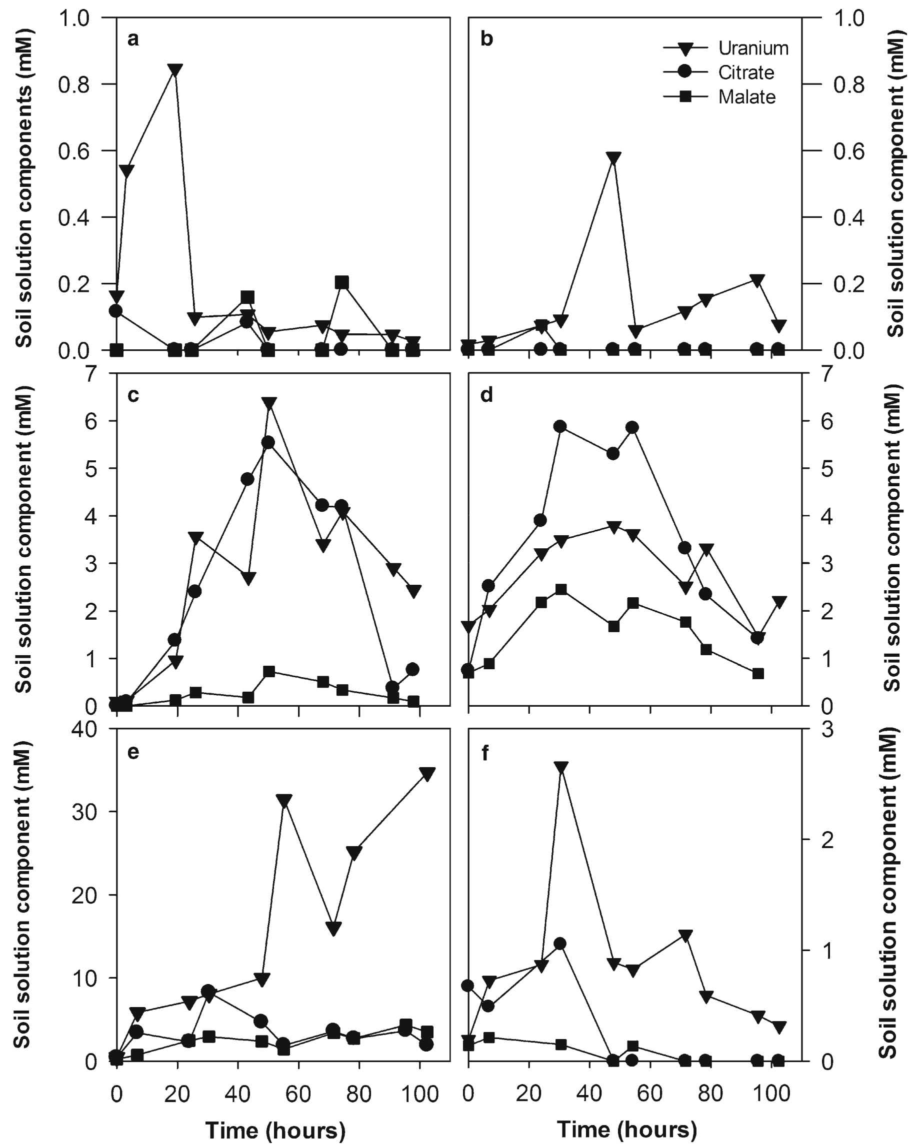

Fig. 3 Synchrony of organic acid release and uranium solubilisation in soil 2. Uranium (▼), citrate (●) and malate (■) soil solution concentrations in control microcosms (**a**–**b**) and plant-soil microcosms (**c**–**f**), (sample from close to DU fragment and functional proteoid root)

to that of citrate; rising and falling in synchrony, peaking between 30 and 60 h after the beginning of sampling. In microcosm 3e, in contrast, the concentration of soluble uranium continued to rise, reaching over 30 mM when sampling ceased. In microcosm 3f, the concentrations of both soluble uranium and citrate where lower than that of the other microcosms. As in microcosms 3c and 3e, however, the concentrations of uranium exceeded those of citrate (and malate) at the end of sampling. This would indicate a phase after soluble organic acid degradation in which uranium retains its elevated solubility and mobility.

Discussion

In the soil mini-microcosms, soluble U concentrations were very low in the control and wheat or lupin conditioned soils despite the fact that rhizosphere soil is said to have a steady state concentration of organic acids of between 1–4 mM (Schwab et al. 2008) which would promote solubilisation of U. As the plant-conditioned soils had soluble citrate and malate concentrations below 0.5 mM this may have been caused by degradation of these organic acids prior to soil use, despite being kept refrigerated. Organic acid degradation in soil is fast, with a reported half-life for citrate of

2.8–10.5 h and of 0.5–3.4 h for malate (Oburger et al. 2009). The degradation may have occurred during the growth period or during refrigerated storage, although cooling would have slowed the degradation rate. As a consequence of this low organic acid concentration the solubilisation of uranium in these and the control treatments was very low.

In the citrate treated mini-microcosms although the dry soil was moistened with 30 mM citrate solution, 22 min after addition, the soluble concentration was only between 9.4 and 17.9 mM. As the half-life of citrate is in the order of hours (Oburger et al. 2009), such rapid disappearance from soil solution may be attributed to processes additional to microbial degradation. Adsorption to the soil is likely as Oburger et al. (2009) found that a mean of 1.6±0.4 mMoles of citrate kg^{-1} of soil were adsorbed, where four typical soils were investigated. This is similar to the amount of citrate lost in our soils. Oburger et al. (2011) also found that a steady state was reached rapidly (within 1 h) in citrate adsorption studies. The concentration in soil solution at the start of sampling was also similar to the maximum found by Dessureault-Rompre et al. (2007) within 1 mm of exuding lupin proteiod roots.

The added citrate had a large solubilising effect on the fragments of DU. Soluble U levels remained high for a considerable time although after 25 h soil solution concentrations decreased in some cases. It is noteworthy that the soluble uranium remained higher for longer than would have been suggested from the half-life of citrate given in literature (Oburger et al. 2009). While this might be partially explained by evaporative concentration due to loss of moisture, it may also indicate a resistance to metabolism of citrate due to its complexation with uranium (Francis et al. 1992; Huang et al. 1998a).

Four soil-plant microcosms showed appreciable concentrations of citrate and malate adjacent to proteoid roots. Citrate concentrations reached maximum values of between 1 and 8 mM, while malate levels were in the range 0.2–4.4 mM. Presumably, the different maxima reached in different microcosms were an indication of variable exudation intensity (Dessureault-Rompre et al. 2007) and/or differential rates of microbial degradation. While citrate is known for its uranium solubilisation properties (Duquène et al. 2008) malate is known to be less effective at solubilising uranium than citrate due to its complexes being weaker than those of citrate (Gunther et al. 2011; Kirishima et al. 2008).

The citrate and malate solubilised U from the DU fragments in the soil-plant microcosms, while solubilisation was minimal in the plant free microcosms (control) with low organic acid concentrations. In general, U concentrations in soil solution were approximately the same as the sum of citrate and malate concentrations, although sometimes U concentrations were higher than organic acid concentrations. At the end of the sampling period, U concentrations exceeded those of citrate (and malate) in most cases indicating a phase after soluble citrate and malate degradation during which uranium retained its elevated concentration in soil solution. This could be due to complexation with degradation products of citrate or malate (the presence of oxalate was sought, but not detected in any of the samples). Equally it could be due to the now soluble U forming complexes with humic or fulvic acids in solution on its release from citrate or malate (Lenhart et al. 2000).

This work indicates that root-derived organic acids are present in sufficient concentrations in the rhizosphere to increase substantially the solubilisation of U from DU anti-tank shell fragments. Moreover, it provides a description of the time course of this process that has not been observed before. The transience of the exudation observed for lupin roots may itself be typical of some plant types, but it also mimics the transient impulse of acid from a moving source, such as a root-tip (Hoffland et al. 1989), or other actively exuding part of the root. This transient wave of organic acid correlates with, and probably causes, a transient solubilisation of uranium. In terms of the large scale behaviour of the soluble U, the key question is how far it will travel during this period of solubilisation before re-precipitating to wait for the next transient event. The relatively short half-life of organic acids would suggest that the uranium will spend much of its time immobile. However, root density and convective flow rates will be important. A high density of roots tips with high efflux rates (Duquène et al. 2009) may well maintain a steady state flow of uranium for considerable distances over which it will be available for uptake by plant roots, microorganisms or small soil animals. Ultimately the organic acid will degrade and its “load” of metal will precipitate. The quantitative relationship between acid and metal has been difficult to define in these experiments, however. In some instances, uranium remained in solution after the level of soluble citrate had declined to control levels. This would suggest secondary

effects such as complexation by degradation products of citrate or malate or by humic or fulvic acids in soil solution. It is clear that the effect of root exudates on uranium solubilisation should be taken into account when assessing the risk posed by fragments of depleted uranium in the environment.

Although this work was carried out with proteoid roots of lupins, where it is common to have 4.7 mM citrate near the roots (Jones 1998) and may not be considered to be 'normal' concentrations, other plants also have been found to release substantial quantities of organic acids. Soil solution in the rhizosphere of *Trifolium* has been found to have concentrations of malate in the region of 1.5 mM (Jones 1998). Ryegrass has also been found to have 2.2–4.4 mM oxalate in the rhizosphere (Gao et al. 2011; Xu et al. 2007) which forms complexes of a similar strength to malate with uranium (Gunther et al. 2011; Kirishima et al. 2008). Although under normal circumstances wheat seems to exude lower concentrations of citrate (47 μM) and malate (23 μM) in rhizosphere soil (Bhattacharyya et al. 2003), under Al stress wheat can exudate malate at rates higher than citrate exudation by lupins. Therefore, we could assume that concentrations in soil solution around wheat roots would be in the range of 5 mM (Jones 1998; Ryan et al. 2001). Often organic acids are extracted from soil by centrifuging. As this is carried out on a large volume of soil, an average concentration is measured. Concentrations near the root tips where organic acids are exudated would therefore be much greater than seen in literature (Jones et al. 2003). It has also been seen that U was greatly enhanced in solution following ryegrass growth of one month (Duquène et al. 2009), backing up this assumption that although other plants may not exude organic acids in such high quantities as lupin, it still will have an effect of DU solubility and transport in soil.

In this work we have accessed soil solution next to plant roots with higher resolution than before. We have also taken samples of very small volumes (~2 nl) and analysed them for organic acid anions and uranium by means of CZE and ICP-MS. With this we have managed to increase the resolution of sampling and analysis of in-situ soil solution in the rhizophere greatly compared to that reported before (Luster et al. 2009). High resolution sampling requires that a lot of samples are taken in order to gain the full picture both spatially and temporally. However, we feel that it would be possible to use these techniques to measure organic acid anion gradients at high spatial resolution in the rhizosphere, which was previously said to be very difficult due to a lack of suitable techniques (Oburger et al. 2011). We also feel that this technique could be invaluable for other rhizosphere investigations that require high spatial resolution of sampling.

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