Uranium fate in wetland mesocosms: effects of plants at two iron loadings with different pH values

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Abstract

Small-scale continuous flow wetland mesocosms (~0.8 L) were used to evaluate how plant roots under different iron loadings affect uranium (U) mobility. When significant concentrations of ferrous iron (Fe) were present at circumneutral pH values, U concentrations in root exposed sediments were an order of magnitude greater than concentrations in root excluded sediments. Micro X-ray absorption near-edge structure (μ -XANES) spectroscopy indicated that U was associated with the plant roots primarily as U(VI) or U(V), with limited evidence of U(IV). Micro X-ray fluorescence (μ -XRF) of plant roots suggested that for high iron loading at

circumneutral pH, U was co-located with Fe, perhaps co-precipitated with root Fe plaques, while for low iron loading at a pH of ~4 the correlation between U and Fe was not significant, consistent with previous observations of U associated with organic matter. Quantitative PCR analyses indicated that the root exposed sediments also contained elevated numbers of *Geobacter* spp., which are likely associated with enhanced iron cycling, but may also reduce mobile U(VI) to less mobile U(IV) species.

Keywords: Uranium fate, uranium sequestration, wetlands, iron, rhizosphere, plants,

1. Introduction

There are significant uncertainties regarding the environmental fate of uranium (U) and efforts to minimize U exposures require understanding of its mobility in environmental systems. One aspect of concern needing further research is the fate of U in wetlands where contaminated groundwater may interface with surface water. For example, groundwater U can enter and in some cases be concentrated in wetlands as observed in Sierra Nevada and Rocky Mountain Regions (Owen and Otton, 1995), as well as at the Department of Energy's Savannah River Site (SRS) in South Carolina (Evans et al., 1992; Sowder et al., 1996). Because the presence of wetland plant roots have been found to facilitate sequestration of other trace metals, including arsenic through sorption with iron (Fe) root plaques (Blute et al., 2004; Seyfferth et al., 2010), and hexavalent chromium through facilitated transformation to less mobile, reduced chromium species (Xu and Jaffé, 2006), the effect of wetland plant roots on U fate are examined here.

Large biogeochemical gradients exist in wetland ecosystems where primary production by wetland plants release large quantities of labile organic matter that often lead to anaerobic wetland sediments. Many wetland plants have evolved specialized tissues named aerenchyma that facilitate transport of oxygen to their roots to maintain conditions appropriate for aerobic

1 respiration. Oxygen diffuses from these wetland plant roots into surrounding sediments, where
2 reactions with ferrous Fe can result in distinctive Fe plaques composed primarily of ferric
3 oxyhydroxides that precipitate on the surface of roots (Blute et al., 2004; Colmer, 2003). The
4 loss of oxygen in the rhizosphere is often termed radial oxygen loss (Colmer, 2003). Due to the
5 general lack of oxygen in wetland sediments, Fe plaques can facilitate active Fe cycling by
6 stimulating Fe reducing bacteria such as *Geobacter* spp. (Weiss et al., 2003).

7 Fe reducing bacteria such as *Geobacter* spp. have been shown to greatly affect the mobility of
8 U by reducing it from mobile U(VI) forms to less mobile U(IV) (Lovley et al., 1991), and
9 enhancing the activity of Fe reducing bacteria is a remediation strategy to prevent migration from
10 contaminated sites (Finneran et al., 2002; Lovley et al., 1991; Wu et al., 2006). As conditions
11 immediate to root plaques enhance Fe reducing activity, U may be immobilized as U(IV) in these
12 regions, and such a mechanism has been previously reported (Chang et al., 2014). Other studies
13 examining the fate of U in the presence of mixed Fe valences suggest an alternate mechanism for
14 lower U mobility. Under conditions of ferrous Fe interacting with ferric oxyhydroxides, Nico et
15 al. (2009) found that U can be immobilized during co-precipitation with newly formed Fe
16 minerals. Such a process was also demonstrated by Duff et al. (2002) who specifically examined
17 the co-precipitation of ferric Fe and U. Because Fe cycling occurs in the vicinity of wetland plant
18 roots resulting in Fe minerals (e.g., Fe plaques), U co-precipitation with newly formed Fe
19 minerals can also lower U mobility. Lastly, U can also sorb with organic and inorganic soil
20 constituents affecting its mobility (Kaplan and Serkiz, 2001; Li et al., 2015, 2014). Based on
21 sequential extraction experiments with SRS wetland soils (pH ~5.5; 0.14% OM; 3.1% CDB
22 extractable reducible Fe-oxides), $86 \pm 7\%$ of U was found bound to an organic matter fraction,
23 while an additional $7 \pm 1\%$ of U was associated with Fe-oxyhydroxides, indicating that OM was

1 particularly effective at binding U (Kaplan and Serkiz, 2001). Other detailed studies with a well
2 characterized ternary system involving NOM, hematite, and U(VI) or Th(IV), showed that the
3 relative importance of OM and Fe oxides were highly pH dependent (Murphy et al., 1999).
4 During the work of Murphy et al. (1999), the binding of U with organic matter slightly decreased
5 and that of U with Fe-oxides sharply increased as pH increased from pH 4 to 6.5. On a mass
6 basis and under acidic conditions, OM may be expected to have an overall greater impact on
7 U(VI) binding than Fe-oxides.

8 Based on mechanisms discussed above, we hypothesized that the presence of wetland plant
9 roots facilitate U sequestration at higher Fe loadings due to U(VI) reduction to less mobile
10 U(IV), co-precipitation of U(VI) or U(V) with Fe(III) on root surfaces, or U(VI) sorption to
11 Fe(III) on root surfaces. To test this hypothesis, we examined U fate in planted and unplanted
12 mesocosms with continuous upward flow (to simulate groundwater to surface water discharge)
13 with high Fe concentrations at circumneutral pH. A second set of planted and unplanted
14 mesocosms was prepared identically and maintained concurrently, but with lower Fe
15 concentrations and lower pH values. The experiments build on different wetland mesocosm
16 experiments with low Fe concentrations, which showed that accumulated U existed primarily as
17 U(VI) bonded to natural organic matter carboxylate sites, with the potential for some U(IV) in
18 the rhizosphere (Chang et al., 2014; Li et al., 2015). The experiments also relate to other
19 previous work examining uranium fate in planted wetland mesocosms, which showed limited
20 uranium remobilization during an oxidation event (i.e., drying and rewetting) (Gilson et al.,
21 2015), indicating that uranium accumulation was likely not linked solely to uranium reduction to
22 U(IV), but perhaps also to some of the other mechanisms discussed above.

2. Materials and Methods

2.1. Experimental Design

Small scale mesocosms (approximately 0.8 L; 9.5 cm diameter, 11 cm depth) were used to investigate U mobility near wetland plant roots and its relationships with geochemical parameters and microbial populations of interest. These mesocosms simulated the discharge of groundwater containing dissolved U through vegetated and non-vegetated sediments, and are of similar design to experiments performed by Gilson et al. (2015). To facilitate water transport (i.e., minimize preferential flow) and control Fe concentrations and speciation, the porous media in the mesocosms consisted almost entirely of ASTM standard C778 20-30 sand (passing 850- μ m sieve (No. 20) and retained on 600- μ m sieve (No. 30) sieve; US Silica, Ottawa, IL). The sand was pre-treated with 1 M HCl for more than five days to remove Fe and other metals and subsequently rinsed with deionized water until the pH was greater than 6. The mesocosm sand was amended with 0.2 wt-% sediment collected from SRS wetlands to provide appropriate microbiological seeding. Due to the small quantity of SRS wetlands introduced, approximately 10 mg/kg of Fe was introduced to the soil media through this amendment. Three small bags (70 μ m nylon mesh) containing 2-4 g of mixed porous media were emplaced in the mesocosms to investigate the influence of sediments physically separated from the roots. These bags were also emplaced in control/unplanted mesocosms to determine whether the nylon material imposed significant transport resistance to sediments. Sediments that were contained in the nylon bags and physically separated from roots are termed root excluded sediments and were assumed to be little impacted by rhizosphere processes. The sediments that were not contained in the nylon bags, and therefore exposed to roots, contained sediments impacted by rhizosphere processes, and are termed root exposed sediments. These root exposed sediments include sediments that

1 were physically separated from roots by shaking at the time of sampling. Sediments outside the
2 nylon bags in the unplanted, control mesocosms are referred to as bulk sediments. The
3 mesocosm setup is illustrated in the Supplemental Material (See Figure S1).

4 Two wetland plants, *Typha latifolia* (common cattail) and *Scirpus acutus* (hardstem bulrush),
5 were used in the mesocosms. These are common wetland plants known to facilitate gas exchange
6 between atmosphere and roots, similar to other wetland plants (e.g., American bur-reed) that
7 contain aerenchyma (Reid and Jaffé, 2012). Two species of plants were used to help evaluate
8 whether observed behaviors may be common among wetland plants and not specific to
9 individual species. The plants were purchased from a commercial nursery (Pinelands Nursery
10 and Supply, Columbus, NJ) and planted by emplacing two to three rhizomes with living stems in
11 each mesocosm. Roots were trimmed before emplacement such that most root mass examined at
12 the termination of the experiment was developed during the experiment. The photoperiod in the
13 greenhouse was extended through the use of growth lamps and greenhouse temperatures ranged
14 from 15 °C to 30 °C.

15 Water containing a minimal set of nutrients for the plants and microbial community was
16 provided in an upflow manner to simulate groundwater to surface water discharge through a
17 wetland. The dilute nutrient solution was similar to that used by Chang et al. (2014) and is
18 described in the Supplemental Material (See Table S1). Briefly, in addition to various
19 micronutrients, nitrogen and phosphorus were added as urea (~300 µM) and monopotassium
20 phosphate (~25 µM, or ~2.5 µM during U addition), respectively. Urea was used to supply
21 nitrogen and helped minimize the introduction of nitrate that may promote U(IV) oxidation.
22 Phosphate was lowered during the period of U addition to minimize the formation of U-
23 phosphate precipitates. Ferrous chloride (~2.5 mM) was also included with the nutrients to

1 encourage Fe cycling in the mesocosms. To minimize abiotic reduction of U, ferrous chloride
2 was not added to the nutrient solution during U addition. The influent nutrient water was pre-
3 purged with $N_{2(g)}$ to reduce the introduction of dissolved oxygen to the mesocosms, and
4 dissolved oxygen concentrations entering mesocosms were measured to be approximately 2
5 mg/L. The flow rate through the mesocosms was 130 mL/day, and the hydraulic residence time
6 in the mesocosms was 1.8 days (assuming an average porosity of 0.3).

7 A slurry of ferrous carbonate ($FeCO_3$) solid was added to one cattail, one bulrush, and one
8 unplanted mesocosm (~0.1 moles to each by syringe injection) four months after planting to
9 buffer the pH and provide an additional long term source of ferrous Fe, as background Fe
10 concentrations in the prepared acid-washed porous media were low. The quantity of ferrous
11 carbonate added was sufficient to ensure buffering and ferrous Fe release for the duration of the
12 experiment. The ferrous carbonate was prepared by mixing anaerobic solutions of Na_2CO_3 and
13 $FeCl_2$ in a manner similar to that described by Ulrich and Sedlak (2010). The addition of
14 carbonate in the $FeCO_3$ amendment may have enhanced U mobility due to strong interactions
15 between carbonate species and U, but this would be evident in both planted and unplanted
16 mesocosms.

17 Approximately two months after the addition of the slurry of $FeCO_3$ solid, uranyl acetate was
18 continuously supplied by peristaltic pump to the influent nutrient solution to yield an influent
19 concentration of approximately 20 μM U. This concentration was selected because SRS aqueous
20 U concentrations can reach 4 μM (Denham and Vangelas, 2010), and slightly higher
21 concentrations were used here to facilitate U analytical detection. The presence of μM -level
22 acetate from the uranium source is unlikely to have affected the microbial activity within the
23 mesocosms, when compared to the effect from root exudates. Previous studies with similar U

1 concentrations showed little toxicity to key organisms responsible for U bioreduction (Komlos et
2 al., 2008b; Moon et al., 2010).

3 *2.2. Experimental Sampling*

4 To assess effluent concentrations and pH values, water in the mesocosms was sampled
5 periodically near the top of the mesocosms through a side sampling port. After 70 days (bulrush
6 mesocosms) or 90 days (unplanted and cattail mesocosms) of continuous U addition, mesocosms
7 were sampled sacrificially in an anaerobic chamber. Three mesocosms (1 unplanted, 1 cattail,
8 and 1 bulrush) had been amended with FeCO_3 , and three (1 unplanted, 1 cattail, and 1 bulrush)
9 had not been amended with FeCO_3 . Roots, root exposed or bulk sediments, and root excluded
10 sediments (those within the nylon bags) were collected, mixed, and subsampled in duplicate to
11 examine U concentrations, U and Fe distribution along roots using X-ray fluorescence (μ -XRF),
12 and U and Fe speciation of locations along roots using X-ray absorption near-edge structure (μ -
13 XANES) spectroscopy. Intact root samples for μ -XRF and μ -XANES were sealed in serum
14 bottles within the anaerobic chamber until mounting in sample holders later under similar
15 anaerobic conditions. Care was taken to ensure that mounted samples for μ -XRF and μ -XANES
16 remained anaerobic until analysis, including their shipment using Mylar bags containing fresh
17 oxygen absorbing packets. Duplicate subsamples for microbial characterization were collected in
18 sterile 1.5 mL micro centrifuge tubes, and shipped on dry ice to Microbial Insights (Rockford,
19 TN).

20 *2.3. U and Fe Concentration Analyses*

21 Dissolved Fe and U concentrations were analyzed by ferrozine and ICP-MS methods,
22 respectively (Arey et al., 1999; Komlos et al., 2007). U concentrations of collected sediment and
23 root samples were determined by extraction of 0.2 to 2 g of sample in 5 mL 1M NaHCO_3 under

aerobic conditions for more than four weeks, similar to extractions performed by Komlos et al. (2008). This extraction procedure yields both oxidizable U(IV), and sorbed U(VI), and digestions of total U were not performed. U concentrations in extracted solutions were quantified using reverse-phase chromatography coupled to postcolumn derivatization with Arsenazo III dye as described by Lack et al. (2002).

2.4. μ -XRF and μ -XANES Analyses

Microspectroscopic X-ray fluorescence (μ -XRF) maps and microspectroscopic X-ray absorption near edge structure (μ -XANES) spectra were collected at Sector 13-ID (GSECARS) at the Advanced Photon Source, Argonne National Laboratory operating at 7 GeV in top-up mode. Sector 13-ID uses a liquid nitrogen cooled Si (111) double crystal monochromator that was calibrated using Y metal foil (17038 eV) for U and Fe metal foil (7112 eV) for Fe with periodic verification during data collection to confirm stability. The root samples were mounted between two pieces of Kapton tape while under anaerobic conditions, and loaded into a cryostat system (to reduce beam-induced artifacts) on an x-y- θ stepping-motor stage. Micro-XRF maps ($1 \times 1 \text{ mm}^2$) of the roots were collected with 3 μm steps at 0.05 s integration for elemental distribution including U and Fe. Once U hotspots were identified using μ -XRF mapping techniques, U L_{III}-edge and Fe K-edge μ -XANES spectra were collected at 0.4 eV steps around the absorption edge -15 through 30 eV (full scans -100 through 500 eV). Three to five μ -XANES spectra were collected for each sample. The μ -XRF maps and μ -XANES spectra were collected in fluorescence mode using a Vortex-ME4 four-element silicon drift detector. The collected μ -XANES spectra for U and Fe speciation of the roots were processed using the IFEFFIT software package (Ravel and Newville, 2005). Principal components analyses (PCAs) were performed in Sixpack (Webb, 2005) on the normalized scans and target factor analyses of each U standard

1 were performed to determine the most appropriate standards to be used for linear combination
2 fitting (LCF) analyses. U standards with SPOIL values less than 3.0 were used in the LCF
3 analyses. LCF standards for U speciation included uraninite (UO_2) and uranyl acetate dihydrate
4 ($\text{UO}_2(\text{CH}_3\text{COO})_2 \cdot 2\text{H}_2\text{O}$). Fe standards included ferrihydrite, goethite, magnetite, vivianite,
5 hematite, marcasite, pyrite, and pyrrhotite. The LCF models were fit using the derivative spectra
6 at -10 to 50 eV relative to the absorption edge and weighting factors were constrained to values
7 between 0 and 1. The results of the LCF analyses generated models with the best fit indicated by
8 the lowest R-factor and reduced chi square values.

9 2.5. Microbial Population Analysis

10 Microbial populations were quantified by qPCR analyses performed by Microbial Insights Inc.
11 (Rockford, TN). The root, root exposed or bulk sediments, and root excluded sediments were
12 separated and shipped overnight on dry ice. DNA was extracted from ~0.5 g soil with a
13 FastDNA® SPIN soil kit, following methods described previously (Komlos et al., 2008b). Each
14 of the TaqMan based 16s quantitative PCR assays contained DNA template, 1X TaqMan
15 Universal PCR Master Mix (Applied Biosystems), TaqMan probe (100 – 500 nM) and forward
16 and reverse primers (300 – 1500 nM). The following groups of bacteria were targeted with the
17 indicated TaqMan probe and forward/reverse primers, respectively: Eubacteria (TM1389,
18 BACT1369/PROK1492R, (Suzuki et al., 2000)); Iron- and sulfate-reducing bacteria (GBC2,
19 361F/685R, (Stults et al., 2001)); and *Geobacteraceae* (GBC2, 561F/825R, (Stults et al., 2001)).

3. Results and Discussion

3.1. Mesocosm Effluent Concentrations

Figure 1 shows dissolved U and Fe concentrations of the mesocosm effluent during the period that uranyl acetate was added to the mesocosms. With one exception, and possible outlier, dissolved U concentrations in all mesocosms where FeCO_3 was added, including the unplanted control mesocosm, were at or below the detection limit of approximately $0.08 \mu\text{M}$ (Figure 1A), whereas U was detected (up to $15 \mu\text{M}$ U) in mesocosms where FeCO_3 was not added (Figure 1B). The lower concentrations of U in mesocosms where FeCO_3 was added was evident despite the presence of carbonate and likelihood of mobile U-carbonate species. These results indicate that U was effectively sequestered in mesocosms where FeCO_3 was added, but does not provide specific information regarding the role of wetland plants. The examination of U concentrations of the sediments described below provides additional insight regarding this role of plants.

Dissolved ferrous Fe concentrations indicate that high concentrations of ferrous Fe (typically $> 400 \mu\text{M}$ Fe) were present in mesocosms where FeCO_3 was added (Figure 1C). One sample from the FeCO_3 amended mesocosms had lower concentrations of Fe, approximately $20 \mu\text{M}$. This sample corresponded with the possible U outlier mentioned above, and suggests these may be sampling artifacts. The presence and stability of high ferrous Fe concentrations at circumneutral pHs provide strong evidence that FeCO_3 mesocosms were anaerobic, as oxidation of Fe should be rapid at circumneutral pHs if oxygen were present. Due to slower Fe oxidation kinetics at the lower pHs observed in the mesocosms where FeCO_3 was not added, the occurrence of ferrous Fe in these mesocosms does not provide as clear evidence of anaerobic conditions. Mesocosms with similarly prepared porous media and plants had oxidation-reduction potentials indicative of anaerobic conditions (Zhang et al., 2016), and anaerobic conditions were likely

present in all mesocosms described here. The effluent pH values of FeCO_3 amended mesocosms were approximately 6.5, while the effluent pH values of mesocosms without the addition of FeCO_3 were approximately 4.

Figure 1. Effluent concentrations of uranium (A, B) and iron (C, D) from Mesocosms amended with FeCO_3 (A, C; pH 6.5) and without FeCO_3 (B, D; pH 4). Note that y-axis scales differ. The dashed lines indicate detection limits, and errors were approximately 10% for U and 20% for Fe.

3.2. Sediment Uranium Concentration

Figure 2 shows U concentrations in root exposed, bulk, and root excluded sediments in the mesocosms. For clarity, Figure 2C shows the ratio of U concentrations between root exposed or

bulk sediments and root excluded sediments. In control unplanted mesocosms, both with and without additional FeCO_3 , U concentrations were generally similar in bulk sediments and in nylon bags. This indicates that the nylon mesh material presented little resistance to U transport. In planted mesocosms without the addition of FeCO_3 , U concentrations in root exposed sediments and in root excluded sediments were also similar. This indicates that the mobility of U was similar near and far from the roots at the lower total Fe ($\sim 20 \mu\text{M Fe}$) and lower pH values (pH ~ 4) of the mesocosms that were not buffered by FeCO_3 . The planted mesocosms amended with FeCO_3 (both bulrush and cattail) contained approximately an order of magnitude more U in root exposed sediments than in root excluded sediments. In fact, U concentrations of these root excluded sediments were at or below detection limits of approximately $0.01 \mu\text{mol/g}$, such that little U was found within the bags. Concentrations in root exposed sediments were also greater than sediments in unplanted mesocosms suggesting U accumulated in the vicinity of the roots. These data indicate that U was less mobile in the plant root exposed sediments of FeCO_3 amended mesocosms.

Figure 2. Uranium concentrations (A,B) in sediments and relative concentrations of uranium (C) between root exposed (or bulk) and root excluded sediments. The asterisk indicates that concentrations in FeCO_3 root excluded soils were below detection, and are represented as having the detection limit with an error of half the detection limit.

3.3. Root Uranium Concentration

Figure 3 shows U concentrations extracted from samples of bulrush and cattail roots in the mesocosms. Concentrations of U associated with plant roots was 50 to 80% greater at higher Fe loadings (at circumneutral pH) than at lower Fe loadings (at lower pH values). As discussed below, μ -XRF analysis indicates that U at higher Fe loadings was co-located with Fe, while U at lower Fe loading was not.

Figure 3. Uranium concentrations of plant roots.

3.4. μ -XRF Analysis

μ -XRF mapping was performed to examine the spatial distribution of U and Fe on plant roots. Figure 4 shows the distribution of Fe and U from cattail mesocosms without FeCO₃ addition (Figure 4A-C) and with FeCO₃ amendments (Figure 4D-F). Figure 4C and Figure 4F show scatter plots of Fe versus U signals observed in the μ -XRF maps. In the FeCO₃ free system, where the aqueous Fe and pH were lower, there was a poor correlation between U and Fe concentrations (Figure 4C). There were many locations of relatively high U accumulation that

was not spatially correlated with Fe, with a spatial correlation R^2 value of 0.29. This is consistent with results reported by Li et al. (2015), who examined U immobilization in a natural acidic wetland, also observed little spatial correlation between Fe and U, and determined by EXAFS analysis that much U was bound as a bidentate to carbon. Roots from the FeCO_3 amended cattail exhibited a much stronger spatial relationship between Fe and U with an R^2 value of 0.89 (Figure 4F). There were a few sparse locations where U appeared enriched and not tightly associated with Fe, but most of the U was co-located with Fe. μ -XRF data for a root from a bulrush mesocosm amended with FeCO_3 is included in the Supplemental Material (See Figure S2), and shows a similar strong relationship between Fe and U, with an R^2 value of 0.91.

Figure 4. μ -XRF maps of uranium and iron on cattail roots. (A) Uranium and (B) iron μ -XRF maps of cattail root sections from a mesocosm study without FeCO_3 ; (C) pixel scatter plot of iron versus uranium signals shown in (A) and (B); (D) uranium and (E) iron μ -XRF maps of cattail root sections from mesocosm amended with FeCO_3 ; and (F) pixel scatter plot of iron versus uranium signals shown in (D) and (E).

The μ -XRF data indicating a close relationship between Fe and U on plant roots in FeCO_3 amended mesocosms suggest a key role for Fe in U sequestration at circumneutral pH values, which was not observed without FeCO_3 amendments at low pH values. The exact cause for the differences in U distribution between the two systems is not known because both Fe

1 concentrations and pH co-varied between these two systems. The stronger correlation of the
2 FeCO_3 system than the FeCO_3 -free system may be attributed to: 1) the stronger tendency for
3 U(VI) or U(IV) to bind to Fe oxides at the higher pH value, 2) the greater tendency for Fe to
4 precipitate at higher pH values, thereby creating more binding sites for the U (Waite et al., 1994),
5 and/or 3) the greater tendency for aqueous U and Fe to co-precipitate on the root surface in the
6 elevated pH 6.5 system (Duff et al., 2002). As discussed below, XANES spectroscopy did not
7 provide much insight in regard to this question and additional research with a more controlled
8 pH/Fe plant system is needed.

9 3.5. μ -XANES

10 μ -XANES was performed to examine the oxidation state of U. Figure 5 shows XANES spectra
11 for U(IV) and U(VI) standard materials along with a μ -XANES spectra for 3 μm resolution
12 locations on intact cattail root samples grown in FeCO_3 amended and FeCO_3 -free mesocosms.
13 The arrows on the figure identify two features: the U L_3 -edge which is an indicator of oxidation
14 state with lower oxidation states having lower energy edges; and a multiple scattering resonance
15 (MSR) “shoulder” feature attributed to the presence of axial oxygen bonds and considered to be
16 indicative of U(VI) uranyl species (Duff et al., 2002). Linear combination fitting (LCF) indicated
17 that between 80 and 100 percent of the U sampled on the roots was in the oxidized, U(VI), state
18 (See Supplemental Material Table S2). Almost all observed U on roots in the FeCO_3 -amended
19 system was characterized as U(VI) ($95 \pm 7\%$ and $93 \pm 5\%$ for cattail and bulrush, respectively),
20 while the percentage was smallest in the low pH system ($82 \pm 10\%$ for cattail w/o FeCO_3 ; pH 4).
21 While minimal U(IV) reoxidation during sample handling cannot be completely discounted
22 despite our efforts to minimize it, the large proportion of U(VI) on the root surfaces strongly
23 suggests that U was found in oxidized states.

One observation of interest with the U XANES is that the MSR shoulder feature in spectra from FeCO_3 amended mesocosms is notably weak or absent. Similar XANES spectra lacking clear MSR shoulders have been observed in previous studies. Duff et al. (2002) performed experiments focused on the co-precipitation of U(VI) species in Fe oxide minerals without any U reducing agents, noted a similar absence of MSR shoulder features, and suggested the presence of non-uranyl oxidized U(VI) species resulting from co-precipitation. O'Loughlin et al. (2010) also noted U(VI) species generally lacking distinctive MSR shoulder features upon U interaction with biogenic siderite (FeCO_3), and suggested that this resulted from site-specific incorporation of U(VI) on the surface of FeCO_3 . Nico et al. (2009) examined the incorporation of oxidized U species into Fe oxides and a μ -XANES spectrum of a U "hotspot" in their work also lacked the distinctive MSR shoulder feature (see Figure S4 in Nico et al. (2009)). Based on LCF with experimentally generated materials as standards (incorporated U, sorbed U, and reduced U), they suggested that 30% of the U in this hotspot was incorporated into Fe oxides with the remainder sorbed onto oxide surfaces (Nico et al., 2009). Interestingly, a synthesized mixed U(V)-U(VI) mineral has also been observed without the MSR shoulder feature (Belai et al., 2008), and hints that an U(V) species may be associated with plant roots in our experiments, but this would need significant further research. The similarities among these literature observations lacking MSR shoulder features and our μ -XANES data indicate that co-precipitated/incorporated U(VI), or U(V), was present on the plant roots, with negligible amounts of U(IV).

The evidence of U immobilization as U(VI) or U(V) near roots in contrast to U(IV) is interesting. Effective U immobilization due to biological reduction to U(IV) has been documented, but previous research indicates that some of these U(IV) phases can be rapidly reoxidized to U(VI) and remobilized in the presence of oxygen or nitrate (Bargar et al., 2008;

Komlos et al., 2008a, 2007; Moon et al., 2009, 2007; Senko et al., 2002; Wu et al., 2007). Wetlands may experience episodic oxidation events associated with wetting and drying cycles, and previous research indicates plant associated U may resist U remobilization during these cycles (Gilson et al., 2015). U immobilization as U(VI) instead of U(IV) near roots offers an explanation for this behavior.

In mesocosms amended with FeCO_3 , the presence of Fe(III) oxyhydroxides (ferrihydrite and goethite) ranged from 65-93% with remaining components of magnetite, vivianite, marcasite, and pyrrhotite. Systems without FeCO_3 additions still observed a majority of Fe(III) oxides (39-69%) and a broader distribution of magnetite, vivianite, hematite, marcasite, pyrite, and pyrrhotite (See Supplemental Material Table S3). As only ferrous Fe was supplied to the mesocosms, the observed Fe(III) oxyhydroxides along root surfaces provides clear evidence of Fe oxidation in the mesocosms and supports hypothesis of active Fe cycling near the roots.

Figure 5. Uranium L₃-edge XANES spectra for cattail roots. Cattails grown in mesocosms with (A) FeCO_3 and (B) without FeCO_3 (MSR = multiple scattering resonance).

3.6. *Microbial Populations*

Quantitative PCR (qPCR) results quantifying different microbial populations found in the mesocosms are shown in Table 1. In each planted mesocosm, total bacterial (i.e., Eubacteria) counts were greater in the root exposed sediments than in root excluded sediments. Not surprisingly, the greatest bacterial counts were found directly on roots. Additionally, bacterial counts were greater in planted mesocosms than in unplanted mesocosms. Together these observations provide evidence that plant root exudates enhanced microbial activity in these planted mesocosms.

1 **Table 1. Microbial gene copy numbers found in mesocosms.** Data are gene counts performed at
2 Microbial Insights using field wet weight materials. Value presented is the average between samples for
3 deep and shallow within the mesocosms and the presented error covers the range of the samples

Gene copies (#/gram field wet weight)										
Mesocosm Type		Eubacteria			Sulfate/Fe Reducing			<i>Geobacter</i> spp.		
		Root Excluded Sediments	Root Exposed / Bulk Sediments	Roots	Root Excluded sediments	Root Exposed / Bulk Sediments	Roots	Root Excluded sediments	Root Exposed / Bulk Sediments	Roots
FeCO ₃	Cattail	3.1x10 ⁶ ±2.8 x10 ⁵	1.1x10 ⁸ ±5.7x10 ⁷	2.9x10 ⁸ ±1.4x10 ⁸	9.3x10 ⁶ ±7.8x10 ⁶	2.5x10 ⁷ ±1.3x10 ⁷	2.9x10 ⁸ ±2.7x10 ⁸	2.5x10 ⁴ ±1.6x10 ⁴	4.7x10 ⁴ ±7x10 ³	2.1x10 ⁶ ±2x10 ⁶
	Bulrush	3.2x10 ⁶ ±1.6x10 ⁶	1.2x10 ⁷ ±5.5x10 ⁶	1.3x10 ⁸ ±2.7x10 ⁷	1.9x10 ⁷ ±5.8x10 ⁶	1.2x10 ⁸ ±1.8x10 ⁷	8.4x10 ⁷ ±3.4x10 ⁷	6.5x10 ⁴ ±1.7x10 ⁴	1.9x10 ⁵ ±6.8x10 ⁴	5.3x10 ⁵ ±1.5x10 ⁵
	Unplanted	1.1x10 ⁶ ±7.2x10 ⁵	6.9x10 ⁵ ±2.4x10 ⁵		1.2x10 ⁵ ±1.2x10 ⁵	3.1x10 ⁶ ±2.3x10 ⁶		ND ^a	1.8x10 ³ ±1.8x10 ³	
No FeCO ₃	Cattail	2.6x10 ⁶ ±2.8x10 ⁵	2.8x10 ⁷ ±2.2x10 ⁷	8.9x10 ⁷ ±4.5x10 ⁷	5.3x10 ⁵ ±3.9x10 ⁵	3.8x10 ⁵ ±8.2x10 ⁴	5.7x10 ⁶ ±4.0x10 ⁶	ND	ND	ND
	Bulrush	3.2x10 ⁷ ±1.1x10 ⁷	1.4x10 ⁸ ±8.2x10 ⁷	3.5x10 ⁷ ±1.4x10 ⁷	2.5x10 ⁵ ±2.5x10 ⁵	2.0x10 ⁵ ±2.5x10 ³	3.7x10 ⁶ ±3.6x10 ⁶	3.6x10 ² ±2.7x10 ²	5.6x10 ² ±1.6x10 ²	2.7x10 ² ±2.7x10 ²
	Unplanted	2.6x10 ⁶ ±1.1x10 ⁵	4.4x10 ⁶ ±4.1x10 ⁶		ND	2.2x10 ³ ±2.2x10 ³		ND	ND	

^a ND = not detected

Microbial gene copy counts of Fe and sulfate reducing bacteria (FeRB/SRB) and *Geobacter* spp. are greater in planted mesocosms where FeCO_3 was amended and are greater in root exposed sediments than in root excluded sediments. The greater populations of FeRB and *Geobacter* spp. nearer roots, along with evidence of Fe(III) oxyhydroxides on roots, suggests active Fe cycling. Such cycling may facilitate ongoing formation of fresh iron oxyhydroxide which can sorb or co-precipitate with U. *Geobacter* spp. and SRB are also known to be capable of reducing U(VI) to U(IV), although, as noted above, not much reduced U was observed in this work. The presence of *Geobacter* spp. and SRB may have contributed to some U(IV) sequestration near roots, as was observed in a previous study (Chang et al., 2014).

4. Conclusions

The mesocosm studies examined in this work suggest that wetland plants increase microbial activity and Fe cycling in the rhizosphere as a result of interactions between organic matter originating from roots and the microbial community including *Geobacter* spp. U accumulated in systems where enhanced Fe cycling occurred, suggesting that similar Fe cycling leads to U sequestration in natural planted wetland systems. X-ray absorption spectroscopy of roots indicated that when Fe precipitated as root plaques at circumneutral pH levels, U, primarily as U(VI) or U(V), was bound or co-precipitated with the Fe. The role of other potential U sequestration mechanisms, including complexation with natural organic matter or the formation of U(IV) materials was not observed, but is difficult to exclude with the data collected during this study. The presence of U reducing microorganisms (e.g., *Geobacter* spp. and SRB) suggests that U reduction to U(IV) was possible.

This study indicates that wetland plants can accumulate U near their roots and impact U attenuation. U was observed in more oxidized states (U(VI) or U(V)) together with Fe plaques on

- 1 plant roots, suggesting that plant associated U in wetlands is less susceptible to remobilization
- 2 during episodic reoxidation events than if U was sequestered as U(IV).

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