**Distributed microbially- and chemically-mediated redox processes controlling arsenic dynamics within Mn-/Fe-oxide constructed aggregates**

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**ABSTRACT**

The aggregate-based structure of soils imparts physical heterogeneity that gives rise to variation in microbial and chemical processes which influence the speciation and retention of trace elements such as As. To examine the impact of distributed redox conditions on the fate of As in soils, we imposed various redox treatments upon constructed soil aggregates composed of ferrihydrite- and birnessite-coated sands presorbed with As(V) and inoculation with the dissimilatory metal reducing bacterium *Shewanella* sp. ANA-3. Aeration of the advecting solution surrounding the aggregates was varied to simulate environmental conditions. We find that diffusion-limited transport allows reducing conditions to persist in the interior of the aggregate despite aerated advecting external solutes, causing As, Mn, and Fe to migrate from the reduced aggregate interiors to the aerated exterior region. Upon transition to anoxic conditions in the external solutes, pulses of As, Mn and Fe are released into the advecting solution, while, conversely, a transition to aerated conditions in the exterior resulted in a cessation of As, Mn, and Fe release. Importantly, we find that As(III) oxidation by birnessite is appreciable only in the presence of O2, where reductive dissolution of Mn oxides inhibits oxidation under anaerobic conditions. Our results demonstrate the importance of considering redox conditions and the physical complexity of soils in determining As dynamics, where redox transitions can either enhance or inhibit As release due to speciation shifts in both sorbents (solubilization versus precipitation of Fe and Mn oxides) and sorbates.

**INTRODUCTION**

Arsenic is a ubiquitous contaminant that jeopardizes water quality as a result of both natural and anthropogenic sources (1). The mobility of As through soils, and eventual contribution to surface or groundwater, is controlled by biological, chemical, and physical processes that are heterogeneously distributed within surface and subsurface environments. Most notable of these processes are those that influence the oxidation state of As and its partitioning between the solid and aqueous phase. Within soil and water systems at circumneutral pH, As(V) predominates under aerated, oxidizing conditions as the oxyanion HxAsO4x-1, while arsenite, as H3AsO30, typically dominates under anoxic, reducing conditions. Arsenic(III), though binding extensively to iron oxides, is generally considered the more mobile species of As (2), while As(V) is less selective and adsorbs appreciably onto a variety of metal oxyhydroxides, hydroxides, and oxides (hereafter collectively referred to as oxides) including Fe, Al, and Mn oxides (3-5).

In many environments, including seasonally saturated soils (6), bioturbated sediments (7), and forest soils (8), temporary O2 depletion within soil aggregates results from redox fluctuations (9, 10), where rapid switches in dominant metabolic processes may occur (7). Iron and Mn oxides are produced by oxidative precipitation under aerated conditions, while reductive dissolution reproduces Fe(II) and Mn(II), both of which tend to be more soluble than their oxidized counterparts, under anaerobic conditions. Rapid oxidation of Fe(II) generally produces ferrihydrite initially, which can transform into more crystalline phases over time including goethite, hematite, and magnetite through abiotic and biotic mechanisms (Schwertmann and Murad, 1983; Hansel et al., 2003). Abiotic oxidation of Mn(II) by molecular oxygen is a thermodynamically favorable, but kinetically limited reaction; a majority of Mn(II) oxidation in natural systems is driven by enzymatic reactions, primarily forming low crystallinity phyllomanganates resembling birnessite (Tebo et al., 2004). Hence, the relative flux of Mn and Fe, and As concomitantly, out of soils and sediments as a consequence of reductive dissolution varies depending upon redox conditions (11).

Physical heterogeneity influences the extent and spatial distribution of oxidative and reductive processes within soils and sediments. Soils are composed of microaggregates typically fused together by labile organic matter into macroaggregates (12, 13), which form a complex matrix of transport pathways comprised of advective flow channels between aggregates combined with diffusion-controlled intra-aggregate transport (14-16). The rate of intra-aggregate transport of chemical species such as oxygen from the aggregate exterior decreases toward the aggregate center due to diminishing pore size, increased tortuosity, and discontinuities (16). Oxygen is further limited within aggregates through microbial respiration, becoming depleted within millimeters of the aggregate exterior (10, 17). Depletion of oxygen initiates microbial anaerobic respiration on alternative terminal electron acceptors, including As(V) and Fe(III) and Mn(IV) oxides that are common to soils (18).

Reductive dissolution and transformation of Fe(III) and Mn(IV/III) oxides and As(V) have been identified as the primary mechanisms controlling As mobilization within soils (1). Anaerobic respiration upon As and Fe and Mn oxides is initiated when anoxic conditions develop. The resulting reductive transformation of Fe(III) oxides decreases oxide surface area, resulting in the release of adsorbed As in to the aqueous phase(3). Microbial reduction of ferrihydrite, however, initially results in sequestration of As concomitant with transformation to magnetite; continued Fe reduction eventually prompts Fe oxide dissolution and As release (3, 19).

Manganese, similar to Fe, is a highly redox active element in natural systems. In its oxidized forms Mn(III/IV) serves as one of nature’s strongest oxidants and a potent adsorbent of many trace metals (20). Higher valent forms of manganese can undergo dissimilatory reduction to Mn(II) under anaerobic conditions, while Mn(II), which is kinetically stabilized towards oxidation at circumneutral pH, is oxidized by molecular oxygen via mineral surface or bacterial catalysis (21).

Here, we examine the combined effects of redox oscillations and physical heterogeneity on transport and transformation of As. Using synthetic aggregates composed of birnessite- and ferrihydrite-coated quartz sands presorbed with As(V), we examined the effects of redox fluctuations on the mobilization and speciation of As within a chemically and physically complex system. The aggregates were inoculated with dissimilatory metal reducing bacteria *Shewanella* sp. ANA-3, capable of respiring on As(V), Fe(III), and Mn(IV) and placed in aerated, anoxic, and redox transitioning environments. We reveal that As release rates and concentrations from the aggregates are highly similar even under different aeration/redox treatments, where As, Fe, and Mn redox cycling differs only within the outer 3 mm of the aggregate exterior. Under aerated conditions, the exterior of the aggregate remains oxic, forming an Fe(III) oxide rich rind proximal to advective flow channel that adsorbs and accumulates As. When the aerated aggregate is transitioned to anoxic conditions, microbial respiration on Fe(III) oxides and As(V) cause an immediate pulse of As(III) to be released from the aggregate. Aerated advective external solutes, Fe migrates from the aggregate interior and accumulates at the exterior relative to initial concentrations. However, Mn(II) elution occurs independent of aeration status, albeit to a lesser extent in the presence of oxygen. Manganese(II) released from the aggregate occurs prior to Fe(II) release, following thermodynamic favorability of microbial electron acceptors.

**MATERIALS AND METHODS**

**Aggregate construction and reactor setup.** Birnessite was synthesized by dissolving 63 g of KMnO4 in 1 L of doubly deionized (DDI) water. The solution was heated to 90˚C and combined with 66 mL concentrated HCl in a separate 4 L flask while being vigorously stirred. The reaction continued at 90˚C for 10 min, then cooled for 30 min before filtering through a vacuum filtration system. Oxides captured by the filter were resuspended in DDI water and filtered repeated to remove entrained KMnO4. Two-line ferrihydrite was synthesized following protocol previously outlined by Schwertmann and Cornell, 2000 (22). A small portion of the birnessite and ferrihydrite was dried and crushed using a mortar and pestle to confirmed oxide identity by powder X-ray diffraction analysis using Cu Kα radiation.

In separate containers, birnessite and ferrihydrite pastes were mixed with quartz sand, allowed to air-dry over 2 d, then rinsed repeatedly with DDI water and air-dried for another 2 d. Birnessite- and ferrihydrite-coated sands were combined to form a 1:10 Mn:Fe molar ratio mixture. Oxide coated sands were sterilized by autoclaving 250 g of sand in 1 L of DDI water. Phosphate was presorbed to sands by decanting DDI water and incubating with 0.26 μM NaH2PO4 in 1 L of autoclaved basal salts medium, BSM (10 mM PIPES, 2.7 mM KCl, 0.3 mM MgSO4, 7.9 mM NaCl and 0.4 mM CaCl2.2H2O, and its pH was adjusted to 7.1 with 3 M HCl) and allowed to incubate at room temperature for 3 d. Phosphate and BSM was decanted and replaced with 2.5 mM Na2HAsO4·7H2O, incubated also at room temperature for 3 d, then decanted and sands were rinsed twice with 250 mL of autoclaved BSM. Arsenate concentration adsorbed to the sands at experiment initiation was 0.0236 moles As(V)/mole Fe or 0.607 moles As(V)/mole Mn.

*Shewanella* sp. ANA-3 was grown aerobically in autoclaved tryptic soy broth (30 g L-1 DDI water) at 30°C until late log phase from frozen seed culture (stored in 20% glycerol at -80°C) in 200 mL of solution. Cells were harvested and washed by centrifuging liquid cultures (5000 x g; 15 min; 25°C) and re-suspended in 30 mL of BSM at pH 7.1 three times.

250 g of As(V)-presorbed oxide-coated sand was inoculated with ~8 x 108 cells g-1 sand, combined with 0.25% agarose (0.25 g UltraPure agarose dissolved in 100 mL DDI water), and mixed thoroughly to ensure homogeneous distribution of bacteria and agarose. The bacteria inoculated agarose sand mixture was poured into sterilized molds to form 2.5 cm diameter spheres. The shaped aggregate had a dry bulk density of 1.21 g cm-3 and porosity of 0.58.

**Flow-through reactor experimental procedure.** Aggregates were placed in the center of flow-through reactor made of polycarbonate (3.7 cm height, 5.1 cm internal diameter) with 0.2 μm filters placed at the inlet (bottom) and outlet (top) of reactor. A total of eight reactors were prepared where four were run under aerated-flow conditions and four run under anoxic-flow. All reactors were initiated with in-flow of BSM amended with 3 mM lactate, 17.8 μM NH4Cl and 1 mL L-1 Wolfe’s mineral solution from bottom of reactors at 1 mL h-1 flow-rate. Aerated-flow reactors were run on bench-top with filtered air continuously purging solution surrounding aggregates. Anoxic-flow reactor experiments were carried out in an anaerobic glove-bag in a 95% N2:5% H2 atmosphere. Effluent was collected from the outlet (top) of reactors at 1 mL h-1.

**Aqueous phase analysis.** AqueousAs, Mn, and Fe concentrations were measured in filtered effluent samples using inductively coupled plasma optical emission spectrometry (ICP-OES). The lower detection limits for measuring As, Mn, and Fe were 5, 1, and 18 μg L-1, respsectively. Another 3 mL of filtrate was used for As(III)/As(V) speciation following the method of Masscheleyn et al. (1991) as modified by Jones et al. (2000) as follows: while purging with N2, 0.6 mL of 2 M Tris (pH 6.0) was added to 3 mL of sample; after Tris is thoroughly mixed into sample, two additions of 0.3 mL of 3% (w/v) NaBH4 in 1 M NaOH is added to sample tube, with 5 min of N2 purging between additions. Lactate and acetate concentrations were determined from1 mL of filtrate stored at -20°C after sampling using ion chromatography.

**Solid phase analyses.** Aggregates were broken down for solid phases analysis after 48 d of flow. Each aggregate was separated into three concentric zones labeled as ‘E’ for exterior (0 to 3.5 mm), ‘M’ for midsection (3.5 to 7.5 mm), and ‘I’ for interior (7.5 to 12.5 mm). Sands from each zone were dried and used for bulk X-ray absorption spectroscopic (XAS) analysis including, X-ray absorption near-edge structure (XANES) spectral collection to determine ratio of As(III) and As(V), Fe extended X-ray absorption fine structure (EXAFS) spectroscopy to quantify Fe phases, and acid digestion with 6 M HCl for quantifying solid phase As, Fe, and Mn concentrations. Triplicate sand digestions were averaged to determine initial solid phase As (17.054±1.23 mmol kg-1 sand), Fe (709.562±27.02 mmol kg-1 sand), and Mn (35.462±6.60 mmol kg-1 sand) concentrations.

Bulk XAS was conducted on beamlines 11-2 and beamline 4-1 at Stanford Synchrotron Radiation Laboratory (SSRL) using method described previously (Masue-Slowey et al., 2011). Dried sands from each aggregate section were sonicated anaerobically in DDI water. Homogenous As-Fe-Mn layers were collected by vacuum filtration of aqueous phase from sonicated samples on cellulose nitrate filters and sealed between Kapton tape. Double-crystal, Si(220) monochromators were used at both beamlines for energy selection. Fe EXAFS spectra were obtained from 100 eV below to 1000 eV above the Fe K-edge at 7111 eV. Fe solid-phase speciation was quantified by performing linear combination fitting on Fe EXAFS collected on bulk samples with *k*3-weighted EXAFS spectra of Fe standard compounds using the SIXPACK interface to IFFEFIT (Webb, 2005). Iron fluorescence spectra were normalized and backscattering contribution isolated by spline function subtraction. Normalized data (eV) were converted to k-space (Å-1), and *k3* weighted. Linear-combination fitting was performed from 3 to 14 Å-1 and results were evaluated based on reduced *X2* values. Ferrihydrite, magnetite, and goethite were chosen as reference compounds for fittings based on reaction products reported in similar past studies (19), and phases identified using scanning electron microscopy (SEM). Arsenic speciation of bulk samples were determined by analyzing the near-edge portion of the As spectra collected from 240 eV below to 430 eV above the As(V) K-edge at 11874 eV. Ratio of As(III) and As(V) adsorbed to solid phase samples were determined with linear-combination fitting of normalized XANES spectra with spectra collected for As(III) and As(V)-sorbed ferrihydrite as fitting standards.

Micro-X-ray fluorescence (μ-XRF) analysis of radial slices of each aggregate was carried out at beamline 2-3 at SSRL and beamline 10.3.2 at ALS to map the spatial distribution of As(III), As(V), Fe, and Mn from the exterior to interior of aggregates. Aggregate slices were dried in anaerobic glove-bag, embedded in EPOTEK301-2FL epoxy, thin-sectioned to 30 μm thickness and mounted on a quartz slide. Maps were taken at three energies (11871, 11874, and 11880 eV) at 6 to 10 μm step size for low-resolution maps and 2 to 5 μm step size for high-resolution maps. Arsenic μ-XANES points were chosen using μ-XRF maps and analyzed for As(V)/As(III) ratio at each location using the same analysis technique described for As speciation on bulk samples. Arsenic speciation across the aggregates was determined by the XANES imaging subroutine SMAK—a subroutine of SIXPAK (23).

**RESULTS**

**Aqueous phase results from aggregate reactors.** Under aerated conditions, effluent As(V) is initially at a concentration >50 μM but undergoes rapid decay to a pseudo-steady-state concentration of ~2 μM (Figure 1A). Arsenic(III) concentration is initially lower than that of As(V), but over the first ~25 d undergoes a more gradual decrease than As(V) and is the dominant form of As in the effluent from 2 to 23 d of reaction; after 25 d of elution, its concentration decreases below our level of detection (Figure 1A). Overall, a greater mass of As(III) was eluted from the reactor than As(V), with a total of 8.26 and 4.57 μmol removed, respectively. Effluent Mn(II) concentrations increase from 2.7 to 130 μM after 6 d of reaction followed by gradual decreased to 40 μM over the remainder of the reaction period (Figure 1B). Aqueous Fe(II) remained at or below the detection limit in the presence of oxygen in the advecting solution (Figure 1B).

Under anoxic advecting solute conditions, As(III) was the dominant As species from the first sampling, with As(V) near or below detection limit throughout the experiment (Figure 2A). Manganese(II) concentrations peaked after 6 d of reaction, as seen under aerated treatment; however, the maximum concentration of Mn(II) measured was nearly three-fold greater (372 μM) than the maximum under aerated conditions (126 μM) (Figure 2B). Effluent Mn(II) concentrations began to decrease after 7 d, with concentrations reaching detection limit at approximately 30 d of reaction. Absent of abiotic oxidation (by O2), Fe(II) concentrations increased over the first 15 d and stabilizing at approximately 180 μMfor the remainder of the reaction period (Figure 2B).

When anoxic reactors are transitioned to oxic conditions in the advecting solution after a 20 d reaction period, As(III) concentrations dominated over As(V) in the effluent throughout the experimental period (Figure 3A). Total As concentrations gradually decreased from 54 μM to 18 μM over the first 17 d of anoxic solution addition; upon switching to aerated conditions, a decrease in total effluent As concentration from 18 μM to 13 μM (Figure 3A) results. Response to the aeration transition was also reflected in Fe(II) concentrations, where effluent Fe(II) concentrations peaked just before the switch to aerated conditions, where upon Fe decreased rapidly to our level of detection. Manganese(II) concentrations are less affected by a transition to aerated conditions owing to effective depletion of Mn in the eluting solution over the first 20 d—a maximum concentration occurs after 6 d of reaction (consistent under all aeration treatments) and then undergoes progressive decay for the next 14 d.

For aggregates first subjected to aerated advecting solutions, a switch to anaerobic conditions after 20 d leads to a nearly immediate pulse (2 d) of As(III) (increase from 10 to 16 μM), a rebound in Mn(II) concentration, and a progressive increase in Fe(II) within effluent solutions (Figure 4). Similar to results from the continually aerated aggregate reactor, during the aeration period As(III) concentrations increase to a maximum of ≈30 uM within the first 5 d and then progressively decreases until the cessation of aeration at day 20. Similarly, Mn(II) concentrations decrease from 200 μM on day 6 to 107 μM over 11 d under aerated advecting solution conditions; upon the transition to anoxic exterior conditions, a second pulse of Mn(II) was released, reaching a comparable magnitude (210 μM) as the first concentration peak and then decaying to 40 μM over the following 11 d. Iron(II) concentrations were below detection limit under aerated conditions (20 d), and then increased rapidly to 201 μM 5 d after the transition to anoxic exterior conditions, reaching a maximum concentration of 225 μM.

**Solid phases analysis.** Redistribution of As, Fe, and Mn content and speciation varied between aggregates under differing redox treatments. Consistent with findings reported by Masue-Slowey et al. (2011), total solid-phase Fe content of aggregate exteriors increased by 49 μmol relative to the initial concentration over 47 d of reaction under aerated conditions, while the mid-section and interior regions lost 30 and 7.7 μmol of Fe (Table 1). Similarly, the highest Fe mass accumulated in the exterior region (only 14 μmol Fe decrease from initial mass) and was depleted from interior regions of anoxic to aerated transitioned aggregate. Anoxic and aerated-to-anoxic transitioned aggregates both lost Fe from all regions relative to the initial mass (Table 1 and Figure 5). Iron EXAFS analyses revealed that ferrihydrite was the dominant (>89 mol-%) Fe solid phase in all aggregates, while magnetite composed a smaller portion (7-11 mol-%) and was evenly distributed across the three regions of the aggregate (Table 2).

The greatest mass of total solid-phase As was lost from the exterior sections while higher amounts were maintained in interior regions of all aggregates (Table 1 and Figure 5). Between 21 to 35 % of As was lost from the exterior, 9.4 to 24 % from the mid-section, and only 3.3 to 6.3 % from the interior regions. μ-X-ray fluorescence (μ-XRF) mapping and μ-XANES analysis (Figures 6 and 7) illustrated that As(V) was present in the exterior of aerated and anoxic-to-aerated aggregates only (Figure 7); As(V) was the dominant As species in the outer 2 mm of the aggregate. A transition zone occurs from 2 to 5 mm into the aggregates where the proportion of As(III) increases and then dominates (~80 % As(III)) the solid phase speciation (Figure 7). By contrast, 70-80 % As(III) and only a maxiumum of 20-30 % As(V) is found in anoxic and oxic-to-anoxic transition aggregates from the exterior to interior regions (Figure 7).

Total solid-phase Mn was greatly depleted (loss of >89 mol-% of initial mass) from all sections of the aggregates except under aerated conditions, which retained 55, 8.5, and 4.7 % Mn in the exterior, mid-section, and interior of the aggregate, respectively, similar to the increased mass near the exterior seen in the redistribution of Fe (Table 1). μ-XRF mapping of aerated aggregate confirms higher concentration of Mn near the exterior of the aggregate and absence of Mn signal as distance from exterior increases (Figure 6). Manganese redistribution in anoxic-to-aerated aggregate mirrored that of the aerated aggregate, with 11.6 % remaining in the exterior, and much less, 2 – 8 % left in the mid and interior sections. Anoxic and aerated-to-anoxic aggregates lost slightly more from the exterior sections than other sections, with losses of 97.4 and 98.4 % from the interior and 98.7 and 98.2 % from the exterior.

**DISCUSSION**

Reduction-oxidation (redox) transitions in soils lead to the redistribution of metal oxides and sorbed trace metals. We examined the effects of prolonged aerated and anoxic conditions on synthetic aggregates composed of As(V) presorbed ferrihydrite- and birnessite coated sands inoculated with dissimilatory metal reducing bacteria, *Shewanella* sp. ANA-3, which are capable of respiring upon all three metals and oxygen while utilizing lactate as a carbon and electron source. Furthermore, a comparison was made between aggregates that undergo single aeration treatment (i.e. continuously anoxic or aerated conditions) and aeration status transitioned aggregates, which were maintained under aerated or anoxic conditions for 20 days then switched to anoxic or aerated environments for another 30 days.

Spatial redistribution and speciation of As, Fe, and Mn within the aggregates are controlled by redox gradients resulting from progressively decreasing oxygen concentrations across the exterior to the interior of the aggregate. As was clearly illustrated by Masue-Slowey et al. (17), despite having ample oxygen supplied at the aggregate exterior under aerated conditions, anaerobic conditions arise within millimeters of the exterior due to greater rate of oxygen respiration by *Shewanella* than rate of oxygen diffusion into the aggregate. Hence, for elements such as Fe and Mn that have more mobile reduced oxidation states, biogeochemical conditions of aggregate interiors leads to mobilization and diffusion toward the exterior. When the advecting solution is aerated, Fe(II) produced within the aggregate interior diffuses toward the exterior where upon it undergoes oxidation. Subsequent precipitation of Fe(III) oxides results in the co-association of As, which also diffuses from the reducing aggregate interior toward the exterior. Upon reaching the metal oxide rind, As(III) is oxidized to As(V) by residual Mn oxides, producing a mixture of As(V) and As(III) associated with the aerated aggregate exterior (Figures 6 and 7). Interestingly, As redistribution within the aggregates was highly similar between all aeration treatments (Figure 5), with increasing As/Fe ratios approaching the interior indicative of As retention concomitant Fe(II) production and ensuing ferrihydrite transformation. Microbial respiration of ferrihydrite is active in the anaerobic center of the aggregate to comparable levels to those exposed to anoxic advecting solutes, leading to production of Fe(II). These results demonstrate that influence of oxygen (or aeration status) on As dynamics is most pronounced in the exterior section, whereas reducing conditions in the interior are similar across various aeration conditions.

Arsenic that is accumulated in the Fe oxide-rich exterior under aerated conditions is predicted to mobilize under anaerobic conditions (Masue-Slowey et al., 2011). Our results support this hypothesis, where As concentrations in the effluent of aerated-to-anoxic aggregate transitions gradually decreased over the first 20 d of aeration, with As being composed of a mixture of As(V) and As(III). When anoxic conditions were imposed upon the aerated aggregate on day 20, a pulse of As was released and As(V) concentrations in the effluent decreased (Figure 4B). Manganese and Fe were also measured in the effluent, though their peak concentrations were delayed by 4 and 13 d, respectively, compared to peak As concentration. Therefore, as the redox status of the exterior environment (advecting solution) shifts from aerobic to anaerobic, an immediate pulse of As release occurs which is then followed by Mn and Fe. Multiple studies support the release of As independent of Fe reduction, consistent with our finding that Fe elution is not a prerequisite for As release from the constructed aggregates (17, 19, 24). Reverting to aerated conditions after anoxic advecting solutions slowly decreases the rate of As release, as demonstrated by the decrease in total As eluted after the onset of aeration, and As(V) re-appears in the effluent concomitant with Fe oxidation and removal from effluent (Figure 2A and 3A).

Regardless of aeration status, As(III) composes approximately 80 mol-% of the total solid phase As within the aggregate at distances greater than ~3 mm from the advective flow channel. Therefore, As redox cycling occurs predominantly in the exterior region of the aggregate, while mid and interior sections are highly reducing. The predominantly As(III)-containing interior shows that As(III) oxidation by Mn oxides is minimal and likely only occurs transiently prior to the onset of anoxic conditions within the aggregate interior;conditions that promote Mn reduction inhibits appreciable As(III) oxidation due to reductive dissolution of Mn oxides. .Our results demonstrate that the presence of O2 is required for Mn oxides to be an effective oxidant of As(III) under conditions operative for dissimilatory reduction of Mn. Similarly, Tokunaga et al. (14) demonstrated that immediately following carbon amendment to sediments containing U(VI) and Fe and Mn oxides, rapid and complete reduction of Mn(III,IV) oxides to Mn(II) was observed indicating Mn oxides were not involved in U(IV) oxidation after dissolution. Instead, As(III) oxidation by Mn oxides likely takes place in the aggregate exterior regions, where As(III) diffused from the interior can be rapidly oxidized by Mn oxides and subsequently adsorbed to the surrounding Fe oxide matrix.

Our results illustrate that oxic and anoxic cycling of soils will alter the retention and distribution of trace elements such as arsenic. Owing to mass transfer limited O2 supply and sustained microbial activity, only the exterior region (outer few millimeters) of soil aggregates may be aerobic even under seemingly well aerated conditions. Owing to the diffusive gradients established from the anaerobic aggregate interior and aerobic exterior, redox active elements such as Fe, Mn, and As, all having generally more soluble/mobile species under reducing conditions, will migrated and build in concentration at aggregate exteriors. Upon a transition from aerobic to anaerobic conditions, the accumulated elements will be released to the aqueous phase and result in a concentration pulse within advecting waters. However, the effluent profiles for Mn and Fe differ considerably. Respiration on Mn oxides is thermodynamically more favorable than reduction of Fe oxides when coupled with most carbon sources (18). Hence, sediment profiles containing both Mn and Fe oxides generally exhibit clearly stratified redox layers where Mn reduction occurs at shallower depths than Fe reduction (25). Similarly, Mn(II) elution from aggregates under anoxic conditions occurred prior to Fe(II) elution. Manganese flux from the aggregates occurred under both aerated and anoxic conditions due to the large activation energy required to oxidize Mn(II) and the requirement for a microbial or mineral catalyst (21, 26, 27); in contrast, abiotic Fe(II) oxidation by molecular oxygen is rapid (28), making Fe flux out of aggregates much more dependent upon aeration status—with aeration resulting in a build-up of Fe in aggregate exteriors and limited release to groundwater.

**CONCLUSIONS**

The fate and transport of As, Fe, and Mn is influenced by both redox transitions and the physical heterogeneity inherent to soils that results in a distribution of biogeochemical conditions. The structure of soil aggregates allows reductive processes to persist within the aggregate interiors even under seemingly aerated conditions, with oxidizing processes being restricted to only a few millimeters of aggregate exteriors. Therefore, reductive dissolution of As, Mn, and Fe (and reduction of a large suite of constituents) actively occur regardless of oxygenation within advective flow channels surrounding soil aggregate. However, release of reduced species from aggregates is highly dependent upon the metal and aeration status; Mn elution occurs prior to the release of Fe and can be operative under aerated or anoxic external (advecting solution) conditions, while Fe is only released out of the aggregate when the Fe oxide rind is reduced. However, rapid Mn(II) oxidation by Mn(II)-oxidizing microorganisms has been observed in many oxic environments (29), a process that can inhibit Mn elution from aggregates in a similar fashion to abiotic oxidation of Fe(II). Interestingly, As elution from the aggregate is continuous and comparable under aerated and anoxic conditions; however, As accumulated on the aggregate exterior (due to adsorption onto Fe oxides formed the oxidation from Fe(II) diffused from aggregate interior), is released when aerated flow channels transition to anoxic conditions, producing a sudden high concentration discharge of As into the advective flow channel. Collectively, our results demonstrate the importance of soil structure and redox transitions when assessing the mobilization, speciation, and flux of metal species from soils and sediments.

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