



Arsenic solubilization and redistribution under anoxic conditions in three aquifer sediments from a basin-fill aquifer in Northern Utah: The role of natural organic carbon and carbonate minerals



Xianyu Meng^{a, b}, R. Ryan Dupont^{a, b}, Darwin L. Sorensen^a, Astrid R. Jacobson^c,
Joan E. McLean^{a, b, *}

^a Utah Water Research Laboratory, 8200 Old Main Hill, Utah State University, Logan, UT 84322-8200, USA

^b Department of Civil and Environmental Engineering, 4110 Old Main Hill, Utah State University, Logan, UT 84322-4110, USA

^c Department of Plants, Soils and Climate, 4820 Old Main Hill, Utah State University, Logan, UT 84322-4820, USA

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ABSTRACT

The basin-fill aquifers of the Western U.S. contain elevated concentrations of arsenic in the groundwater due to ancient volcanic deposits that host arsenic minerals. Microcosms were constructed using two oxidized sediments and, by contrast, a reduced sediment collected from a shallow basin-fill aquifer in the Cache Valley Basin, Northern Utah to evaluate the fate of geologic arsenic under anoxic conditions. Sequential extractions indicated the primary arsenic host mineral was amorphous iron oxides, but 13%–17% of the total arsenic was associated with carbonate minerals. Arsenic was solubilized from the sediments when incubated with groundwater in the presence of native organic carbon. Arsenic solubilization occurred prior to iron reduction rather than the commonly observed co-reactivity. Arsenic(V) associated with carbonate minerals was the main source of arsenic released to solution and redistributed onto less soluble minerals, including FeS and siderite as defined by chemical extraction. Arsenic reduction occurred only in the site-oxidized sediments. The addition of a carbon and energy source, glucose, resulted in enhanced arsenic solubilization, which was coupled with iron reduction from the site-oxidized sediments. Adding glucose promoted iron reduction that masked the role of carbonate minerals in arsenic solubilization and retention as observed with incubation with groundwater only.

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1. Introduction

Using statistical models the USGS estimated that 43% of the groundwater in basin-fill aquifers of the Southwestern U.S. has As concentrations that exceed the USEPA maximum contaminant level (MCL) for drinking water (10 µg/L) (Anning et al., 2012). A survey of domestic wells in the Cache Valley Basin located in Northern Utah on the eastern edge of the Basin and Range Province, showed that 23 of the 157 wells tested (15%) had As greater than the MCL (Lowe et al., 2003). Concentrations of As above the MCL ranged from 11 to 100 µg/L, with a mean concentration (\pm SD) of 24.2 ± 18 µg/L.

Arsenic released to groundwater has been attributed to the

* Corresponding author. Utah Water Research Laboratory, 8200 Old Main Hill, Utah State University, Logan, UT 84322-8200, USA.

E-mail addresses: xianyu.meng@aggiemail.usu.edu (X. Meng), ryan.dupont@usu.edu (R.R. Dupont), Darwin.sorensen@usu.edu (D.L. Sorensen), astrid.jacobson@usu.edu (A.R. Jacobson), joan.mclean@usu.edu (J.E. McLean).

microbial reductive dissolution of host iron (Fe) oxide minerals in the deltaic sediments in Bangladesh and West Bengal (Islam et al., 2004; McArthur et al., 2004; Neidhardt et al., 2014; Nickson et al., 2000; Ravenscroft et al., 2001; Swartz et al., 2004). Dissimilatory iron respiring bacteria (DIRB) are diverse and widespread throughout the environment. These redox dissolution processes are not limited to deltaic sediments of Southern and Southeastern Asia but are also attributed to As solubilization in the Hetao Basin, Inner Mongolia (Guo et al., 2008), and in the Pannonian Basin underlying Hungary, Romania, Croatia, and Serbia (Rowland et al., 2011). Arsenic can also be solubilized through direct microbial reduction of As(V) to the potentially more mobile As(III) (Ahmann et al., 1997; Tufano et al., 2008). The diversity of dissimilatory arsenate respiring bacteria (DARB) has been described in ecosystems from Mono and Searles Lakes, CA (Kulp et al., 2006), aquifers in Cambodia (Lear et al., 2007; Pederick et al., 2007) and West Bengal (Hery et al., 2010), estuarine sediments from Chesapeake Bay (Song et al., 2009), stream sediments from the Inner Coastal Plains, NJ

(Lear et al., 2007), and in the Cache Valley basin-fill aquifer, UT (Mirza et al., 2014).

Elevated As in groundwater has been studied in several locations in the arid and semi-arid Western U.S., where processes other than reductive dissolution of Fe oxides and reduction of sorbed As(V) were reported to result in elevated As concentrations. Evaporative concentration was concluded to cause the high As concentration in groundwater in the Carson Desert, NV (Welch and Lico, 1998) and Tulare Basin, CA (Fujii and Swain, 1995). Busbee et al. (2009) excluded reductive dissolution of Fe oxides but concluded that the As in surficial sediments was leached to the groundwater by infiltrating irrigation waters in the Western Snake River Plain, ID. These previous studies suggest that As solubilization in the West is not controlled exclusively by redox processes.

Basin-fill aquifers, underlying the American West, contain As-bearing sulfides derived from volcanic rock (Welch et al., 2000). Contact of As-bearing pyritic materials with the atmosphere and surface water or groundwater results in the oxidative dissolution of As-bearing sulfides (Polizzotto et al., 2006, 2005), and repartitioning of As with Fe oxides and other minerals with increasing depth. Variation in redox conditions, due to groundwater fluctuation, inevitably releases this fraction of As, through reductive dissolution of Fe oxides and reduction of sorbed As(V). Carbonate minerals, such as calcite and siderite, also sequester As through adsorption, co-precipitation, and substitution (Bardelli et al., 2011; Costagliola et al., 2013; Jonsson and Sherman, 2008; Roman-Ross et al., 2006; So et al., 2008). Although carbonate minerals sequester much less As than oxides on a weight basis (Smedley and Kinniburgh, 2002), they are substantial soil components and are widely distributed in semi-arid subsurface systems, including in the Cache Valley Basin. Our study site provided a unique opportunity to explore the role of carbonate minerals in controlling As solubilization.

The aim of this study was to identify geochemical processes that influence As solubilization and redistribution among aquifer solids under anoxic conditions, simulating a rise in the water table. Irrigation and recharge from the surrounding mountain ranges causes groundwater fluctuations. Groundwater fluctuation causes not only the cycling of redox conditions but also the formation and dissolution of carbonate minerals. Microcosms were set up under anoxic conditions using three sediments, two site-oxidized and one site-reduced, with site groundwater from an As-bearing aquifer in the Cache Valley Basin. The microbial reduction of Fe and/or As requires organic carbon (OC) to be bioavailable rather than high in concentration (Fendorf et al., 2010). An external carbon source is commonly used in microcosm studies to amplify and expedite microbial processes while exploring As solubilization mechanisms (Islam et al., 2004; Liao et al., 2011). This present study emphasized As solubilization in the presence of native OC (NOC), whereas the influence of the external carbon source on the mechanisms of solubilization was evaluated for comparison. Since the As entering groundwater is a health concern, the As in solution in relation to Fe and carbonate chemistry was first explored. Arsenic speciation was then investigated in the solid phase because what is re-adsorbed and precipitated could provide mechanistic information about As biogeochemistry. We revealed decoupled As solubilization and Fe reduction when only NOC was present. The external carbon source stimulated As solubilization from the site-oxidized sediments but overwhelmed the naturally dominant mechanism. The shift in As mineralogy determined from sequential extractions further revealed mechanisms of As solubilization and in particular the role of carbonate minerals in determining the long-term fate of As in these sediments.

2. Materials and methods

2.1. Study site

Detailed geology of the Cache Valley Basin has been described by Evans and Oaks (1996) and Inkenbrandt (2010). The Cache Valley Basin is located in the northeastern edge of the Basin and Range Physiographic Province. The basin sits on the Utah/Idaho border and is approximately 80 km long and 24 km wide. The valley is bound on the east by the Bear River Range and on the west by the Wellsville Mountains and the Bannock Range. The Tertiary aged Wasatch Formation and the later Salt Lake Formation were deposited over the Proterozoic and Paleozoic rocks (limestone, dolostone, sandstone, and shale) after these deposits underwent Sevier–Laramide orogeny. The Wasatch Formation is composed of fluvial, colluvial and lacustrine deposits whereas the Salt Lake Formation consists of tuff, tuffaceous sandstone, sandstone, tuffaceous limestone, limestone and conglomerate. Quaternary unconsolidated lacustrine and fluvial deposits (Alpine, Bonneville, and Provo Formations) overlay the Tertiary formations in the Cache Valley Basin (Evans and Oaks, 1996). Much of the Quaternary sediments in the valley are associated with lake cycles within the Great Basin. Most recently, Lake Bonneville filled the valley from about 30,000 to 16,400 years ago. The Lake Bonneville shoreline was at an elevation of 1550 m above sea level for about 500 years. A catastrophic failure of a natural spillway at Red Rock Pass, Idaho, and climatic changes caused Lake Bonneville to retreat from Cache Valley about 14,000 years ago. The Quaternary sediments extend to a depth of 150 m. The source of As may be associated with the Salt Lake Formation that are exposed at high elevations in the Bear River Range east of the basin and on the eastern side of the Wellsville Range and along the margins of the valley. Moving from the outer edges of the valley to the valley floor, sediments change from gravels to sands to fine lacustrine deposits. The Bear River Range was mined for copper, lead and silver and contains copper arsenic sulfosalts, including enargite and tennantite.

Typical of basin-fill systems in the Western U.S., the aquifer is recharged by precipitation in the surrounding mountain ranges rather than by the limited rainfall and snowmelt in the valley. Annual precipitation near the study site ranged from 217 to 833 mm, while annual evapotranspiration ranged from 987 to 1190 mm (1981–2010). Temperature varied from a minimum of -34.4°C to a maximum 39.4°C . The study area is on city-owned property near the Logan City Municipal Landfill ($41^{\circ}44'03\text{N}$ and $111^{\circ}52'22\text{W}$) in the center of Cache Valley (Fig. 1), where a network of monitoring wells has been sampled for As concentration for over 10 years.

2.2. Sample collection

Thirteen aquifer cores (Fig. 1) were collected using a Geoprobe driller by direct push technology. Cores were collected from 0.9 m above the water table to 0.6 m below the water table. Water depths at the time of sampling ranged from 0.2 to 2.2 m below ground surface. The plastic sleeves were immediately capped and taped, stored on ice and returned to the Utah Water Research Laboratory, Utah State University, within 2 h of collection. Each core was divided into three or four sections based on the observed changes in texture and visible redoximorphic features generating a total of 43 sediments samples. Each section was placed into a ziplock plastic bag and thoroughly mixed by massaging the sediment within the bag. All processing was performed in a glove bag filled with 95% $\text{N}_2/5\%\text{H}_2$. Sediments were stored under field-moisture conditions in

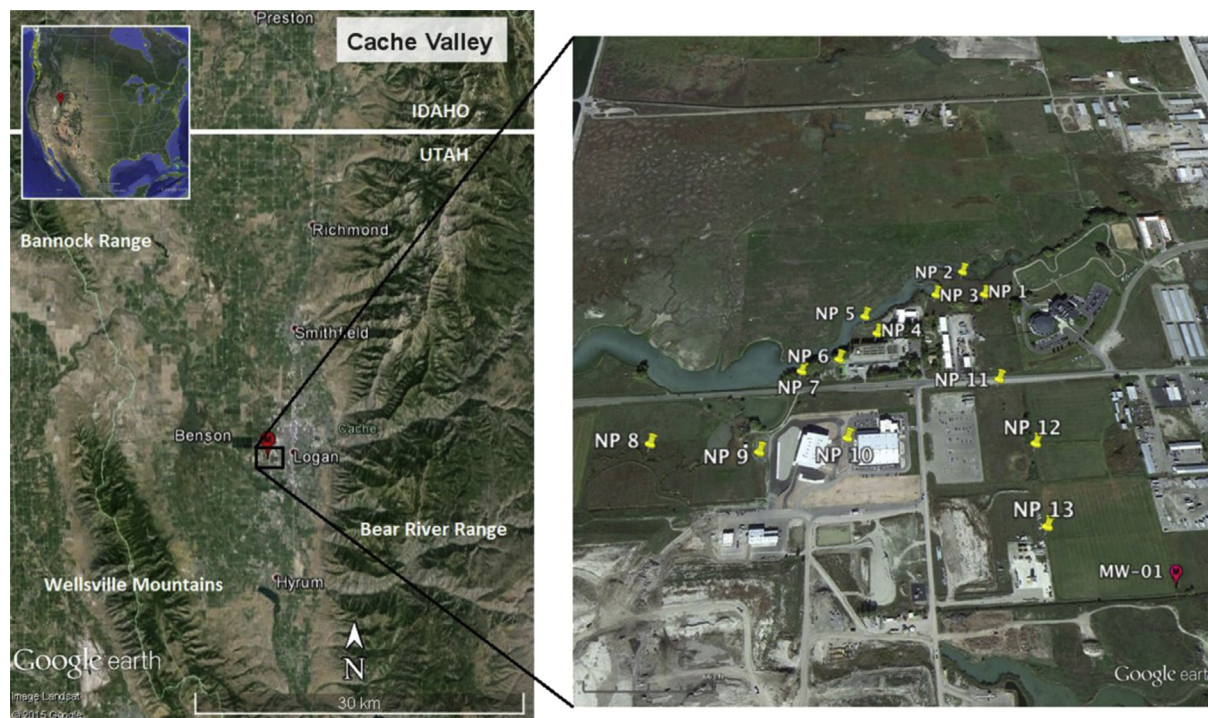


Fig. 1. Map of the Cache Valley Basin (red pin denotes study site location). Insert shows sampling locations of the 13 cores collected. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

the glove bag in a 15 ± 1 °C constant temperature room during preliminary sediment analyses.

2.3. Sediment and water analyses

An HCl extraction procedure was performed in the glove bag using 1 g of solids with 20 mL of 0.5 M HCl to recover Fe from FeCO_3 , FeS, and HCl soluble Fe oxides (Heron et al., 1994), and As associated with these minerals and other minerals that have similar solubility. The HCl-extractable Fe_T (Fe(II)+Fe(III)) and As_T (As(III)+As(V)) were analyzed using Inductively Coupled Plasma Mass Spectrometry (ICP-MS) with a helium collision cell to minimize polyatomic interferences from ArCl (ICP-MS, Agilent 7500C). The As(III) concentration was determined by Hydride Generation Atomic Absorption Spectrometry (HG-AAS) (Perkin Elmer FIAS mercury/hydride flow injection system with a Perkin Elmer Analyst 800 AAS). Although this method is not exclusively determining As(III), the method has limited sensitivity to As(V) (<10%). The Fe(II) concentration was determined using a Genesys 10Vis spectrophotometer (Thermo Scientific) at a wavelength of 562 nm after complexing with ferrozine (Lovley and Phillips, 1986). Analysis for Fe(II) was performed in the glove bag within minutes of sample preparation. As(III) was analyzed within 6 h of sample preparation; As(III) oxidation is kinetically limited.

A series of extractants developed by Amacher (1996) was used to recover ionically bound As and As associated with carbonates, Mn oxides and very amorphous Fe oxides, amorphous Fe oxides, crystalline Fe oxides, and the residual phase (Table 1). For all sequential extraction methods, there are limitations due to non-selectivity of reagents for a specific solid phase, potential re-adsorption of elements onto other solids phases, and other considerations as discussed by Bacon and Davidson (2008). Although researchers that utilize sequential extraction methods recognize these limitations, many report consistent results in abundance of elements in the operationally defined mineral phases and other

techniques such as x-ray absorption fine structure spectroscopy (Lowers et al., 2007) and geochemical modeling (Onstott et al., 2011). Some extraction procedures for As include a step for removal of ligand-exchangeable As using a phosphate solution. Instead we used a calcium chloride solution that would not remove ligand-exchangeable As. We emphasized here mineral-associated As and Fe; As and Fe associated with procedure-defined mineral phases may be surface-bound.

There are a number of sequential extraction schemes that have been developed for defining cationic metals and oxyanion association with mineral phases. Although the specific reagents used differ, the concept of using weak to strong reagents to remove surface bound and solid phase associated trace elements is the same. Several schemes developed for As have not included an extraction step for carbonate minerals, since the aquifer materials used in these studies did not contain carbonates (Huang and Kretzschmar, 2010; He et al., 2010; Rowland et al., 2007; Keon et al., 2001). Carbonate minerals are selectively soluble at a pH value of 5, hence the use of an acetate buffer at this pH (Tessier et al., 1979). Costagliola et al. (2013) examined the use of the acetate buffer versus the initial extraction for As sorbed to Fe oxides using NaOH at pH 12 then dissolving carbonates with aqua regia. Calcite was the main mineral phase in these travertine deposits and both extraction procedures were adequate for defining the concentration of As associated with carbonate minerals (Costagliola et al., 2013). Others have likewise used 1 M acetate buffer at pH 5 for the extraction of As associated with carbonates (Ryu et al., 2010; Romero et al., 2003; Sharif et al., 2008; Wenzel et al., 2001).

The Fe and As in the extracts were determined using ICP-MS. Fe(II) and As(III) were analyzed for the first two extraction steps only. Hydroxylamine-hydrochloride used in steps F3 and F4 and ascorbic acid used in step F5 leach Mn or Fe oxides by reducing these metals (Tessier et al., 1979) therefore these reagents would also affected the oxidation state of As during the extraction. The first two extraction steps were performed in the glove bag to avoid

Table 1
Sequential extraction procedure for determination of As fractionation in the sediments.

Fraction	Extractant	Volume	Extraction conditions	Target phase
F1	0.05 M CaCl ₂	20 mL	24 h, shaking, room temperature	Ionically bound
F2	pH 5, 1 M NH ₄ OAc	25 mL	24 h, shaking, room temperature	Carbonates
F3	pH 2, 0.01 M NH ₂ OH·HCl	25 mL	30 min, shaking, room temperature	Very amorphous Fe oxides and Mn oxides
F4	0.25 M NH ₂ OH·HCl + 0.25 M HCl	25 mL	2 h, shaking, 50 °C	Amorphous Fe oxides
F5	0.2 M ammonium oxalate + 0.2 M oxalic acid + 0.1 M ascorbic acid (fresh)	35 mL	15 min in boiling bath followed by 15 min in ice bath, 1 repetition	Crystalline Fe oxides
F6	HNO ₃ and H ₂ O ₂		USEPA 3050B	Insoluble As mainly associated with silicates and sulfides

changes in Fe and As oxidation state. Analyses for As(III) and Fe(II) were performed immediately after extraction to preserve oxidation states. Triplicate analysis on select cores resulted in less than 10% error among the replicates for As and Fe analyses of the different extracting solutions; the samples after mixing were homogenous and extraction and analysis were within quality control limits. Variance among replicate microcosms however was highly influenced by the inconsistency of microbial processes.

The Walkley–Black method was performed to determine the sediment NOC content, and acid addition with CO₂ evolution was used to determine carbonate content (Sparks et al., 1996). pH of the sediments was determined on a 1:1 ratio of solid to 0.01 M CaCl₂ (Sparks et al., 1996). Particle size distribution was determined by the hydrometer method (Klute, 1986). Water analyses were performed using Standard Methods for the Examination of Water and Wastewater Analyses (APHA et al., 2014). pH (Method 4500H) and EC (Method 2510B) were determined using probes, sulfate by ion chromatography (Method 4500 – SO₄²⁻-B), alkalinity by titration (Method 2320B), dissolved organic carbon (DOC) by combustion with IR detection (Method 5310B), macro cations and trace elements by ICPMS using Method 6020 (USEPA, 1996), and Fe(II) and As(III) as described above.

2.4. Microcosm study configuration

Cluster analysis, using JMP statistical software (JMP 5.01 Statistical Software; SAS Institute, Cary, NC) was used to separate the

43 sediments into three groups based on similar physicochemical properties (Fig. 2A). All parameters listed in Table S1 were used in this analysis. Discriminating variables were HCl extractable As(III), arsenic associated with carbonates and amorphous Fe oxides, Fe associated with carbonates and very amorphous Fe oxides/Mn oxides and soil properties including NOC and sand content (Fig. 2B, Table 2). Three sediments, one from each of the clusters, were selected for use in this study (Fig. 2A and in Table S1). Arsenic associated with carbonate minerals and amorphous Fe oxides are pools of primary interest in this study. Therefore the aquifer solids with carbonates and amorphous Fe oxides associated As within the 95% confidence interval of each cluster (Cluster 1 NP3-1B, Cluster 2 NP8-3B and Cluster 3 NP1-2) were selected as representative of each cluster for the microcosm study.

Microcosms were constructed using groundwater collected from MW-01 (Fig. 1). The groundwater from MW-01 had low As concentration compared with the other wells (0.96 µg/L As with 100% as As(III)), but similar general water quality properties as groundwater across the site: 193 µg/L dissolved Fe (100% as Fe(II)), bicarbonate alkalinity of 290 mg/L as CaCO₃, 8.6 mg/L SO₄²⁻, 6.9 mg/L dissolved organic carbon (DOC), a pH of 7.4, and an EC of 483 µS/cm. The groundwater was collected using a peristaltic pump after pH, electrical conductivity, and dissolved oxygen readings reached steady-state conditions. The water was used to construct the microcosms within 24 h of collection. To construct a microcosm unit, 10-g (equivalent dry weight) of the sediment was mixed with 40 mL of autoclaved and oxygen-free groundwater in a 50 mL

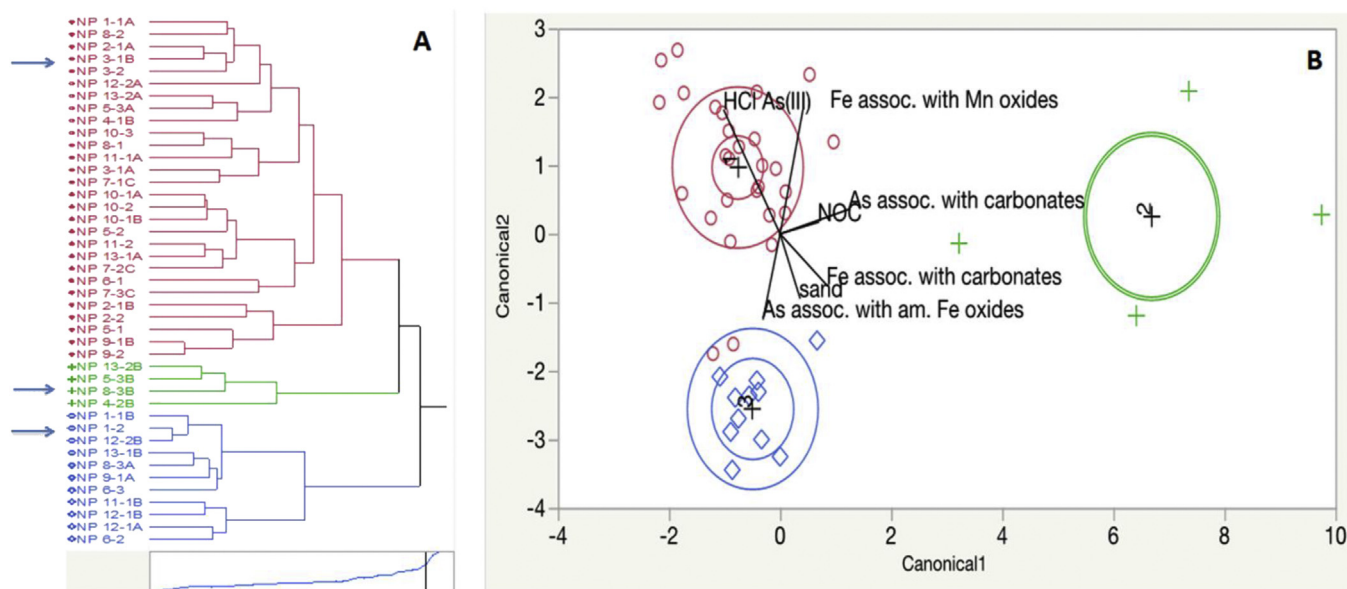


Fig. 2. Cluster analysis (using all parameters in Table S1) of the 43 sediment samples. Arrows indicate samples selected for this study (A) and canonical plot with the seven most discriminant variables (B).

Table 2
Major physicochemical characteristics of the clustered aquifer solids.

Cluster no.	Fe associated with carbonates (mg/kg)	HCl extractable As(III) ($\mu\text{g}/\text{kg}$)	Fe associated with am. Fe oxides (mg/kg)	As associated with carbonates ($\mu\text{g}/\text{kg}$)	NOC (w/w%)	Sand (w/w%)	As associated with am. Fe oxides ($\mu\text{g}/\text{kg}$)
1 (n = 28)	99.3 \pm (17.1)	225 \pm (33.0)	35.5 \pm (5.7)	908 \pm (150)	0.18 \pm (0.02)	17.6 \pm (5.1)	2789 \pm (561)
2 (n = 4)	1124 \pm (698)	38.2 \pm (9.0)	65.0 \pm (22.8)	1106 \pm (335)	0.42 \pm (0.07)	5.83 \pm (5.0)	1070 \pm (302)
3 (n = 11)	91.2 \pm (31.3)	78.2 \pm (16.3)	19.1 \pm (3.5)	468 \pm (82)	0.14 \pm (0.06)	32.6 \pm (5.6)	825 \pm (215)

sterile polypropylene centrifuge tube (Fisherbrand®). The experimental treatments were groundwater and groundwater with glucose, in triplicate. Filtered-sterilized glucose was added to the microcosms at a final concentration of 2000 mg C/L (167 mM C). The selection of glucose as a carbon source was based on the results of a previous study in which we used a number of different carbon sources and the highest As reduction was detected in response to glucose addition (McLean et al., 2006). Abiotic controls were generated by autoclaving individual centrifuge tubes with 10 g of field moist sediment at 121 °C at 1.1 atm for 1 h, with this procedure repeated after 48 h (Trevors, 1996). Groundwater and glucose were added to these microcosms. The microcosms were capped and placed horizontally in the glove bag at 15 °C in the dark. They were sacrificed at discrete time points over 54 days by separating the aqueous and solid phases by centrifugation at 7000 \times g for 20 min. The supernatant was decanted in the glove bag and filtered through a 0.2 μm nylon filter (Life Science Products, Inc.) for aqueous phase analyses including As and Fe species, as described above. Concentrations of aqueous phase As and Fe were expressed as $\mu\text{g}/\text{kg}$ or mg/kg, dry weight basis of the sediment instead of on a volume basis, to normalize the data. The aqueous phase was also analyzed for sulfate, pH, and EC using methods as described above.

After decanting the aqueous phase, the remaining solids were mixed to ensure homogeneity. Arsenic and Fe speciation in the incubated solids was determined after 0.5 M HCl extraction at each sampling interval, while the sequential extraction procedure was performed at Day 0 and Day 54 to evaluate changes in As mineralogy over the course of the experiment. As(III) and Fe(II) were determined in the independent HCl extraction and the first two sequential extraction steps. Huang and Kretzschmar (2010) have discussed the necessity of preserving the oxidation state of As through the extraction procedure by adding chemicals including mercury chloride. In this present study, to avoid the use of toxic chemical but aid in preserving oxidation state, all processing was performed in the glove bag except centrifugation. Fe(II) was analyzed using ferrozine immediately in the glove bag. Extracts for As(III) analysis were stored in the glove bag until analyzed by HGAA within 6 h. All data are reported on a dry weight basis.

A one-way ANOVA was used to determine significant differences in Fe and As solubilization and reduction with time using JMP (JMP 5.01 Statistical Software; SAS Institute, Cary, NC). Post-hoc testing was performed using Tukey's Honestly Significant Difference (HSD) ($\alpha = 0.05$). Since the data analysis involves a multiple comparison, a common, family error rate as described by the HSD is more appropriate than using a standard deviation for determining differences. The common Tukey error rate is a scaled average of the individual values.

Results reported in Table S1 were on the sediments analyzed immediately after sample collection using the referenced standard methods of analyses; these values however are not directly comparable to reported values at Day 0 of the microcosm study. When added to the microcosms, sediments were suspended in four times the mass of groundwater as solids, a ratio not encountered in the field, but necessary for the type of microcosm study used here and reported in the literature. All microcosms were established under

the same conditions therefore all comparisons over time were with the Day 0 characteristics of the sediments.

3. Results and discussion

3.1. Sediment properties

The three representative sediment samples, NP1, NP3, and NP8 were calcareous (30–55% CaCO_3), with pH values from 7.2 to 7.7 (Table 3). NP1 and NP3 were site-oxidized sediments, indicated by the orange-brown to dark brown Fe oxides patches and the presence of HCl-extractable Fe(III) (78.9 mg/kg and 400 mg/kg) (Table 3). These sediments were low in NOC (0.09% and 0.12%) and high in sand content (33% and 42%). The greenish coloration in NP8 indicated that NP8 was site-reduced. The NOC content of NP8 (0.31%) was approximately three times higher than of NP1 and NP3. Total As content in NP3 (8.28 mg/kg) was approximately twice that in NP1 (3.15 mg/kg) and approximately three times that in NP8 (3.91 mg/kg) (Table 3). Across the study site total solid phase As ranged from 2.0 to 36.7 mg/kg, with a mean concentration of 7.8 ± 1.9 mg/kg ($\pm 95\%$ CI) (Table S1).

The majority of total As in both NP1 and NP3 was associated with Fe oxides (amorphous and crystalline), while the residual phase was the dominant As pool in NP8 (Table 4). Although carbonate minerals host less As than Fe oxides, the amounts of total As associated with carbonate minerals were high compared with sediments collected from other semi-arid regions (Bhattacharya et al., 2006; Busbee et al., 2009). Arsenic sequestered by natural calcite occurs primarily as As(V) (Bardelli et al., 2011), in agreement with the dominance of As(V) in the carbonate fraction of these sediments (Table 4). As(V) was always the dominant oxidation state in HCl-soluble minerals, even for the site-reduced NP8 (Table 3). Crystalline Fe oxides and the residual phase constituted over 86% of the Fe in the three sediments; the remaining Fe was primarily distributed in amorphous Fe oxides.

3.2. As solubilization and Fe reduction during incubation with groundwater

Addition of groundwater (Day 0) to the sediments resulted in immediate release of As_T (As(III) + As(V)) to solution (Fig. 3A–C) equivalent in concentration to the ionically bound As_T , as defined by chemical extraction (Table 4). With incubation, the concentration of As_T and As(III) in solution increased by Day 6 compared to the concentration on Day 0 (Fig. 3A–C). This increase, however, was not accompanied by discernible Fe reduction, since there was no detectable increase of Fe(II) associated with the solids or in solution, indicating the release of As_T and As(III) was not due to the reductive dissolution of Fe minerals. Others reporting the decoupling of As and Fe reactivity have ignored the amount of Fe(II) produced that is re-sorbed to surfaces (McLean et al., 2006; Van Geen et al., 2004). The solution phase Fe(II) was never more than 10% of the Fe(II) reported in Fig. 3. Without analysis of the solid phase the rate and extent of Fe reduction would have been greatly underestimated as also pointed out in the study by Weber et al.

Table 3
Major chemical characteristics of selected sediments.

Sample	Depth m	pH	NOC (w/w%)	Carbonate (w/w%)	Clay (w/w%)	Sand (w/w%)	Total		HCl extraction			
							As (mg/kg)	Fe (mg/kg)	As(III) ($\mu\text{g}/\text{kg}$)	As(V) ($\mu\text{g}/\text{kg}$)	Fe(II) (mg/kg)	Fe(III) (mg/kg)
NP1 (Cluster 3)	3.1–3.7	7.2	0.09	28.8	21.7	33.2	3.15	13,100	57.2	811	129	78.9
NP3 (Cluster 1)	2.7–3.4	7.4	0.12	39.3	19	41.8	8.28	12,000	294	2550	14.2	400
NP8 (Cluster 2)	4.7–4.9	7.7	0.31	55.1	30	15.6	3.91	14,700	38	499	2420	ND ^a

^a ND, non-detectable.**Table 4**
The distribution of arsenic among solid phases in selected sediments (Day 0).

Sample	Ionically bound			Carbonates			Very am. Fe oxides and Mn oxides		Am. Fe oxides		Crystalline Fe oxides		Residual	
	As(III) $\mu\text{g}/\text{kg}$	As(V) $\mu\text{g}/\text{kg}$	% of total As	As(III) $\mu\text{g}/\text{kg}$	As(V) $\mu\text{g}/\text{kg}$	% of total As	$\mu\text{g}/\text{kg}$	% of total As	$\mu\text{g}/\text{kg}$	% of total As	$\mu\text{g}/\text{kg}$	% of total As	$\mu\text{g}/\text{kg}$	% of total As
NP1	1.56	10.7	0.30%	33.9	499	13%	222	5.4%	1160	28%	1390	34%	791	19%
NP3	1.1	63.3	0.68%	39.7	1300	14%	597	6.3%	3280	34%	3420	36%	775	8.2%
NP8	27.8	32.7	1.60%	131	529	17%	99	2.6%	1010	26%	581	15%	1470	38%

(2010). However, in the present study, there was no discernible Fe reduction in the solution or on the solid phase until Day 12 (NP1), Day 25 (NP3), and Day 54 (NP8), but As_T was released to solution by Day 6. The decoupled As solubilization and Fe reduction indicated that As solubilization was not exclusively limited by Fe reduction in these studied sediments. The concentration of aqueous Fe(III) was below the detection limit ($<1 \mu\text{g}/\text{L}$).

The extent of As release and Fe reduction was dependent on sediment type (Fig. 3). NP1 and NP3 were both oxidized under field conditions, as defined by the low proportion of Fe(II) in the sediment, whereas NP8 was reduced under field conditions (Table 3). NP3 contained the highest concentration of total As of the three sediments tested (Table 3), with 5300 $\mu\text{g}/\text{kg}$ associated with the more reactive pools (F1–F4). Thus NP3 had the highest aqueous and HCl concentration of As in this microcosm study (Fig. 3).

The initial release of As_T was probably due to desorption of As or dissolution of As-containing non Fe minerals. Bicarbonate ion is reported to result in As solubilization through competitive sorption (Appelo et al., 2002; Holm, 2002). The desorption of As by bicarbonate present in these incubated calcareous sediments is a possible mechanism of As release to solution.

The pH of the groundwater added to the sediments was 7.4 and the sediment pH values were 7.2–7.7. The assembly of the microcosms, with initial dissolution of soluble constituents with the 4:1 water:solids ratio resulted in initial pH values of 8.7 ± 0.02 . Over the 54-day incubation period, the pH decreased from 8.7 ± 0.02 to 7.7 ± 0.03 in the microcosms of the three sediments (Fig. 4A). Geochemical modeling (MINEQL+ 4.6, Environmental Research Software) with solution phase ion constituents indicated carbonates and Fe oxides remained as solid phases in this pH range; the As released to solution was not exclusively due to dissolution of minerals with decreasing pH.

The increase in As(III) in solution by Day 6 was not due exclusively to the desorption of surface-bound As(III) or release of As(III) associated with carbonate minerals, since the concentration of As(III) in solution exceeded the concentration of As(III) associated with these two pools in the NP1 and NP3 sediments (Table 4). NP3, for example, had $40.8 \pm 5.1 \mu\text{g}/\text{kg}$ As(III) associated with the first two extraction steps; by Day 6 the concentration of As(III) in solution was equivalent to $131 \pm 13.0 \mu\text{g}/\text{kg}$. From Day 0 to Day 6, the percentage of As(III) in solution increased from 32% for NP1 and 18% for NP3 to 100% (Fig. 3A and B). Microbial activity has been shown to be necessary for As(V) reduction even when the Eh is sufficiently

low for As(III) dominance (Yamaguchi et al., 2011). We attempted to establish abiotic controls but the autoclaving process was not successful in eliminating all spore-forming bacteria. Other microcosm studies have shown that As reduction is minimal in sterilized control samples (Lear et al., 2007; Percy et al., 2011), although there have been reports of limited abiotic reduction of As(V) under specific conditions (Kocar et al., 2006; Zobrist et al., 2000). However, even without abiotic controls, the microbial reduction of As(V), independent of Fe reduction, was evident (Mirza et al., 2014). Dissimilatory arsenate respiring bacteria (DARB) were present in these microcosms (Mirza et al., 2014). DARB carrying the arsenate respiring reductase coding gene (*arrA*) are capable of reducing As in both solution and solid phases (Malasarn et al., 2004; Oremland and Stolz, 2005).

The solubilization of carbonate minerals, unlike Fe oxides, is not a direct microbially driven process. The release of As(V) from association with carbonate minerals through this incubation may be an abiotic process, but the reduction of As(V) to As(III) must be microbially mediated. Although we failed to provide sterile controls for this present study, further evaluation of sterilization methods using mercuric chloride with sediments from NP9 at this site (Fig. 1), showed that As(V) was solubilized in the abiotic control at the same concentrations as observed in the biotic microcosms. As(III) and Fe(II) however were not produced without microbial activity. Whereas the reduction of As is a microbially driven process, the desorption or dissolution of As within the microcosms may, in part, be an abiotic process.

After the initial lag phase for Fe reduction, NP1 and NP3 showed co-occurrence of Fe reduction and As solubilization supporting the mechanism of As release with the reductive dissolution of Fe oxides (Nickson et al., 2000; Smedley and Kinniburgh, 2002). Microcosm studies using sediments from West Bengal and Taiwan have shown increased Fe reduction with increased solution phase As(III) (Hery et al., 2010; Islam et al., 2004; Liao et al., 2011). In this study, solution phase As(V) increased, but the As(III) reached either steady-state conditions (NP1 Fig. 3A) or decreased (NP3 Fig. 3B); As(III) was either precipitated or sequestered by the solid phase, or production of As(III) stopped. Further exploration of solid phase As will distinguish these processes, as discussed below.

In NP8, the concentration of As(III) in solution equaled the ionically bound As(III) and approximately half of the carbonate-bound As(III), suggesting that the occurrence of As(III) in the solution may be due to As(III) released from these pools. Although

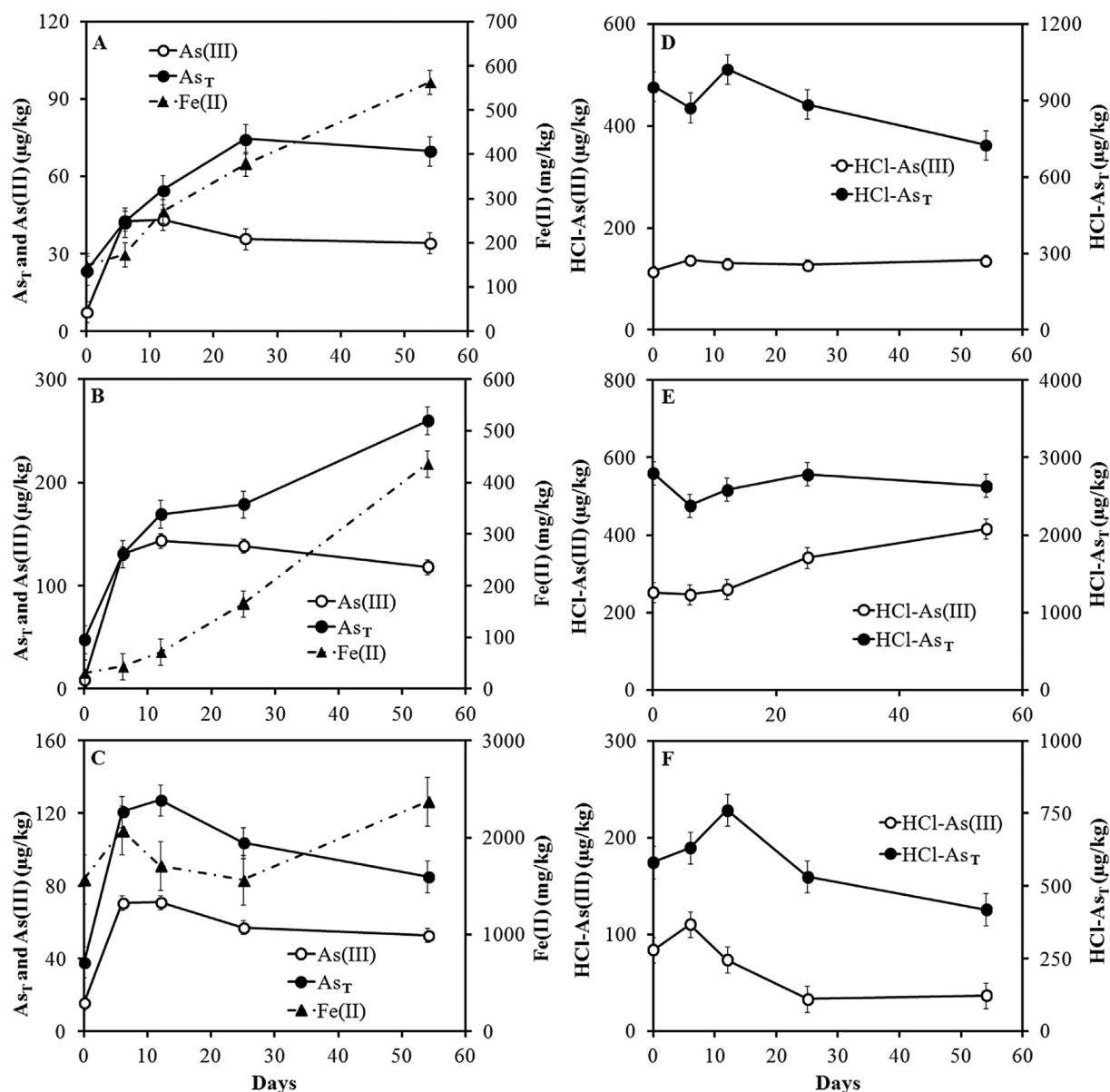


Fig. 3. Arsenic solubilization and Fe reduction (Fe(II) in solution + extractable with HCl) in microcosms containing the three sediments A) NP1, B) NP3, and C) NP8 incubated with groundwater. Arsenic speciation in the 0.5 M HCl extractable solid phase in the three sediments D) NP1, E) NP3, and F) NP8. Error bars represent Tukey HSD ($\alpha = 0.05$; $n = 3$).

laboratory-imposed reducing conditions produced Fe(II) between Day 25 and Day 54 in this site-reduced sediment, there was no evidence of further As reduction with incubation. There were bacteria with the *arrA* gene in NP8 after incubation, as in NP1 and NP3 (Mirza et al., 2014); the potential for As reduction existed but was not observed.

Arsenic solubilization, and reduction in NP1 and NP3, without adding a carbon source observed in this study has also been observed in the field and in other laboratory microcosm studies (Islam et al., 2004; Liao et al., 2011; Rowland et al., 2011, 2007). The NOC content in these sediments was low ($\leq 0.3\%$), but the bioavailability was sufficient to drive the microcosms to sulfate reducing conditions (Fig. 4B). In NP3 the decrease in sulfate was not as rapid as in NP1 and NP8 because NP3 was relatively rich in HCl extractable Fe(III), presumably bioavailable, which suppresses sulfate reduction in sediments (Lovley and Phillips, 1987). The NOC at the study site was, at least partially, labile, driving Fe, As, and sulfate

reduction reactions and As solubilization. The concentration of sulfate in groundwater used in the microcosm studies was 8.6 mg/L whereas the aqueous sulfate concentration at Day 0 for all sediments was approximately 17 mg/L, the higher concentration due to the presence of water-soluble sulfate associated with the sediments.

3.3. Changes in solid phase As with groundwater treatment

Solution phase As(V) and As(III) may be re-adsorbed by the solid phase or may form precipitates. Solid phase As must be investigated over time to understand the fate of As in groundwater systems, although such studies have seldom been reported in previous microcosm studies (Weber et al., 2010). Aqueous phase As(III) in NP3 reached steady-state conditions (Fig. 3B) and may lead one to conclude that reduction of As(V) only occurred within the first sampling interval. HCl extractable As(III), however, continued to

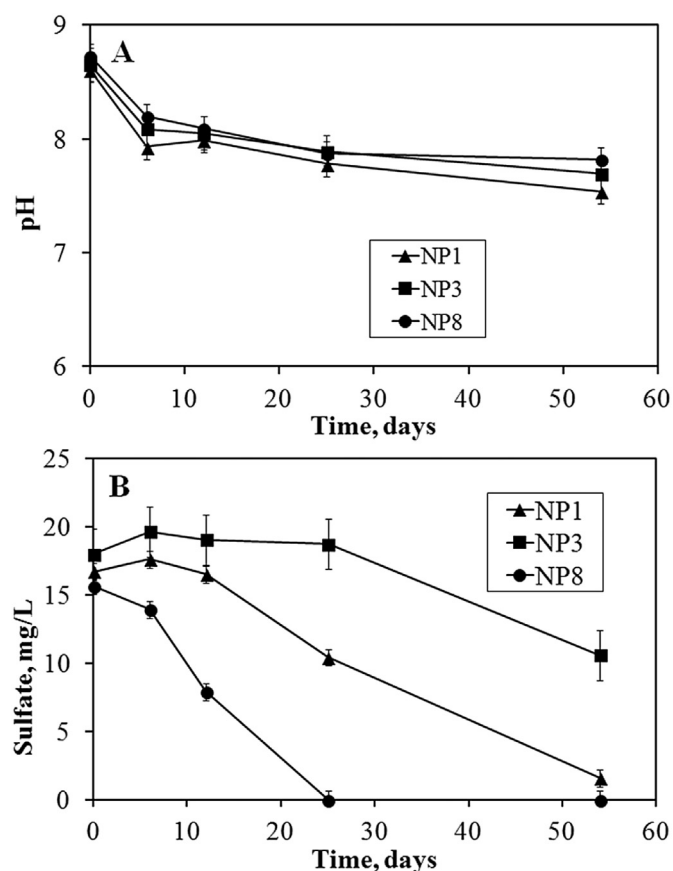


Fig. 4. The changes in pH and sulfate concentration in the aqueous phase of microcosms containing the three sediments incubated with groundwater: A) pH and B) sulfate. Error bars represent Tukey HSD ($\alpha = 0.05$; $n = 3$).

increase with time (Fig. 3E); As(III) was produced but was accumulated in the solid phase. Because this accumulation co-occurred with the production of Fe(II), between Day 12 and Day 25, the As(III) sink may be newly formed Fe(II) minerals. Ahmann et al. (1997) suggested that the solid sink of aqueous As(III) may be siderite, FeCO_3 . The accumulation of As(III) in the solid phase may also be due to the direct enzymatic reduction of As(V) on the solid phase by DARB (Ohtsuka et al., 2013). The reduction of As(V) at the mineral surfaces facilitates As solubilization (Ahmann et al., 1997; Ohtsuka et al., 2013; Tufano et al., 2008), but the As(III) produced in NP3 remained on the solid phase. In contrast, no discernible change occurred in the HCl-extractable As(III) in NP1, the other site-oxidized sediment (Fig. 3D), indicating As reduction was limited to the aqueous phase for this sediment. The As(V) associated with minerals in NP1 was not as bioavailable to its native microbe community as that in NP3, as observed by the lack of As(III) production in the solid phase of NP1 (Fig. 3D).

The HCl-extractable As_T and As(III) followed a similar pattern with time in NP8 (Fig. 3F). In this site-reduced sediment, As_T had precipitated as non-HCl-soluble minerals, such as crystalline Fe oxides and/or pyrite (FeS_2), supported by sequential extraction (Table 4). The depleted sulfate concentration (Fig. 4B) indicated more complete bacterial sulfate reduction in NP8. Sulfide may occur in the NP8 microcosm, thereby forming more FeS_2 which was reported to sequester As (Bostick and Fendorf, 2003).

3.4. The redistribution of As and Fe in the mineral phase

The redistribution of As(III) and As(V) was examined by

comparing significant changes, as determined using t-test ($\alpha = 0.05$), in aqueous and mineral phases defined by sequential extraction and 0.5 M HCl extraction between Day 0 and Day 54 (Fig. 5A–C). Distinguishing the oxidation state of As was possible in extracts for ionically bound, acetate-, and HCl-soluble defined fractions. The other extractants used reducing reagents for dissolving Mn and Fe oxides thus also affecting the oxidation state of As.

The 0.5 M HCl solution extracts As associated with the first two steps of the sequential extraction and As associated with FeS, FeCO_3 , and HCl-soluble Fe and Mn oxides, defined as the portion of Mn and Fe oxides that are solubilized without addition of a reducing reagent such as hydroxylamine hydrochloride (Heron et al., 1994). Acid volatile sulfides, such as FeS, are extracted with 0.5 M–1 M HCl so would be included in this extraction. Pyritic minerals (FeS_2) would not be solubilized by this extraction (Tessier et al., 1979). Arsenic associated with FeS, FeCO_3 , and HCl-soluble Mn and Fe oxides was therefore operationally defined as the difference between As(III) or As(V) in the HCl extract and in the sum of the ionically bound and carbonate-associated extracts. Amorphous Fe oxides not solubilized by 0.5 M HCl, but requiring the addition of hydroxylamine-hydrochloride (HA-HCl) for reduction of Fe(III) for dissolution, were determined as the difference between HA-HCl extractable Fe (the sum of the first four extractants) minus 0.5 M HCl extractable Fe. Arsenic associated with this amorphous Fe(III) oxides, crystalline Fe oxides and the residual fraction were therefore also reported. For the defined mineral phases that required the combination of several extraction steps, the overall error was determined by summing the variance from each extraction step. To determine whether Day 0 and Day 54 values were statistically different, 95% confidence intervals, calculated from the variance, were compared. The mass balance of As and Fe, as loss and gain from the different mineral phases, was within 15%, except for Fe in NP1 (Fig. 5A–F).

The As, all as As(V), was removed from mineral phases soluble in 1 M acetate buffer (pH 5) (Fig. 5A–C). The dissolution of carbonate minerals with the acetate buffer would remove As associated with the surface of carbonate minerals and As incorporated into the structure of carbonate minerals. The acetate extraction can also dissolve Ca and Mg arsenate (Ryu et al., 2010), but the procedure is relatively specific to the dissolution of carbonate minerals (Tessier et al., 1979), with an exception of siderite (FeCO_3) (Heron et al., 1994). Acetate is not an effective ligand for removal of As from ligand exchange sites; As(V) surface bound to non-acetate soluble minerals, such as Fe oxides, would not be removed by this extractant. These observations defined a major portion of As that is subject to solubilization in these sediments as carbonate-associated As. There was also loss of As from amorphous Fe(III) oxides in NP1 and NP3, but not from the site-reduced NP8.

As(V) was reduced and the produced As(III) was associated with the aqueous phase, ionically bound to solid surfaces, and also associated with carbonate minerals (NP3). A portion of the As(V) from the carbonates and/or amorphous Fe(III) oxide was retained by the FeS/ FeCO_3 /HCl–Fe oxide pool. Arsenic was also redistributed into the more insoluble crystalline Fe oxides (NP1, NP3, NP8) and the residual phase (NP1), but the oxidation state of this As could not be distinguished.

Fe was redistributed within the FeS/ FeCO_3 /HCl–Fe oxide pool with a shift in oxidation state from Fe(III) to Fe(II) (Fig. 5D–F), indicating Fe(II)-bearing FeS and/or FeCO_3 formed with incubation. FeS and/or FeCO_3 accumulated As in the solid phase as illustrated by the gain of As(V) in this extracted phase (Fig. 5). The immobilization of solubilized As by siderite (Jonsson and Sherman, 2008) and other newly formed Fe(II) and Fe(II)/Fe(III) mineral phases (Neidhardt et al., 2014) buffers the resulting solution concentration

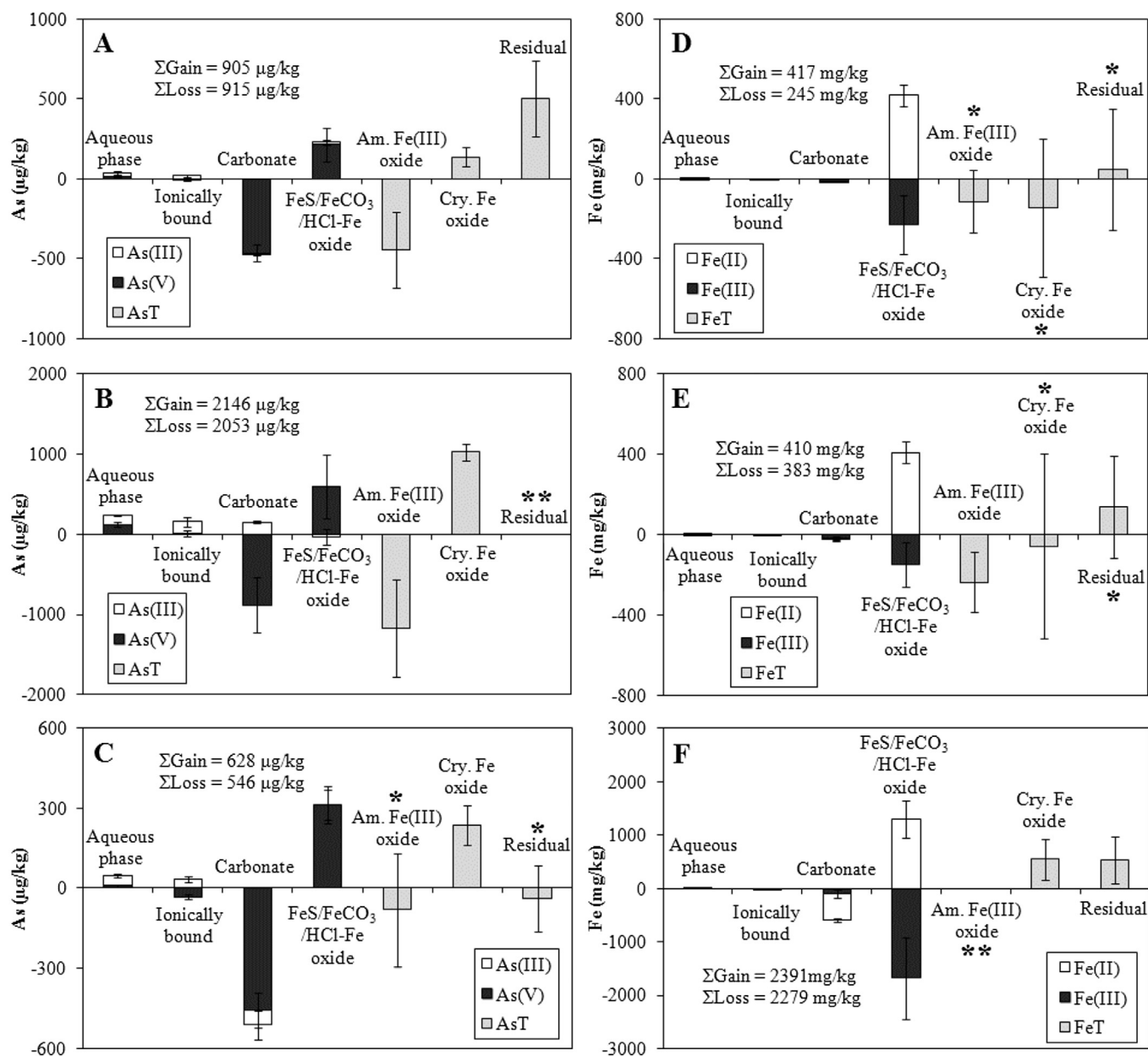


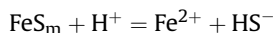
Fig. 5. Redistribution of As and Fe in microcosms made from three sediments and groundwater after 54 days of incubation. The column shows the change of As or Fe in each pool after 54-day of incubation; a bar above the x-axis indicates a gain and a bar below the x-axis indicates a loss that is statistically significant (t-test or 95% CI, $\alpha = 0.05$; $n = 3$). As: A) NP1, B) NP3, and C) NP8, and Fe: D) NP1, E) NP3, and F) NP8. Error bars are ± 1 standard deviation derived from the summed variance of values used to calculate gains/losses in triplicate microcosms. Asterisks indicate no significant difference between Day 54 and Day 0. Double asterisks indicate no detectable As or Fe in this phase at Day 54 and Day 0.

of As. [Burnol and Charlet \(2010\)](#) likewise concluded that poorly crystalline mackinawite (FeS_m) and siderite were the main solid phases for As retention in an aquifer in India under reducing conditions. [Lowers et al. \(2007\)](#) reported that framboidal and massive pyrite was the principal arsenic pool in sediments from boreholes in Bangladesh.

Table 5
Calculation for ion activity product of Fe^{2+} and S^{2-} in the three microcosms at Day 54.

Sample	$[\text{Fe}^{2+}] \text{ M}$	Estimated $[\text{HS}^-] \text{ M}$	pH	$\log Q_r$
NP1	1.86×10^{-3}	7.89×10^{-5}	7.54	0.71
NP3	1.81×10^{-3}	3.85×10^{-5}	7.83	0.67
NP8	3.58×10^{-3}	8.16×10^{-5}	7.69	1.16

FeS_m , a precursor for the formation of pyrite, was predicted to precipitate in all three microcosms by the end of the study ([Table 5](#)). Since the pK_a values for H_2S are 7 ([Morse et al., 1987](#)) and >14 , sulfide in solution at the pH of these microcosms exists dominantly as HS^- . The formation of FeS_m is therefore represented by the bisulfide reaction,



with reported $\log K_{sp}$ values between -3.5 and -3.0 ([Rickard, 2006](#); [Davison et al., 1999](#)). FeS_m will form when the ion activity product, $\log Q_r$ ($Q_r = [\text{Fe}^{2+}] \times [\text{HS}^-]/[\text{H}^+]$), is greater than $\log K_{sp}$. By assuming that half of the decreased sulfate ([Fig. 4](#)), which is the difference between Day 0 and Day 54, was converted to HS^- , FeS_m

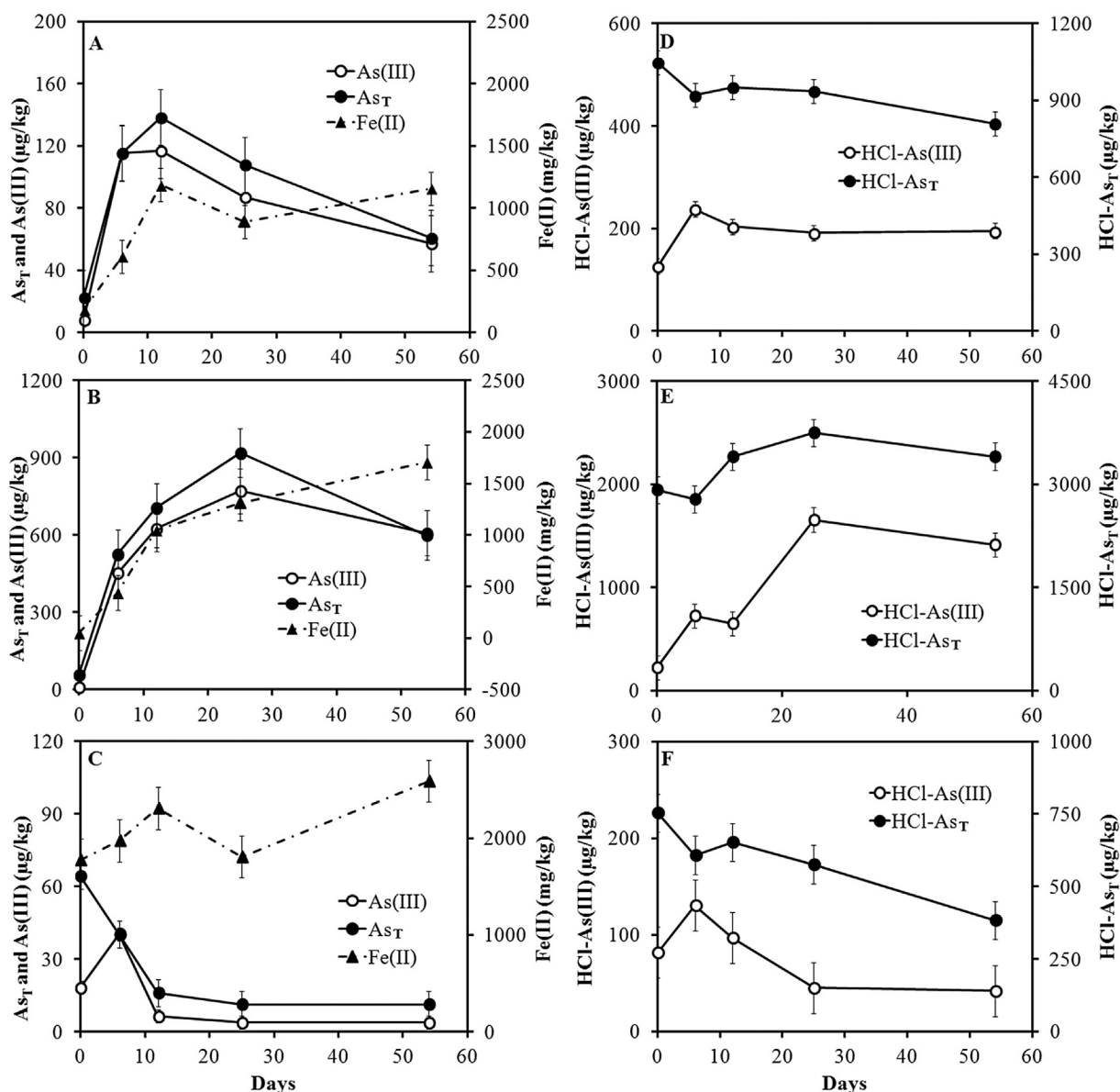


Fig. 6. Arsenic solubilization and Fe reduction (Fe(II) in solution + extractable with HCl) in microcosms containing the three sediments A) NP1, B) NP3, and C) NP8 incubated with groundwater plus glucose amendment. Arsenic speciation in the 0.5 M HCl extractable solid phase in the three sediments D) NP1, E) NP3, and F) NP8. Error bars represent Tukey HSD ($\alpha = 0.05$; $n = 3$).

was supersaturated in all three microcosms (Table 5). At the beginning of the study, the site-oxidized NP1 and NP3 did not produce any HS^- . The Fe(II) produced was precipitated as siderite in these calcareous sediments (as determined by chemical equilibrium modeling, MINEQL+, input parameters included Ca, Mg, alkalinity, Cl, pH, total Fe(II) produced and HS^-). With time HS^- production would favor the formation of FeS_m . For NP8, the site reduced sediment, HS^- was produced by the first sampling interval, with FeS_m as the dominant mineral phase for Fe(II) throughout the study. At the end of the study, these mineral phases provide surfaces for the retention of As, as delineated by the sequential extraction.

The formation of FeS has also been reported in other microcosm studies, in which the microcosms were amended with sulfate and lactate (Omeregie et al., 2013; Kocar et al., 2010). The excessive sulfate and simulated reducing conditions did not result in FeS_2 in these studies. FeS is a precursor for the formation of FeS_2 but the

formation of pyrite would not occur under the time and conditions of this present study, and similar microcosm studies.

The major solid phase loss of Fe(III) was from the HCl–Fe oxide for all three sediments. This process, however, was not accompanied by a discernible loss of As from this pool. Arsenic was however removed from the amorphous Fe(III) oxide fraction. The microbial dissolution of Fe(III) oxides, with the metabolism of NOC, was however not the exclusive mechanism contributing to solubilize As from the sediments; carbonate minerals contributed equal or greater concentrations of As for redistribution.

3.5. Influence of glucose addition on As solubilization

The addition of glucose stimulated As solubilization from NP1 (Fig. 6A); the solution phase As reached 138 ± 32.0 µg/kg at Day 6, compared to the maximal solution As_T concentration of 74.6 ± 3.9 µg/kg without glucose addition (Fig. 3A). The stimulated

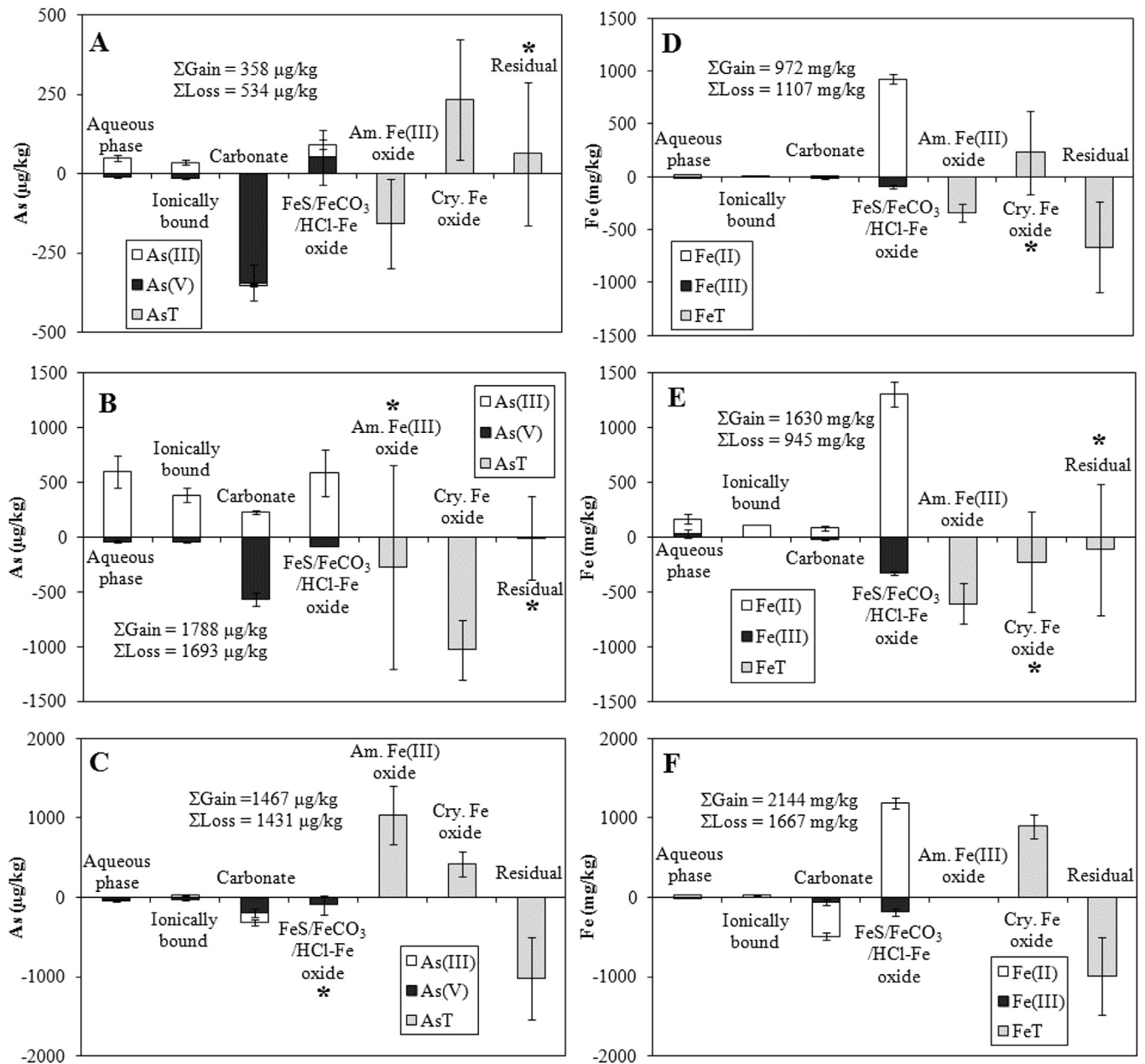


Fig. 7. Redistribution of As and Fe in microcosms made from three sediments and groundwater plus glucose after 54 days of incubation. The column shows the change of As or Fe in each pool after 54-day of incubation; a bar above the x-axis indicates a gain and a bar below the x-axis indicates a loss that is statistically significant (t-test or 95% CI, $\alpha = 0.05$; $n = 3$). As: A) NP1, B) NP3, and C) NP8, and Fe: D) NP1, E) NP3, and F) NP8. Error bars are ± 1 standard deviation derived from the summed variance of values used to calculate gains/losses in triplicate microcosms. Asterisks indicate no significant difference between Day 54 and Day 0. Double asterisks indicate no detectable As or Fe in this phase at Day 54 and Day 0.

As_T solubilization was concomitant with enhanced Fe reduction. Similarly in NP3, the As_T solubilization and Fe(III) reduction were both stimulated by glucose (Fig. 6B), with three times the soluble As_T concentration and four times the Fe reduction observed as compared to the microcosms without glucose addition. The solubilized As_T was dominated by As(III) at all sampling intervals after Day 0 in both NP1 and NP3 (Fig. 6A and B). Glucose has been observed to successfully stimulate As solubilization (Corsini et al., 2010; Duan et al., 2009), as do other carbon sources, including acetate, lactate, and whey (Islam et al., 2004; McLean et al., 2006; Pederick et al., 2007; Rowland et al., 2007). The lag between As_T solubilization and Fe reduction observed without glucose was

overwhelmed by glucose addition, that significantly promoted Fe reduction. The glucose addition produced coupled As_T solubilization and Fe reduction which would fit the most-described mechanism of As solubilization in the literature (McArthur et al., 2004; Nickson et al., 2000; Ravenscroft et al., 2001; Swartz et al., 2004), but it concealed As solubilization independent of Fe as observed in this study in the absence of glucose. The addition of glucose in microcosm studies may result in overestimating the importance of Fe oxides in As solubilization and over-predicting As solubilization, because high microbial dissolution of Fe oxides would be rare under typical subsurface environments where labile carbon sources are limited. Glucose stimulated a population of fermenting bacteria

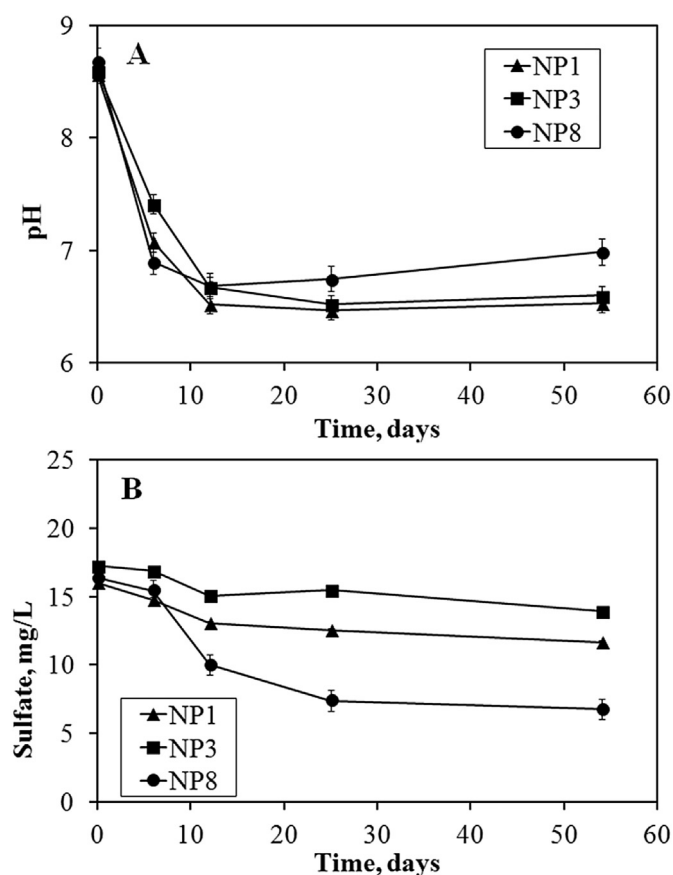


Fig. 8. The changes in pH and sulfate concentration in the aqueous phase of microcosms containing the three sediments incubated with groundwater plus glucose: A) pH and B) sulfate. Error bars represent Tukey HSD ($\alpha = 0.05$; $n = 3$).

in this microcosm study (Mirza et al., 2014). Fermenting bacteria have been shown to reduce Fe(III) via electron shuttling compounds (Akob et al., 2008; Gerlach et al., 2011; Kappler et al., 2004), increasing the bioavailability of Fe(III) within sediments.

As observed by others (Hery et al., 2010; Islam et al., 2004; Rowland et al., 2007), addition of a carbon source enhanced the proportion of As(III) in solution, whereas As(V) was the dominant species with only groundwater treatment (Fig. 3A and B). Enhanced production of solid phase As(III) (Fig. 6D and E) in NP1 and NP3 may contribute to As solubilization. Microbial reduction of As(V) to As(III) potentially facilitated As solubilization since As(III) has a lower affinity for some solid surfaces (Ahmann et al., 1997; Ohtsuka et al., 2013; Tufano et al., 2008), however the relative affinity is surface type dependent (Dixit and Hering, 2003). Another explanation is that the solubilized As(V) was reduced to As(III) in solution and then proportionally and consistently partitioned between the solid and solution phases. The enhanced reducing conditions following glucose addition support the microbially mediated reduction of As, which is further supported by the increasing population of DARB after 54 days of incubation (Mirza et al., 2014).

Glucose addition caused an unanticipated attenuation of As solubilization from the NP8 sediment. Soluble As decreased after incubation started, reaching a concentration as low as $11.2 \pm 3.8 \mu\text{g/kg}$ at Day 25 (Fig. 6C). The amount of ionically bound As (Table 4) was equal to the maximal amount of As in the aqueous phase. The simplest assumption would be that only the ionically bound As was released and glucose addition inhibited further solubilization (Fig. 6F). The HCl extractability of As_T and As(III) in NP8 decreased to

the level observed in the groundwater alone treatment (Fig. 3F); the cessation of As solubilization was not due to the association of As with HCl-soluble minerals but with the more insoluble minerals.

The loss of As from carbonate minerals in the three sediments was not enhanced by the addition of glucose (Fig. 7A–C) compared to groundwater (Fig. 5A–C); solubilization of As(V) from the carbonates was not associated with the stimulation of the microbial population. The enhanced As solubilization in NP1 and NP3 (Fig. 6A and B) therefore involved the loss of As from other mineral phases dependent on microbial redox cycling. Amorphous Fe(III) oxides (NP1) and crystalline Fe oxides (NP3) were sources for release of As that would only be possible through microbial reductive dissolution processes. The microbially produced As(III) was redistributed onto minerals surfaces and associations with carbonates and/or the FeS/FeCO₃/HCl–Fe oxide pool in NP1 and NP3.

Although enhanced microbial activity caused a two-fold decrease in the pH of the microcosms after 54 days (Fig. 8A), this circumneutral pH should not significantly contribute to the dissolution of Fe oxides. Furthermore, pH-related processes do not explain the dramatic increases in aqueous and solid phase Fe(II) and As(III). The utilization of glucose by fermenting microbes produced electron shuttles and chelating agents that enable the microbes to utilize Fe in more crystalline Fe oxides or even in the residual phase (McLean et al., 2006). An indicator of redox conditions was sulfate reduction (Fig. 8B). With groundwater only, the microcosms moved beyond Fe reduction to sulfate reducing conditions by Day 25; the Fe was not bioavailable in these carbon-limited systems. With glucose addition, sulfate reduction was delayed, as microbes accessed Fe(III) minerals through utilization of electron shuttles. The utilization of Fe(III) within amorphous Fe(III) oxides (NP1 and NP3) and the residual phase (NP1) in the site-oxidized sediments caused loss of Fe in this pool (Fig. 7D–E). Incubation of NP8 with glucose caused dissolution of insoluble Fe-bearing minerals, i.e., in the residual phase, which was accompanied by a major loss of As (Fig. 7C and F). The As was sequestered by minerals defined as the crystalline Fe oxide pool, limiting solubilization of As. Under the reducing conditions developed with NP8 with the addition of glucose, this extracted pool may also contain freshly precipitated sulfides.

4. Conclusions

Carbonate associated As contributed significantly to As solubilization in solids from this basin-fill aquifer. Aquifers that contain As-bearing deposits in semi-arid environments enriched in carbonate minerals are at risk of producing groundwater that contain As at concentrations exceeding the MCL without the input of an exogenous carbon source, even in systems that have low organic carbon. Adding a carbon and energy source stimulates microbial activity and promotes extensive Fe reduction, masking the mechanisms of As solubilization and reduction that would occur under natural conditions of seasonal groundwater fluctuations. In this semi-arid region, groundwater recharge occurs due to snowmelt and spring rains with groundwater elevations at the ground surface in the spring. The water table lowers by as much as 300 cm at this site during the summer due to high evapotranspiration demand, low rainfall and withdrawal for irrigation. Changes in groundwater elevation not only influence redox conditions but also dissolution and precipitation of carbonate minerals, altering mineral composition for As release and retention.

Carbon addition dramatically increased the solution concentration of As and to the potentially more mobile As(III). Soil-based waste treatment, biostimulation of aquifer remediation, domestic development, and irrigation can increase carbon loading in the subsurface, resulting in inadvertent As solubilization. The

vulnerability of an aquifer to As reduction and mobilization needs to be investigated before any carbon-importing practice is performed.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.apgeochem.2016.01.004>.

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