



Geochemical, mineralogical and microbiological characteristics of sediment from a naturally reduced zone in a uranium-contaminated aquifer

K.M. Campbell^{a,b,*}, R.K. Kukkadapu^c, N.P. Qafoku^c, A.D. Peacock^d, E. Leshner^e, K.H. Williams^f, J.R. Bargar^g, M.J. Wilkins^{h,c}, L. Figueroa^e, J. Ranville^e, J.A. Davis^{b,f}, P.E. Long^{c,f}

^a U.S. Geological Survey, Boulder, CO, United States

^b U.S. Geological Survey, Menlo Park, CA, United States

^c Pacific Northwest National Laboratory, Richland, WA, United States

^d Haley and Aldrich, Oak Ridge, TN, United States

^e Colorado School of Mines, Golden, CO, United States

^f Lawrence Berkeley National Laboratory, Berkeley, CA, United States

^g Stanford Synchrotron Radiation Laboratory, Menlo Park, CA, United States

^h University of California, Berkeley, CA, United States

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ABSTRACT

Localized zones or lenses of naturally reduced sediments have the potential to play a significant role in the fate and transport of redox-sensitive metals and metalloids in aquifers. To assess the mineralogy, microbiology and redox processes that occur in these zones, several cores from a region of naturally occurring reducing conditions in a U-contaminated aquifer (Rifle, CO) were examined. Sediment samples from a transect of cores ranging from oxic/suboxic Rifle aquifer sediment to naturally reduced sediment were analyzed for U and Fe content, oxidation state, and mineralogy; reduced S phases; and solid-phase organic C content using a suite of analytical and spectroscopic techniques on bulk sediment and size fractions. Solid-phase U concentrations were higher in the naturally reduced zone, with a high proportion of the U present as U(IV). The sediments were also elevated in reduced S phases and Fe(II), indicating it is very likely that U(VI), Fe(III), and SO₄ reduction has occurred or is occurring in the sediment. The microbial community was assessed using lipid- and DNA-based techniques, and statistical redundancy analysis was performed to determine correlations between the microbial community and the geochemistry. Increased concentrations of solid-phase organic C and biomass in the naturally reduced sediment suggests that natural bioreduction is stimulated by a zone of increased organic C concentration associated with fine-grained material and lower permeability to groundwater flow. Characterization of the naturally bioreduced sediment provides an understanding of the natural processes that occur in the sediment under reducing conditions and how they may impact natural attenuation of radionuclides and other redox sensitive materials. Results also suggest the importance of recalcitrant organic C for maintaining reducing conditions and U immobilization.

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1. Introduction

Uranium-contaminated groundwater is a long-term environmental problem resulting from the legacy of U mining, ore processing, and radioactive waste disposal. Even after extensive clean-up efforts, groundwater U concentrations can still exceed levels acceptable for site closure. The need for additional remediation at these sites often stems from the inability of natural attenuation to decrease dissolved U concentrations within a reasonable time-frame (Curtis et al., 2006). One such site is the Old Rifle Mill Processing site (Rifle, CO, Fig. 1), where *in situ* bioreduction is being

explored as a potential strategy for the long-term remediation of low-level U contamination in the groundwater as part of the U.S. Department of Energy's Integrated Field Research Challenge (IFRC) Site in Rifle, CO. Although U attenuation in the form of natural flushing was originally predicted to be sufficient for the site (DOE, 1999), elevated U concentrations have persisted, making pilot-scale testing of alternative treatment methods necessary. The processes resulting in residual U in the aquifer are not completely understood, but may be due in part to zones or lenses of naturally reduced sediments with elevated concentrations of U.

Naturally reduced sediments may be common in alluvial aquifers (Bargar et al., 2011) and because of their elevated concentrations of trace elements and redox-active phases, are likely to be important for accurate estimation of natural attenuation capacity. Although the reduced sediments may be a relatively small fraction

* Corresponding author. Address: USGS, 3215 Marine Street, Suite E127, Boulder, CO 80303, United States. Tel.: +1 303 541 3035; fax: +1 303 541 3084.

E-mail address: kcampbell@usgs.gov (K.M. Campbell).

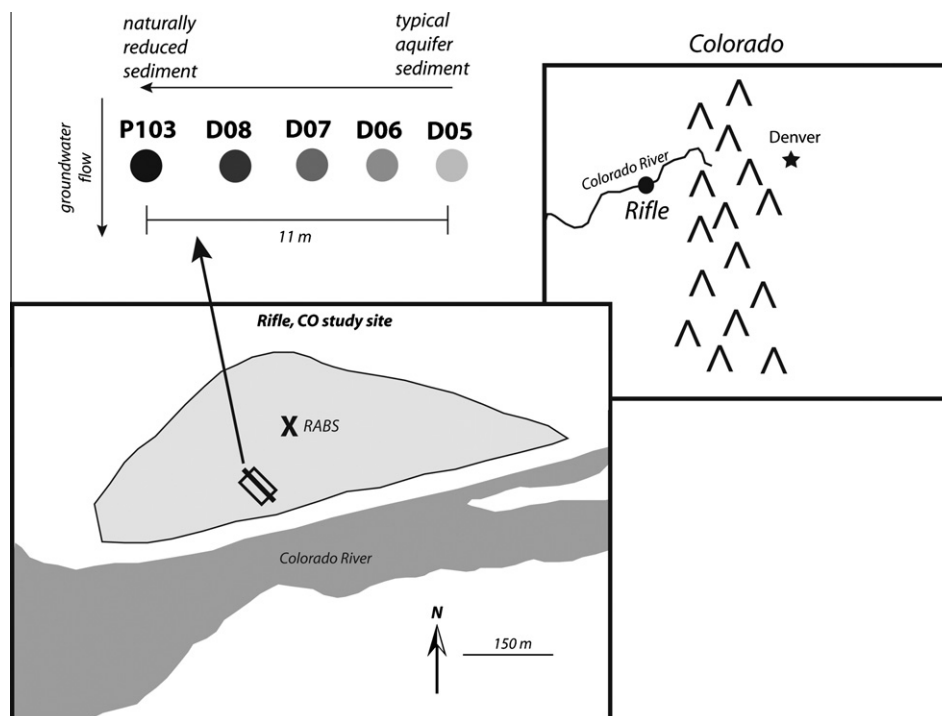


Fig. 1. Location of the Old Rifle Mill Processing site (Rifle, CO) and schematic of core samples analyzed in this study. Groundwater flow direction is approximate. D05 represents typical aquifer sediment and D08 is extensively naturally bioreduced. The location of background sample RABS is denoted with an "X".

of the total mass of the aquifer sediment, their ability to seasonally sequester and/or release U may affect the overall behavior of the contaminant in the aquifer (Davis et al., 2006). Estimates of sediment composition, mineralogy, and natural attenuation capacity are often based on representative aquifer sediment samples (e.g., Hyun et al., 2009), but the heterogeneity caused by naturally reduced zones may be an overlooked yet important component in understanding long-term U behavior.

In addition to assessing natural attenuation capacity of an aquifer, the biogeochemical processes active within naturally reduced sediments can yield insight into strategies for stabilizing reduced phases produced during stimulated bioremediation. In field-scale bioremediation experiments conducted at the site, dissolved U(VI) is reduced to insoluble U(IV) by amending groundwater with acetate to stimulate indigenous metal- and SO_4 -reducing bacteria. The efficacy of this method has been demonstrated in recent studies (Anderson et al., 2003; Vrionis et al., 2005; N'Guessan et al., 2008; Williams et al., 2011), where maximum precipitation of dissolved U occurred concomitantly with metal reduction, particularly Fe reduction. However, the long-term success of *in situ* bioremediation relies on the stability of the resulting U(IV) phase(s) to re-oxidation. Biogenic UO_2 has been shown to be susceptible to oxidation by a variety of oxidants commonly found in groundwater aquifer systems such as dissolved O_2 , NO_3 , and solid-phase Fe(III) (e.g., Ginder-Vogel et al., 2006; Hee et al., 2007; Moon et al., 2007; Senko et al., 2007), although the rate of oxidation in the field is ultimately controlled by many factors such as diffusional barriers (Campbell et al., 2011) and redox-active mineral phases (Abdelouas et al., 1999). Since the mechanism of U(IV) formation and association with mineral phases and biomass can affect the susceptibility of reduced U phases to oxidation during and after bioremediation, a more complete understanding of the mechanisms of U(VI) reduction in natural subsurface media will help in exploring possible strategies for stabilization of reduced phases for long-term sequestration of U.

The purpose of this study is to identify the mineralogical, geochemical, and microbiological characteristics in a localized zone of naturally reduced sediments, and to identify possible redox processes that contribute to the creation and stability of these zones. The sediment used in this study was never exposed to exogenous C amendments. The samples analyzed were from a transect of cores including both typical oxic/suboxic aquifer sediments and naturally reduced sediments (Fig. 1). The naturally reduced zone is chemically, mineralogically and biologically distinct from the typical aquifer sediments at the site. Characterization of the naturally reduced sediment will ultimately provide an understanding of the natural reductive processes that occur in the sediment, an assessment of the stability of the reduced phases to oxidation and the ability of the reduced zones to act as a source or sink for U, as well as possible strategies for artificial immobilization of U(VI) and stabilization of U(IV) in contaminated subsurface environments.

2. Materials and methods

2.1. Site description

The Old Rifle site is part of the U.S. Department of Energy's Uranium Mill Tailings Remedial Action (UMTRA) program, and was formerly a V and U ore processing facility. The mill tailings and directly impacted alluvial sediment have been removed from the site and the surface capped with a clay layer. However, the shallow, unconfined aquifer has residual U contamination from the tailings pile in excess of the EPA drinking water standard and the UMTRA statutory standard. The saturated thickness of the aquifer is about 4 m, depending upon Colorado River stage, and is underlain by an impermeable formation at approximately 8 m below ground surface. The aquifer sediment is primarily an alluvial deposit from the nearby Colorado River, containing unconsolidated clay, silt, sand, gravel and cobbles (DOE, 1999).

Average groundwater composition has been reported previously (DOE, 1999; Anderson et al., 2003; Yabusaki et al., 2007).

In general, the aquifer has elevated concentrations of SO_4 (≈ 10 mM) and alkalinity (≈ 10 meq/L), and is close to equilibrium with calcite. The pH is typically between 7.2 and 7.4. Dissolved Fe(II) can be quite variable across the site, while dissolved O_2 is often low (0–0.6 mg/L). Uranium concentrations in the groundwater are between 0.4 and 1.8 μM , and the primary aqueous species are U(VI)-carbonate and Ca-U(VI)-carbonate ternary species.

2.2. Sediment collection and preservation

Sediment core samples were taken along a transect 11 m wide across the edge of the zone of natural bioreduction and approximately perpendicular to the groundwater flow direction (Fig. 1). The cores were labeled D05–D08 and P103, with D05 representing typical, oxic to suboxic aquifer conditions with the sediment becoming increasingly reduced toward D08 and P103. The water table was approximately 3–4 m (10–13 feet) below ground surface depending on the time of sampling (June 2007 and September 2007), although the water table can be significantly higher when the Colorado River stage is elevated due to spring runoff. Samples were taken from 3.1 m (P103 only), 4.0 m, 4.9 m, 5.8 m, and 6.1 m (P103 only), respectively (10, 13, 16, 19 and 20 feet) below ground surface. All samples were obtained from the saturated zone except for the 3.1 m depth, which was obtained from the capillary fringe/vadose zone. Whereas the sediment from the shallowest depth (3.1 m) was only moist at the time of sampling, seasonal fluctuations in groundwater elevation suggest this depth is periodically below the water table.

Sediment cores were taken using a N_2 gas borehole flush to prevent O_2 contamination during core extraction. The core was immediately processed in a N_2 -filled field-portable glove bag. Each sediment sample was triple-bagged with a chemical O_2 -scrubber between the first and second bags and immediately placed on ice or frozen for overnight shipping. Upon arrival at the laboratory, the samples were either frozen or processed in a glove bag. The sample notation convention used is “core-depth” (e.g., D08-16 denotes core D08, 16 feet (4.9 m) below ground surface). A table of all samples, elevations, and corresponding analyses is listed in Tables S1 and S2.

Two background sediment samples were collected, one in December 2001 (Rifle Aquifer Background Sediment, RABS) and one in August 2007 (BKG-A). RABS was collected on the Rifle UMTRA site north of the core transect (Fig. 1). Although RABS is a site background sample, it contains slightly elevated U (4.1 ± 0.4 mg/kg total U, <2 mm size fraction, by gamma spectrometry) because the sample location had been subject to leaching from the tailing pile and processing facility. BKG-A was collected approximately 2 km upstream of the Rifle UMTRA site in a location geologically similar to the Rifle site but that had not been exposed to U mill tailings. The overall mineralogy of BKG-A is similar to RABS, and the total concentration of U in BKG-A (<2 mm size fraction) was 3.2 ± 0.1 mg/kg measured by gamma spectrometry.

2.3. Size fractions

A subset of selected samples were dried and sieved into seven fractions using a standard series of grid sizes. Size fractions of background sample RABS were collected and analyzed for a limited number of parameters.

2.4. Chemical extractions

2.4.1. Reagents

All chemicals were reagent grade and were used without further purification unless otherwise noted. All water used was 18 M Ω cm deionized water. Nitric acid solutions were made with

trace metal grade HNO_3 . Extractions were performed in glass or plastic containers that had been acid washed in 10% HCl.

2.4.2. Bicarbonate/carbonate extractions

Bicarbonate/carbonate extractions were used to determine the labile U(VI) content of the sediments, based on the method of Kohler et al. (2004). Carbonate is a strong complexing agent for U(VI) and the extraction conditions are optimized to promote desorption of U(VI) from the sediment. The extraction solution was composed of 144 mM NaHCO_3 and 28 mM of Na_2CO_3 , and was adjusted to pH 9.4 with 1 M NaOH (<1% of the total volume was added as NaOH). Before extraction, the sediment was air-dried in a laminar-flow hood for at least 2 weeks, or until the dry weight did not change with additional drying. During this step, any easily oxidizable U(IV) in the sediment was oxidized to U(VI); the labile U(VI) content as measured by this extraction technique is the sum of native U(VI) plus oxidized U(IV). The solid to solution ratio of the extraction was 100 g dry sediment/L. The extractions were performed in duplicate or triplicate. Aqueous U (<0.45 μM) was analyzed by a Kinetic Phosphorescence Analyzer (Chemcheck Instruments, Richland, WA), with pretreatment with hot concentrated HNO_3 and 1% H_2O_2 to remove analytical interferences.

2.4.3. Nitric acid extractions

Air-dried sediment samples were extracted with 0.5 M HNO_3 for 24 h at a solid to solution ratio of 167 g dry sediment/L. After allowing the slurry to degas for 2 h under atmospheric conditions, the reaction vessels were placed on an end-over-end rotator for the remaining 22 h. The supernatant was diluted to 1% HNO_3 (0.16 M) and analyzed for a suite of elements by ICP-OES (Thermo Jarrell-Ash, IRIS). The HNO_3 extractions provide an estimate of total acid extractable metal concentrations after mild oxidation of the sediments (air-drying the sediments and exposure to HNO_3).

2.4.4. HCl-hydroxylamine extractions

The hydroxylamine (HA)-reducible Fe(III) content of the sediment (HA-extractable Fe(III)) was determined by difference between a 0.5 N HCl/0.25 N hydroxylamine hydrochloride extraction (extractable Fe(II) + Fe(III)) and a 0.5 N HCl extraction (extractable Fe(II)); the difference is an estimate of the amount of solid phase Fe(III) in Fe (oxyhydr)oxides that may be available for dissimilatory Fe-reducing bacteria (Lovley et al., 1991). The 0.5 N HCl extraction step also yields the acid-extractable Fe(II) content of the sediment. Although 0.5 N HCl can also dissolve poorly crystalline Fe(III)-oxide phases such as ferrihydrite, Rifle sediment has relatively little amorphous Fe(III) phases. Therefore, this method presents a reasonable determination of acid extractable Fe(II) and HA extractable Fe(III). Both extractions were performed on ~ 1 g wet, anoxically preserved sediment which was incubated anaerobically in the extraction solutions for 1 h. Iron(II) concentrations were then determined by the Ferrozine spectroscopic method (Stookey, 1970).

2.4.5. Acid volatile sulfides (AVS)

All reduced S extractions were performed on moist, anaerobically preserved bulk sediments samples. The acid volatile sulfide (AVS) content of the sediment was determined using a modified version of the method described by Ulrich et al. (1997). Rather than using a mixture of 1 M Cr(II) and 12 N HCl to quantify the pool of total reduced inorganic S, only 12 N HCl was added to the sealed serum vials. As a result, only the acid soluble fraction (e.g., FeS) of the selected sediment samples was assessed.

2.4.6. Sodium hydroxide extractions

Sodium hydroxide extractions were used to determine the labile and humic organic C in the sediment, based on the method

of Stevenson (1994). Air dried sediments were extracted with 0.5 N NaOH for 20 h at a solid to solution ratio of 0.25 g dry sediment/mL. Ten ml of centrifuged and filtered extract was passed through a cation exchange column (H^+ form) to reduce the salt content and lower the pH of the sample prior to triplicate TOC analysis on a Sievers 800 Turbo TOC Analyzer.

2.5. Mineral characterization

2.5.1. BET surface area

A standard BET method was used to determine surface areas in the <2 mm size fraction sediment samples using N_2 adsorption/desorption collected with a QUANTACHROME AUTOSORB 6-B gas sorption system on degassed samples (outgassed at 150 °C for 16 h followed by 515 min analysis time).

2.5.2. X-ray diffraction (XRD)

Bulk samples were prepared for XRD by crushing 1 g of homogenized sample before packing into Al sample holders. Each specimen was analyzed using a Scintag XRD unit equipped with a Peltier thermoelectrically cooled detector and a Cu X-ray tube, with individual scans from 2° to 65° 2 θ (0.01° step) and a dwell time of 2 s. Scans were collected electronically and processed using the JADE® XRD pattern processing software. Identification of the mineral phases was based on mineral powder diffraction files published by the Joint Committee on Powder Diffraction Standards (JCPDS) International Center for Diffraction Data (ICDD) (Newtown Square, Pennsylvania).

The clay fraction for XRD analysis was prepared by dispersing approximately 100 g of the <2 mm size fraction sample in 1.0 L of 0.001 M sodium hexametaphosphate. The sand fraction was separated from the dispersed sample by wet sieving. The sand and silt fractions were separated from the clay fractions by using Stoke's Law settling; the lower limit of the silt fraction was taken at >1.4 μ m. Each clay suspension was concentrated to approximately 30 mL, and several drops of 10 N $MgCl_2$ were added to the dispersed slurry. Slides of preferentially oriented clay were scanned from 2° to 45° 2 θ with a dwell time of 2 s. Data were processed as described for the bulk powdered samples. Pure mineral reference phases of illite, smectite, kaolinite and chlorite were obtained from the Clay Mineral Society's source clays repository (University of Missouri, MO), and analyzed under the same conditions as the sediment samples.

2.5.3. XRF

X-ray fluorescence (XRF) analyses were conducted at Washington State University, Pullman WA. Briefly, the samples were fused into Li-tetraborate beads and analyzed for 27 elements by comparing the X-ray intensity of each element to a standard sample. The method is fully described in the following link: <http://www.sees.wsu.edu/Geolab/note/xrf.html> (accessed November 2011).

2.5.4. Gamma spectrometry

Total solid-phase U was determined on selected samples by counting the ^{234}Th gamma emission (63 keV) using a gamma spectrometer with a high-resolution coaxial Ge detector. This method is described in Fuller et al. (1999) and assumes equilibrium between the ^{234}Th daughter nuclide and its ^{238}U parent nuclide. Approximately 20–30 g of dry sediment was counted in triplicate to ensure constant counting geometry. Self-adsorption was corrected based on the method of Cutshall et al. (1983).

2.5.5. X-ray absorption spectroscopy (XAS)

X-ray absorption spectroscopy (XAS) was used to determine the bulk U oxidation state and molecular coordination environment in

P103-10. Due to detection limit constraints, this was the only sample analyzed by XAS; all other samples had insufficient U for XAS analysis. The sample was placed in a sealed jar and was kept under vacuum during transport to the Stanford Synchrotron Radiation Lightsource (SSRL), where the sample was stored under vacuum in a glove bag. Immediately prior to analysis, each sample was loaded inside a glove bag into a sealed chamber with Kapton windows to prevent exposure to O_2 during analysis. Uranium L_{III} -edge fluorescence spectra were collected using a 30-element Ge detector (Canberra) and a typical beam size of 0.2–0.5 mm at SSRL beamline 11-2, using detuned Si(220) double-crystal monochromators. A Y foil was used for energy calibration. The EXAFS data were processed and fit using the SixPACK software package (Webb, 2005). The spectrum was fit using a theoretical backscattering phase and amplitude functions obtained from FEFF8 (Rehr and Zabinsky, 1992) calculations of the minerals andersonite, autunite, and $UFeO_4$.

2.5.6. Mössbauer spectroscopy

Mössbauer spectra of selected sediment fractions (RABS < 53 μ m, D08-16 < 53 μ m, and P103-10 < 53 μ m) were collected using either a WissEl Elektronik (Germany) or Web Research Company (St. Paul, MN) instrument that included a closed-cycle cryostat SHI-850 obtained from Janis Research Company, Inc. (Wilmingon, MA), a Sumitomo CKW-21 He compressor unit, and an Ar–Kr proportional counter detector with WissEl or a Ritvec (St. Petersburg, Russia) NaI detection system. A $^{57}Co/Rh$ source (50–75-mCi, initial strength) was used as the gamma source. With the WissEl setup, the transmitted counts were stored in a multi-channel scalar (MCS) as a function of energy (transducer velocity) using a 1024-channel analyzer. The setup data were folded to 512 channels to provide a flat background and a zero-velocity position corresponding to the center shift (CS) of a metal Fe foil at room temperature (RT). Calibration spectra were obtained with a 25- μ m-thick Fe foil (Amersham, England). The Mössbauer data were modeled with Recoil software (University of Ottawa, Canada) using a Voigt-based structural fitting routine (Rancourt et al., 1994).

2.6. Solid-phase organic carbon analysis

Solid-phase C was measured in bulk samples using a CM150 Total Carbon Analyzer (UIC, Joliet, IL, USA). The sediment samples were sieved to less than 2 mm and prior to analysis were dried and ground. The instrument was checked daily with a $CaCO_3$ standard. The CM150 utilizes an acidification module for measuring total inorganic C (TIC), and a furnace combustion module for measuring total C (TC). Both modules generate CO_2 , which is detected and measured by a CM5014 Coulometer. Subtracting the average TIC from the average TC gave total organic C (TOC).

The inorganic and organic C content was determined in different size-fractions with a Shimadzu solid sample combustion unit, Model SSM-5000A, connected to a TOC-5000A Shimadzu carbon analyzer. Separate samples were analyzed for total C and for inorganic C. The organic C value was determined by subtracting the inorganic C value from the total C value. Total C was determined by heating the solid sample to 900 °C and measuring the evolved CO_2 gas. Inorganic C was measured by heating the sample to 250 °C and adding 1 mL 25% H_3PO_4 and measuring the evolved CO_2 . The instrument was calibrated by using solid D-(+)-glucose for total C and $NaHCO_3$ for inorganic C.

2.7. Phospholipid Fatty Acid (PLFA) and DNA analysis

2.7.1. Quantitative polymerase chain reaction (PCR)

DNA was extracted using the MoBio (Carlsbad, CA) PowerSoil extraction kit following the manufacturer's recommended

protocol. Quantitative PCR (qPCR) was performed on each sediment sample using oligonucleotides that were designed to target *Geobacteraceae* (Stultz et al., 2001), *Deltaproteobacteria* (Stultz et al., 2001), methanogens (Dhillon et al., 2005; Juottonen et al., 2006), SO_4 -reducing bacteria (Tang et al., 2004) and total bacteria (Marcelino et al., 2000). For TaqMan based assays, one of the oligonucleotides is a probe containing 6-carboxy-fluorescein (FAM) as a reporter fluorochrome on the 5' end, and N,N,N',N'-tetramethyl-6-carboxy-rhodamine (TAMRA) as a quencher on the 3' end. Each 30 μL reaction contained $1\times$ TaqMan Universal PCR Master Mix (Applied Biosystems, Foster City, CA), forward primer, reverse primer, TaqMan probe and DNA template from the extracted samples. The PCR conditions were as follows: 2 min at 50 °C and 10 min at 95 °C, followed by 50 cycles of 15 s at 95 °C and 1 min at 58 °C. The PCR reaction was carried out in an ABI Prism 7300 Sequence Detection System (Applied Biosystems). For SYBR green based assays, real-time PCR was performed on an ABI 7000 Sequence Detection System (Applied Biosystems). PCR mixtures contained $1\times$ Cloned Pfu Buffer (Stratagene, La Jolla, CA), 0.2 mM of each of the four deoxynucleoside triphosphates, SYBR green (diluted 1:30,000; Molecular Probes, Eugene, OR), and 1 unit of Pfu Turbo HotStart DNA polymerase (Stratagene). Annealing temperatures, primer concentrations, and MgCl_2 concentrations varied dependent on the primer set used. A calibration curve was obtained by using a serial dilution of a known concentration of positive control DNA. The C_T values that are obtained from each sample were compared with the standard curve to determine the original sample DNA concentration.

2.7.2. PLFA

Total lipids were extracted from the sediment samples using a modified Blyer and Dyer method (White and Ringelberg, 1998) for PLFA analysis. Silicic acid chromatography was used to separate the total lipids into polar, neutral and glycolipid fractions (Guckert et al., 1985). The polar lipid fraction was subsequently transesterified using mild alkaline methanolysis to form fatty acid methyl esters (FAMES) and convert plasmalogen ethers to dimethylacetals (DMAs) (Guckert et al., 1985), with modifications (Mayberry and Lane, 1993). The different lipid fractions were quantified as previously described (Michalsen et al., 2007).

2.8. Statistical analysis

Redundancy analysis (RDA), a linear canonical community ordination method, was calculated and graphed using CANOCO for Windows, version 4.5 (Ter Braak, 1994), and was used to visualize and explore the relationships between microbiological parameters (response variables), the geochemical parameters and sediment samples. In RDA, the ordination axes are constrained to be linear combinations of the variables, thus allowing the relationships between all of the geochemical and the microbiological parameters to be directly compared on one plot. The microbiological parameters (qPCR and PLFA data) and geochemical parameters (e.g., HNO_3 -extractable U, HCl-extractable Fe(II), etc.) are represented by arrows projecting from the origin; the arrows are representations of all of the data for a particular parameter (Ter Braak and Prentice, 1988). Individual sediment samples are represented by points in the ordination space. The focus of the ordination was on correlations between geochemical and microbiological parameters, using the method of Ter Braak (1998). The arrows in the resulting ordination diagram point in the direction of maximum variation in the microbiological parameters, and the arrow length is proportional to the rate of change. Microbiological parameter arrows pointing in the same general direction as environmental arrows can be interpreted as correlating well with that variable (Ter Braak, 1994). Geochemical arrows that are longest allow more confidence in

the inferred correlations, and roughly indicate a larger effect of that parameter on the microbial parameter variation (Ter Braak, 1998).

3. Results and discussion

3.1. Sediment mineralogy and composition

3.1.1. Mineralogy

In general, there was very little difference between the composition of the bulk sediment samples (Tables S3 and S4). The bulk sediments were dominated by quartz and feldspar (both plagioclase and alkali-feldspar), with lesser amounts of amphiboles and clay. Clay minerals identified in the <2 mm fraction by XRD were limited to mica as other clay minerals were not easily detectable. However, a separation of the clay fraction shows illite as the dominant clay fraction with smectite, chlorite and kaolinite also present. The amount of total HNO_3 -extractable Fe ($[\text{Fe}(\text{T})_{\text{HNO}_3}]$) was variable but showed no systematic trend across the core transect or as a function of depth (Fig. 2). The XRF data also show that the total amount of Fe was relatively constant across all sediment samples (Table S4).

A previous mineralogical analysis of RABS sediments (<2 mm fraction) has demonstrated the presence of clays (clinocllore, illite and smectite) and Fe oxides (hematite, magnetite and goethite) (Komlos et al., 2008). Detailed Mössbauer spectroscopy measurements of RABS (<53 μm fraction) in this study indicated that different types of Fe(II)/(III)-(oxyhydr)oxides (crystalline, poorly ordered and Al-substituted) and phyllosilicates (as a mixture of clays) are present in the sediment (Fig. 3). The presence of hematite and magnetite is apparent from the RT spectrum of the bulk sample as distinct sextet features. However, Mössbauer parameters of these oxides are slightly different to their pure mineral counterparts, most likely due to minor substitution of Fe by other metals such as Al or Ti (Fysh and Clark, 1982; Hamadeh et al., 1999; Stucki et al., 2007). Partial transformation of the RT goethite broad feature to a well-defined sextet at 77 K suggests goethite of different particle sizes, including nano-goethite, and/or Al-substitution; nano- and Al-substituted goethite has been observed in other shallow aquifer systems (Zheng et al., 2011). Even though it is not possible to unambiguously differentiate between nano- and Al-substituted phases, the data indicate that a substantial portion of the Fe-oxide pool is goethite, with small amounts of ferrihydrite (~5%). Modeled Mössbauer spectral parameters, along with the relative contributions of various phases, are included in Table S5.

3.1.2. U, Fe, S and organic carbon content

Although the major components of the bulk sediment mineralogical and chemical composition were constant across the samples analyzed, substantial differences in the U content and Fe and S redox state were apparent from the chemical extractions. Solid-phase U concentrations were elevated in the naturally reduced zone, particularly in samples D08-13, D08-16, P103-10, P103-13 and P103-16, compared to typical unreduced Rifle sediment (D05, RABS) (Fig. 2). The HNO_3 extractions recovered between 40% and 89% of the total amount of U determined by gamma spectrometry, with the highest recovery in P103-10. There are more fine particles (<53 μm) present in D08 than D05 and RABS (Table 1), suggesting higher clay content. This is also reflected in the substantially higher specific surface area in the <2 mm size fraction of D08-13 and D08-16 (Table 2). In general, the fine fractions contain more U/g of dry sample than the coarser fractions, similar to other sediments exposed to U mill tailings (Davis et al., 2004). While there is a higher proportion of fines by weight, there is also more HNO_3 -extractable U and bicarbonate/carbonate-extractable U in the fines of D08 than D05. The amount of bicarbonate/carbonate-extractable U is 82% of the HNO_3 -extractable U in the fine fraction

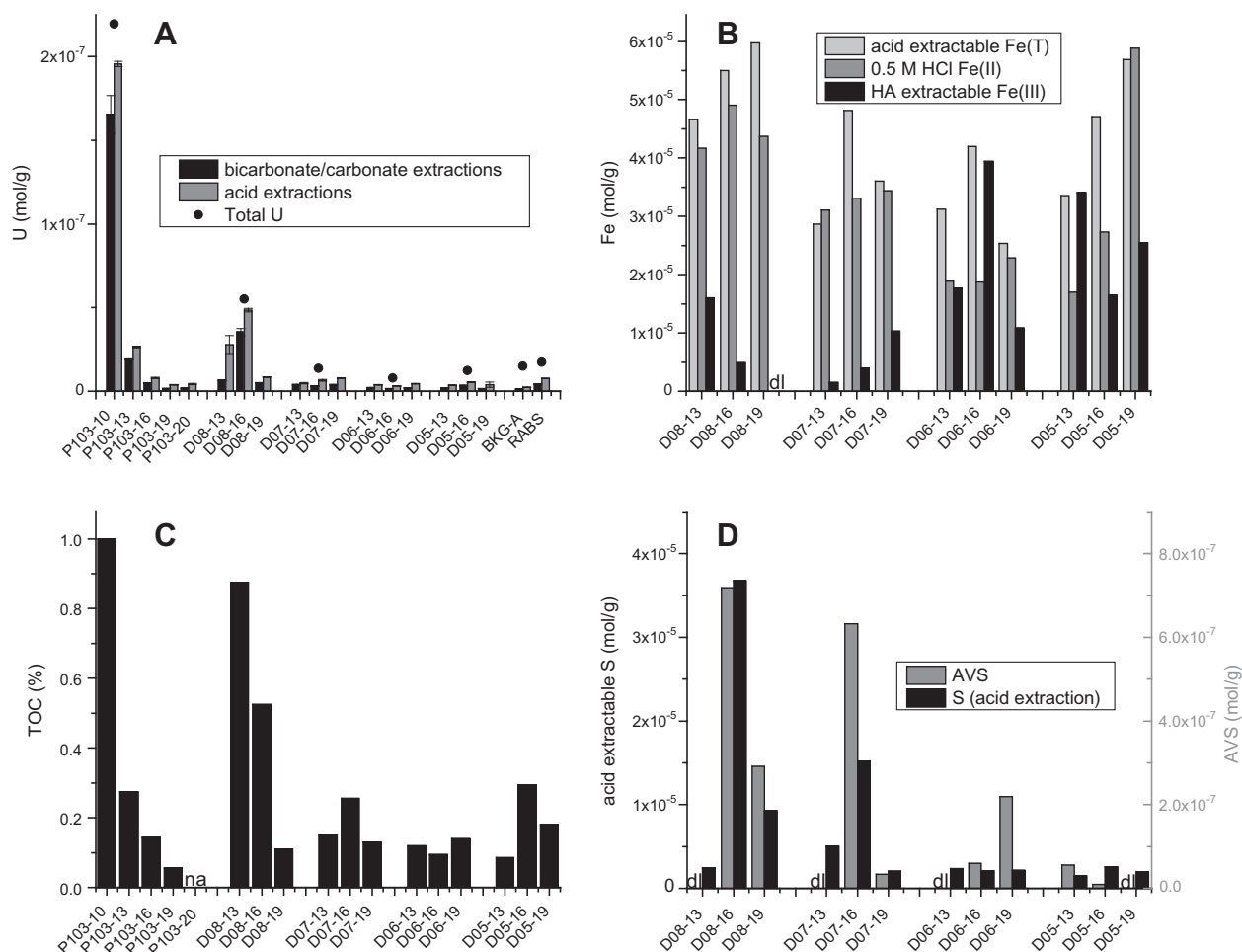


Fig. 2. (A) Amount of U in each sediment sample measured by bicarbonate/carbonate extraction and HNO_3 extractions. The total amount of U was measured in selected samples by gamma spectrometry. (B) Bulk Fe extractions in cores D05–D08: HNO_3 extractable total Fe, HCl extractable Fe(II), and hydroxylamine/hydrochloric (HA) acid extractable Fe(III). (C) Bulk total organic C content for cores P103, D05–D08. Size fraction organic C data for D08-16 are presented in Table 1. (D) Bulk acid volatile sulfide (AVS) and HNO_3 -extractable S content for cores D05–D08. If a sample was not analyzed for a particular parameter, “na” appears in the figure. If the analysis was less than the detection limit of the technique, “dl” appears in the figure.

of samples from D08-16, suggesting that most of the U is present as labile U(VI) and/or easily oxidized U(IV). The combination of higher specific surface area, smaller particle size, elevated clay content, and higher U concentrations suggest that the naturally reduced zones are likely associated with regions of lower permeability in the aquifer that accumulate U.

Whereas the total HNO_3 -extractable Fe ($(\text{Fe(T)})_{\text{HNO}_3}$) and total Fe (XRF) were relatively constant across the sediment transect, the concentration of HCl-extractable Fe(II) ($[\text{Fe(II)}_{\text{HCl}}]$) was higher in samples from D07 and D08 than from D05 and D06, with the exception of D05-19 (Fig. 2). Conversely, the amount of hydroxylamine extractable Fe(III) ($[\text{Fe(III)}_{\text{HA}}]$) was lowest in samples from D07 and D08, indicating that reduction of Fe(III) had occurred in these sediments, particularly in D08-16, D08-19, D07-13, and D07-16. When comparing these extraction results, the sum of $[\text{Fe(II)}_{\text{HCl}}]$ and $[\text{Fe(III)}_{\text{HA}}]$ often does not equal $[\text{Fe(T)}_{\text{HNO}_3}]$. This may be due to several effects, including sample heterogeneity, variations in the pools of Fe accessed in the hydroxylamine and HNO_3 extractions, and/or differences in sample treatment (i.e., air drying of sediment for HNO_3 extraction). For example, the hydroxylamine extraction may dissolve more crystalline Fe oxides and clays than the HNO_3 extraction, resulting in larger $[\text{Fe(III)}_{\text{HA}}]$ than expected based on Fe(II) and Fe(T) results (see, for example, D06-13,16,19, and D05-13). However, the relative trends within each extraction data set are robust.

Both AVS and acid extractable S are elevated in cores D07 and D08, particularly in samples D08-16, D08-19, and D07-16 (Fig. 2). The groundwater at Rifle contains a relatively high concentration of SO_4 , and the enrichment of S and reduced sulfide phases indicates that SO_4 reduction has occurred in the vicinity of wells D07 and D08.

The sediment in the upper sections of cores P103 and D08 were highest in total solid-phase organic C (Fig. 2). The upper-most sections of the P103 and D08 cores (P103-10 and D08-13) contained nearly 1% organic C while sediments from the lower sections (4.9–5.8 m; 16–19 feet) averaged less than 0.2% organic C. The size fractions from D08-16 were also analyzed for solid-phase organic C (Table 1). The organic C was 8 times higher in the fine fraction ($<53 \mu\text{m}$) relative to the coarse fractions (particles between 500 and 2000 μm). A NaOH extraction was performed on a set of nearby (within 1–2 m of P103 and D08) sediment samples within the natural reduced zone (data not shown). These samples were also enriched in TOC (1.7–1.9%), and showed that base extractable organic C was only 0.06–0.13% of the total sediment sample mass, suggesting that the organic C in the Rifle sediment was relatively recalcitrant and low in humic acid content.

3.1.3. Detailed characterization of D08-16 and P103-10

Because of the elevated concentrations of U, organic C, reduced S phases and Fe(II), D08-16 and P103-10 were chosen for detailed characterization. In general, the D08-16 ($<53 \mu\text{m}$ fraction, data not

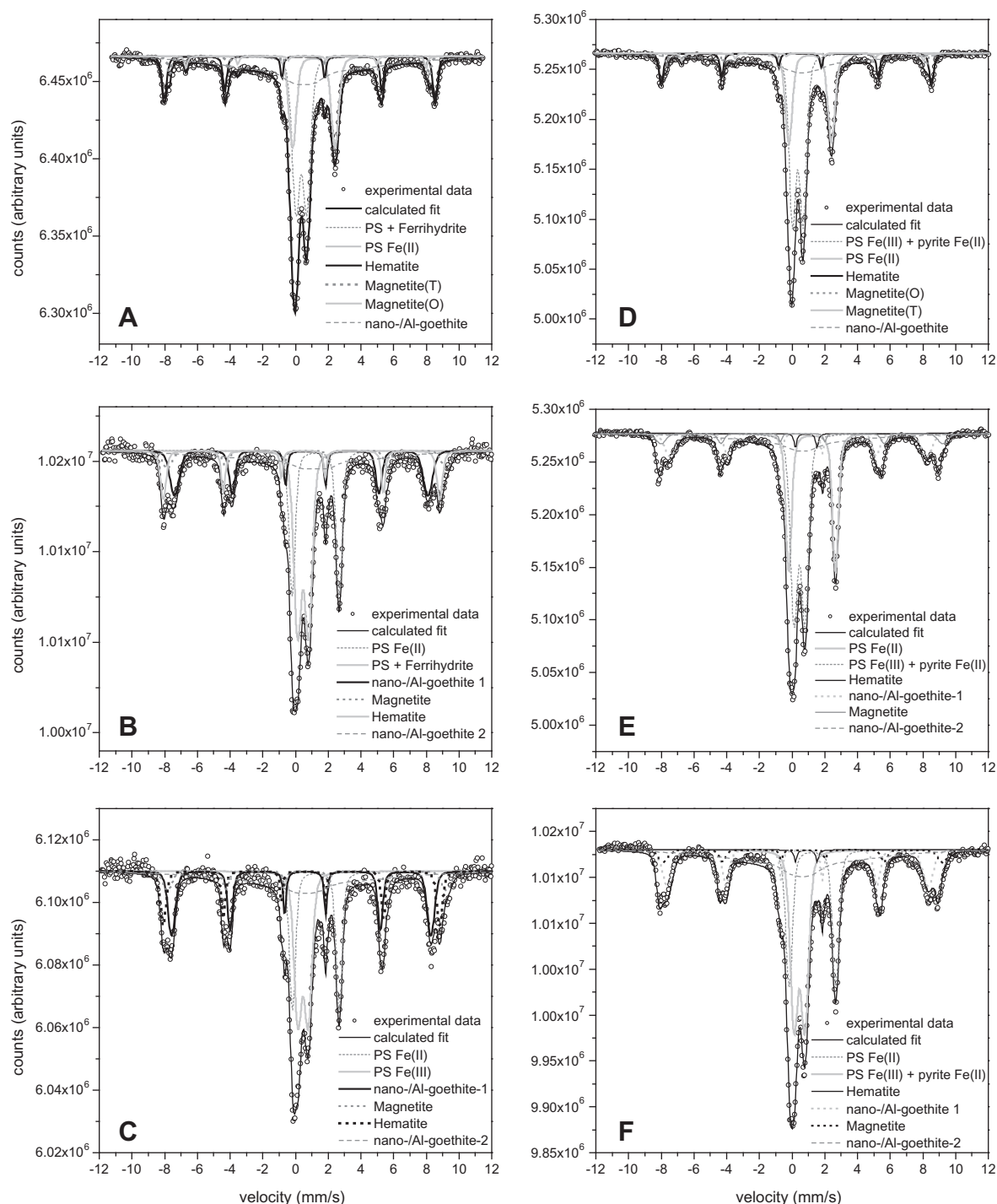


Fig. 3. Variable temperature ^{57}Fe -Mössbauer spectra of RABS and P103-10 sediment samples. (A) RABS room temperature (RT, 298 K) spectrum, with ~50% of the total Fe as hematite, magnetite and goethite, and the remainder due to phyllosilicate (PS) Fe. (B) RABS 77 K spectrum. The main difference between (A) and (B) is the resolution of the nano-/Al-goethite sextet. (C) RABS 12 K spectrum. (D) P103-10 RT spectrum. (E) P103-10 77 K spectrum. (F) P103-10 12 K spectrum. P103-10 shows a lower Fe oxide content (~40%) than RABS. Ilmenite was not plotted because its contribution was <3%. Abbreviations in legend are: phyllosilicate (PS), tetrahedral Fe in magnetite [magnetite(T)], and octahedral Fe in magnetite [magnetite(O)].

shown) and P103-10 Mössbauer spectra (Fig. 3) are qualitatively similar to each other but are considerably different from RABS (<53 μm fraction, Fig. 3). The differences between RABS and P103-10 are evident from their RT and 12 K modeled spectra: the relative combined Fe-oxide content (hematite, magnetite, goethite) is lower (40% vs. 50%), and the clay Fe(II) content is higher (22% vs. 18%) in the P103-10 sample than in the RABS. It is not possible to estimate the clay Fe(III) fraction from the P103-10 spectra

obtained at various temperatures because, unlike RABS, the sample contains pyritic S that contributes to the central doublet. A comparison of RT and 12 K spectra also indicated that the sample contains little or no siderite or ferrihydrite.

Although the age of the naturally bioreduced zone is unknown, the absence of magnetic griegite (Fe_3S_4), a metastable intermediate product of mackinawite (FeS) conversion to pyrite suggests that the reduced zones are in a mature state. Magnetite grains within

Table 1
Nitric acid extractable U, bicarbonate/carbonate extractable U, and selected organic C contents in size fractions in cores D05–D08 and one background sample (RABS). Weight percent of each size fraction is reported as a fraction of the <2 mm size fraction.

Sediment sample	Particle size fraction (μm)	Weight % of the <2000 μm fraction	Acid extractable U (μg g ⁻¹)	Bi/carbonate extractable U(VI) (μg g ⁻¹)	Organic carbon (%)
D-08-16'	<53	4.1	19.0	18.9	0.9
	<106 > 53	14.6	14.5	11.9	–
	<149 > 106	7.6	10.4	8.5	0.4
	<250 > 149	14.8	9.2	7.8	0.3
	<500 > 250	23.0	7.5	5.6	–
	<1000 > 500	26.3	3.9	2.9	0.1
	<2000 > 1000	9.7	4.3	5.2	0.1
	<2000	–	9.3	–	0.4
D-05-16'	<53	3.0	3.3	2.2	–
	<106 > 53	5.6	2.0	1.1	–
	<149 > 106	4.1	1.4	0.8	–
	<250 > 149	11.6	1.0	0.6	–
	<500 > 250	29.9	0.8	0.4	–
	<1000 > 500	36.1	0.6	0.3	–
	<2000 > 1000	9.9	0.8	0.4	–
	<2000	–	1.0	–	–
RABS	<53	3.1	4.9	–	–
	<106 > 53	8.3	3.7	–	–
	<149 > 106	4.4	2.9	–	–
	<250 > 149	10.5	2.7	–	–
	<500 > 250	27.5	1.7	–	–
	<1000 > 500	34.1	1.2	–	–
	<2000 > 1000	12.2	0.9	–	–
	<2000	–	1.7	–	–

Table 2
Specific surface area measured by BET analysis of <2 mm samples from D05–D08 and background samples RABS and BKG-A.

Sediment	Specific surface area (m ² g ⁻¹)
BKG-A	4.1
RABS	4.9
D-05-13'	2.7
D-05-16'	3.8
D-05-19'	3.5
D-06-13'	4.6
D-06-16'	2.5
D-06-19'	3.2
D-07-13'	2.6
D-07-16'	3.8
D-07-19'	3.2
D-08-13'	6.9
D-08-16'	5.9
D-08-19'	3.8

the sediment are likely detrital in origin; biogenic and/or authigenic magnetites that are products of biotransformation of Fe(III)-oxides by dissimilatory Fe-reducing bacteria are small-particle and exhibit Mössbauer signatures that are distinct from those in the samples examined in this study (Kukkadapu et al., 2005).

EXAFS and Fourier transform (FT) of sample P103-10 are shown in Fig. 4. Initial fits were performed with up to 10% of U as uranyl, as constrained by the XANES results. Fits to the EXAFS were performed over the data range 3–7 Å⁻¹. Addition of 10% uranyl using parameters typical of uranyl to the fits resulted in a significant decrease of the statistical R factor. EXAFS fits to the first coordination shell using O atoms indicated the presence of 7 ± 1 atoms at 2.32 Å, consistent with values expected for U(IV), which is often (pseudo-) cubically coordinated to O (Bernier-Latmani et al., 2010; Schofield et al., 2008). A small but clear second shell frequency can be seen in the FT at ca. 2.8 Å (Fig. 4B). Fits to this shell were attempted using a single shell of atoms that could be present in the sediments, including phosphate or carboxylate functional groups in biomass or refractory organic material derived by biomass (Bernier-Latmani

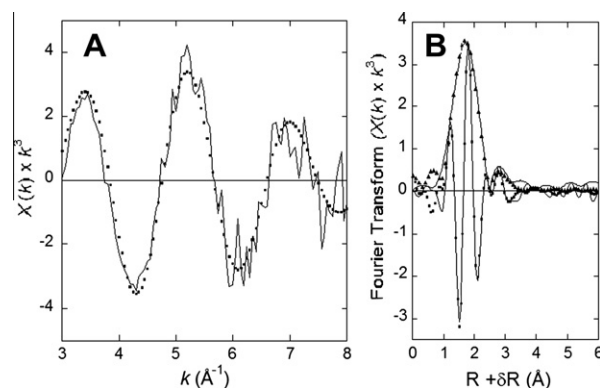


Fig. 4. EXAFS (A) and corresponding Fourier transform (FT) (B) from P103-10 bulk sample. Solid lines are data, and dotted lines are fits.

et al., 2010; Fletcher et al., 2010), and Fe or Si functional groups on mineral surfaces. The simple EXAFS model used here assumed that U was coordinated to a single phosphate group, or to a single Fe or Si surface functional group. For carboxylate groups, it was assumed that two such groups were bonded to each U(IV). Addition of 2nd frequency shell to O-shell-only fits produced a decrease in the statistical R significant at the 90% confidence interval, as judged by the Hamilton test (Hamilton, 1965). Fit-derived interatomic distances to U were: 3.60 Å (U–P), 3.41 Å (U–C), 3.41 Å (U–Fe), and 3.73 Å (U–Si). Whereas P or C atoms provided the lowest R factors (0.0129 and 0.0118, respectively), the R factors for the fits using Fe and Si (0.0145 and 0.0135, respectively) were not significantly different from those obtained using P/C. It is, therefore, not possible to distinguish the identity of the neighboring atoms from the EXAFS fits. Fits using P to model the 2nd shell are presented in Table S6.

The EXAFS data can be used to exclude several U(IV) phases from consideration. The lack of U–U pair correlations at 3.85 Å allows concluding that uraninite and coffinite are at most minor phases, and could be present only below the detection limit

Table 3

Descriptions of PLFA and qPCR parameters used in Fig. 5.

Abbreviation	Name	qPCR or PLFA	Description
Geo	Geobacter	qPCR	Assay for <i>Geobacter</i> -type bacteria
Monos	Monoenoic	PLFA	Abundant in gram-negative bacteria
Cells (pmol/g)	Cells in pmol/g	PLFA	Biomass
Polys	Polyenoic	PLFA	Found in eukaryotes (fungi and protozoa)
MGN	Methyl coenzyme M reductase	qPCR	Assay for archaeal methanogens
NSats	Normal saturated	PLFA	High proportions often indicate less diverse populations
Ebac	Bacterial biomass	qPCR	Biomass
TbSats	Terminally branched saturated	PLFA	Characteristic of firmicutes and gram-negative bacteria; indicates presence of anaerobic fermenting bacteria
T/C	Trans to cis ratio	PLFA	The higher the ratio, the less fluid in bacterial membrane; a measure of microbial stress
MBSats	Mid-chain branched saturated	PLFA	Often associated with anaerobic sulfate and iron reducing bacteria
Hydroxy	Hydroxy	PLFA	Indicative of iron reducing bacteria
Cy/mono	Cyclopropyl to monounsaturate ratio	PLFA	High ratio representative of an older population; indicates how active microbial population is
Dioic	Dioic	PLFA	Marker for iron reducing bacteria
Proteo	Includes iron and sulfate reducing bacteria	qPCR	Cells/g delta proteobacteria
DSR	Dissimilatory sulfite reductase	qPCR	Assay for sulfate reducing bacteria

(15–20% of total U). The absence of a 3.13 Å Si shell further suggests that coffinite is not present. The absence of P atoms at interatomic distances of 3.1–3.2 Å argues against the presence of U(IV) phosphate minerals containing U–P pairs at these distances, such as ningyoite ($\text{UCa}(\text{PO}_4)_2(\text{H}_2\text{O})_x$), but the presence of other U(IV) phosphate minerals cannot be excluded. Uranium–P/C interatomic distances similar to those observed for the P103-10 sample also have been observed for monomeric complexes of U(IV) bound to biomass (Bernier-Latmani et al., 2010; Fletcher et al., 2010). It is, therefore, possible that some of the U(IV) in the sediment could be complexed by organic matter.

In a previous study focused on framboidal pyrites from P103-10, <53 µm fraction, U was found to be associated with Fe and S by electron microscopy and electron microprobe analysis (Qafoku et al., 2009). Although it is not possible to quantify how much of the U is associated with reduced Fe–S phases from the present analyses, the direct evidence of U–Fe–S co-occurrence in Qafoku et al. (2009) is consistent with bulk data presented in this study.

The absence of uraninite suggests that the mechanism of U(VI) reduction is not similar to that of metal-reducing bacteria cultured in a laboratory batch environment (e.g., Lovley et al., 1991; Suzuki et al., 2002; Sharp et al., 2009). Although it is still possible that U(VI) reduction may be enzymatic, it has also been shown in laboratory studies that adsorbed Fe(II) on Fe oxide surfaces and Fe(II)-containing mineral phases (e.g., green rust, magnetite, Fe(II)-sulfides) can also reduce adsorbed U(VI) (Wersin et al., 1994; Liger et al., 1999; Missana et al., 2003; O'Loughlin et al., 2003; Jeon et al., 2005; Boyanov et al., 2007; Hua and Deng, 2008). Iron(II)-containing clays are another possible reducing phase for U(VI); when U diffuses into clay interlayers, electron transfer with Fe(II) can occur (Ilton et al., 2006). Several possible reactive phases for U are present in the natural reduced zone, and include pyrite, magnetite and Fe(II)-containing clays. However, the relative contribution of enzymatic and various possible abiotic reductants is currently not known.

3.2. Correlating microbial community analysis to sediment geochemistry

The microbial community varied across the sediment transect (D05–D08), correlating to changes in the sediment composition, particularly the abundance of biomass, Fe- and SO_4 -reducing bacteria, and indicators of diversity (Table 3). The results of the statis-

tical redundancy analysis calculations are presented in Fig. 5, with the gradient from oxidizing to reducing conditions marked by an arrow superimposed on the data from the upper left to lower right of the plot. The advantage of RDA for this system is in the ability to simultaneously and quantitatively compare multiple microbial and geochemical parameters along a redox gradient. In the RDA plot, the AVS, U, and to a lesser degree the organic C and Fe(II), are correlated, defining the most reducing sediments, located in the lower right of Fig. 5. The HA-extractable Fe(III) is negatively correlated to AVS and U, as expected for the more oxidizing sediments. Nitric acid-extractable $\text{Fe}(\text{T})_{\text{HNO}_3}$ does not lie on the gradient because it was relatively constant in samples taken from cores D05–D08. The total amount of biomass (pmol PLFA/g sediment) is highly correlated to organic C. PLFA is a better estimate of live/active bacteria than total DNA (Ebac) since lipids are readily degraded in the environment after cell death, whereas DNA may be relatively stable to degradation resulting in an overestimation of the active microbial population. The normal saturated and terminally branched saturated PLFA (NSats and TBSats) were correlated to AVS and U, suggesting that a less diverse, possibly slower growing, gram positive bacterial community exists in the reduced sediments. Moreover, the methyl coenzyme-M reductase (*mcrA*, MGN in Fig. 5) gene, indicative of methanogens, was highly correlated with the reduced sediments. Although methanogens probably do not directly influence the redox state of U, Fe or S, the data suggest that they be one of the important groups of organisms in the naturally reduced zone. It is possible that methanogens are important in metabolism for the overall community structure given the relative scarcity of Fe(III) and SO_4 as electron acceptors. The presence of proteobacteria (Proteo), which includes Fe- and SO_4 -reducing bacteria, and the dissimilatory sulfite reductase (DSR) gene targets are correlated to HA-extractable Fe(III) in the oxidizing portion of the redox gradient. The correlation of dioic PLFA, indicative of Fe-reducing bacteria, supports this observation, although other indicators of Fe- and SO_4 -reducing bacteria are uncorrelated (mid-chain branched saturated and hydroxy PLFAs, MBSats and Hydroxy in Fig. 5). In addition, the total cyclopropyl to monounsaturated precursor ratio (Cy/mono in Fig. 5) in the more oxidized region of the RDA plot suggests that the microbial community may be relatively active compared to the natural reduced zone. Iron/metal-reducing bacteria are dominant in the more oxidizing sediments possibly because there are more abundant terminal electron acceptors available for metabolism. Sulfate-reducing bacteria have a weaker correlation

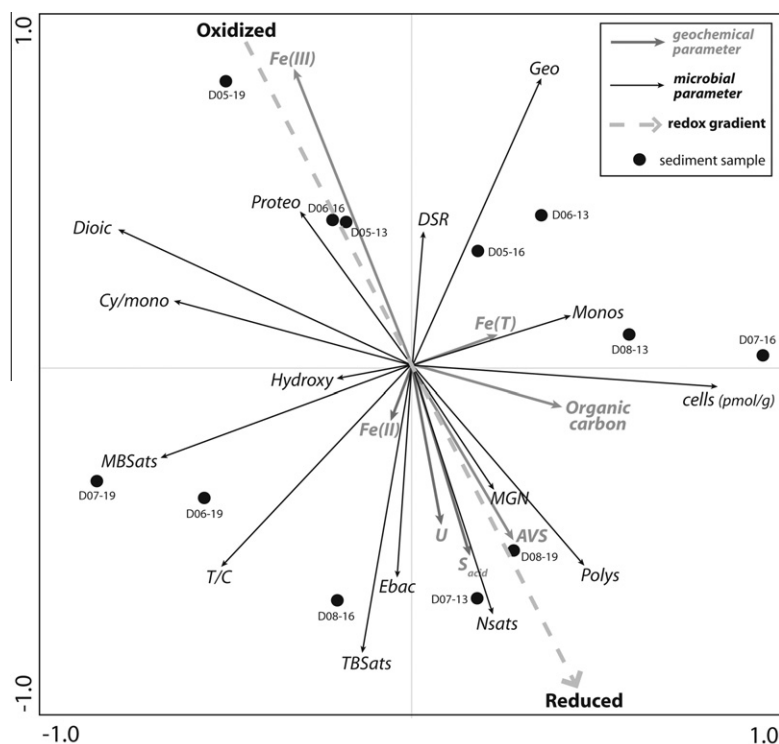


Fig. 5. Redundancy analysis plot of microbial (Phospholipid Fatty Acid (PLFA) and quantitative polymerase chain reaction (qPCR)) and selected geochemical parameters. The analysis calculates the response of the microbial parameters to the geochemical data. Each parameter is represented by a vector projected onto a two-dimensional plot with arbitrary axes. The arcsine of the angle between two vectors is the correlation coefficient of those two parameters; parallel vectors are highly correlated, opposing vectors are highly anti-correlated, and orthogonal vectors have no correlation. Sediment samples are included as circles (●) on the plot. A key to the notation and a description of the microbial parameters is presented in Table 3. S_{acid} represents the acid extractable S content, AVS is acid volatile sulfide content, and Fe(T) is the acid extractable total Fe content. The gradient from oxidized to reduced conditions is marked with a dashed arrow.

with oxidizing conditions, possibly because they can employ alternate electron acceptors besides SO_4 .

Fig. 5 also shows how the geochemical and microbial composition of each sediment sample maps onto the gradient defined by the geochemical parameters. D08-19, D08-16, and D07-13 lie in the reducing quadrants of the plot, while D05-13, D05-19 and D06-16 fall in the more oxidizing portion of the plot. The location of other samples with respect to the RDA gradient is more ambiguous. However, the tendency for D08 to correlate to the reducing zone while D05 is associated with the oxidizing zone supports the physical location of the reducing sediments to be in the vicinity of D08, while D05 is more typical of more oxidized Rifle aquifer sediments.

The correlation between microbial parameters and geochemical parameters along the oxidized–reduced gradient suggests that the enrichment of reduced Fe, S and U are linked to microbially mediated processes. The RDA analysis suggests that a less diverse, slower growing population has found a metabolic niche, causing the accumulation of reduced S, U and Fe in the sediments. The correlation between biomass and organic C supports the hypothesis that alluvial deposition of organic matter from the Colorado River has stimulated microbial activity in the sediment, including Fe(III), U(VI) and SO_4 reduction. Discounting U-bearing groundwaters entering the aquifer from off-site locations, the bulk of the U accumulation in these sediments has occurred since the opening of the mill, resulting in approximately 80 a of reaction time for U reduction in the subsurface.

The organic C, U and S vectors are only moderately correlated on the RDA plot, suggesting that the successful reduction and long-term mobilization of U(VI) and SO_4 in the sediments is not simply a function of organic C content. The successful accumula-

tion of U(IV) may also be linked to an optimization of organic C and groundwater flow by a slow growing, specialized microbial community adapted to the particular conditions in the sediment. The higher fraction of fines, including clays, may make the sediments in the natural reduced zone less permeable to groundwater flow. Growth of microbes utilizing recalcitrant organic C may be better suited to a less porous material with a smaller flux of groundwater. Decreased flux may also allow the development of locally reducing conditions conducive to the reduction of Fe, U and SO_4 ; a lower permeability zone may provide an important microbial ecosystem that stabilizes reduced phases, including U, in the aquifer.

3.3. Role of natural organic carbon

Natural organic C has been shown to serve as an electron donor for microbial U(VI) reduction in batch experiments with natural sediments and a mixed microbial community (Dong and Brooks, 2006) as well as pure microbial cultures (Gu et al., 2005). Some U roll-front ore deposits are associated with natural organic debris (Reynolds and Goldhaber, 1983). It has also been hypothesized that natural organic C can act as an electron shuttle between bacteria and adsorbed U(IV) (Fredrickson et al., 2000), which could increase the rate and extent of U(VI) reduction. In addition, U tends to partition into organic C-containing phases, due to the affinity of various aqueous, adsorbed, and solid-phase organic C–U complexes (Mason et al., 1997; Gu et al., 2005; Dong et al., 2006; Křepelová et al., 2006). Substantial accumulation of U in wetlands, peat deposits and other organic-rich, reducing formations from low-concentration source rock has been observed in many environments (Read et al., 1993; Owen and Otton, 1995; Regenspurg

et al., 2010). In an organic-rich soil in Switzerland, U accumulated as a mixture of U(VI) and U(IV), suggesting that both complexation with organic C and reductive processes were important in sequestering U (Regenspurg et al., 2010). The role of natural organic C in the redox cycle of U is complex, affecting microbial activity, U adsorption, aqueous complexation, and electron transfer processes. Although the affinity of U for organic matter may be an important component of U enrichment in naturally reduced sediments at Rifle, the RDA analysis shows that U has a stronger correlation to reduced Fe and S phases than to organic C, suggesting that association with organic matter is not the only process controlling higher U concentrations.

The creation of reducing conditions (reduced Fe and S phases) by microbial consumption of organic C is a probable explanation for the conditions observed in the naturally reduced sediments. This is similar to a mechanism for creating roll-front deposits, one of the most economically important sources of U ore (Plant et al., 1999). The deposits are created by fluid transport of dissolved U in oxidized groundwaters to a redox front where oxidized waters meet reducing conditions, in some cases produced by heterotrophic microbial metabolism. At the redox front, U(IV) and other reduced Fe and S phases precipitate. As the oxidized front moves through reduced sandstone, U accumulates, in a band, to ore-grade concentrations at the redox front (e.g., Granger and Warren, 1969, 1974). The mechanism of U accumulation in Rifle sediments appears to be analogous to roll-front deposits, on a smaller, more localized scale. However, further characterization of the organic C in the naturally bioreduced sediments at Rifle is needed to understand the mechanisms by which U(VI) is reduced in the subsurface, and if the organic C contributes to the redox activity of U phases in any capacity other than as an electron donor for microbial metabolism.

The relative importance of biotic and abiotic reduction of U(VI) in the naturally reduced zone of the Rifle aquifer cannot be determined at present, but the co-occurrence of elevated concentrations of U(IV), Fe(II), and reduced S phases suggest that there may be a complex interaction among bioreduction, sorption, complexation and abiotic reduction reactions in the sediments from the naturally reduced zone. It is clear that the activity of the microbial community, the resulting changes in mineralogy, and reactions involving Fe oxide, Fe(II)-rich clay, and Fe sulfide phases are critically important to the long-term fate of U.

4. Conclusions and environmental implications

The lenses of higher U in the natural reduced zone exist on the border of highly variable redox conditions in the aquifer. Sample P103-10 was from the vadose zone/capillary fringe at the time of sampling, although it is seasonally saturated as the water table increases with Colorado River stage during peak runoff in the spring and early summer. The presence of significant amounts of U(IV) in P103-10 not only confirms the reduction of U(VI), but also suggests that the U(IV) in the naturally reduced zone is stabilized to re-oxidation when seasonally placed in more oxidizing conditions in the vadose zone. The lower permeability sediment and higher organic C content may have created a reducing microenvironment where U(IV) may be protected from the more oxidizing conditions. The lower permeability zones may create biogeochemically favorable conditions to stabilize reduced phases and associated constituents, such as U, in the alluvial aquifer. Association with biomass and clays may also serve to stabilize the U(IV) to oxidation by providing a diffusive boundary for oxidants like dissolved O₂ (Ilton et al., 2006; Campbell et al., 2011). In addition, the minerals closely associated with U(IV) may also play a stabilizing role (e.g., oxidative buffer capacity provided by Fe sulfide mineral phases). The accu-

mulation of U(IV) in these localized lenses may also have implications for larger-scale aquifer loading of U; slow release from these lenses may explain the persistence of dissolved U in the aquifer long after natural flushing was predicted to decrease the concentration of U below applicable standards. Lenses of naturally reduced sediments may be common in alluvial aquifers (Bargar et al., 2011) and because of their capacity to accumulate and stabilize trace elements and redox-active phases, they are important for accurate estimation of natural attenuation capacity.

It has been demonstrated here that U(VI)-, Fe(III)- and SO₄-reduction driven by microbial processes stimulated by deposition of organic matter has likely occurred in localized zones of sediment in the Rifle aquifer. The conditions in the naturally reduced sediment are analogous to the artificially biostimulated conditions created upon injection of acetate; the reducing conditions created during biostimulation are designed to be favorable for long-term sequestration of U(IV). Characterization of the mineralogy and natural organic C in the zone of natural reduction suggests that addition of recalcitrant organic C may help to sustain low-level, long-term reducing conditions. By understanding these processes in the naturally reduced sediment in more detail, the overall design and long-term strategy for artificially stimulated reducing conditions can be optimized to maximize U removal from the groundwater and stabilize U(IV) products and other reduced phases to oxidation.

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Appendix A. Supplementary material

Summary table of sample names, size fractions, and analyses, complete elemental results from HNO₃ extractions, XRF and XRD results, modeled Mössbauer parameters, EXAFS and FT with fit results, and pPCR data. Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.apgeochem.2012.04.013>.

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