Environ. Sci. Technol. **2010,** *44,* 177–183

Impact of Biostimulated Redox Processes on Metal Dynamics in an Iron-Rich Creek Soil of a Former Uranium Mining Area

EVA-MARIA BURKHARDT,[†] DENISE M. AKOB,[†,‡] SEBASTIAN BISCHOFF,[†] JANA SITTE,[†] JOEL E. KOSTKA,[‡] DIPANJAN BANERJEE,[§,∥] ANDREAS C. SCHEINOST,[§,∥] AND KIRSTEN KÜSEL*[,†]

Institute of Ecology, Friedrich Schiller University Jena, Dornburger Straβe 159, D-07743 Jena, Germany, Department of Oceanography, Florida State University, 255 Atomic Way, Tallahassee, Florida 32306, Institute of Radiochemistry, Forschungszentrum Dresden-Rossendorf, D-01314 Dresden, Germany, and The Rossendorf Beamline at ESRF, F-38043 Grenoble, France

Received July 8, 2009. Revised manuscript received October 9, 2009. Accepted October 28, 2009.

Understanding the dynamics of metals and radionuclides in soil environments is necessary for evaluating risks to pristine sites. An iron-rich creek soil of a former uranium-mining district (Ronneburg, Germany) showed high porewater concentrations of heavy metals and radionuclides. Thus, this study aims to (i) evaluate metal dynamics during terminal electron accepting processes (TEAPs) and (ii) characterize active microbial populations in biostimulated soil microcosms using a stable isotope probing (SIP) approach. In biostimulated soil slurries, concentrations of soluble Co, Ni, Zn, As, and unexpectedly U increased during Fe(III)-reduction. This suggests that there was a release of sorbed metals and As during reductive dissolution of Fe(III)-oxides. Subsequent sulfate-reduction was concurrent with a decrease of U, Co, Ni, and Zn concentrations. The relative contribution of U(IV) in the solid phase changed from 18.5 to 88.7% after incubation. The active Fe(III)-reducing population was dominated by *δ-Proteobacteria* (*Geobacter*) in ^{13}C-ethanol amended microcosms. A more diverse community was present in ^{13}C-lactate amended microcosms including taxa related to *Acidobacteria*, *Firmicutes*, *δ-Proteobacteria*, and *β-Proteobacteria*. Our results suggested that biostimulated Fe(III)-reducing communities facilitated the release of metals including U to groundwater which is in contrast to other studies.

Introduction

Natural attenuation, enhanced or mediated by microbial activities, can immobilize heavy metals and radionuclides and prevent their transport by different mechanisms, such as sorption and precipitation processes, and redox state transformations (*1, 2*). Metal- and sulfate-reducing microorganisms can immobilize uranium (U) by direct enzymatic reduction to an insoluble and less mobile form (*3, 4*), or indirectly via production of reducing agents, e.g., Fe(II) and sulfide (*5, 6*). Reductive immobilization of U was observed among a phylogenetically diverse assemblage of respiratory and fermentative microorganisms. In particular, members of the Fe(III)-reducing *Geobacteraceae* family (*δ-Proteobacteria*) have been demonstrated to be important for bioremediation at contaminated subsurface sites (*7, 8, 2*). However, metal-reducing microorganisms also can cause metal release by reductive dissolution of iron- or manganese-oxides which are important metal scavengers (*9, 10*).

In the most productive mining district of the former German Democratic Republic, Ronneburg (Thuringia, Germany), uranium mining caused severe environmental contamination (*11*). The creek Gessenbach was one of the main drainage systems for the former mining sites (*12*). In contrast to other well investigated U contaminated sites (Rifle, CO and Oak Ridge, TN), the remediated Ronneburg mining district contains high concentrations of a variety of heavy metals, e.g., Cu, Ni, Cd, As, Zn, Pb, Cr, and Co, which raises the question how multiple metal contaminants are effected by microbial activities under changing redox conditions. This study aims to link microbial activities with metal dynamics in an iron-rich creek soil at the bank of the Gessenbach in biostimulated microcosms incubated first under anoxic conditions and then during reoxidation. The active microbial populations during terminal electron accepting processes (TEAPs) in these microcosms were identified with stable isotope probing (SIP).

Materials and Methods

Study Site and Sampling. The study site is the bank of the creek Gessenbach downstream of the former mining sites near Ronneburg (Thuringia, Germany, location: E 4510121, N 5635807, Gauss/Krueger Potsdam coordinate system), which receives contaminated groundwater. Addition of HCl did not indicate the presence of carbonate in the soil. The groundwater influenced oxidized horizon Btlc contained the highest concentrations of U, Cu, Zn, Ni, Pb, and As in the solid phase that approximated 1.57, 644, 466, 205, 191, and 85 μg g^{-1}, respectively (*13*). The organic carbon, Fe, and Mn contents were 6%, 65 and 0.4 mg g^{-1}, respectively.

Soil−water was sampled monthly in ∼10 cm depth intervals from June to November 2007 using Rhizons according to ref *14*. Btlc soil samples for microcosm experiments were taken in May and June 2007 (electron donor experiments), June 2007 (SIP experiment), and April 2008 (metal dynamics experiment) to ensure fresh material for each experiment. Soil was obtained from different sites of the outcrop, pooled, collected in plastic bags, and stored at 15 °C overnight.

Microcosm Experiments. Twenty or 80 g of fresh weight soil (41−47% water content) was placed into sterile 150- or 500-mL incubation flasks, respectively, under a continuous flow of sterile argon and mixed with either 80 or 320 mL of water (amended with 50 μM nitrate, 70 μM ammonium, pH 5.5, according to soil−water concentrations). Flasks were closed, shaken for 1 h, and incubated in the dark at 15 °C with an initial overpressure of 800 mbar. Triplicate microcosms were amended with 10 mM ethanol, 10 mM lactate, 10 mM acetate, 5 mM glucose, or left unamended, respectively. For the SIP experiment ^{13}C-labeled carbon sources (>99 atom % ^{13}C; Cambridge Isotopes, USA) were used. For the metal dynamics experiment, sterile (autoclaved) unamended controls were also prepared. For the reoxidation

* Corresponding author phone: +49-3641-949461; fax: +49-3641-949462; e-mail: Kirsten.Kuesel@uni-jena.de.
[†] Friedrich Schiller University Jena.
[‡] Florida State University.
[§] Forschungszentrum Dresden-Rossendorf.
[∥] The Rossendorf Beamline at ESRF.

10.1021/es902038e
Published on Web 11/16/2009

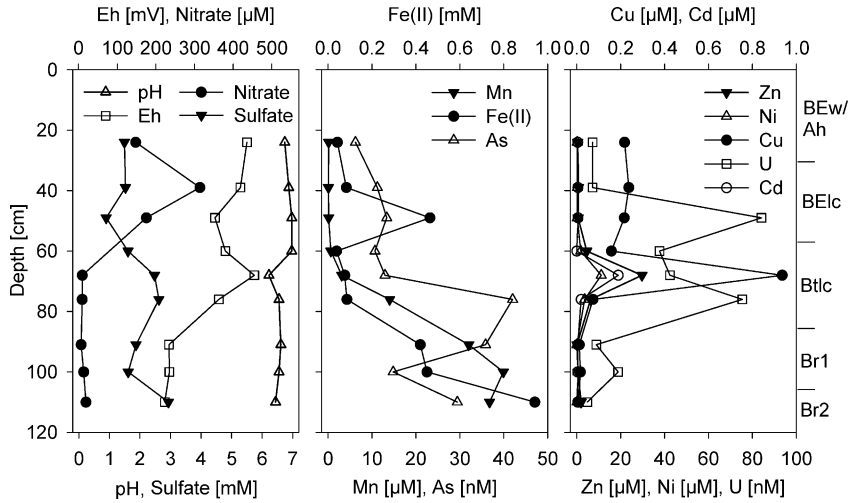

FIGURE 1. Geochemistry of the pore water in the soil profile at the bank of the contaminated creek Gessenbach in August 2007. The corresponding soil horizons are given at the right side of the graphs.

experiment, microcosms of the metal dynamics experiment were opened, closed with sterile cotton stoppers, and incubated on a horizontal shaker. Samples for cultivation-independent community characterization were collected and pooled from each replicate microcosm, centrifuged at 2300*g* for 3 min, and the pellets were frozen at −20 °C until DNA extraction. Procedures used for DNA extraction, separation of ^{13}C- and ^{12}C-labeled DNA, SSU rRNA gene PCR amplification, and terminal restriction fragment length polymorphism (TRFLP) analysis are described in the Supporting Information.

Analytical Techniques. Soil samples for total Fe and Fe(II) determination were extracted in oxalate (0.2 M ammonium oxalate, 0.2 M oxalic acid, pH 2.5) for 4 h in the dark, shaking, as previously described (*15*), and Fe was measured with the phenanthroline method (*16*). Nitrate, ammonium, sulfate, Fe(II), and Fe(III) in the soil solution were measured as described elsewhere (*17*−*21*). Metals and As were measured in acidified, 0.45 μM filtered samples using ICP-MS (inductively coupled plasma−mass spectrometry, X-Series II, Quadrupol ICP-MS, Fa. Thermo Electron, Bremen, Germany). In the microcosm experiments, pH and Eh were measured in the soil suspension. Then, soil suspensions were centrifuged at 2300*g* for 3 min, and the supernatant was analyzed for nitrate and sulfate using ion chromatography (*14*). Iron(II) and total Fe [Fe(II) and Fe(III)] in the soil suspensions were measured after extraction in 0.5 N HCl for 1 h (*20*). Carbon concentrations were determined with high-performance liquid chromatography (*14*).

X-ray Absorption Spectroscopy. X-ray Absorption Near-Edge Structure (XANES) and Extended X-ray Absorption Fine-Structure (EXAFS) spectra were collected at the Rossendorf Beamline at the European Synchrotron Radiation Facility. Btlc soil samples and microcosm solid phase material from triplicate lactate microcosms sampled at the end of incubation were placed on a SH 01 B polyethylene sample holder under anoxic conditions. Additional details are described in the Supporting Information.

Results

Biogeochemistry of the Soil Solution. The geochemistry of the soil solution was comparable at all sampling times. Nitrate was present only in upper horizons (<320 μM) (Figure 1A). Eh declined from 450 to 220 mV, and concentrations of Mn and Fe(II) increased with increasing soil depth (Figure 1B). High sulfate concentrations were observed over the whole soil profile. Concentrations of Cd, Zn, Ni, Cu, and Co peaked in soil solution of horizon Btlc (Figure 1C), and average U concentrations reached 30−80 nM in the BElc and Btlc horizons.

Metal Dynamics under Reducing Conditions. Although the total iron content approximated 323 ± 66 mM with 94% as Fe(III), low to negligible Fe(II) formation rates (≤1.2 μmol g^{-1} d^{-1}) were observed in unamended anoxic soil microcosms. Amendments with lactate or ethanol enhanced rates after a lag phase of 8 days to 3.6 or 4.4 μmol g^{-1} d^{-1}, respectively, whereas amendments with acetate or glucose had no stimulatory effect. Thus, dynamics of TEAPs and soluble metal and As concentrations were studied in lactate (Figure 2) or ethanol (data not shown) stimulated microcosms. Lactate and ethanol supplemented microcosms were also used to identify the active microbial communities. The pattern and kinetics of TEAPs and supplemental carbon consumption were similar in all studies. Nitrate was completely consumed within 2 days (data not shown) followed by an increase in soluble Mn concentrations (Figure 2). Fe(II) concentrations in lactate microcosms increased after 6 days paralleling Mn-reduction accompanied by an increase in soluble metal and As concentrations. Co and Ni concentrations increased immediately, whereas U was released after the onset of Fe(III)-reduction (9 days). Sulfate increased from 0.4 to 0.8 mM during the initial incubation period and decreased after 18 days, paralleling the decrease of Co, Ni, and U. In unamended controls, little to no change in metal or As concentrations were observed and autoclaved controls showed no change. Microcosms amended with ethanol showed patterns similar to those of lactate microcosms. TEAPs observed explained 30−50% of carbon consumption.

XANES. The increase of soluble U under Fe(III)-reducing conditions was unexpected. X-ray absorption near-edge region (up to 17.25 KeV) of the samples fitted with a linear combination fitting approach using a U(IV) and U(VI) component (data not shown) showed that the Btlc soil prior to incubation predominantly contained U(VI) species (81.5%). Soil after anoxic incubation was enriched in reduced U(IV) (88.7%). The linear combination approach provided excellent fits to the data and the obtained values were correct to ±1%. A comparison with a reference spectrum of UO_2 showed a lack of features in the post-edge part of the incubated soil spectrum, suggesting that U(IV) in the incubated soil did not occur in a crystalline form similar to UO_2, but was more likely to be present as a sorbed complex (Figure S1).

Metal Dynamics under Oxidizing Conditions. During the reoxidation experiment, Mn concentrations decreased rapidly within 3 days in the biostimulated microcosms (Figure 3). Concurrently, there was a rapid increase in U concentrations and a rapid decrease in Co and As. Fe(II) concentrations decreased slowly parallel to the increase in

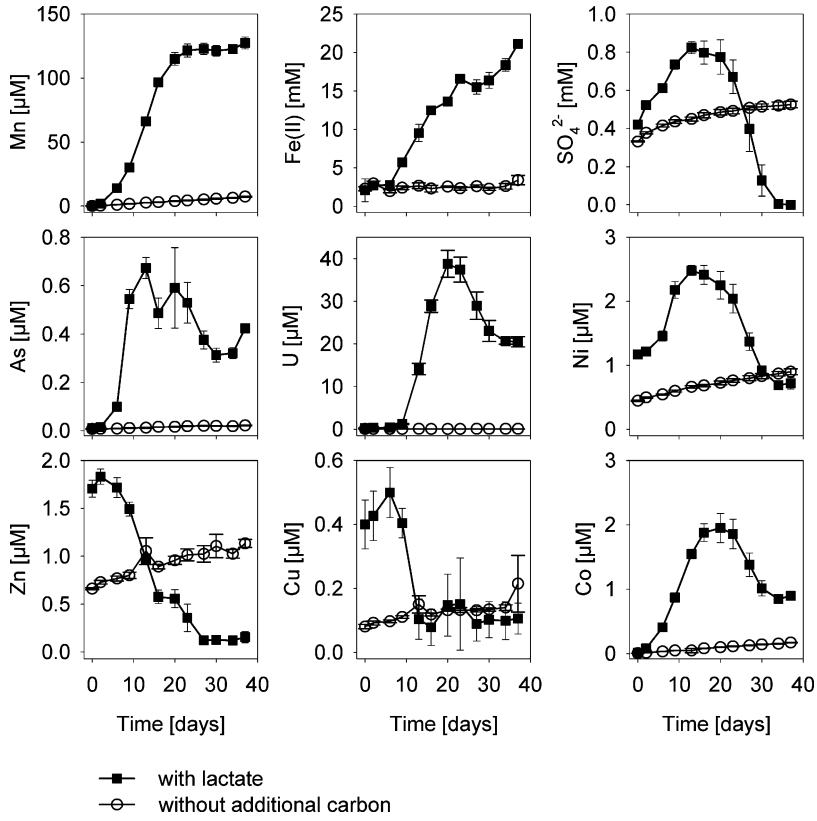

FIGURE 2. Dynamics of Fe(II), soluble Mn, sulfate, and soluble metal concentrations (mean of triplicates ± standard deviations) in anoxic soil microcosms amended with lactate and in controls without additional carbon.

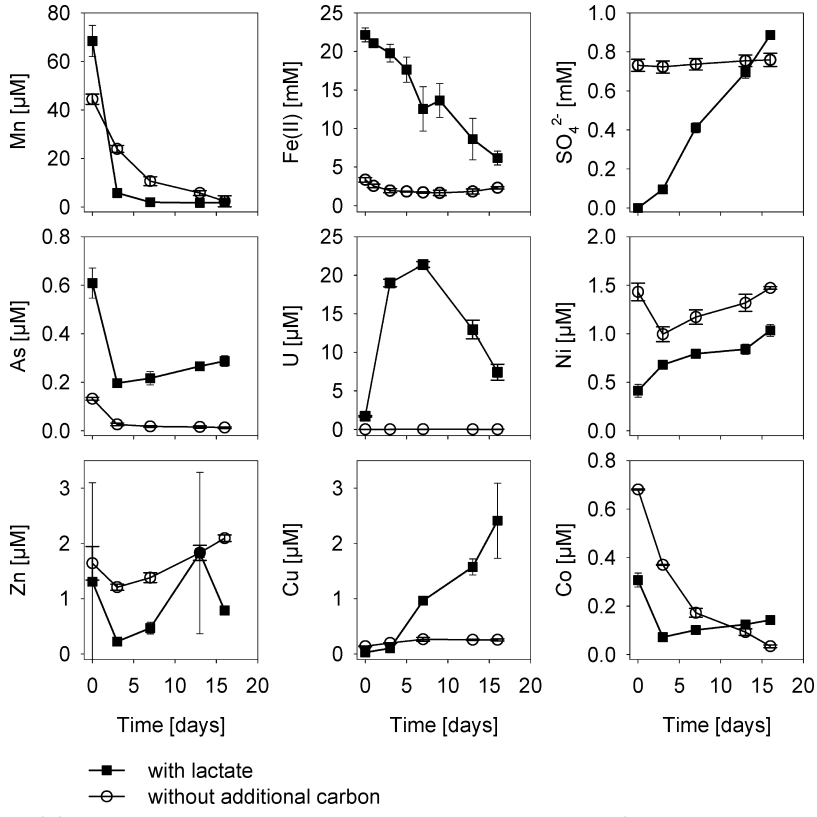

FIGURE 3. Dynamics of Fe(II), soluble Mn, sulfate, and soluble metal concentrations (mean of triplicates ± standard deviations) during reoxidation of formerly reduced soil microcosms amended with lactate at the beginning of the reduction and in controls without additional carbon.

sulfate. After 8 days of reoxidation, U concentrations decreased again.

Characterization of Active Microbial Communities in Anoxic Microcosms. With the next microcosms experiments,

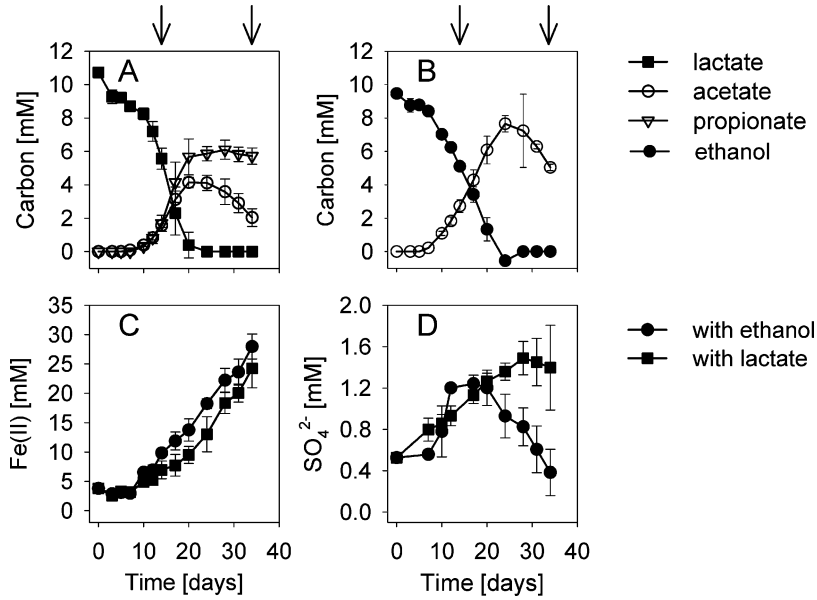

FIGURE 4. Consumption of ^{13}C-lactate (A) or ^{13}C-ethanol (B) in the SIP experiment soil microcosms during Fe(III)- (C) and sulfate-reduction (D) (mean of triplicates ± standard deviations). Arrows indicate time of sampling for SIP.

we wanted to identify the active biostimulated microorganisms with ^{13}C-labeled carbon sources during the periods of Fe(III)- and sulfate-reduction which paralleled changes in heavy metal concentrations. SIP and community characterization were performed at days 14 and 34 corresponding to ^{13}C-carbon usage and TEAPs (Figure 4). Lactate was consumed within 20 days yielding propionate and acetate (Figure 4A) and consumption was linked to Fe(II) formation (Figure 4C). After 20 days, acetate concentrations declined in parallel to an increase in Fe(II) that was followed by a slight decrease of sulfate after 28 days (Figure 4D). Ethanol was completely consumed within 24 days and yielded acetate in parallel to an increase of Fe(II) (Figure 4B and C). After 24 days, acetate declined from 8 to 5 mM in parallel to an Fe(II) increase and a decrease in sulfate from 1.2 to 0.4 mM (Figure 4D). Assuming an 8:1 ratio for acetate oxidation and Fe(III)-reduction and a 1:1 ratio for acetate oxidation and sulfate-reduction, both processes appeared to be stimulated by acetate.

DNA was detected in gradient fractions with buoyant densities ranging from 1.52 to 1.62 g mL^{-1} (Figure S2). The highest density DNA fractions represented the microbial community with the highest ^{13}C incorporation. Comparison of TRFLP patterns generated for each of the DNA fractions revealed a distinct clustering of the heavy fractions only in the ^{13}C-lactate treatment (Figure S3). A total of 40 or 48 clones were screened, yielding coverage of 0.51 or 0.19 for the ethanol or lactate treatments, respectively. The community of the ^{13}C-lactate treatment was dominated by members of the *Acidobacteria*, whereas, the ^{13}C-ethanol treatment was dominated by members of the *δ-Proteobacteria* (Table S1). Additional clones were related to the *Acidobacteria* and *β-Proteobacteria* in the ethanol or *β-Proteobacteria* and *Firmicutes* in the lactate treatments. TRFs of phylotypes were determined by *in silico* and *in vitro* cutting of representative clones and compared to peaks in the TRFLP patterns (Figure S4, Table S1).

TRFs of *Geobacter*-related clones dominated TRFLP patterns from ^{13}C-enriched fractions of the ethanol treatment (days 14 and 34). *Acidobacteria*, *Geothrix fermentans* (phylotypes 1OE20, 2OE14) and *Firmicutes*, *Desulfosporosinus* (phylotype 1OE44) TRFs were detected at a lower abundance at 14 days (Figure S4). After 34 days the abundance of *Acidobacteria* and *Firmicutes* increased in the ^{13}C-enriched and intermediate fractions. After 34 days of incubation, a TRF corresponding to a *δ-Proteobacteria*-phylotype with 87% identity to *Pelobacter* was observed in the active DNA fractions indicating a shift in the microbial community over time (Figure S4, Table S1).

In the TRFLP patterns of the ^{13}C-lactate treatment, TRFs corresponding to clones belonging to the *Acidobacteria*, *Firmicutes*, and *δ-Proteobacteria* were present in similar abundance at 14 days (Figure S4, Table S1). In the clone library, *Acidobacteria* and *Firmicutes* were abundant, but in contrast, *δ-Proteobacteria* had low abundance. *β-Proteobacteria* TRFs, related to *Dechloromonas* and *Janthinobacterium*, were in low abundance in the ^{13}C-lactate treatment (Figure S4). After 34 days, *Acidobacteria* were the most abundant TRFs in the ^{13}C-enriched and intermediate fractions of the lactate treatment, whereas, the abundance of *Firmicutes*, *β-Proteobacteria*, and *δ-Proteobacteria* decreased (Figure S4).

Discussion

Metal Dynamics during Reductive and Oxidative Periods in Soil Microcosms. This study linked biostimulated Fe(III)-reduction with the release of heavy metals from contaminated creek soil and identified the biostimulated, active microbial communities. Despite the high amount of Fe(III) and organic carbon content (6%) present in the Btlc horizon, this soil showed low to negligible Fe(III)-reduction without supplemental carbon. However, biostimulated Fe(II) formation rates were at least three times higher compared to other studies (*22, 23*). In those studies, ethanol and glucose but not lactate had biostimulatory effects.

The peak of heavy metal concentrations in soil porewater of the Btlc horizon was corroborated with sequential extraction data that demonstrated the presence of high proportions of these metals in the water-soluble or exchangeable fraction ((*13*), and data not shown). Similarly, high proportions of metals were present in the amorphous Fe(III)-oxide fraction, while Mn-oxides were of minor importance. Thus, the reductive dissolution of Fe(III)-oxides might have caused the release of adsorbed or coprecipitated metals (*9, 10, 24*). Concentrations of dissolved Cd and Cu increased prior to Fe(III)-reduction suggesting a release from Mn-oxides. In contrast, Co, Ni, Zn, and As were released during both processes. Differences in metal dynamics could be due to different metal bonding strengths to Fe(III)- or Mn-oxides. Similarly, the increase of sulfate during the beginning of incubation might be due to the release of sulfate adsorbed

to Mn- or Fe(III)-oxides as shown in other studies (*25*). The increase in As could be due to direct reduction as well as reductive dissolution of sorbents (*26, 27*). Initial metal concentrations were enhanced in the autoclaved controls apparently due to a changed geochemistry. However, no concentration changes over time were observed.

The release of U during Fe(III)-reduction was surprising as Fe(III)-reducing bacteria (FeRB) are known to remove U(VI) from solution through precipitation of the insoluble U(IV) mineral phase uraninite [UO_2(s)] (*28, 3, 4*). Unfortunately, we were unable to differentiate the redox state of U in the liquid phase. Release of complexed U(VI) due to carbon amendments is unlikely due to the long lag phase observed prior to U mobilization. The formation of soluble Ca−uranyl−carbonate complexes that would be persistent to enzymatic reduction (*29, 30*) might be insignificant due to the lack of carbonates in the soil and the lack of CO_2 in the microcosm headspace at the beginning of incubation. Sequential extractions showed that ~30% of U is present in the amorphous Fe(III)-oxide fraction (*13*) and 81% of the solid phase-associated U was U(VI). These results indicate that U was sequestered in Btcl horizon that represents a redox transition boundary with likely active Fe remineralization processes. The incorporation of U into newly formed minerals was supported by the dip in U concentrations in the porewater in the Btlc horizon (Figure 1). Our results suggest that solid-phase associated U was not enzymatically reduced by FeRB but was released during reductive dissolution of Fe(III)-oxides. The partial decrease of U started with the onset of sulfate-reduction, although a contribution of FeRB cannot be ruled out. Several sulfate-reducing bacteria (SRB) reduce U enzymatically (*4*), although only a few have been reported to gain energy for growth from this process (*31*). In addition, sorption of U to metal sulfides, such as pyrite, might be possible (*32*). Comparative spectra demonstrated that no UO_2(s) was formed but apparently a sorbed complex of U(IV).

Metal immobilization during sulfate-reduction was probably due to formation of metal sulfides with Co, Ni, Zn, Cd, and As (*33*−*37*), although precipitation onto newly formed Fe(II) phases can not be excluded (*28, 38*). Since As concentrations were below those previously reported to facilitate arsenic sulfide precipitation (*35*), formation of As-bearing pyrite might have occurred (*39*).

The initial release of U during reoxidation is likely due to chemical oxidation by oxygen (*40*) followed by the sorption on or coprecipitation with newly formed Fe(III)- and Mn-oxides acting as metal scavengers (*41, 42*). Solubilization of Ni and Cd could be associated with the dissolution of metal sulfides (*43*). The oxidation of Fe(II) proceeded rather slowly.

Active Microbial Communities. In lactate-stimulated soil microcosms a diverse community dominated by *Acidobacteria* (*Geothrix*) and *Firmicutes* (*Pelosinus*) was detected. The genus *Geothrix* is known to reduce nitrate, Mn(IV), Fe(III), and humic acids (*44*), and although it is not known to reduce U, it might promote indirect reduction by formation of Fe(II) (*45*). *Geothrix* has been detected in biostimulated uranium-contaminated sediments, although its specific role in bioremediation is unclear (*45*−*47*). Members of the *Firmicutes* are able to transfer electrons from fermentation processes to Fe(III) (*48*). In addition, this group was suggested to play a role in bioremediation (*49*) due to observed reduction and increased sorption of U(VI) by *Firmicutes* (*50, 51*). A *δ-Proteobacteria* clone related to *P. propionicus*, and *β-Proteobacteria* clones related to *Dechloromonas* and *Janthinobacterium* were detected in the active FeRB community. Members of the genus *Pelobacter* are known to reduce Fe(III) and elemental sulfur, although it was suggested recently that Fe(III)-reduction only occurred indirectly via sulfide production (*52, 53*). The genus *Dechloromonas* has been observed before in uranium-contaminated soil microcosms (*49, 54*), and can reduce nitrate, sulfate, and apparently Cr(VI) and Se(VI) (*55, 56*). Dissimilatory Fe(III)-reduction has not been observed in pure cultures of *Dechloromonas* but by *Ferribacterium limneticum* which shared >96% identity to the *Dechloromonas*-related clones (*57*). Members of the genus *Janthinobacterium* have been reported to reduce nitrate (*58, 59*) and may be contributing to nitrate-, Fe(III)- and sulfate-reduction in the soil microcosms. Only a minor shift in the microbial community was detected between the sampling times. However, sulfate concentrations indicated the onset of sulfate-reduction starting at the time of sampling, whereas reduction of millimolar amounts of Fe(III) continued. Thus, the labeling of the FeRB community continued, while the labeling of the SRB community may have been below detection.

In agreement with former studies, addition of ethanol stimulated, in particular, the activity of *Geobacter*, a genus of the *δ-Proteobacteria* important in Fe(III)- and U(VI)-reducing communities (*60, 7, 8, 49, 54*). In ethanol microcosms, *Acidobacteria* (*Geothrix*) and *Firmicutes* (*Desulfosporosinus*) were detected in lower abundance after 14 days of incubation but became important members of the community after 34 days. *Desulfosporosinus* species are able to reduce sulfate and As(V) (*61*) and these organisms may have been involved in As solubilization at the beginning of the experiment. The increased TRF intensity of *Desulfosporosinus*-related clones during sulfate-reduction suggests that this organism contributed to this process. *Geothrix* can utilize acetate but not ethanol as electron donor, which explains its low abundance during ethanol consumption after 14 days of incubation (*44*). However, *Geothrix*, in addition to *Pelobacter*-related organisms who were detected after 34 days, might have contributed to Fe(III)-reduction during the later period of the incubation.

In contrast to biostimulation studies at uranium-contaminated sites in the United States (*7, 28, 51, 60*), uranium release was observed under reducing conditions in conjunction with activity of the *Geobactereaceae* family. Our results suggested that biostimulated FeRB facilitated the release of heavy metals, including U, and metal-enriched soil horizons could potentially be a contaminant source to groundwater and adjacent creek waters. Thus, no single bioremediation strategy, e.g., addition of identical carbon sources, can be applied to environments regardless if the same groups of FeRB are present.

Acknowledgments

We thank Michael Humphrys (Department of Oceanography, Florida State University) and Ingo Schöning (Institute of Ecology, Friedrich Schiller University Jena) for technical assistance. The study was supported by the German Science Foundation (DFG: Graduate research school 1257: "Alteration and Element Mobility at the Microbe Mineral Interface").

Supporting Information Available

Methods for XANES and fitted XANES spectra (Figure S1); identification of metabolically active bacteria; DNA concentrations in gradient fractions (Figure S2); similarity analysis of TRFLP patterns from SIP soil microcosms (Figure S3); TRFLP profiles from soil microcosms (Figure S4); and SSU rRNA gene cloning (Table S1). This information is available free of charge via the Internet at http://pubs.acs.org.

Literature Cited

(1) Picardal, F.; Cooper, D. G. Microbially mediated changes in the mobility of contaminant metals in soils and sediments, chapter 3. In *Heavy Metal Contamination of Soil: Problems and Remedies*; Ahmad, I., Hayat, S., Pichtel, J., Eds.; Science Publishers, Inc.: Enfield, NH, 2005; pp 43−88.

(2) Wilkins, M. J.; Livens, F. R.; Vaughan, D. J.; Lloyd, J. R. The impact of Fe(III)-reducing bacteria on uranium mobility. *Biogeochemistry* **2006**, *78*, 125–150.
(3) Lovley, D. R. Microbial reduction of iron, manganese, and other metals. *Adv. Agron.* **1995**, *54*, 175–218.
(4) Wall, J. D.; Krumholz, L. R. Uranium reduction. *Annu. Rev. Microbiol.* **2006**, *60*, 149–166.
(5) Liger, E.; Charlet, L.; Van Cappellen, P. Surface catalysis of uranium(VI) reduction by iron(II). *Geochim. Cosmochim. Acta* **1999**, *63*, 2939–2955.
(6) Hua, B.; Xu, H. F.; Terry, J.; Deng, B. L. Kinetics of uranium(VI) reduction by hydrogen sulfide in anoxic aqueous systems. *Environ. Sci. Technol.* **2006**, *40*, 4666–4671.
(7) Anderson, R. T.; Vrionis, H. A.; Ortiz-Bernad, I.; Resch, C. T.; Long, P. E.; Dayvault, R.; Karp, K.; Marutzky, S.; Metzler, D. R.; Peacock, A.; et al. Stimulating the in situ activity of *Geobacter* species to remove uranium from the groundwater of a uranium-contaminated aquifer. *Appl. Environ. Microbiol.* **2003**, *69*, 5884–5891.
(8) North, N. N.; Dollhopf, S. L.; Petrie, L.; Istok, J. D.; Balkwill, D. L.; Kostka, J. E. Change in bacterial community structure during in situ biostimulation of subsurface sediment cocontaminated with uranium and nitrate. *Appl. Environ. Microbiol.* **2004**, *70*, 4911–4920.
(9) Francis, A. J.; Dodge, C. J. Anaerobic microbial remobilization of toxic metals coprecipitated with iron oxide. *Environ. Sci. Technol.* **1990**, *24*, 373–378.
(10) Cambier, P.; Charlatchka, R. Influence of reducing conditions on the mobility of divalent trace metals in soils, chapter 8. In *Fate and Transport of Heavy Metals in the Vadose Zone*; Selim, H. M., Iskandar, I. K., Eds.; Lewis Publishers: Boca Raton, FL, 1999; pp 159−175.
(11) Jakubick, A. T.; Gatzweiler, R.; Mager, D.; Robertson, A. M. The Wismut waste rock pile remediation program of the Ronneburg mining district, Germany. In *Proceedings of the Fourth International Conference on Acid Rock Drainage, Vancouver, BC, Canada, May 31-June 6, 1997*; 1997; pp 1285−1301.
(12) *Umweltbericht WISMUT 2005*; Wismut GmbH: Chemnitz, Germany, 2006.
(13) Küsel, K.; Burkhardt, E.-M.; Sitte, J. Effect of metal-reducing microorganisms on element fluxes in a former uranium-mining district. In *Microbes in the Environment: Perspectives and Challenges*; Liu, S.-J., Drake, H. L., Eds.; Science Press: Beijing, 2008; pp 128−137.
(14) Reiche, M.; Torburg, G.; Küsel, K. Competition of Fe(III) reduction and methanogenesis in an acidic fen. *FEMS Microbiol. Ecol.* **2008**, *65*, 88–101.
(15) Kostka, J. E.; Luther, G. W., III. Partitioning and speciation of solid phase iron in saltmarsh sediments. *Geochim. Cosmochim. Acta* **1994**, *58*, 1701–1710.
(16) Tamura, H.; Goto, K.; Yotsuyan, T.; Nagayama, M. Spectrophotometric determination of iron(II) with 1,10-phenanthroline in presence of large amounts of iron(III). *Talanta* **1974**, *21*, 314–318.
(17) Velghe, N.; Claeys, A. Rapid spectrophotometric determination of nitrate in mineral waters with resorcinol. *Analyst* **1985**, *110*, 313–314.
(18) Harwood, J. E.; Kühn, A. L. A colorimetric method for ammonia in natural waters. *Water Res.* **1970**, *4*, 805–811.
(19) Tabatabai, M. A. Determination of sulfate in water samples. *Sulphur Inst. J.* **1974**, *10*, 11–13.
(20) Küsel, K.; Wagner, C.; Trinkwalter, T.; Gössner, A. S.; Bäumler, R.; Drake, H. L. Microbial reduction of Fe(III) and turnover of acetate in Hawaiian soils. *FEMS Microbiol. Ecol.* **2002**, *40*, 73–81.
(21) Reiche, M.; Hädrich, A.; Lischeid, G.; Küsel, K. Impact of manipulated drought and heavy rainfall events on peat mineralization processes and source-sink functions of an acidic fen. *J. Geophys. Res.* **2009**, *114*, doi:10.1029/2008JG000853.
(22) Akob, D. M.; Mills, H. J.; Kostka, J. E. Metabolically active microbial communities in uranium-contaminated subsurface sediments. *FEMS Microbiol. Ecol.* **2007**, *59*, 95–107.
(23) Edwards, L.; Küsel, K.; Drake, H.; Kostka, J. E. Electron flow in acidic subsurface sediments co-contaminated with nitrate and uranium. *Geochim. Cosmochim. Acta* **2007**, *71*, 643–654.
(24) Zachara, J. M.; Fredrickson, J. K.; Smith, S. C.; Gassman, P. L. Solubilization of Fe(III)-oxide-bound trace metals by a dissimilatory Fe(III) reducing bacterium. *Geochim. Cosmochim. Acta* **2001**, *65*, 75–93.
(25) Peine, A.; Tritschler, A.; Küsel, K.; Peiffer, S. Electron flow in an iron-rich acidic sediment-evidence for an acidity-driven iron cycle. *Limnol. Oceanogr.* **2000**, *45*, 1077–1087.
(26) Tufano, K. J.; Reyes, C.; Saltikov, C. W.; Fendorf, S. Reductive processes controlling arsenic retention: Revealing the relative importance of iron and arsenic reduction. *Environ. Sci. Technol.* **2008**, *42*, 8283–8289.
(27) Islam, F. S.; Boothman, C.; Gault, A. G.; Polya, D. A.; Lloyd, J. R. Potential role of the Fe(III)-reducing bacteria *Geobacter* and *Geothrix* in controlling arsenic solubility in Bengal delta sediments. *Mineral. Mag.* **2005**, *69*, 865–875.
(28) Gorby, Y. A.; Lovley, D. R. Enzymatic uranium precipitation. *Environ. Sci. Technol.* **1992**, *26*, 205–207.
(29) Brooks, S. C.; Fredrickson, J. K.; Carroll, S. L.; Kennedy, D. W.; Zachara, J. M.; Plymale, A. E.; Kelly, S. D.; Kemner, K. M.; Fendorf, S. Inhibition of bacterial U(VI) reduction by calcium. *Environ. Sci. Technol.* **2003**, *37*, 1850–1858.
(30) Neiss, J.; Stewart, B. D.; Nico, P. S.; Fendorf, S. Speciation-dependent microbial reduction of uranium within iron-coated sands. *Environ. Sci. Technol.* **2007**, *41*, 7343–7348.
(31) Tebo, B. M.; Obraztsova, A. Y. Sulfate-reducing bacterium grows with Cr(VI), U(VI), Mn(IV), and Fe(III) as electron acceptors. *FEMS Microbiol. Lett.* **1998**, *162*, 193–198.
(32) Wersin, P.; Hochella, M. F., Jr.; Persson, P.; Redden, G.; Leckie, J. O.; Harris, D. W. Interaction between aqueous uranium (VI) and sulfide minerals: Spectroscopic evidence for sorption and reduction. *Geochim. Cosmochim. Acta* **1994**, *58*, 2829–2843.
(33) Ehrlich, H. L. Microbes as geologic agents: Their role in mineral formation. *Geomicrobiol. J.* **1999**, *16*, 135–153.
(34) Krumholz, L. R.; Elias, D. A.; Suflita, J. M. Immobilization of cobalt by sulfate-reducing bacteria in subsurface sediments. *Geomicrobiol. J.* **2003**, *20*, 61–72.
(35) O'Day, P. A.; Vlassopoulos, D.; Root, R.; Rivera, N. The influence of sulfur and iron on dissolved arsenic concentrations in the shallow subsurface under changing redox conditions. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 13703–13708.
(36) Mandal, D.; Bolander, M. E.; Mukhopadhyay, D.; Sarkar, G.; Mukherjee, P. The use of microorganisms for the formation of metal nanoparticles and their application. *Appl. Microbiol. Biotechnol.* **2006**, *69*, 485–492.
(37) Gramp, J. P.; Bigham, J. M.; Sasaki, K.; Tuovinen, O. H. Formation of Ni- and Zn-sulfides in cultures of sulfate-reducing bacteria. *Geomicrobiol. J.* **2007**, *24*, 609–614.
(38) Cooper, D. C.; Picardal, F. F.; Coby, A. J. Interactions between microbial iron reduction and metal geochemistry: effect of redox cycling on transition metal speciation in iron bearing sediments. *Environ. Sci. Technol.* **2006**, *40*, 1884–1891.
(39) Saunders, J. A.; Lee, M. K.; Shamsudduha, M.; Dhakal, P.; Uddin, A.; Chowdury, M. T.; Ahmed, K. M. Geochemistry and mineralogy of arsenic in (natural) anaerobic groundwaters. *Appl. Geochem.* **2008**, *23*, 3205–3214.
(40) Wu, W. M.; Carley, J.; Luo, J.; Ginder-Vogel, M. A.; Cardenas, E.; Leigh, M. B.; Hwang, C.; Kelly, S. D.; Ruan, C.; Wu, L.; et al. In situ bioreduction of uranium (VI) to submicromolar levels and reoxidation by dissolved oxygen. *Environ. Sci. Technol.* **2007**, *41*, 5716–5723.
(41) Lack, J. G.; Chaudhuri, S. K.; Kelly, S. D.; Kemner, K. M.; O'Connor, S. M.; Coates, J. D. Immobilization of radionuclides and heavy metals through anaerobic bio-oxidation of Fe(II). *Appl. Environ. Microbiol.* **2002**, *68*, 2704–2710.
(42) Webb, S. M.; Fuller, C. C.; Tebo, B. M.; Bargar, J. R. Determination of uranyl incorporation into biogenic manganese oxides using x-ray absorption spectroscopy and scattering. *Environ. Sci. Technol.* **2006**, *40*, 771–777.
(43) Zehl, K.; Einax, J. W. Influence of atmospheric oxygen on heavy metal mobility in sediment and soil. *J. Soils Sed.* **2005**, *5*, 164–170.
(44) Coates, J. D.; Ellis, D. J.; Gaw, C. V.; Lovley, D. R. *Geothrix fermentans* gen. nov., sp. nov., a novel Fe(III)-reducing bacterium from a hydrocarbon-contaminated aquifer. *Int. J. Syst. Bacteriol.* **1999**, *49*, 1615–1622.
(45) Cardenas, E.; Wu, W. M.; Leigh, M. B.; Carley, J.; Carroll, S.; Gentry, T.; Luo, J.; Watson, D.; Gu, B.; Ginder-Vogel, M.; et al. Microbial communities in contaminated sediments, associated with bioremediation of uranium to submicromolar levels. *Appl. Environ. Microbiol.* **2008**, *74*, 3718–3729.
(46) Brodie, E. L.; DeSantis, T. Z.; Joyner, D. C.; Baek, S. M.; Larsen, J. T.; Andersen, G. L.; Hazen, T. C.; Richardson, P. M.; Herman, D. J.; Tokunaga, T. K.; et al. Application of a high-density oligonucleotide microarray approach to study bacterial population dynamics during uranium reduction and reoxidation. *Appl. Environ. Microbiol.* **2006**, *72*, 6288–6298.
(47) Luo, W.; Wu, W. M.; Yan, T.; Criddle, C. S.; Jardine, P. M.; Zhou, J.; Gu, B. Influence of bicarbonate, sulfate, and electron donors on biological reduction of uranium and microbial community composition. *Appl. Microbiol. Biotechnol.* **2007**, *77*, 713–721.

(48) Lovley, D. Dissimilatory Fe(III)- and Mn(IV)-Reducing Prokaryotes. In *The Prokaryotes, Volume 2: Ecophysiology and Biochemistry*; Dworkin, M., Falkow, S., Rosenberg, E., Schleifer, K.-H., Stackebrandt, E., Eds.; Springer: New York, 2006; pp 635−658.
(49) Akob, D. M.; Mills, H. J.; Gihring, T. M.; Kerkhof, L.; Stucki, J. W.; Anastácio, A. S.; Chin, K. J.; Küsel, K.; Palumbo, A. V.; Watson, D. B.; Kostka, J. E. Functional diversity and electron donor dependence of microbial populations capable of U(VI) reduction in radionuclide-contaminated subsurface sediments. *Appl. Environ. Microbiol.* **2008**, *74*, 3159–3170.
(50) Gao, W.; Francis, A. J. Reduction of uranium(VI) to uranium(IV) by *Clostridia*. *Appl. Environ. Microbiol.* **2008**, *74*, 4580–4584.
(51) N'Guessan, A. L.; Vrionis, H. A.; Resch, C. T.; Long, P. E.; Lovley, D. R. Sustained removal of uranium from contaminated groundwater following stimulation of dissimilatory metal reduction. *Environ. Sci. Technol.* **2008**, *42*, 2999–3004.
(52) Lovley, D. R.; Phillips, E. J. P.; Lonergan, D. J.; Widman, P. K. Fe(III) and S0 reduction by *Pelobacter carbinolicus*. *Appl. Environ. Microbiol.* **1995**, *61*, 2132–2138.
(53) Haveman, S. A.; DiDonato, R. J., Jr.; Villanueva, L.; Shelobolina, E. S.; Postier, B. L.; Xu, B.; Liu, A.; Lovley, D. R. Genome-wide gene expression patterns and growth requirements suggest that *Pelobacter carbinolicus* reduces Fe(III) indirectly via sulfide production. *Appl. Environ. Microbiol.* **2008**, *74*, 4277–4284.
(54) Mohanty, S. R.; Kollah, B.; Hedrick, D. B.; Peacock, A. D.; Kukkadapu, R. K.; Roden, E. E. Biogeochemical processes in ethanol stimulated uranium-contaminated subsurface sediments. *Environ. Sci. Technol.* **2008**, *42*, 4384–4390.
(55) Horn, M. A.; Ihssen, J.; Matthies, C.; Schramm, A.; Acker, G.; Drake, H. L. *Dechloromonas denitrificans* sp. nov., *Flavobacterium denitrificans*, sp. nov., *Paenibacillus anaericanus* sp. nov. and *Paenibacillus terrae* strain MH72, N_2O-producing bacteria isolated from the gut of the earthworm *Aporrectodea caliginosa*. *Int. J. Syst. Evol. Microbiol.* **2005**, *55*, 1255–1265.
(56) Chung, J.; Ryu, H.; Abbaszadegan, M.; Rittmann, B. E. Community structure and function in a H_2-based membrane biofilm reactor capable of bioreduction of selenate and chromate. *Appl. Microbiol. Biotechnol.* **2006**, *72*, 1330–1339.
(57) Cummings, D. E.; Caccavo, F., Jr.; Spring, S.; Rosenzweig, R. F. *Ferribacterium limneticum*, gen. nov., sp. nov., an Fe(III)-reducing microorganism isolated from mining-impacted freshwater lake sediments. *Arch. Microbiol.* **1999**, *171*, 183–188.
(58) Shivaji, S.; Ray, M. K.; Kumar, G. S.; Reddy, G. S. N.; Saisree, L.; Wynn-Williams, D. D. Identification of *Janthinobacterium lividum* from the soils of the island of Scotia Ridge and from Antarctic peninsula. *Polar Biol.* **1991**, *11*, 267–271.
(59) Hashidoko, Y.; Takakai, F.; Toma, Y.; Darung, U.; Melling, L.; Tahara, S.; Hatano, R. Emergence and behaviors of acid-tolerant *Janthinobacterium* sp. that evolves N_2O from deforested tropical peatland. *Soil Biol. Biochem.* **2008**, *40*, 116–125.
(60) Holmes, D. E.; Finneran, K. T.; O'Neil, R. A.; Lovley, D. R. Enrichment of members of the family *Geobacteraceae* associated with stimulation of dissimilatory metal reduction in uranium-contaminated aquifer sediments. *Appl. Environ. Microbiol.* **2002**, *68*, 2300–2306.
(61) Spring, S.; Rosenzweig, F. The genera *Desulfitobacterium* and *Desulfosporosinus*: Taxonomy. In *The Prokaryotes, Volume 4: Bacteria: Firmicutes, Cyanobacteria*; Dworkin, M., Falkow, S., Rosenberg, E., Schleifer, K.-H., Stackebrandt, E., Eds.; Springer: New York, 2006; pp 771−786.

ES902038E