

# Nitrate-Stimulated Release of Naturally Occurring Sedimentary Uranium

Jeffrey P. Westrop, Pooja Yadav, PJ Nolan, Kate M. Campbell, Rajesh Singh, Sharon E. Bone, Alicia H. Chan, Anthony J. Kohtz, Donald Pan, Olivia Healy, John R. Bargar, Daniel D. Snow, and Karrie A. Weber\*



Cite This: <https://doi.org/10.1021/acs.est.2c07683>



Read Online

ACCESS |



Metrics & More



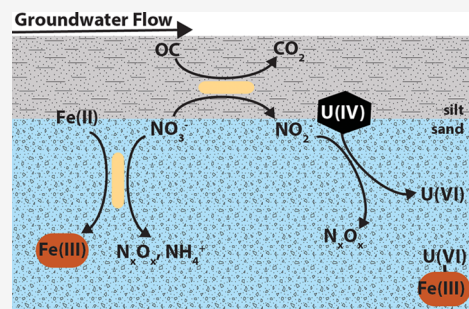
Article Recommendations



Supporting Information

**ABSTRACT:** Groundwater uranium (U) concentrations have been measured above the U.S. EPA maximum contaminant level (30  $\mu\text{g/L}$ ) in many U.S. aquifers, including in areas not associated with anthropogenic contamination by milling or mining. In addition to carbonate, nitrate has been correlated to uranium groundwater concentrations in two major U.S. aquifers. However, to date, direct evidence that nitrate mobilizes naturally occurring U from aquifer sediments has not been presented. Here, we demonstrate that the influx of high-nitrate porewater through High Plains alluvial aquifer silt sediments bearing naturally occurring U(IV) can stimulate a nitrate-reducing microbial community capable of catalyzing the oxidation and mobilization of U into the porewater. Microbial reduction of nitrate yielded nitrite, a reactive intermediate, which was further demonstrated to abiotically mobilize U from the reduced alluvial aquifer sediments. These results indicate that microbial activity, specifically nitrate reduction to nitrite, is one mechanism driving U mobilization from aquifer sediments in addition to previously described bicarbonate-driven desorption from mineral surfaces, such as Fe(III) oxides.

**KEYWORDS:** microbial nitrate reduction, uranium oxidation



## INTRODUCTION

Alluvial aquifers serve as municipal and private drinking water sources for populations world-wide<sup>1</sup> and are subject to anthropogenic and geogenic contamination, resulting in decreased drinking water quality. One such geogenic contaminant recently recognized in groundwater is uranium.<sup>2</sup> Uranium is a naturally occurring trace element within the Earth's crust (3 mg/kg average abundance<sup>3</sup>) that is released geogenically from subsurface sediments into groundwater.<sup>4–7</sup> In alluvial aquifers, U tends to be deposited in fine-grained, organic-rich sediment lithologies such as silts or clays, as reduced U(IV) oxide minerals such as uraninite ( $\text{UO}_2$ ), or as amorphous U(IV) phases.<sup>8,9</sup>

Alluvial aquifer sediments are physically and chemically heterogeneous owing to fluvial depositional processes. This setting creates a heterogeneous subsurface with sharp boundaries between fine-grained, organic-rich sediments and coarser-grained sediments.<sup>10</sup> Due to their high organic carbon content and low permeability, fine-grained sediments tend to become anoxic once they become waterlogged during burial<sup>8,9</sup> often leading to the accumulation of U during diagenesis. U predominantly exists as U(VI) in oxic sediments. In the presence of minerals, U(VI) adsorbs to the surface and is easily solubilized in the presence of carbonate.<sup>11</sup> Under reducing conditions, U forms sparingly soluble phases such as uraninite, amorphous  $\text{UO}_2$  or U–organic matter complexes.<sup>12,13</sup> As a

result, environmental redox state has a strong impact on mobility of U in natural systems owing to the control in the U valence state and species solubility. At circumneutral pH and under oxidizing conditions, U generally exists as a soluble U(VI) species.<sup>14</sup> However, it is worth noting that high cation (i.e., calcium) and anion (i.e., carbonate or phosphate) concentrations in natural groundwaters can favor the precipitation of solid-phase uranyl minerals.<sup>15,16</sup> Conversely, when U(VI) encounters reducing conditions in low-permeability, organic-rich clay layers, it will precipitate as reduced U(IV) phases.<sup>8,12,17</sup> In addition, U(VI) will strongly adsorb onto reactive sediment surfaces, such as Fe(III) oxides or aluminosilicate clays.<sup>18–22</sup> However, as groundwater ages, dissolution of carbonate minerals and microbial respiration reactions may increase the bicarbonate alkalinity, resulting in the formation of U(VI)–carbonate and ternary U(VI)–Ca–carbonate complexes,<sup>23–27</sup> which are readily soluble in groundwater and thus highly mobile. Therefore, the combina-

**Received:** October 18, 2022

**Revised:** February 10, 2023

**Accepted:** February 13, 2023



ACS Publications

© XXXX American Chemical Society

A

<https://doi.org/10.1021/acs.est.2c07683>  
Environ. Sci. Technol. XXXX, XXX, XXX–XXX

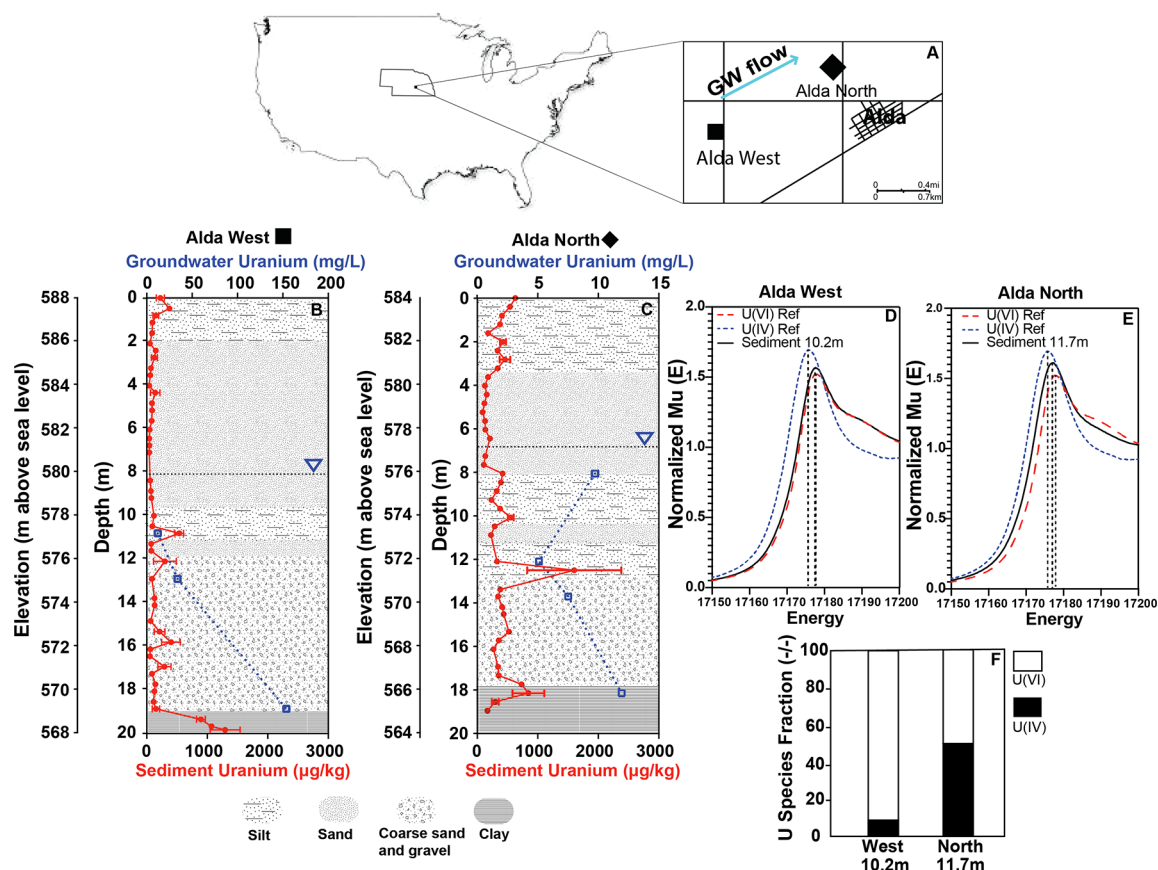


**Figure 1.** Uranium distribution in a shallow alluvial aquifer. (A) Intact sediment cores and groundwater collected from two locations within an alluvial aquifer in the Platte River Valley, located near Alda, Nebraska (USA). Direction of groundwater flow is denoted by the arrow (SW to NE). (B) Sediment cores collected from the SW location denoted as west and (C) NE location denoted as North determined the subsurface lithology and sediment associated total uranium (red circles) with the collection of groundwater from the borehole to determine dissolved uranium (blue squares). Dashed line and blue triangle denote the water table level at the time of sampling. (D,E) Sediments with the highest measured U concentration (10.2 m bgs) were selected to determine the valence state of sediment-associated uranium using XANES analysis and used in laboratory experiments. (F) Fraction of U(IV) and U(VI) identified in the sediments was determined using linear combination fitting of XANES spectra.

tion of oxidation and the formation of bicarbonate species has the potential to mobilize naturally occurring U in alluvial aquifers and is recognized as playing an important role in the mobility of naturally occurring uranium.<sup>15,28,29</sup>

In reduced, organic-rich lithologies where U(IV) phases are present, the influx of oxidants will influence U mobility. Nitrate is a strong oxidant and a common anthropogenic groundwater contaminant.<sup>30</sup> To date, published experiments that have investigated nitrate driving uranium mobilization have been conducted either at anthropogenically contaminated sites impacted by U mining,<sup>31,32</sup> milling,<sup>33–35</sup> and nuclear waste processing sites<sup>36</sup> or in laboratory batch culture studies amended with uranium.<sup>37–40</sup> These studies have demonstrated that abiotic oxidation of U by nitrate is kinetically hindered, but through the production of microbial nitrate reduction intermediate species, such as nitrite or nitrous oxide,<sup>37,39,41</sup> or through the formation of Fe(III)-(hydr)oxides,<sup>42,43</sup> U(IV) can be oxidized and mobilized. Given the prevalence of anthropogenic nitrate contamination in groundwater passing through alluvial aquifer sediments, this process has the potential to mobilize and release naturally sequestered U into groundwater. Here, we experimentally test the hypothesis that microbial nitrate reduction stimulates the mobilization of naturally occurring uranium from reduced sediments in

addition to bicarbonate leaching. A series of upflow column reactors were packed with alluvial aquifer sediment containing naturally occurring U(IV)-bearing minerals and amended the bicarbonate buffered influent with nitrate or nitrite, nitrate or nitrite and a microbial respiratory inhibitor, azide, or remained unamended. Metagenome assembled genomes (MAGs) were used to identify microbial taxa and the metabolic potential for nitrogen and metal metabolisms within the microbial community to further support the observed geochemical data. Together, the geochemical data and metabolic potential data further suggested that the precipitation of Fe(III) oxides could lead to U retention in the sediments via adsorption. Uranium retention on reduced and oxidized sediments was assessed through a series of batch adsorption experiments and the development of a surface complexation model (SCM). Given the prevalence of anthropogenic nitrate contamination in groundwater passing through alluvial aquifer sediments, this process has the potential to periodically mobilize and release naturally sequestered U into groundwater.

## MATERIALS AND METHODS

**Alluvial Aquifer Site Geological Description.** Alluvial aquifer subsurface sediment cores and groundwater were collected from two sites within the Platte River drainage basin

in the High Plains aquifer located in Central Nebraska, USA (Figure 1). The High Plains aquifer is an unconfined aquifer composed of multiple hydrologically connected Oligocene to Holocene alluvial aquifer units.<sup>44</sup> Groundwater flows from west to east and discharges into streams and major rivers, including the Platte River.<sup>44</sup> Previous work in the region demonstrated that these undifferentiated sands, silts, and gravels are laterally continuous in the study area.<sup>45</sup> Cores were collected from the study area, where the most significant water-bearing formations are Pleistocene to Holocene-aged undifferentiated gravels, sands, and silts deposited by Platte River migration.<sup>44</sup> Groundwater flows northeast following the Platte River channel,<sup>44,45</sup> and the water table depth at the time of sampling was 7.01 m below ground surface (bgs) and 8.22 m bgs at Alda North (40.877763, -98.475085) and West (40.869933–98.493119), respectively.

**Sediment Core and Groundwater Collection.** One 18 meter core from each site was collected in 1.5 m sections using a direct-push technique via Geoprobe coring into sterile (70% ethanol rinse followed by a bleach rinse, 0.6% hypochlorite final concentration) core liners. Upon collection cores were immediately capped, paraffin wax sealed, and placed into gas tight PVC tubes flushed with 100% Ar gas to minimize oxidation of reduced chemical species, esp. Fe(II) and U(IV). Cores were transported on ice to the Weber Laboratory at the University of Nebraska Lincoln (UNL) for processing under an anoxic atmosphere (85:15:5; N<sub>2</sub>/CO<sub>2</sub>/H<sub>2</sub>) in a vinyl anaerobic chamber instrumented with a H<sub>2</sub> and O<sub>2</sub> meter (Coy Instruments, Glass Lake MI). Any core in which the core liner cracked was disposed and omitted as oxidation of Fe(II) and precipitation of Fe(III) oxides was observed.

The aquifer water level in the borehole was determined using a capacitive water level probe (Solnist, Georgetown, ON). Groundwater was collected by bailing immediately after core collection.<sup>46</sup> Bailing was chosen as the fastest method to obtain samples in order to avoid redox changes and to comply with the sampling permit. No organoleptic peculiarities, such as the smell of sulfur, were observed at the time of sampling. ORP,<sup>47</sup> pH,<sup>48</sup> and dissolved oxygen (DO)<sup>49</sup> were immediately measured using calibrated field meters and electrodes. Samples collected for measurement of cations and metals/radionuclides were filtered (0.2 μm nylon) and immediately acidified. Both filtered (0.2 μm nylon) and unfiltered samples were acidified with 0.5 M HCl (pH < 2) in the field for determination of dissolved Fe(II) and total iron, as previously described.<sup>50</sup> Additional groundwater samples were filtered through 0.2 μm nylon filters and transported on ice for analysis of dissolved ions, including nitrate, nitrite, and ammonium. Samples collected for dissolved organic carbon and dissolved inorganic carbon (DIC) were filtered [0.2 μm polyvinylidene difluoride (PVDF) filters], and transported on ice to the UNL.

Cores were removed from the argon atmosphere and immediately placed in the anaerobic chamber. Once O<sub>2</sub> concentrations within the anaerobic chamber were measured at 0 ppm, cores were processed. Sediment lithologic properties (color, grain size, and sorting) were logged, and 1.25-foot sections were homogenized and placed in autoclaved mason jars. Homogenized sediments were immediately analyzed for pH, ORP, sediment carbon (total and organic), iron (Fe(II) and total Fe), and total U contents.<sup>15</sup> The remaining sediment was vacuum-sealed and stored in the dark at 4 °C. The pH and ORP were measured using calibrated electrodes in a slurry made with anoxic (sparged with 100% N<sub>2</sub>) double-deionized

(DDI) water.<sup>15</sup> Sediment carbon content was measured using an elemental analyzer (Shimadzu).<sup>15</sup> Iron and U content were also measured via inductively coupled plasma mass spectrometry (ICP–MS) from triplicate aliquots of microwave-digested sediment.<sup>15</sup>

**Geochemical Methods.** Field-collected water samples were analyzed to quantify DO using an InLab 605 electrode (Mettler Toledo, Columbus, Ohio), and pH and ORP were collected using field meters (VWR H30CO; Mettler-Toledo SevenGo pro) by correction to the standard hydrogen electrode potential. Experimental effluent water samples were analyzed for ORP and pH using a calibrated meter with a microelectrode (M-414, Microelectrodes Inc., Bedford, NH) inside the anaerobic chamber. Aqueous U(VI) concentration (0.2 μm nylon filter) was measured by kinetic phosphorescence analysis (KPA, Chemchek Inc., Richmond, WA)<sup>51</sup> with a detection limit of 1 μg/L. Total aqueous U concentration was measured on filtered nitric acid-acidified (pH < 2) samples using an iCAP RQ ICP–MS (Thermo Scientific, Sunnyvale, CA) at the UNL Water Sciences Laboratory (WSL)<sup>52</sup> with a detection limit of 0.1 μg/L. Anions, including chloride, bromide, nitrate, sulfate, and phosphate concentrations, were measured via ion chromatography using a Dionex ICS 3000 with IonPac AS9-HC column (Thermo Scientific, Sunnyvale, CA).<sup>53</sup> Nitrite concentration was measured using the sulfamide colorimetric assay,<sup>54</sup> aqueous Fe(II) concentration was determined following filtration (0.2 μm nylon filter) directly into ferrozine,<sup>55</sup> and the total Fe concentration was measured following acidification in 0.5 N HCl on a CARY 50 UV/VIS Spectrophotometer. Sediment Fe content was determined by adding samples to 3 N HCl and extracting for 48 h at room temperature.<sup>56</sup> The extract was removed and added to ferrozine<sup>55</sup> to determine solid-phase Fe(II) and total Fe and was measured following a hydroxylamine reduction step as previously described<sup>57–59</sup> on a Varian CARY 50 UV/VIS (Agilent technologies, Santa Clara CA). DIC was determined as previously described.<sup>53</sup> Dissolved organic carbon was measured by wet chemical oxidation<sup>60</sup> at the UNL WSL. Sediment U content was determined by digesting samples in 10% nitric acid for 72 h at 80 °C<sup>61</sup> and measuring the U concentration via ICP–MS, as described above.<sup>52</sup>

**U Valence State Analysis Using X-Ray Absorption Near-Edge Structure Spectroscopy.** Sediment samples determined to contain the highest concentration of U were selected for further valence state characterization. Sediment from one core representative of each site (silt layers at 10.2–10.7 m depth from the Alda West and 11.7–12.1 m from the Alda North sites) was sonically sieved (Advantech L3P, Advantech Manufacturing) under anoxic conditions (85:15:5; N<sub>2</sub>/CO<sub>2</sub>/H<sub>2</sub>) to collect a fine fraction (<0.02 μm), stored under a vacuum, and sent to the Stanford Synchrotron Radiation Lightsource (SSRL) for uranium L<sub>III</sub>-edge X-ray absorption near-edge structure (XANES) spectroscopy, which was measured under vacuum in a liquid nitrogen cryostat (77 K), as previously described.<sup>15</sup> The averaged, calibrated absorption spectra were normalized using ATHENA<sup>62,63</sup> by fitting a linear function to the region between –120 and –65 eV below the edge and a linear function to the region between 100 and 300 eV above the edge. The normalized XANES spectra (μE) were fit from 17,151 to 17,201 eV using linear combinations of reference spectra to determine the oxidation state of U in the samples. U(VI) adsorbed to ferrihydrite was

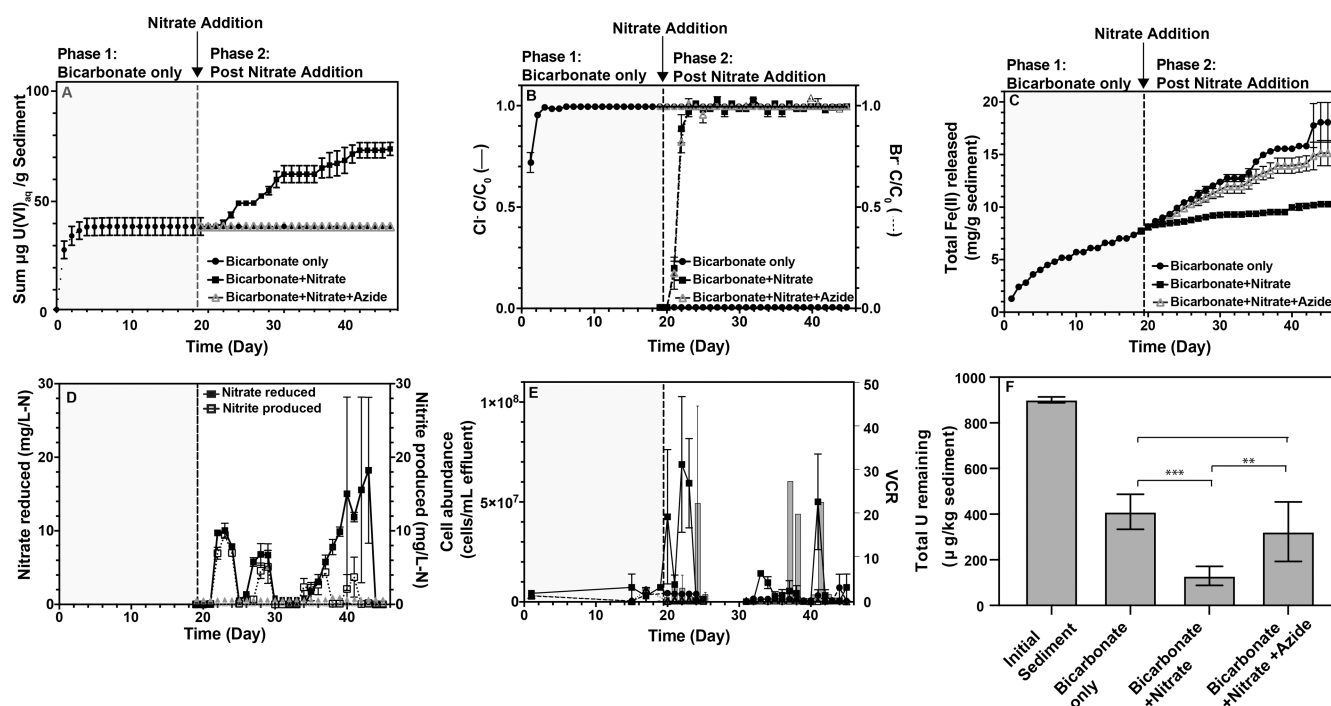


**Figure 2.** Mobilization of naturally occurring uranium from upflow-column reactors in response to a nitrate amendment replicating the sand and silt lithologic interface at the Alda North site (1.7–12.2 m). After 19 days of bicarbonate-buffered artificial groundwater medium inflow (Phase 1), two columns were amended with nitrate, two columns were amended with nitrate and sodium azide, and two columns remained unamended (Phase 2, denoted by arrow and dashed line). (A) Total cumulative (sum) mass of dissolved uranium eluted from the reactor (measured by KPA) normalized to sediment mass across time. (B) Conservative tracers chloride (Phase 1; solid line) and bromide (Phase 2; dashed line). (C) Total cumulative (sum) mass of Fe(II) eluted from the column normalized to sediment mass. (D) Mass of measured nitrate lost (nitrate<sub>influent</sub> – nitrate<sub>effluent</sub>) and nitrite effluent concentrations. Nitrate solid symbols and nitrite open symbols. (E) Microbial cell and virus abundance, where the VCR is depicted as bar graphs from the nitrate-amended column. Samples below detection, where samples are not reported, including control treatments. (F) Total sediment-associated uranium from the silt after 45 days of treatment, relative to the total sediment associated uranium initially measured in the sediments. Statistically significant differences between treatments are denoted by asterisks (\*\*\*)  $p < 0.001$ ; (\*\*)  $p < 0.01$ ; (\*)  $p < 0.05$  as determined by one-way ANOVA followed by a Tukey post-hoc analysis. (a–f) Error bars denote the standard error of measurement for duplicate treatments.

used as the reference for U(VI), and biogenic uraninite (UO<sub>2</sub>) produced by *Shewanella oneidensis* MR-1 was used for U(IV) to determine the U oxidation state.<sup>64</sup>

**Experimental Up-Flow Column Reactors.** Sediment collected at Alda North at a depth of 11.7 m was selected for further experimentation based on the measured U sediment concentration and XANES confirmation of U(IV) (Figure 1). Six glass chromatography columns (Santa Cruz Biotechnology, 10 cm × 2.54 cm for internal volume of ~49 cm<sup>3</sup>) were packed in the anaerobic chamber with 6 N hydrochloric acid-washed combusted (450 °C for 4 h), autoclaved sand (50/70-mesh quartz sand; BET surface area = 2.0 cm<sup>2</sup>/g), and sediments bearing naturally reduced U(IV) to develop an interface between sand and sediment representative of lithologic interfaces in the aquifer (Figure S1). Approximately 33.7 (±0.6) g of sediment was added to each column. All column materials were sterilized prior to packing with sediment by autoclaving, where possible, or 70% ethanol followed by ultraviolet light sterilization.

To determine the effect of bicarbonate driven desorption of U(VI) from the sediment surface, columns were pre-equilibrated in bicarbonate buffered artificial groundwater (Phase 1) prior to nitrate or nitrite amendments (Phase 2). Anoxic (80:20; N<sub>2</sub>/CO<sub>2</sub>) bicarbonate-buffered (1220 mg/L) artificial groundwater medium (AGWM; 0.235 g/L KH<sub>2</sub>PO<sub>4</sub>, 0.09 g/L KHPO<sub>4</sub>, 2.52 g/L NaHCO<sub>3</sub>, 0.1 g/L NH<sub>4</sub>Cl, 0.0248

g/L MgCl<sub>2</sub>, 0.141 g/L CaCl<sub>2</sub>, 5 mL vitamin, and 5 mL mineral mix<sup>65</sup>) influent was delivered at a net 10 cm/day, equivalent to the natural groundwater flow rate in the High Plains aquifer in this region<sup>66</sup> to columns in the anaerobic chamber with an oxygen-free atmosphere to maintain a CO<sub>2</sub> headspace (85:15:5; N<sub>2</sub>/CO<sub>2</sub>/H<sub>2</sub>) to maintain pH. Once bicarbonate-driven U desorption was complete and effluent U levels stabilized below 1 µg/L (Phase 1), duplicate columns received AGWM amended with 28 mg/L-N nitrate (as sodium nitrate) or 15 mg/L-N nitrite (as sodium nitrite) in Phase 2 of the experiment. Another set of duplicate columns received AGWM amended with 28 mg/L-N nitrate or 15 mg/L-N nitrite and 42 mg/L azide. Azide is an electron transport chain inhibitor and will suppress respiratory metabolisms, including nitrate reduction.<sup>67–69</sup> The two remaining duplicate columns remained unamended. Concentrations of nitrate were added based on field measured concentrations, and nitrite was added at levels observed during Phase 2 of the experiments presented here (Figure 2). Chloride served as the conservative tracer during the pre-equilibration bicarbonate-driven desorption phase, whereas added bromide served as a conservative tracer during the second phase in all columns. Columns were maintained in the anaerobic chamber under an anoxic atmosphere (85:15:5; N<sub>2</sub>/CO<sub>2</sub>/H<sub>2</sub>) for 45 days. Effluent was collected daily (when possible) in sterile 50 mL serum vials to measure geochemical parameters and collect samples for cell

and virus abundance. Briefly, unfiltered samples were measured for pH and ORP and preserved for cell and virus enumeration. Effluent filtered through a 0.2  $\mu\text{m}$  nylon filter was measured for DIC, aqueous Fe(II), major ions, U(VI), and total aqueous U.

Effluent samples were collected and filtered through low-protein binding PVDF filters (0.45 and 0.2  $\mu\text{m}$  for cells and viruses, respectively), fixed with electron microscopy grade glutaraldehyde (0.5% final concentration) for 15–30 min at 4  $^{\circ}\text{C}$ , and flash frozen in liquid  $\text{N}_2$  before storage at  $-80^{\circ}\text{C}$ . Cells and viruses were enumerated using epifluorescence microscopy. Frozen samples were thawed, diluted in TE buffer (pH 8.0), and filtered onto 0.2  $\mu\text{m}$  black polycarbonate filters. Viruses (<0.2  $\mu\text{m}$ ) were collected onto a 0.02  $\mu\text{m}$  Anodisc filter.<sup>70,71</sup> Filters were stained with SYBR Green I (1:80 dilution)<sup>70</sup> and enumerated as previously described.<sup>70,71</sup>

Columns were destructively sampled in the anaerobic chamber under an anoxic atmosphere upon conclusion of the experiment. A 1.5 cm-diameter mini-core was taken through the center of the column. Sediment and sand were digested<sup>72</sup> for total U and total Fe analysis,<sup>52</sup> as described above. In the nitrate experiment, the core was divided in half, and 5 g was preserved at  $-80^{\circ}\text{C}$  for microbial community analysis.

**U Adsorption and SCM.** Equilibrium adsorption experiments were conducted using naturally occurring reduced and air-oxidized sediments from the Alda North (11.7 m) site to measure changes in sedimentary adsorption capacity resulting from oxidation and oxide precipitation. Molecular oxygen was added as the oxidant owing to its rapid oxidation kinetics and ease of removal following mineral oxidation. The surface area of dried sediments was measured using a BET nitrogen adsorption isotherm method at  $-196.2^{\circ}\text{C}$  (Micromeritics ASAP 2020, Micromeritics, Inc), as previously described.<sup>15</sup> Sieved (2 mm) sediment were added to acid-washed serum bottles in the anaerobic chamber with 10 mL of anoxic AGWM (pH 7.20, 54,000 ppm  $\text{CO}_2$ ).<sup>73</sup> Ionic strength was set at a value of (0.04 mol/kgw) by adding an anoxic (100%  $\text{N}_2$  as atmosphere) solution of dissolved 1 M KCl. Sediments in serum bottles for anoxic experiments were sparged with  $\text{N}_2/\text{CO}_2$  (85:15) for 5 min, while sediments in oxic experiments were continuously sparged with compressed Air/ $\text{CO}_2$  (85:15) for 5 min. DIC was immediately measured,<sup>53</sup> and atmospheric  $\text{CO}_2$  was adjusted to achieve a final DIC concentration of 0.5 g/L. DIC was measured by adding 1 mL of filtered sample (0.2  $\mu\text{m}$ , nylon membrane) to a degassed serum vial (100% Ar atmosphere) containing 1 mL 3 N HCl, and the amount of headspace  $\text{CO}_2$  was measured via GC-TCD (Varian GC-3900) and used to calculate DIC. Sediments were pre-equilibrated in AGWM for 36 h on an orbital shaker (30 rpm) at 21  $^{\circ}\text{C}$ . After pre-equilibration, samples were collected and filtered (0.2  $\mu\text{m}$  nylon membrane) for measurement of pH, anions, DIC, and total U via ICP-MS as described above. Pre-equilibrated bottles were spiked with an anoxic solution of U(VI) (100%  $\text{N}_2$  atmosphere) prepared from  $\text{UO}_2\text{Cl}_2$  (SPI supplies) to achieve a final concentration ranging from 0.010 to 0.285 mg/L U. Similar to pre-equilibration, bottles were incubated on an orbital shaker (30 rpm, 21  $^{\circ}\text{C}$ ) for 36 h. After 36 h samples were anoxically removed and filtered (0.2  $\mu\text{m}$ , nylon). The filtrate was measured for pH, DIC, and total U. Unfiltered samples were extracted for sediment Fe and U in either 3 N HCl for 24 h or 10% nitric acid for 72 h, respectively.

A nonelectrostatic-generalized SCM<sup>74,75</sup> was developed using the equilibrium adsorption experimental data. Data were fit using PHREEQC,<sup>76</sup> with sorption mass action

constants optimized by coupling PHREEQC to UCODE<sup>77</sup> as described in the [Supporting Information](#).

**DNA Extraction, Sequencing, and Bioinformatic Analyses.** DNA from a known sediment mass was collected following column deconstruction, extracted as previously described,<sup>78</sup> purified with polyethylene glycol 6000, and precipitated with ultrapure glycogen.<sup>78</sup> DNA concentration was quantified using the Qubit DNA 1 $\times$  HS assay according to the manufacturer's specifications, and DNA purity was determined using the 260/280 (nm) ratio in a Hellma TrayCell on a CARY 50. All samples yielded sufficient DNA for shotgun metagenomic sequencing, with the exception of one replicate column treated with the electron transport inhibitor, azide. A shotgun metagenomic sequencing library was prepared using the Nextera XT DNA library kit. Equimolar pooling of libraries was performed based on QC values and sequenced on an Illumina NovaSeq S4 (Illumina, California, USA) with a read length configuration of 150 PE for 40 M PE reads (20 M in each direction) per sample. The quality of shotgun metagenomic raw sequence reads was checked using FastQC. Low quality bases and Illumina adapters were filtered using Trimmomatic (v.0.39)<sup>79</sup> using default parameters and contigs (1000 bp in length) coassembled by MEGAHIT (v.1.2).<sup>80</sup> Clean reads were mapped to assembled contigs utilizing Bowtie (v.2.3).<sup>81</sup> Binning was performed using Metabat2 (v2.15),<sup>82</sup> Maxbin2 (v2.2),<sup>83</sup> and CONCOCT (v1.1)<sup>84</sup> followed by dereplication using DasTools.<sup>85</sup> MAGs comprising contamination were manually curated with Anvi'o (v.7).<sup>86</sup> Completion and contamination of MAGs were assessed by CheckM (v.1.1).<sup>87</sup> MAG genome coverage was normalized against the total number of metagenomic reads in each sample, as previously described.<sup>88</sup> MAGs were then taxonomically assigned using the GTDB database (2021.04.26)<sup>89</sup> and annotated using DRAM (v1.2),<sup>90</sup> and FeGenie (v1.0).<sup>91</sup> Putative virus contigs in the metagenome were identified using VirSorter2 (v2.1) in MAGs (--high-confidence-only--hallmark-required--prep-for-dramv).<sup>92</sup> Virus infection in the MAGs was validated using clustered regularly interspaced short palindromic repeat (CRISPR) sequences utilizing CRISPRCasFinder (v.2.20).<sup>93</sup>

**Statistical Analysis.** Differences between the mean sediment uranium concentrations between treatments were assessed using a one-way ANOVA test followed by a Tukey post-hoc analysis in R (CRAN project) using the vegan package.<sup>94</sup>

## RESULTS AND DISCUSSION

**Microbial Nitrate Reduction-Stimulated Mobilization of Naturally Occurring Uranium from Alluvial Aquifer Sediments.** In this region of the High Plains aquifer, where sediment and groundwater were collected, groundwater flows from SW to NE (Figure 1).<sup>44</sup> A redox gradient was observed spatially, with higher DO, nitrate, and U(VI) concentrations and low Fe(II) concentrations in groundwater at Alda West (Figure 1, Table S1; Snow, 1996). Elevated levels of groundwater U (max 147.1  $\mu\text{g/L} \pm 0.8$ ) and nitrate (27 mg/L-N  $\pm 0.4$ ) concentrations were measured upgradient (Figure 1, Table S1), which is consistent with historic measurements.<sup>2,45</sup> The collected cores and groundwater captured the established redox gradient, which decreased in the direction of groundwater flow from Alda West to Alda North. Measured sedimentary U was highest in fine-grained silt and clay layers (Figure 1). XANES analysis of U valence state

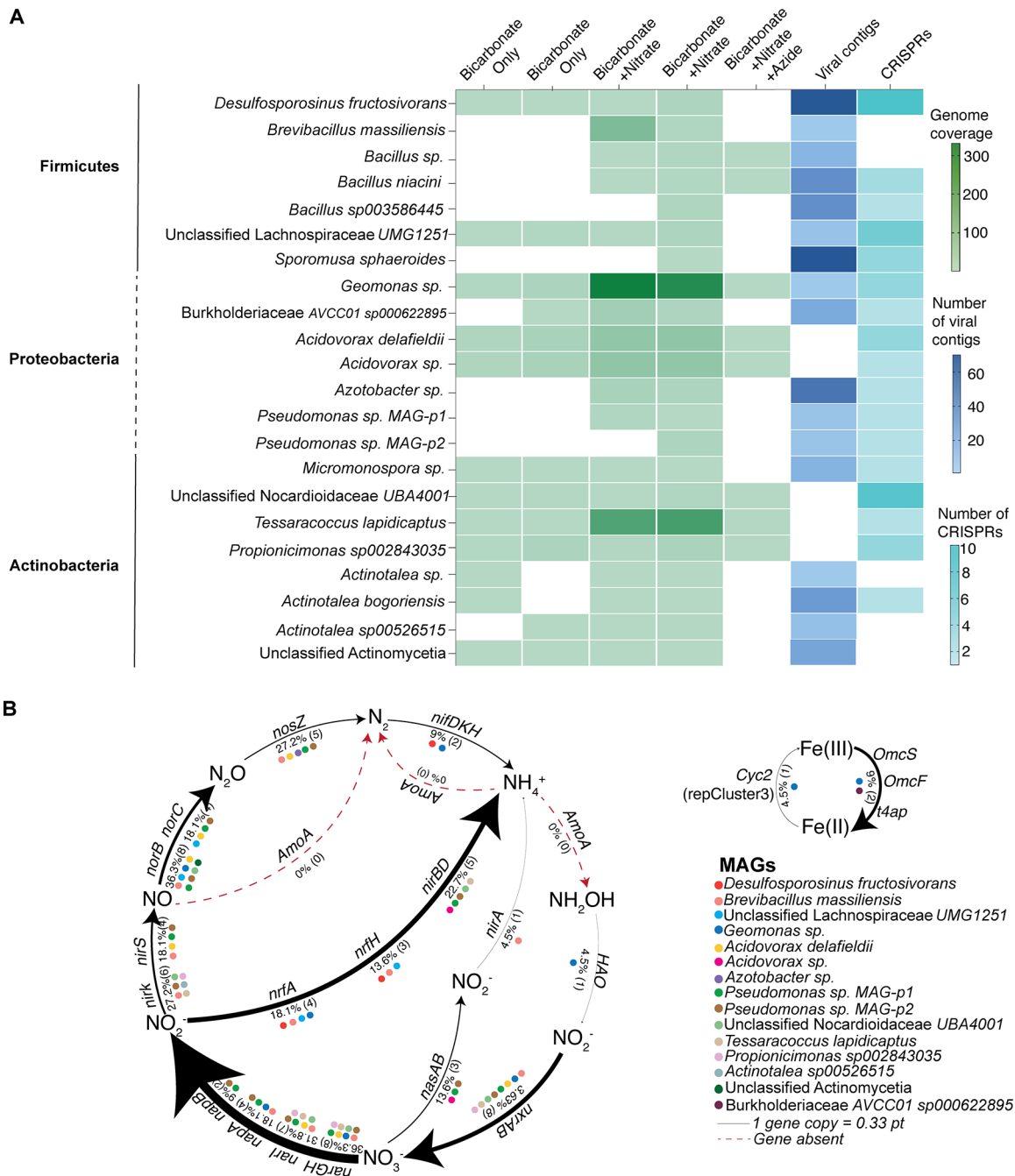


**Figure 3.** (A) MAGs from the sediment microbial and viral (viral contigs) communities following 45 days of reactor incubation with the addition of nitrate, nitrate and azide relative to the unamended (bicarbonate only) reactors. Taxa were identified using GTDB to the species level where possible, with the phylum level denoted on the left. Unclassified taxa are described to the lowest possible classification. (B) Genes associated with nitrogen and iron redox (and metal) cycling were identified from within the MAGs identified in the nitrate-amended column using DRAM and FeGenie, where gene copy number determined the weight and size of arrows in each biogeochemical cycle. Identification of the microbiota with the identified metabolic potential is denoted by each gene identified in the MAGs.

from stratigraphically equivalent sediments, where sediment-associated uranium concentrations were relatively high and revealed an oxidizing front or gradient in the direction of groundwater flow; upgradient sediments harbored 90% U(VI) and 10% U(IV) with more reduced sediments downgradient (50% U(VI); 50% U(IV)). Sediments bearing 50% U(IV) were selected to experimentally test the role of nitrate stimulated oxidative dissolution of sedimentary U(IV) and the subsequent mobilization of naturally occurring uranium under advective flow.

Naturally occurring uranium was mobilized (Figure 2) upon influx of bicarbonate buffered artificial groundwater medium in a series of six upflow column reactors packed with the U(IV) and U(VI)-bearing aquifer sediments (Figures 1 and 2A). The column reactors were packed to simulate the subsurface silt and sand lithologic interface (Figures 1B,C and S1); all solutions were prepared anoxically, and experiments were conducted under anoxic conditions. The mobilization of U at the introduction of influent is consistent with uranyl carbonate formation and desorption of U(VI) from sediments upon

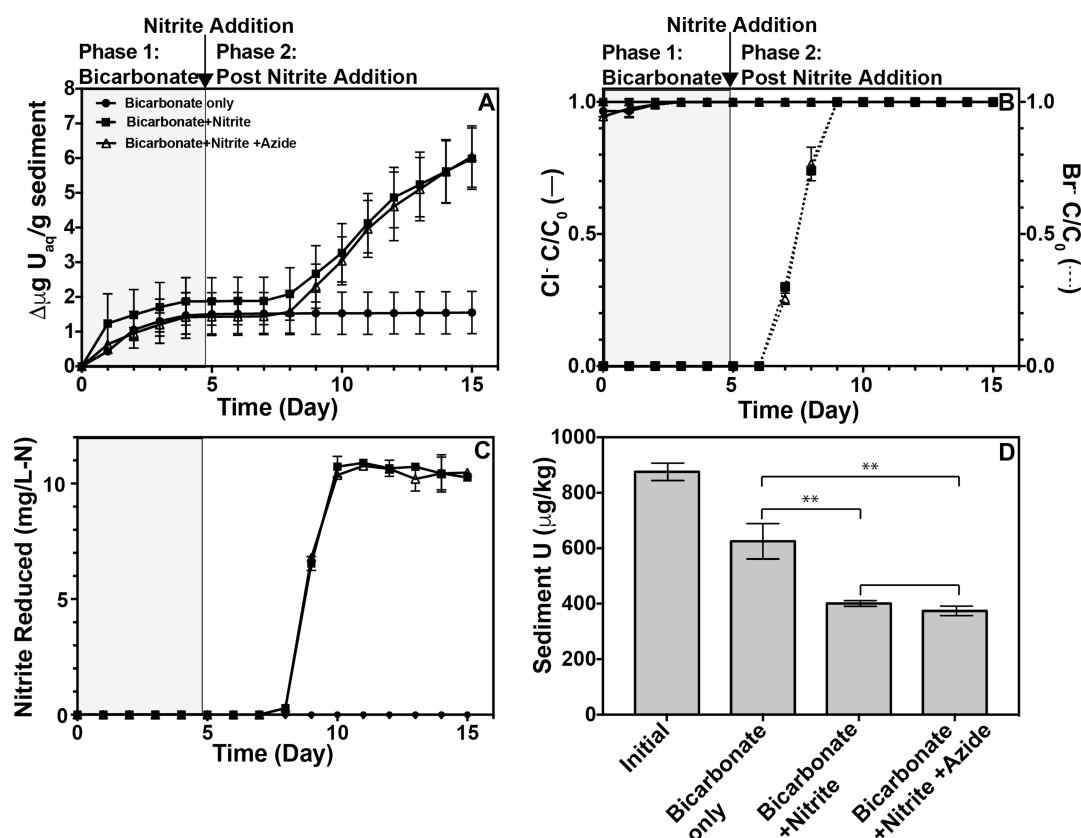


**Figure 4.** Nitrite-stimulated mobilization of naturally occurring sedimentary uranium from the Alda North site (1.7–12.2 m). After five days of inflow of bicarbonate buffered artificial groundwater medium into two upflow-column reactors two columns were amended with nitrite, two columns amended with nitrite and sodium azide, and two columns remained unamended (denoted by arrow and dashed line). (A) Total cumulative mass of dissolved uranium eluted from the reactor (measured by KPA) normalized to sediment mass across time, the same as ICP–MS (Figure S2B). (B) Conservative tracers chloride (Phase 1) and bromide (Phase 2). (C) Mass of nitrite lost (nitrite<sub>inflow</sub> – nitrite<sub>effluent</sub>), as measured by analyte concentrations in the effluent. (D) Total sediment-associated uranium after 15 days of treatment, relative to the total sediment-associated uranium initially measured in the sediments. Statistically significant differences between treatments are denoted by asterisks (\*\* $p < 0.01$ ; \*\*\* $p < 0.001$ ), as determined by one-way ANOVA, followed by a Tukey post-hoc analysis. (A–D) Error bars denote the standard error of measure for duplicate treatments.

addition of DIC.<sup>23</sup> Within Phase 1 of the experiment,  $43.75 \pm 1.90 \mu\text{g U}$  was recovered as bicarbonate desorbed U (Figure 2A) and measured as U(VI) using KPA.<sup>51</sup> Pore water U concentrations measured via KPA and ICP–MS were correlated (Pearson correlation,  $r = 0.9966$ ,  $p < 0.001$ ) with each other and revealed no difference between U concentrations collected using the two methods (Figures 2A and S3B). Following the cessation of bicarbonate-driven U elution, nitrate, and a conservative tracer, bromide, were amended into the influent of two column reactors. Two replicate column reactors were also amended with nitrate, bromide, and sodium azide (final concentration 1 mM) to inhibit microbial nitrate respiration, and two replicates amended with bromide only served as negative controls. The nitrate addition stimulated the mobilization of U from sediments into the column effluent, whereas no significant elution of U was observed in controls where nitrate was omitted or nitrate and sodium azide were amended together (Figure 2A,B). Nitrate stimulated microbial growth and nitrate reduction to nitrite in columns receiving the nitrate amendment in the absence of azide (Figure 2D,E). No significant microbial growth or nitrate reduction was observed in columns in which nitrate was omitted or amended with azide. Given that azide, an inhibitor of electron transport and respiration, inhibited nitrate reduction and mobilization of naturally occurring uranium, the results demonstrate that

microbial nitrate reduction plays a role in the mobilization of naturally occurring uranium from sediments. This was further supported by the increase in cell number during periods of nitrate reduction (Figure 2E). Cell counts remained low in azide-amended and unamended columns, indicating that microbial activity was suppressed (Figure 2E). Nitrite was transiently measured in the column effluent of nitrate-amended columns (Figure 2D), which could play a role in the oxidation of uranium.<sup>41,95</sup> No nitrite was detected in the azide-amended columns or unamended controls (Figure 2B). During this period, pH did not significantly change (Figure S3E). During the nitrate stimulated U mobilization phase an additional  $43.75 \pm 1.90 \mu\text{g}$  or  $50 \pm 5\%$  of measured total aqueous uranium eluted over days 19–45. Total U mass removed or lost from column sediments as determined from extractions of the naturally occurring sediment in the columns at the conclusion of the experiment revealed  $85.24 \pm 1.61\%$  of the sediment associated U was lost from the nitrate-amended columns relative to the unamended ( $55.52 \pm 3.03\%$  total U) and nitrate and azide-amended treatments ( $60.09 \pm 5.13\%$  total U) (Figure 2F). Together, these results indicate that microbially stimulated nitrate reduction is one mechanism for mobilization of naturally occurring uranium from alluvial aquifer sediments in addition to bicarbonate-mediated desorption.

Identification of the sediment microbial community by shotgun metagenomic sequencing and MAGs at the conclusion of the experiment revealed the enrichment of microorganisms capable of reducing nitrate to nitrite (Figure 3). Bacteria within the phyla Firmicutes, Proteobacteria, and Actinobacteria were enriched in nitrate-amended sediments relative to sediments amended with nitrate and azide or where nitrate was omitted (Figure 3A). Specifically, *Brevibacillus massiliensis*, *Bacillus* sp003586445, *Sporomusa sphaeroides*, *Geomonas* sp., unclassified Burkholderiaceae, *Acidovorax* spp., *Azotobacter* sp., *Pseudomonas* spp., and *Tessaracoccus lapidicaptus* were enriched in nitrate-amended treatments (Figure 3A). These taxa recovered from the nitrate-amended column all have the metabolic potential to reduce nitrate (Figure 3B). A review of metabolic potential further revealed the prevalence of the genomic capability for the reduction of nitrate to nitrite, which was consistent with the observed production of nitrite through the experiment (Figure 2D). However, after day 37, nitrite was consumed. The potential for dissimilatory nitrate reduction to ammonia (DNRA) as well as denitrification was also identified and could explain community enrichment toward these processes (Figure 3B). However, it is also possible that the community could have shifted over the course of the experiment as a result of viral predation, where shifts in the virus-to-cell ratio (VCR) were observed (Figure 2E) and the identification of viral contigs within the MAGs in nitrate-amended treatments (Figure 3A). This may explain the shift to lower concentrations of nitrite observed after day 37 (Figure 2D), where *Geomonas* sp. was identified with the potential to favor DNRA in the sediments at the conclusion of the study.

The observed increase in Fe(II) in both Phase 1 and Phase 2 of the study (Figure 2C) indicated the potential for Fe(III) reduction and was supported by the identification of a recognized Fe(III) reducing bacterium, *Geomonas* sp. (closely related to *Geomonas bemidjensis* formerly *Geobacter bemidjensis*), and an unclassified Burkholderiaceae harboring the metabolic potential for dissimilatory Fe(III) reduction (Figure 2C). However, during Phase 2, the elution of the total sum of Fe(II) was substantially lower in columns amended with nitrate relative to the nitrate- and azide-amended column as well as the bicarbonate only amended columns. The potential for nitrate-dependent Fe(II) oxidation exists given that *Geomonas* sp. identified in this study harbors the metabolic potential to oxidize Fe(II) (Figure 3B). Taxa recognized to oxidize iron coupled to nitrate reduction, *Acidovorax* spp. and *Pseudomonas* spp., were also identified.<sup>50,96</sup> While the metabolic potential was identified, the decreased amount of Fe(II) eluting from the nitrate-amended columns suggests active Fe(II) oxidation. However, it is noted that there remains an increase in the total amount of Fe(II) eluting from the column indicating both the potential for Fe(III) reduction and nitrate-dependent Fe(II) oxidation. Both *Acidovorax* sp. and *Pseudomonas* sp. have also been demonstrated to catalyze nitrate-dependent U(IV) oxidation in addition to iron oxidation.<sup>97</sup> While the potential for nitrate-dependent U(IV) oxidation exists within the microbial community, the production of significant concentrations of nitrite indicate a possible role of abiotic nitrite driven oxidation of U(IV) (Figure 3B).

**Nitrate-Reduction Intermediate, Nitrite, Stimulates Mobilization of Naturally Occurring U.** Nitrite is a reactive intermediate of nitrate reduction which can abiotically oxidize reduced chemical species, such as U(IV) and Fe(II).<sup>37,41,95,98</sup>

The role nitrite plays in uranium mobilization was tested in upflow column reactor experiments under the same experimental conditions as described for nitrate-amended columns replacing nitrate with nitrite. Following the bicarbonate desorption phase, nitrite was injected into the influent of duplicate reactors at a concentration observed in the preceding experiments (15 mg/L). Duplicate columns were also run where nitrite was omitted (control columns) or where nitrite and azide were coinjected. Nitrite stimulated the mobilization of naturally occurring uranium in two treatments: nitrite only and nitrite plus azide amendments (Figure 4). Nitrite decreased relative to the concentration of a conservative tracer, bromide (Figure 4B,C), with more sedimentary U lost from the nitrite-amended columns ( $475 \pm 5 \mu\text{g U/kg sed}$ ; Figure 4D) than columns where nitrite was omitted ( $277 \pm 29 \mu\text{g U/kg sed}$ ; Figure 4D). Nitrite decreased similarly in azide-amended treatments, which demonstrated the role of an abiotic reaction in these sediments. More sediment U was lost from the nitrate-amended experiments ( $771 \pm 15 \mu\text{g U/kg sediment}$ ) than from the nitrite-amended experiments ( $475 \pm 5 \mu\text{g U/kg sediment}$ ). However, this may be a result of the increased duration of the nitrate-amended experiment or total amount of nitrite produced over the course of the study. Overall, these results indicate that nitrite, a nitrate reduction intermediate, can oxidize naturally occurring U(IV) and indicates this reaction can play an important role in environmental U mobilization. The prevalence of gene copy numbers associated with nitrate reduction to nitrite (Figure 3B) supports this as a predominant pathway within the microbial community. Nitrite production could also cause the abiotic oxidation of Fe(II) and the precipitation of Fe(III) oxides.<sup>98</sup>

**U Retention on Sedimentary Fe(III) Oxides.** Mobilization of U from sediments in the presence of nitrate has been linked to the formation of Fe(III)-oxides by nitrate-dependent Fe(II) oxidation.<sup>41,42,95</sup> Analysis of aqueous Fe(II) data from the effluent revealed that less cumulative Fe(II) was released from columns amended with nitrate than the unamended controls (Figure 2D). This could be due to Fe(II) oxidation occurring in the nitrate-amended columns, although we cannot rule out another mechanism, such as fermentative Fe(III) reduction, occurring in the azide-amended and unamended columns. Fe(III)-oxides are reactive surfaces that serve as adsorptive sites for U(VI). There is the potential for Fe(III) oxides to catalyze U(IV) oxidation.<sup>42,43</sup> Therefore, they could either increase or decrease dissolved U depending on the aqueous chemistry or surface chemistry of Fe(III) oxide minerals.

The precipitation of Fe(III) oxides can increase U retention in the subsurface via adsorption. In order to determine if U would be retained by adsorption in association with the precipitation of Fe(III) oxides generated in the study, a series of equilibrium adsorption experiments were performed. Sediments containing reduced elements, such as Fe(II) and U(IV), were maintained under an anoxic atmosphere (control for a final DIC concentration of  $0.55 \pm 0.1 \text{ g/L}$ ; 54,000 ppm CO<sub>2</sub> atmosphere balanced with Ar) and replicates were air oxidized in air with a 54,000 ppm CO<sub>2</sub> atmosphere. Following air oxidation the majority of Fe in the sediments was oxidized with only  $12.4 \pm 4.0\%$  of the Fe remaining as Fe(II) in the air oxidized treatments (Table S3), whereas the Fe(II) comprised  $75 \pm 6.9\%$  of the unamended or reduced treatment (Table S3). Together, these data indicate the precipitation of Fe(III) oxide

minerals. More U was adsorbed onto the air-oxidized sediment surface ( $48.6 \pm 6.5\%$  U adsorbed; Table S4) relative to the sediments that remained reduced ( $23.7 \pm 6.8\%$  U adsorbed; Table S4; Figure 5). Development of a generalized composite

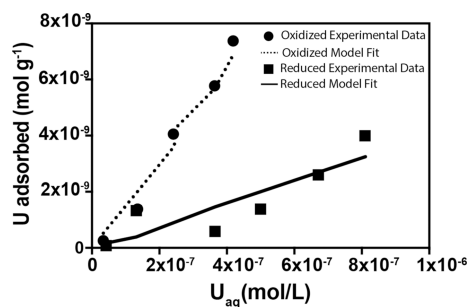


**Figure 5.** U adsorption to reduced and air-oxidized sediments collected from the Alda North site (1.7–12.2 m). Artificial groundwater containing reduced (squares) or air oxidized (circles) sediment was spiked with U(VI) under controlled  $pCO_2$  (54,000 ppm) conditions. The dashed line denotes adsorption predicted by the SCM in which Fe(III) oxides formed following air oxidation and accounted for U(VI) adsorption. The solid line describes surface complexation results modeled using measured iron oxide values in the reduced sediment.

SCM indicated that the precipitation of ferrihydrite could account for the U adsorbed to the Fe(III) oxide surfaces (Figure 5). This result is consistent with retention of U by the precipitation of Fe(III) oxide minerals<sup>99–101</sup> and suggests the capacity of iron oxides generated in the column study to sequester some U even in the presence of bicarbonate.

**Long-Term Implications for Nitrate-Mediated Uranium Mobilization in Alluvial Aquifers.** The release of U(VI) associated with complexing ligands such as carbonate has been recognized as a factor in the mobilization of U in alluvial aquifer sediments.<sup>2,102,103</sup> Here, we demonstrate that in addition to bicarbonate-driven U desorption, U deposited in fine-grained, low-permeability silts and clays within alluvial aquifers as reduced U(IV) phases is subject to oxidation following the influx of nitrate into the reduced aquifer. The potential exists for direct oxidation of U(IV) coupled to nitrate reduction by microbial taxa, such as *Acidovorax* sp.<sup>97</sup> In addition, microbial production of nitrite can abiotically drive U(IV) oxidation and mobilize U into the groundwater. Not all of the U would remain mobile as both biological and abiotic Fe(II) oxidation reactions can lead to the precipitation of a high reactive surface area mineral for U(VI) to absorb, thus removing it from groundwater.<sup>42,43</sup> Under increasing alkalinity and carbonate concentrations, U(VI) would be prone to desorption and mobilization. A redox gradient is established in this region of the High Plains aquifer, where nitrate-bearing groundwater moves from an oxidized region of an aquifer, where U(VI) is adsorbed to iron oxide surfaces into a region of the aquifer with reduced low permeability silty clay deposited at 11.7–12.1 m depth bgs (Figure 1). Within this region nitrate reduction is coupled to the oxidation of U(IV) and generation of U(VI), which can be retained on Fe(III) oxide surfaces. The continued influx of nitrate into zones containing reduced minerals may eventually overcome the reducing potential of the sediments and oxidize reduced minerals and organic carbon, resulting in the oxidation of reduced U(IV) and Fe(II) into soluble U(VI) and ferric Fe(III) oxide minerals, respectively. Newly generated Fe(III) oxides may

then serve as a temporary sink for U(VI) through adsorption; however, natural increases in bicarbonate through processes such as carbonate mineral dissolution or heterotrophic microbial metabolisms can release the adsorbed U(VI) back into the groundwater.

## ■ ASSOCIATED CONTENT

### Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acs.est.2c07683>.

Groundwater geochemical data; descriptive statistics for XANES linear combination fits; measured geochemical parameters from batch adsorption experiments; upflow-column reactor experimental design replicating the sand and silt lithologic interface; ORP, pH, Fe(II), U, and viruses/mL measured from upflow-column reactor effluent; upflow reactor sediment U and Fe; and surface complexation model (PDF)

## ■ AUTHOR INFORMATION

### Corresponding Author

**Karrie A. Weber** – Department of Earth and Atmospheric Sciences, University of Nebraska-Lincoln, Lincoln, Nebraska 68588, United States; School of Biological Sciences, University of Nebraska-Lincoln, Lincoln, Nebraska 68588, United States; Daugherty Water for Food Global Institute, University of Nebraska, Lincoln, Nebraska 68588, United States; [orcid.org/0000-0002-0457-658X](https://orcid.org/0000-0002-0457-658X); Phone: (402) 472-2739; Email: [kweber@unl.edu](mailto:kweber@unl.edu)

### Authors

**Jeffrey P. Westrop** – Department of Earth and Atmospheric Sciences, University of Nebraska-Lincoln, Lincoln, Nebraska 68588, United States; Present Address: Conservation and Survey Division, School of Natural Resources, Hardin Hall, University of Nebraska-Lincoln, Lincoln, NE 68583-0996, United States

**Pooja Yadav** – School of Biological Sciences, University of Nebraska-Lincoln, Lincoln, Nebraska 68588, United States

**PJ Nolan** – Department of Earth and Atmospheric Sciences, University of Nebraska-Lincoln, Lincoln, Nebraska 68588, United States; Present Address: WSP Golder, Farmington Hills, MI 48331, United States.

**Kate M. Campbell** – Geology, Geophysics, and Geochemistry Science Center, Denver Federal Center, U.S. Geological Survey, Denver, Colorado 80225, United States; [orcid.org/0000-0002-8715-5544](https://orcid.org/0000-0002-8715-5544)

**Rajesh Singh** – Environmental Hydrology Division, National Institute of Hydrology, Roorkee 247667, India

**Sharon E. Bone** – Stanford Synchrotron Radiation Lightsource, SLAC National Accelerator Laboratory, Menlo Park, California 94025, United States; [orcid.org/0000-0002-7521-9627](https://orcid.org/0000-0002-7521-9627)

**Alicia H. Chan** – Department of Earth and Atmospheric Sciences, University of Nebraska-Lincoln, Lincoln, Nebraska 68588, United States; School of Biological Sciences, University of Nebraska-Lincoln, Lincoln, Nebraska 68588, United States

**Anthony J. Kohtz** – Department of Earth and Atmospheric Sciences, University of Nebraska-Lincoln, Lincoln, Nebraska 68588, United States

**Donald Pan** – School of Biological Sciences, University of Nebraska-Lincoln, Lincoln, Nebraska 68588, United States; Present Address: School of Oceanography, Shanghai Jiao Tong University, 200240 Shanghai, China.

**Olivia Healy** – School of Biological Sciences, University of Nebraska-Lincoln, Lincoln, Nebraska 68588, United States

**John R. Bargar** – Stanford Synchrotron Radiation Lightsource, SLAC National Accelerator Laboratory, Menlo Park, California 94025, United States; Present

Address: Environmental Molecular Sciences Laboratory, Pacific Northwest National Laboratory, Richland, WA, 99352 United States.; [orcid.org/0000-0001-9303-4901](https://orcid.org/0000-0001-9303-4901)

**Daniel D. Snow** – School of Natural Resources and Nebraska Water Center, Part of the Daugherty Water for Food Global Institute, Water Sciences Laboratory, University of Nebraska-Lincoln, Lincoln, Nebraska 68588, United States

Complete contact information is available at:

<https://pubs.acs.org/10.1021/acs.est.2c07683>

### Author Contributions

J.P.W., P.J.N., and K.A.W. designed the study, and together with D.D.S., they coordinated fieldwork and collected and analyzed samples. A.J.K., D.P., and O.H. assisted with the collection and analysis of field samples. J.P.W. conducted experiments and analyzed data, and with assistance from A.H.C., R. S., and K.A.W. P.Y., extracted DNA and conducted sequence and bioinformatic analyses. K.M.C. developed the SCM. S.E.B. conducted XANES analysis with J.R.B. All authors contributed extensively to the analysis, interpretation of the data, and preparation of the manuscript.

### Notes

The authors declare no competing financial interest.

Shotgun metagenomic sequence data has been deposited in the NCBI BioProject ID PRJNA875447 and PRJNA875080. Experimentally derived data reported in this study have been deposited in the University of Nebraska-Lincoln Data Repository (<https://doi.org/10.32873/unl.dr.20221104>).

### ACKNOWLEDGMENTS

This research was supported by the U.S. Geological Survey (USGS) 104g Program (2014NE265G) to K.A.W., D.D.S., and K.M.C., Daugherty Water for Food Institute to K.A.W. and J.P.W. and student research grants to J.P.W. from the Nebraska Geological Society (NGS), Geological Society of America (GSA) Exxon Mobil Research Grant and P.J.N. NGS, GSA, and American Association of Petroleum Geologists Jay M. McMurray Memorial Research Grant. R.S. was supported by the Water Advanced Research and Innovation (WARI) Fellowship Program. S.B. was supported by the U.S. Department of Energy (DOE), Office of Science, Office of Biological and Environmental Research, Subsurface Biogeochemistry Research program through the SLAC National Accelerator Laboratory (SLAC) SFA program. Use of the Stanford Synchrotron Radiation Lightsource, SLAC, is supported by the DOE, Office of Science, Office of Basic Energy Sciences under contract no. DE-AC02-76SF00515. K.M.C. was supported by the USGS Toxic Substances Hydrology Program. Any use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the U.S. Government.

### REFERENCES

- (1) DeSimone, L. A.; Hamilton, P. A.; Gilliom, R. J. *Quality of Water from Domestic Wells in Principal Aquifers of the United States, 1991–2004—Overview of Major Findings*; U.S. Geological Survey Circular, 2009.
- (2) Nolan, J.; Weber, K. A. Natural Uranium Contamination in Major U.S. Aquifers Linked to Nitrate. *Environ. Sci. Technol. Lett.* **2015**, *2*, 215–220.
- (3) Herring, J. S. Uranium and Thorium Resources. In *Encyclopedia of Sustainability Science and Technology*; Meyers, R. A., Ed.; Springer New York, 2012; pp 11201–11219.
- (4) Coyte, R. M.; Jain, R. C.; Srivastava, S. K.; Sharma, K. C.; Khalil, A.; Ma, L.; Vengosh, A. Large-Scale Uranium Contamination of Groundwater Resources in India. *Environ. Sci. Technol. Lett.* **2018**, *5*, 341–347.
- (5) Steffanowski, J.; Banning, A. Uraniferous dolomite: a natural source of high groundwater uranium concentrations in northern Bavaria, Germany? *Environ. Earth Sci.* **2017**, *76*, 508.
- (6) Wu, Y.; Wang, Y. X.; Xie, X. J. Occurrence, behavior and distribution of high levels of uranium in shallow groundwater at Datong basin, northern China. *Sci. Total Environ.* **2014**, *472*, 809–817.
- (7) Liesch, T.; Hinrichsen, S.; Goldscheider, N. Uranium in groundwater - Fertilizers versus geogenic sources. *Sci. Total Environ.* **2015**, *536*, 981–995.
- (8) Janot, N.; Lezama Pacheco, J. S.; Pham, D. Q.; O'Brien, T. M.; Hausladen, D.; Noël, V.; Lallier, F.; Maher, K.; Fendorf, S.; Williams, K. H.; Long, P. E.; Bargar, J. R. Physico-Chemical Heterogeneity of Organic-Rich Sediments in the Rifle Aquifer, CO: Impact on Uranium Biogeochemistry. *Environ. Sci. Technol.* **2016**, *50*, 46–53.
- (9) Noël, V.; Boye, K.; Kukkadapu, R. K.; Bone, S.; Lezama Pacheco, J. S.; Cardarelli, E.; Janot, N.; Fendorf, S.; Williams, K. H.; Bargar, J. R. Understanding controls on redox processes in floodplain sediments of the Upper Colorado River Basin. *Sci. Total Environ.* **2017**, *603*–*604*, 663–675.
- (10) Blazejewski, G. A.; Stolt, M. H.; Gold, A. J.; Gurwick, N.; Groffman, P. M. Spatial Distribution of Carbon in the Subsurface of Riparian Zones. *Soil Sci. Soc. Am. J.* **2009**, *73*, 1733–1740.
- (11) Davis, J. A.; Curtis, G. P.; Wilkins, M. J.; Kohler, M.; Fox, P.; Naftz, D. L.; Lloyd, J. R. Processes affecting transport of uranium in a suboxic aquifer. *Phys. Chem. Earth* **2006**, *31*, 548–555.
- (12) Bone, S. E.; Cliff, J.; Weaver, K.; Takacs, C. J.; Roycroft, S.; Fendorf, S.; Bargar, J. R. Complexation by Organic Matter Controls Uranium Mobility in Anoxic Sediments. *Environ. Sci. Technol.* **2020**, *54*, 1493–1502.
- (13) Aeppli, M.; Babey, T.; Engel, M.; Lacroix, E. M.; Tolar, B. B.; Fendorf, S.; Bargar, J. R.; Boye, K. Export of Organic Carbon from Reduced Fine-Grained Zones Governs Biogeochemical Reactivity in a Simulated Aquifer. *Environ. Sci. Technol. Lett.* **2022**, *56*, 2738–2746.
- (14) Langmuir, D. Uranium solution-mineral equilibria at low temperatures with applications to sedimentary ore deposits. *Geochim. Cosmochim. Acta* **1978**, *42*, 547–569.
- (15) Nolan, P. J.; Bone, S. E.; Campbell, K. M.; Pan, D.; Healy, O. M.; Stange, M.; Bargar, J. R.; Weber, K. A. Uranium(VI) attenuation in a carbonate-bearing oxic alluvial aquifer. *J. Hazard Mater.* **2021**, *412*, 125089.
- (16) Kanematsu, M.; Perdrial, N.; Um, W.; Chorover, J.; O'Day, P. A. Influence of Phosphate and Silica on U(VI) Precipitation from Acidic and Neutralized Wastewaters. *Environ. Sci. Technol.* **2014**, *48*, 6097–6106.
- (17) Bone, S. E.; Dynes, J. J.; Cliff, J.; Bargar, J. R. Uranium(IV) adsorption by natural organic matter in anoxic sediments. *Proc. Natl. Acad. Sci. U.S.A.* **2017**, *114*, 711–716.
- (18) Das, D.; Sureshkumar, M. K.; Koley, S.; Mithal, N.; Pillai, C. G. S. Sorption of uranium on magnetite nanoparticles. *J. Radioanal. Nucl. Chem.* **2010**, *285*, 447–454.
- (19) Echevarria, G.; Sheppard, M. I.; Morel, J. Effect of pH on the sorption of uranium in soils. *J. Environ. Radioact.* **2001**, *53*, 257–264.

- (20) Katsoyiannis, I. A.; Werner Althoff, H. W.; Bartel, H.; Jekel, M. The effect of groundwater composition on uranium(VI) sorption onto bacteriogenic iron oxides. *Water Res.* **2006**, *40*, 3646–3652.
- (21) Payne, T. E.; Davis, J. A.; Ochs, M.; Olin, M.; Tweed, C. J. Uranium adsorption on weathered schist - intercomparison of modelling approaches. *Radiochim. Acta* **2004**, *92*, 651–661.
- (22) Tsunashima, A.; Brindley, G. W.; Bastovanov, M. Adsorption of Uranium from Solutions by Montmorillonite; Compositions and Properties of Uranyl Montmorillonites. *Clay Clay Miner.* **1981**, *29*, 10–16.
- (23) Dong, W. M.; Brooks, S. C. Determination of the formation constants of ternary complexes of uranyl and carbonate with alkaline earth metals (Mg<sup>2+</sup>, Ca<sup>2+</sup>, Sr<sup>2+</sup>, and Ba<sup>2+</sup>) using anion exchange method. *Environ. Sci. Technol.* **2006**, *40*, 4689–4695.
- (24) Fox, P. M.; Davis, J. A.; Zachara, J. M. The effect of calcium on aqueous uranium(VI) speciation and adsorption to ferrihydrite and quartz. *Geochim. Cosmochim. Acta* **2006**, *70*, 1379–1387.
- (25) Kelly, S. D.; Kemner, K. M.; Brooks, S. C. X-ray absorption spectroscopy identifies calcium-uranyl-carbonate complexes at environmental concentrations. *Geochim. Cosmochim. Acta* **2007**, *71*, 821–834.
- (26) Tokunaga, T. K.; Kim, Y.; Wan, J.; Yang, L. Aqueous uranium(VI) concentrations controlled by calcium uranyl vanadate precipitates. *Environ. Sci. Technol.* **2012**, *46*, 7471–7477.
- (27) Zheng, Z.; Tokunaga, T. K.; Wan, J. Influence of calcium carbonate on U(VI) sorption to soils. *Environ. Sci. Technol.* **2003**, *37*, 5603–5608.
- (28) Jurgens, B. C.; Fram, M. S.; Belitz, K.; Burow, K. R.; Landon, M. K. Effects of groundwater development on uranium: Central Valley, California, USA. *Ground Water* **2010**, *48*, 913–928.
- (29) Stewart, B. D.; Mayes, M. A.; Fendorf, S. Impact of Uranyl–Calcium–Carbonate Complexes on Uranium(VI) Adsorption to Synthetic and Natural Sediments. *Environ. Sci. Technol.* **2010**, *44*, 928–934.
- (30) Burow, K. R.; Nolan, B. T.; Rupert, M. G.; Dubrovsky, N. M. Nitrate in Groundwater of the United States, 1991–2003. *Environ. Sci. Technol.* **2010**, *44*, 4988–4997.
- (31) Dam, W. L.; Campbell, S.; Johnson, R. H.; Looney, B. B.; Denham, M. E.; Eddy-Dilek, C. A.; Babits, S. J. Refining the site conceptual model at a former uranium mill site in Riverton, Wyoming, USA. *Environ. Earth Sci.* **2015**, *74*, 7255–7265.
- (32) Bone, S. E.; Cahill, M. R.; Jones, M. E.; Fendorf, S.; Davis, J.; Williams, K. H.; Bargar, J. R. Oxidative Uranium Release from Anoxic Sediments under Diffusion-Limited Conditions. *Environ. Sci. Technol.* **2017**, *51*, 11039–11047.
- (33) Bopp, C. J.; Lundstrom, C. C.; Johnson, T. M.; Sanford, R. A.; Long, P. E.; Williams, K. H. Uranium 238U/235U Isotope Ratios as Indicators of Reduction: Results from an in situ Biostimulation Experiment at Rifle, Colorado, U.S.A. *Environ. Sci. Technol.* **2010**, *44*, 5927–5933.
- (34) Williams, K. H.; Long, P. E.; Davis, J. A.; Wilkins, M. J.; N'Guessan, A. L.; Steefel, C. I.; Yang, L.; Newcomer, D.; Spane, F. A.; Kerkhof, L. J.; McGuinness, L.; Dayvault, R.; Lovley, D. R. Acetate Availability and its Influence on Sustainable Bioremediation of Uranium-Contaminated Groundwater. *Geomicrobiol. J.* **2011**, *28*, 519–539.
- (35) Pan, D.; Williams, K. H.; Robbins, M. J.; Weber, K. A. Uranium Retention in a Bioreduced Region of an Alluvial Aquifer Induced by the Influx of Dissolved Oxygen. *Environ. Sci. Technol.* **2018**, *52*, 8133–8145.
- (36) Moon, H. S.; Komlos, J.; Jaffé, P. R. Biogenic U(IV) oxidation by dissolved oxygen and nitrate in sediment after prolonged U(VI)/Fe(III)/SO<sub>4</sub><sup>2-</sup> reduction. *J. Contam. Hydrol.* **2009**, *105*, 18–27.
- (37) Asta, M. P.; Beller, H. R.; O'Day, P. A. Anaerobic Dissolution Rates of U(IV)-Oxide by Abiotic and Nitrate-Dependent Bacterial Pathways. *Environ. Sci. Technol.* **2020**, *54*, 8010–8021.
- (38) Beller, H. R. Anaerobic, nitrate-dependent oxidation of U(IV) oxide minerals by the chemolithoautotrophic bacterium *Thiobacillus denitrificans*. *Appl. Environ. Microbiol.* **2005**, *71*, 2170–2174.
- (39) Finneran, K. T.; Housewright, M. E.; Lovley, D. R. Multiple influences of nitrate on uranium solubility during bioremediation of uranium-contaminated subsurface sediments. *Environ. Microbiol.* **2002**, *4*, 510–516.
- (40) Weber, K. A.; Thrash, J. C.; Van Trump, J. I.; Achenbach, L. A.; Coates, J. D. Environmental and taxonomic bacterial diversity of anaerobic uranium(IV) bio-oxidation. *Appl. Environ. Microbiol.* **2011**, *77*, 4693–4696.
- (41) Senko, J. M.; Suflita, J. M.; Krumholz, L. R. Geochemical controls on microbial nitrate-dependent U(IV) oxidation. *Geomicrobiol. J.* **2005**, *22*, 371–378.
- (42) Ginder-Vogel, M.; Criddle, C. S.; Fendorf, S. Thermodynamic constraints on the oxidation of biogenic UO<sub>2</sub> by Fe(III) (Hydr)-oxides. *Environ. Sci. Technol.* **2006**, *40*, 3544–3550.
- (43) Ginder-Vogel, M.; Stewart, B.; Fendorf, S. Kinetic and mechanistic constraints on the oxidation of biogenic uraninite by ferrihydrite. *Environ. Sci. Technol.* **2010**, *44*, 163–169.
- (44) Korus, J.; Howard, L.; Young, A.; Divine, D.; Burbach, M.; Jess, J.; Hallum, D. *The Groundwater Atlas of Nebraska*; University of Nebraska–Lincoln. Conservation and Survey Division, 2013.
- (45) Snow, D. D. Geochemistry, hydrology, and environmental applications of uranium-series nuclides in the Platte River drainage basin. Ph.D. Thesis, University of Nebraska-Lincoln, Lincoln, NE, 1996. <https://digitalcommons.unl.edu/dissertations/AAI9703790>.
- (46) Wilde, F. D. *Water-Quality Sampling by the U.S. Geological Survey-Standard Protocols and Procedures*; U.S. Geological Survey, 2011. <https://pubs.usgs.gov/fs/2010/3121>
- (47) Nordstrom, D. K.; Wilde, F. D. Chapter A6. Section 6.5. *Reduction-Oxidation Potential (Electrode Method)*, Reston, VA, 2005. <http://pubs.er.usgs.gov/publication/twri09A6.5>.
- (48) USGS. Chapter A6.4. *Measurement of pH*, Reston, VA, 2021. <http://pubs.er.usgs.gov/publication/tm9A6.4>.
- (49) USGS. Chapter A6.2. *Dissolved Oxygen*, Reston, VA, 2020. <http://pubs.er.usgs.gov/publication/tm9A6.2>.
- (50) Byrne-Bailey, K. G.; Weber, K. A.; Chair, A. H.; Bose, S.; Knox, T.; Spanbauer, T. L.; Chertkov, O.; Coates, J. D. Completed Genome Sequence of the Anaerobic Iron-Oxidizing Bacterium *Acidovorax ebreus* Strain TPSY. *J. Bacteriol.* **2010**, *192*, 1475–1476.
- (51) Brina, R.; Miller, A. G. Direct Detection of Trace Levels of Uranium by Laser-Induced Kinetic Phosphorimetry. *Anal. Chem.* **1992**, *64*, 1413–1418.
- (52) US EPA. *EPA Method 200.8: Determination of Trace Elements in Waters and Wastes by Inductively Coupled Plasma Mass Spectroscopy. Revision 5.4*, Cincinnati OH, 1994.
- (53) Pan, D.; Watson, R.; Wang, D.; Tan, Z. H.; Snow, D. D.; Weber, K. A. Correlation between viral production and carbon mineralization under nitrate-reducing conditions in aquifer sediment. *ISME J.* **2014**, *8*, 1691–1703.
- (54) US EPA. *Determination of Nitrate-Nitrite Nitrogen by Automated Colorimetry*; US Environmental Protection Agency, 1993.
- (55) Stookey, L. L. Ferrozine—a new spectrophotometric reagent for iron. *Anal. Chem.* **1970**, *42*, 779–781.
- (56) Fredrickson, J. K.; Zachara, J. M.; Kennedy, D. W.; Dong, H. L.; Onstott, T. C.; Hinman, N. W.; Li, S. M. Biogenic iron mineralization accompanying the dissimilatory reduction of hydrous ferric oxide by a groundwater bacterium. *Geochim. Cosmochim. Acta* **1998**, *62*, 3239–3257.
- (57) Viollier, E.; Inglett, P. W.; Hunter, K.; Roychoudhury, A. N.; Van Cappellen, P. The ferrozine method revisited: Fe(II)/Fe(III) determination in natural waters. *Appl. Geochem.* **2000**, *15*, 785–790.
- (58) Zimmermann, C. F. *Method 440.0: Determination of Carbon and Nitrogen in Sediments and Particulates of Estuarine/Coastal Waters Using Elemental Analysis*; United States Environmental Protection Agency, Office of Research and Development, National Exposure Research Laboratory, 1997.
- (59) Schumacher, B. *Methods for the Determination of Total Organic Carbon (TOC) In Soils and Sediments. Ecological Risk Assessment Support Center Office of Research and Development*; United States Environmental Protection Agency: Washington, DC, 2002.

- (60) US EPA. *Determination of Total Organic Carbon and Specific UV Absorbance at 254 nm in Source Water and Drinking Water*; US Environmental Protection Agency, 2009.
- (61) Kohler, M.; Curtis, G. P.; Meece, D. E.; Davis, J. A. Methods for estimating adsorbed uranium(VI) and distribution coefficients of contaminated sediments. *Environ. Sci. Technol.* **2004**, *38*, 240–247.
- (62) Ravel, B.; Newville, M. ATHENA, ARTEMIS, HEPHAESTUS: data analysis for X-ray absorption spectroscopy using IFEFFIT. *J. Synchrotron Radiat.* **2005**, *12*, 537–541.
- (63) Ravel, B.; Newville, M. ATHENA and ARTEMIS Interactive Graphical Data Analysis using IFEFFIT. *Phys. Scr.* **2005**, *2005*, 1007–1010.
- (64) Alessi, D. S.; Uster, B.; Veeramani, H.; Suvorova, E. I.; Lezama-Pacheco, J. S.; Stubbs, J. E.; Bargar, J. R.; Bernier-Latmani, R. Quantitative Separation of Monomeric U(IV) from UO<sub>2</sub> in Products of U(VI) Reduction. *Environ. Sci. Technol.* **2012**, *46*, 6150–6157.
- (65) Lovley, D. R.; Phillips, E. J. Novel mode of microbial energy metabolism: organic carbon oxidation coupled to dissimilatory reduction of iron or manganese. *Appl. Environ. Microbiol.* **1988**, *54*, 1472–1480.
- (66) Gutentag, E. D. *Geohydrology of the High Plains Aquifer in Parts of Colorado, Kansas, Nebraska, New Mexico, Oklahoma, South Dakota, Texas, and Wyoming: High Plains RASA Project*; United States Geological Survey, 1984.
- (67) Hewitt, E. J.; Nicholas, D. J. D. Cations and anions: inhibitions and interactions in metabolism and in enzyme activity. In *Metabolic Inhibitors: A Comprehensive Treatise*; Hochster, R. M., Quastel, J. H., Eds.; Academic Press, Inc., 1963; Vol. 2, pp 311–436.
- (68) Elliott, S. J.; Hoke, K. R.; Heffron, K.; Palak, M.; Rothery, R. A.; Weiner, J. H.; Armstrong, F. A. Voltammetric Studies of the Catalytic Mechanism of the Respiratory Nitrate Reductase from *Escherichia coli*: How Nitrate Reduction and Inhibition Depend on the Oxidation State of the Active Site. *Biochemistry* **2004**, *43*, 799–807.
- (69) Tsubaki, M.; Yoshikawa, S. Fourier-transform infrared study of azide binding to the Fea3-CuB binuclear site of bovine heart cytochrome c oxidase: new evidence for a redox-linked conformational change at the binuclear site. *Biochemistry* **1993**, *32*, 174–182.
- (70) Noble, R. T.; Fuhrman, J. A. Use of SYBR Green I for rapid epifluorescence counts of marine viruses and bacteria. *Aquat. Microb. Ecol.* **1998**, *14*, 113–118.
- (71) Pan, D.; Nolan, J.; Williams, K. H.; Robbins, M. J.; Weber, K. A. Abundance and Distribution of Microbial Cells and Viruses in an Alluvial Aquifer. *Front. Microbiol.* **2017**, *8*, 1199.
- (72) US EPA. *Method 3050B: Acid Digestion of Sediments, Sludges, and Soils Revision 2*, Cincinnati OH, 1996.
- (73) Miller, T. L.; Wolin, M. J. A serum bottle modification of the Hungate technique for cultivating obligate anaerobes. *Appl. Microbiol.* **1974**, *27*, 985–987.
- (74) Davis, J. A.; Meece, D. E.; Kohler, M.; Curtis, G. P. Approaches to surface complexation modeling of uranium(VI) adsorption on aquifer sediments. *Geochim. Cosmochim. Acta* **2004**, *68*, 3621–3641.
- (75) Hyun, S. P.; Fox, P. M.; Davis, J. A.; Campbell, K. M.; Hayes, K. F.; Long, P. E. Surface Complexation Modeling of U(VI) Adsorption by Aquifer Sediments from a Former Mill Tailings Site at Rifle, Colorado. *Environ. Sci. Technol.* **2009**, *43*, 9368–9373.
- (76) Parkhurst, D. L.; Appello, C. A. J. Description of input and examples for PHREEQC version 3—A computer program for speciation, batch- reaction, one-dimensional transport, and inverse geochemical calculations. *U.S. Geological Survey Techniques and Methods*; USGS, 2013; Vol. 6, p 497.
- (77) Poeter, E. P.; Hill, M. C. UCODE, a computer code for universal inverse modeling. *Comput. Geosci.* **1999**, *25*, 457–462.
- (78) Griffiths, R. L.; Whiteley, A. S.; O'Donnell, A. G.; Bailey, M. J. Rapid method for coextraction of DNA and RNA from natural environments for analysis of ribosomal DNA- and rRNA-based microbial community composition. *Appl. Environ. Microbiol.* **2000**, *66*, 5488–5491.
- (79) Bolger, A. M.; Lohse, M.; Usadel, B. Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics* **2014**, *30*, 2114–2120.
- (80) Li, D.; Liu, C. M.; Luo, R.; Sadakane, K.; Lam, T. W. MEGAHIT: an ultra-fast single-node solution for large and complex metagenomics assembly via succinct de Bruijn graph. *Bioinformatics* **2015**, *31*, 1674–1676.
- (81) Langdon, W. B. Performance of genetic programming optimised Bowtie2 on genome comparison and analytic testing (GCAT) benchmarks. *BioData Min.* **2015**, *8*, 1.
- (82) Kang, D. D.; Li, F.; Kirton, E.; Thomas, A.; Egan, R.; An, H.; Wang, Z. MetaBAT 2: an adaptive binning algorithm for robust and efficient genome reconstruction from metagenome assemblies. *PeerJ* **2019**, *7*, No. e7359.
- (83) Wu, Y. W.; Simmons, B. A.; Singer, S. W. MaxBin 2.0: an automated binning algorithm to recover genomes from multiple metagenomic datasets. *Bioinformatics* **2016**, *32*, 605–607.
- (84) Alneberg, J.; Bjarnason, B. S.; de Bruijn, I.; Schirmer, M.; Quick, J.; Ijaz, U. Z.; Lahti, L.; Loman, N. J.; Andersson, A. F.; Quince, C. Binning metagenomic contigs by coverage and composition. *Nat. Methods* **2014**, *11*, 1144–1146.
- (85) Sieber, C. M. K.; Probst, A. J.; Sharrar, A.; Thomas, B. C.; Hess, M.; Tringe, S. G.; Banfield, J. F. Recovery of genomes from metagenomes via a dereplication, aggregation and scoring strategy. *Nat. Microbiol.* **2018**, *3*, 836–843.
- (86) Eren, A. M.; Esen, Ö. C.; Quince, C.; Vineis, J. H.; Morrison, H. G.; Sogin, M. L.; Delmont, T. O. Anvi'o: an advanced analysis and visualization platform for omics data. *PeerJ* **2015**, *3*, No. e1319.
- (87) Parks, D. H.; Imelfort, M.; Skennerton, C. T.; Hugenholtz, P.; Tyson, G. W. CheckM: assessing the quality of microbial genomes recovered from isolates, single cells, and metagenomes. *Genome Res.* **2015**, *25*, 1043–1055.
- (88) Emerson, J. B.; Roux, S.; Brum, J. R.; Bolduc, B.; Woodcroft, B. J.; Jang, H. B.; Singleton, C. M.; Solden, L. M.; Naas, A. E.; Boyd, J. A.; Hodgkins, S. B.; Wilson, R. M.; Trubl, G.; Li, C.; Frolking, S.; Pope, P. B.; Wrighton, K. C.; Crill, P. M.; Chanton, J. P.; Saleska, S. R.; Tyson, G. W.; Rich, V. I.; Sullivan, M. B. Host-linked soil viral ecology along a permafrost thaw gradient. *Nat. Microbiol.* **2018**, *3*, 870–880.
- (89) Parks, D. H.; Chuvochina, M.; Rinke, C.; Mussig, A. J.; Chaumeil, P.-A.; Hugenholtz, P. GTDB: an ongoing census of bacterial and archaeal diversity through a phylogenetically consistent, rank normalized and complete genome-based taxonomy. *Nucleic Acids Res.* **2022**, *50*, D785–D794.
- (90) Shaffer, M.; Borton, M. A.; McGivern, B. B.; Zayed, A. A.; La Rosa, S. L.; Solden, L. M.; Liu, P.; Narrowe, A. B.; Rodríguez-Ramos, J.; Bolduc, B.; Gazitúa, M. C.; Daly, R. A.; Smith, G. J.; Vik, D. R.; Pope, P. B.; Sullivan, M. B.; Roux, S.; Wrighton, K. C. DRAM for distilling microbial metabolism to automate the curation of microbiome function. *Nucleic Acids Res.* **2020**, *48*, 8883–8900.
- (91) Garber, A. I.; Nealson, K. H.; Okamoto, A.; McAllister, S. M.; Chan, C. S.; Barco, R. A.; Merino, N. FeGenie: a comprehensive tool for the identification of iron genes and iron gene neighborhoods in genome and metagenome assemblies. *Front. Microbiol.* **2020**, *11*, 37.
- (92) Guo, J.; Bolduc, B.; Zayed, A. A.; Varsani, A.; Dominguez-Huerta, G.; Delmont, T. O.; Pratama, A. A.; Gazitúa, M. C.; Vik, D.; Sullivan, M. B.; Roux, S. VirSorter2: a multi-classifier, expert-guided approach to detect diverse DNA and RNA viruses. *Microbiome* **2021**, *9*, 37.
- (93) Couvin, D.; Bernheim, A.; Toffano-Nioche, C.; Touchon, M.; Michalik, J.; Néron, B.; Rocha, E. P. C.; Vergnaud, G.; Gautheret, D.; Pourcel, C. CRISPRCasFinder, an update of CRISPRFinder, includes a portable version, enhanced performance and integrates search for Cas proteins. *Nucleic Acids Res.* **2018**, *46*, W246–W251.
- (94) Dixon, P. VEGAN, a package of R functions for community ecology. *J. Veg. Sci.* **2003**, *14*, 927–930.
- (95) Senko, J. M.; Istok, J. D.; Sufita, J. M.; Krumholz, L. R. In-situ evidence for uranium immobilization and remobilization. *Environ. Sci. Technol.* **2002**, *36*, 1491–1496.

- (96) Kappler, A.; Bryce, C.; Mansor, M.; Lueder, U.; Byrne, J. M.; Swanner, E. D. An evolving view on biogeochemical cycling of iron. *Nat. Rev. Microbiol.* **2021**, *19*, 360–374.
- (97) Weber, K. A.; Thrash, J. C.; Van Trump, J. I.; Achenbach, L. A.; Coates, J. D. Environmental and Taxonomic Bacterial Diversity of Anaerobic Uranium(IV) Bio-Oxidation. *Appl. Environ. Microbiol.* **2011**, *77*, 4693–4696.
- (98) Weber, K. A.; Picardal, F. W.; Roden, E. E. Microbially catalyzed nitrate-dependent oxidation of biogenic solid-phase Fe(II) compounds. *Environ. Sci. Technol.* **2001**, *35*, 1644–1650.
- (99) Waite, T. D.; Davis, J. A.; Payne, T. E.; Waychunas, G. A.; Xu, N. Uranium(VI) adsorption to ferrihydrite: Application of a surface complexation model. *Geochim. Cosmochim. Acta* **1994**, *58*, 5465–5478.
- (100) Bargar, J. R.; Reitmeyer, R.; Lenhart, J. J.; Davis, J. A. Characterization of U(VI)-carbonate ternary complexes on hematite: EXAFS and electrophoretic mobility measurements. *Geochim. Cosmochim. Acta* **2000**, *64*, 2737–2749.
- (101) Moyes, L. N.; Parkman, R. H.; Charnock, J. M.; Vaughan, D. J.; Livens, F. R.; Hughes, C. R.; Braithwaite, A. Uranium uptake from aqueous solution by interaction with goethite, lepidocrocite, muscovite, and mackinawite: An X-ray absorption spectroscopy study. *Environ. Sci. Technol.* **2000**, *34*, 1062–1068.
- (102) Burow, K. R.; Belitz, K.; Dubrovsky, N. M.; Jurgens, B. C. Large decadal-scale changes in uranium and bicarbonate in groundwater of the irrigated western U.S. *Sci. Total Environ.* **2017**, *586*, 87–95.
- (103) Lopez, A. M.; Wells, A.; Fendorf, S. Soil and Aquifer Properties Combine as Predictors of Groundwater Uranium Concentrations within the Central Valley, California. *Environ. Sci. Technol.* **2021**, *55*, 352–361.