Advection of surface-derived organic carbon fuels microbial reduction in Bangladesh groundwater

Brian J. Mailloux[a,1], Elizabeth Trembath-Reichert[a], Jennifer Cheung[a], Marlena Watson[a], Martin Stute[a], Greg A. Freyer[b], Andrew S. Ferguson[c], Kazi Matin Ahmed[d], Md. Jahangir Alam[d], Bruce A. Buchholz[e], James Thomas[f], Alice C. Layton[g], Yan Zheng[h], Benjamin C. Bostick[h], and Alexander van Geen[h]

[a]Environmental Science Department, Barnard College, New York, NY 10027; [b]Environmental Health Science, Columbia University, New York, NY 10032; [c]Department of Civil Engineering and Engineering Mechanics, Columbia University, New York, NY 10027; [d]Department of Geology, University of Dhaka, Dhaka 1000, Bangladesh; [e]Center for Accelerator Mass Spectrometry, Lawrence Livermore National Laboratory, Livermore, CA 94551-9900; [f]Hydrologic Sciences, Desert Research Institute, Reno, NV 89512; [g]Department of Microbiology and Center for Environmental Biotechnology, University of Tennessee, Knoxville, TN 37922; and [h]Lamont–Doherty Earth Observatory, Columbia University, Palisades, NY 10964

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Chronic exposure to arsenic (As) by drinking shallow groundwater causes widespread disease in Bangladesh and neighboring countries. The release of As naturally present in sediment to groundwater has been linked to the reductive dissolution of iron oxides coupled to the microbial respiration of organic carbon (OC). The source of OC driving this microbial reduction—carbon deposited with the sediments or exogenous carbon transported by groundwater—is still debated despite its importance in regulating aquifer redox status and groundwater As levels. Here, we used the radiocarbon (^{14}C) signature of microbial DNA isolated from groundwater samples to determine the relative importance of surface and sediment-derived OC. Three DNA samples collected from the shallow, high-As aquifer and one sample from the underlying, low-As aquifer were consistently younger than the total sediment carbon, by as much as several thousand years. This difference and the dominance of heterotrophic microorganisms implies that younger, surface-derived OC is advected within the aquifer, albeit more slowly than groundwater, and represents a critical pool of OC for aquifer microbial communities. The vertical profile shows that downward transport of dissolved OC is occurring on anthropogenic timescales, but bomb ^{14}C-labeled dissolved OC has not yet accumulated in DNA and is not fueling reduction. These results indicate that advected OC controls aquifer redox status and confirm that As release is a natural process that predates human perturbations to groundwater flow. Anthropogenic perturbations, however, could affect groundwater redox conditions and As levels in the future.

Aquifer redox status is a major factor affecting groundwater composition and its suitability for human consumption. The most egregious example is the dire health impact of elevated levels of arsenic (As) in groundwater drawn with inexpensive hand-pumped tube wells by more than 100 million villagers across South, Southeast, and East Asia (1, 2). Aquifer redox status is largely controlled by microbial respiration of organic carbon (OC) coupled to the utilization of terminal electron acceptors. Reduction of iron (Fe) oxides containing As coupled with the oxidation of OC is the dominant process causing the accumulation of As in groundwater (1), but there is no consensus on the source of OC that fuels these transformations. Such information is critical to understanding subsurface carbon cycling, redox reactions, and the vulnerability of groundwater aquifers to perturbation.

Human perturbations have long been suggested to exacerbate the problem of elevated As in groundwater in several ways. Given the connectivity between contaminated surface waters and aquifers, widespread pumping could redistribute As from the surface to depth (3, 4) or between aquifers (5). This pumping could potentially also deliver reactive OC from ponds and latrines to depth, causing microbial reduction and As release (3, 4). Fluorescent spectra of dissolved organic carbon (DOC), correlations between sedimentary organic carbon and As levels, and stratigraphy suggest, however, that reactive OC is derived from the sediment within the aquifer and therefore is primarily of geologic origin (6–8).

The traditional view is that molecular properties of OC control its reactivity (9). Young OC is the most labile and becomes more recalcitrant over time as microbes continually use the OC. This view of carbon reactivity implies that recharge of anthropogenic waste fuels microbial reduction in As-rich aquifers, even if the amount of young carbon is small compared with other pools and difficult to detect. However, recent direct observations indicate the OC in the subsurface exists in smaller, simpler molecular structures than originally thought based on bulk extractions, and that often these reactive molecules persist in sedimentary systems much longer than previously believed (10, 11). This different perspective suggests that the molecular properties do not in themselves control reaction rates but OC decomposition rates are affected by ecosystem properties such as nutrient limitation, energy scarcity, or carbon bioavailability due to physical limitation such as reactions with mineral surfaces (12–14). These recent observations suggest that sedimentary organic matter or older OC pools may also be available for microbial respiration. These conflicting data imply that both young and old carbon sources could potentially contribute to redox reactions in aquifer systems.

Compound-specific radiocarbon analysis of cellular biomass can be compared with the radiocarbon content of different OC sources that have a unique radiocarbon signature to provide a direct measure of carbon substrate utilization in the environment (15–21). For example, this method has been used to document the preference of microbes for degradation of petroleum hydrocarbons and utilization of organic matter derived from shale weathering. In subsurface environments, a range of OC pools exist, each of which have their own radiocarbon signature. Surficial carbon advected from recent recharge would have a modern radiocarbon signature. Decades-old OC would have elevated radiocarbon from nuclear testing. Older OC of geologic origin would be depleted in radiocarbon, the age of which would depend on sediment depositional ages. Radiocarbon analysis of cellular biomass could differentiate these sources, but it can be difficult to collect, purify, and measure from aquifers. Radiocarbon analysis of lipids is usually performed on sediment samples but drilling methods to obtain large amounts of pristine sediments are not available for aquifers in Southeast Asia. Radiocarbon analysis has been performed on DNA extracted

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[1]To whom correspondence should be addressed. E-mail: bmaillou@barnard.edu.

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from human cells (22) and surface water samples (19, 21) but not groundwater, in part because it is difficult to collect sufficient DNA and to remove humic substances when purifying the DNA. The radiocarbon signature of DNA is a direct measure of the carbon used during microbial respiration and growth. In this study, we developed a method to filter, extract, and purify DNA from groundwater aquifers for radiocarbon analysis to determine the OC pools fueling microbial reduction.

Surprisingly, given the important role of OC in controlling groundwater quality and affecting the health of such a large population, no attempt has been made to unequivocally determine the source of reactive OC in aquifers in Southeast Asia or worldwide. Here, we use natural abundance radiocarbon analysis of microbial DNA to directly identify the OC source being used by microorganisms in a high-As aquifer. The DNA represents the OC used by microbes for cellular division. We chose a well-studied high-As site in Bangladesh where groundwater is pumped on a large scale during the dry season for irrigation, sand extends to the surface, and recharge is rapid (Fig. 1). If human activities are significant and influence redox reactions in these aquifers, it is likely to be detected at this site. Our results indicate that the OC used to fuel microbial reduction is advected from the surface to the aquifer over hundreds to thousands of years at a rate ~100 times slower than groundwater flow.

Results

Radiocarbon Analysis of DNA. To establish that microbes record the radiocarbon (^{14}C) signature of the substrates they use, three samples were analyzed as controls, a blank filter, DNA extracted from *Escherichia coli* grown on petroleum-derived acetate, and DNA extracted from *E. coli* grown on present-day LB media. No DNA was detected by UV/Vis nor carbon by accelerator mass spectrometry (AMS) in the control blank filter sample, and all samples had absorbance spectra consistent with pure DNA (Table 1). The close correspondence between the $\Delta^{14}C$ of the media on which the cells were grown and the $\Delta^{14}C$ of the cellular DNA indicates that the method developed to obtain and purify DNA for radiocarbon analysis from drinking water aquifers can be applied to understand OC dynamics in the subsurface. Radiocarbon measurements for DNA extracted from Bangladesh groundwater can therefore be compared directly with radiocarbon data for sediment, groundwater DOC, and dissolved inorganic carbon (DIC).

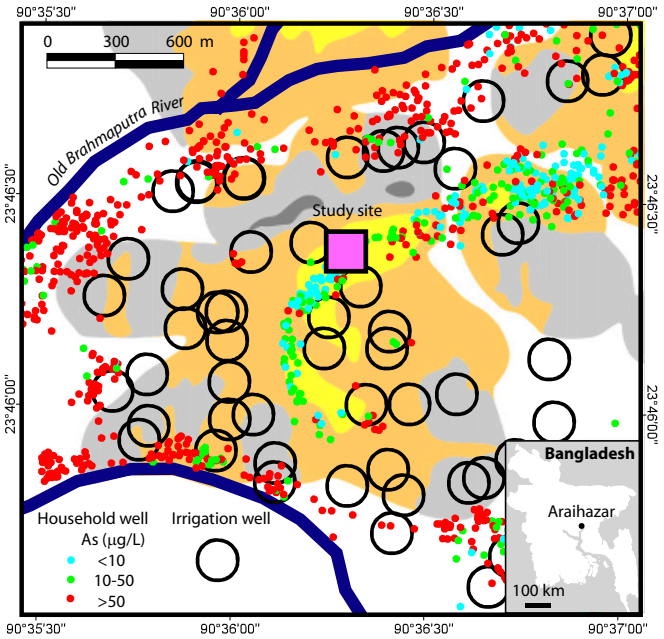

Fig. 1. Map of study location in Bangladesh (*Inset*) and area surrounding study location at site F. The small circles indicate As concentrations in private tube wells and delineate the villages (34). The larger circles represent high-capacity irrigation wells (25). The background color is interpolated EM31 resistivity data (31), and the yellow shades represent more permeable surface sediments, and the gray shades represent fine-grained surface sediment. The white background indicates no EM31 data are available.

Bangladesh Aquifer Analysis. The vertical profile in Lashkardi village represents the evolution of groundwater geochemistry from the surface to depth, even if it does not directly trace a groundwater flow path (Figs. 1 and 2) (23). The five shallow wells ranging between 6 and 25 m in depth below ground tap a Holocene (<10,000-y-old) aquifer that has developed the characteristic vertical As gradient with concentrations increasing from 0.007 to 2.7 μM (<1–203 μg/L) (24, 25). Within the same depth interval, concentrations of Fe, methane (CH_4), and DIC increase with depth while redox potential (Eh) and sulfate concentrations decrease, consistent with microbial respiration of OC coupled to reductive dissolution of Fe oxides, sulfate reduction, and methanogenesis (Fig. 2 and Tables S1–S5). There is a thin clay layer separating the two wells at 20- and 25-m depth within the Holocene aquifer. Above this thin clay layer, the aquifer contains ^{3}H and has been recharged since atmospheric testing of nuclear bombs began in the 1950s, whereas deeper groundwater has no ^{3}H and contains older (>50 y) groundwater. The deepest monitoring well extends beyond 15 m of clay and reaches a Pleistocene (>10,000 y) aquifer at 57 m containing orange oxidized sands and groundwater with <5 μg/L As that is likely much older than the shallower water (Fig. 2). The radiocarbon signature of the microbial DNA was measured in four samples, three from the Holocene aquifer and one from the Pleistocene aquifer (Tables 1 and 2).

Starting from the Pleistocene aquifer, the radiocarbon age of microbial DNA at 57 m is 4,680 ± 60 ^{14}C y, which is equivalent to $\Delta^{14}C$ −446 ± 4‰ or 0.56 fraction modern (Fm) in other notations (Tables 1 and 2, and Figs. 2 and 3). This DNA is considerably younger than the Pleistocene sediment (>10,000 y). Therefore, the carbon used by the resident microbes does not originate solely from the sediment and a significant portion has to be supplied from younger, exogenous sources through advection. Advection also explains why DOC is considerably younger than the sediment. In fact, the DNA age is similar to that of DOC and DIC, which were dated at 5,560 ± 42 ^{14}C y (−502 ± 3‰) and 6,240 ± 30 ^{14}C y (−543‰), respectively.

The age of the OC in the sediment corresponding to the next deepest Holocene interval in the profile (25 m) is about 9,200 ^{14}C y and contrasts with that of the groundwater which is >50 y old (based on lack of ^{3}H) and <540 ± 25 y (based on DIC ^{14}C age; Figs. 2 and 3, Tables 1 and 2, and Tables S1–S3). In this depth interval, the DNA (1,185 ± 40 ^{14}C y or $\Delta^{14}C$ −143 ± 4‰) is also much younger than the sediment and also much closer to the age of the DOC (865 ± 35 ^{14}C y) (Figs. 2 and 3). The deeper depth intervals (25 and 57 m) of the profile constrain the source of reactive carbon driving reduction. The DNA ages suggest that advected OC contributes considerably to the reactive OC pool. This surprising result indicates that carbon is effectively advected to depth, even when separated from the surface by low permeability units.

Metagenomic analysis of DNA from the 25-m Holocene well is useful to characterize the microbial populations and properly interpret the radiocarbon data in the context of the broader microbial community. In this complex community with over 900 genera identified encompassing all known domains, heterotrophic Proteobacteria represented >50% of the total classified sequences, as found in both shotgun metagenomic and amplicon-based 16S

Table 1. Summary of radiocarbon data for DNA

Sample	CAMS ascension no.	Volume filtered, L	A260/280	A260/230	Δ^{14}C ‰*	Radiocarbon age, y*
Blank filter			nd	nd		
LB media	143162				54 ± 4	Modern
LB-DNA	143159		1.88	2.33	6 ± 6	Modern
Acetate media	149428				−860 ± 1	15,715 ± 45
Acetate DNA	151193		1.82	2.13	−849 ± 3	15,170 ± 170
F2-11.1m	141040	3,780	1.93	2.02	−56 ± 10	410 ± 90
F4-19.4m	143158	11,103	1.83	2.12	−117 ± 5	940 ± 50
F6-25.4m	147445	24,000	1.87	2.19	−143 ± 4	1,185 ± 40
F5-56.8m	147444	17,464	1.86	2.24	−446 ± 4	4,680 ± 60

CAMS, Lawrence Livermore National Laboratory's Center for Accelerator Mass Spectrometry; nd, not detected. Blank cells represent where data could not be measured.
*Errors are reported AMS uncertainties.

rDNA libraries, whereas Achaea represented about 9% of the sequences in the shotgun metagenomic library (Figs. S1 and S2, and Table S6). The microbial community is also metabolically diverse and dominated by genomes associated with metal reduction, nitrogen fixation, nitrate reduction, sulfur cycling, fermentation, and utilization of complex organic compounds (Table S6). With respect to metabolism, analysis of the subsystems indicates that genes for the utilization of multiple forms of organic carbon were prevalent including sugars, organic acids, and aromatic carbons commonly associated with DOC (26, 27) (Fig. S3). Although methanogenesis and autotrophy could potentially lead to the incorporation of younger DIC into DNA, this does not appear to be a major factor as Archaea and autotrophic genes represented only a small fraction of the total sequences within this zone. Thus, methanogenesis and autotrophic processes probably have a minimal effect on DNA ages. Given that the DNA age reflects the most abundant organisms and genes, the DNA analyzed is most likely associated with organisms performing heterotrophic respiration. Paired with the younger and similar ^{14}C ages of DNA and DOC relative to sedimentary OC, we conclude that advected OC largely sustains microbial metabolism in this aquifer.

Groundwater from depths <19 m contains bomb-produced ^{3}H; this young groundwater is the most likely to be impacted by recent human perturbation. The presence of a strong bomb-^{14}C spike (Δ^{14}C 370 ± 5‰; Fig. 2) for DOC at 11 m confirms that young DOC has been transported to this depth. This elevated radiocarbon in DOC is consistent with DOC formation from ~1963 to 1977 when atmospheric radiocarbon was above 350‰ (28). In contrast, there is no indication of a significant bomb-carbon input in the DOC at 19 m (Δ^{14}C −205 ± 4‰; Fig. 2). These observations combined indicate that the advection of DOC is delayed relative to that of groundwater. We estimate the lower limit of the retardation factor for DOC transport of 5.5 by dividing the ratio of the vertical groundwater velocity at this site (29) to the fastest potential vertical penetration of bomb-labeled DOC (11 m in 30 y). The ^{14}C content of the DNA extracted at 11m (410 ± 90 ^{14}C y or

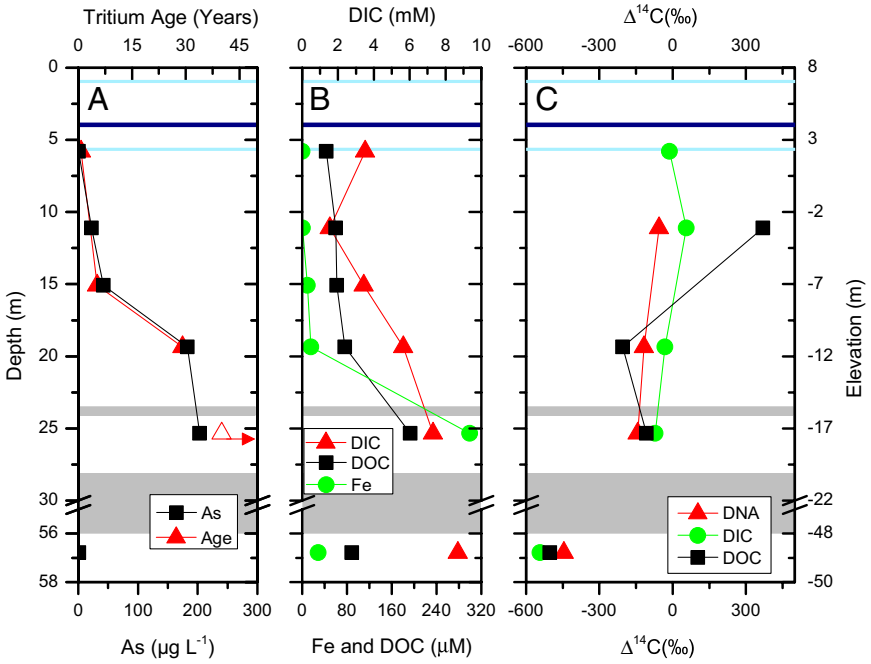

Fig. 2. Depth profiles of aqueous parameters from the site F well nest in Lashkardi village, Bangladesh. There is a break in the depth from 30 to 55 m, which is a continuous clay/silt unit. Silt/clay units are represented by a gray background. The dark blue line at 4.0 m on each panel is the average water table elevation, and the lighter blue lines are the observed minimum and maximum water table elevations. (*A*) Arsenic (As) concentrations and tritium ages of the water (29). The sample at 25 m was greater than 40 y old and is represented by an open triangle with an arrow. (*B*) Dissolved inorganic carbon (DIC), total iron (Fe), and dissolved organic carbon (DOC) concentrations. (*C*) Δ^{14}C (‰) of DNA, DOC, and DIC. Data are also presented in years and Fm in *SI Text*.

Table 2. Summary of radiocarbon data for well water samples

Well	Well depth, m	Tritium age, y	DNA		DIC		DOC	
			$\Delta^{14}C$ ‰	Radiocarbon age, y	$\Delta^{14}C$ ‰	Radiocarbon age, y	$\Delta^{14}C$ ‰	Radiocarbon age, y
F1	5.8	0.79			−12 ± 4	45 ± 30		
F2	11.1		−56 ± 10	410 ± 90	56 ± 5	Modern	370 ± 5	Modern
F3	15.1	5.28						
F4	19.4	29.10	−117 ± 5	940 ± 50	−31 ± 4	195 ± 25	−205 ± 4	1,759 ± 38
F6	25.4	>40	−143 ± 4	1,185 ± 40	−72 ± 6	540 ± 25	−108 ± 4	865 ± 35
F5	56.8	>40	−446 ± 4	4,680 ± 60	−543 ± 2	6,240 ± 30	−502 ± 3	5,560 ± 42

Blank cells indicate that no data are available. Errors are reported AMS uncertainties.

$\Delta^{14}C$ −56 ± 10‰) indicates that little bomb-spiked DOC has been metabolized by the resident microbes. A bomb spike is also not observed in the DIC, indicating that microbes are not respiring bomb-spiked DOC. Thus, DOC is effectively retarded relative to advection and its utilization by resident microbes appears to be quite limited.

The radiocarbon age of DNA at 19 m (940 ± 50 ^{14}C y or $\Delta^{14}C$ −117 ± 5‰) is younger than the sediment and intermediate and collinear between DNA ages at 11 and 25 m (Fig. 3). The combination of all three ages extrapolates to approximately zero age at the water table surface and therefore indicates an effective rate of reactive OC transport from the surface of 0.018 m·y^{-1}. This is over 100-fold slower than the vertical groundwater velocity, and roughly 20-fold slower than the estimated DOC velocity. Thus, advected surface-derived OC is not quickly consumed and can persist for decades without being metabolized in the aquifer.

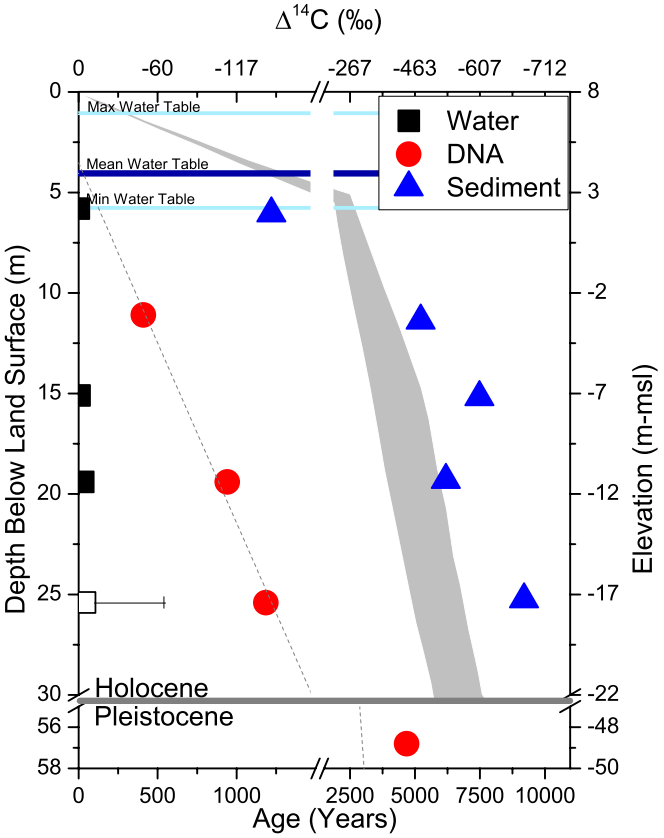

Fig. 3. Vertical profiles comparing water ages, DNA ages, and sediment ages. Note that there is a break in the depths from 30 to 55 m, and the scale on the ages changes at 1,500 y. The water ages are based on tritium–helium ($^3H/^3He$) groundwater dating. The sample at 25 m contained no tritium and is older than 40 y but less than 540 y based on DIC. The sediment ages are bulk organic carbon analyses of sediment cores. The gray zone represents predicted ages based on a depositional model (35). The dashed line is the best fit interpolation of the three shallowest DNA ages. The slope is 0.018 m·y^{-1}, and the intercept is 3.45 m (r^2 = 0.99).

Discussion

The generation, delivery, and transformation of surficial OC regulates aquifer redox status and the utilization of this OC plays a central role in regulating As levels at this site. Although advected OC is used in the aquifer, the data reported here challenge the notion that young OC drawn down by irrigation pumping over the last 50 y has affected water quality in a rapidly recharged aquifer (3, 4). In addition, the data place strict limits on the role that very old OC contained in petroleum (30), isolated peat strata (6), or ambient sedimentary carbon (7) play in driving microbial activity in reducing aquifers. In all sampled intervals of Holocene and Pleistocene age, the DNA is considerably younger than the sediment. Advection of OC from a younger interval, most likely shallow soils based on the extrapolated depth trend of DNA age, plays a central role.

An alternative explanation of the DNA radiocarbon results is the simultaneous utilization by microbes of two end members, rapidly recharged anthropogenic OC and sedimentary OC producing an intermediate age. The microbes probably use a diverse range of OC that are sourced from both the sediment and the water and it is clear that the advected DOC interacts with minerals as indicated by its retardation. However, the presence of bomb-spiked DOC but not bomb-spiked DNA at 11.1-m depth rules out the contribution of a young carbon source because the DNA age data versus depth remains linear and does not contain this large bomb spike.

The ^{14}C data for DNA imply that advected OC is both reactive and transported to depth over hundreds of years, significantly more slowly than the rate of groundwater flow. These seemingly conflicting observations indicate that the OC that recharges the aquifer remains labile during transport but is not immediately metabolized by the microbial communities at depth. Young OC (<100 y) penetrates only a few meters into the sediments and is not associated with the current locations of elevated As. Over time (>100 y), exogenous OC is advected throughout the aquifer system, is the main driver of redox reactions, and is associated with high As. During transport, decomposition of surficial OC is inhibited, but our data cannot be used to determine the ecosystem property limiting the decomposition of the OC.

The data show that, even at a site where downward flow was greatly enhanced by groundwater pumping and enhanced recharge, anthropogenic factors do not appear to have significantly influenced groundwater redox status. These results indicate that As release to date is primarily a natural process fueled by the slow advection of surface-derived OC. The advection of OC including anthropogenic sources could eventually affect groundwater redox

conditions and As concentrations, but this has not yet occurred at this particular site and therefore probably elsewhere. Aquifers that are low in As and relatively isolated from OC recharge are therefore less likely to be vulnerable in the long term.

Methods

Field Area. The study site (site F) has been the focus of extensive investigations and is located in Lashkardi village of Araihazar upazila, ~20 km east of the capital Dhaka (24, 25, 29) (Fig. 1). The site has six wells ranging in depth from 6 to 57 m. The water table at the site rises by over 3 m during the summer monsoon (Fig. 2). The surface soil and sediment surrounding the village is particularly sandy (31), and the area contains a large number of high-capacity irrigation wells (Fig. 1). These characteristics contribute to an unusually rapid groundwater recharge rate of 0.5 $m{\cdot}y^{-1}$ (corresponding to a vertical velocity of ~2 $m{\cdot}y^{-1}$ near the water table, porosity of 0.25) inferred from ^{3}H-^{3}He groundwater dating (29).

Sample Collection and Analysis. All samples were collected using submersible pumps at ~4.2 $L{\cdot}min^{-1}$, and the wells were purged three well volumes before sampling. New tubing was used during each sampling trip, and tubing was never allowed to touch surface sediment and was wiped with disinfecting wipes before placement into a well. We concentrated bacteria from over 3,000 L (equivalent to a 1.8-m radius around a 1-m well screen) of groundwater by filtering onto a 0.2-μm nylon filter; this will capture planktonic cells and not detrital DNA, which will pass through the filter. It is not possible to collect enough sterile sediment to perform radiocarbon analysis of the DNA, and therefore it is assumed that the planktonic community is representative. Filters were frozen on dry ice in the field. DNA was extracted and purified using phenol/chloroform and CsCl. Graphite targets were made from the DNA and were measured at the Center for AMS at Lawrence Livermore National Laboratory. Multiplex tag-encoded pyrosequencing (32, 33) and 454 shotgun metagenomic sequencing were performed to examine the microbial community of the high As well (F6-25.4m). Complete methods are available and all data are described in *SI Text*.

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