

Sulfate reduction accelerates groundwater arsenic contamination even in aquifers with abundant iron oxides

Received: 19 August 2022

Accepted: 19 December 2022

Published online: 16 February 2023

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Groundwater contamination by geogenic arsenic is a global problem affecting nearly 200 million people. In South and Southeast Asia, a cost-effective mitigation strategy is to use oxidized low-arsenic aquifers rather than reduced high-arsenic aquifers. Aquifers with abundant oxidized iron minerals are presumably safeguarded against immediate arsenic contamination, due to strong sorption of arsenic onto iron minerals. However, preferential pumping of low-arsenic aquifers can destabilize the boundaries between these aquifers, pulling high-arsenic water into low-arsenic aquifers. We investigate this scenario in a hybrid field-column experiment in Bangladesh where naturally high-arsenic groundwater is pumped through sediment cores from a low-arsenic aquifer, and detailed aqueous and solid-phase measurements are used to constrain reactive transport modelling. Here we show that elevated groundwater arsenic concentrations are induced by sulfate reduction and the predicted formation of highly mobile, poorly sorbing thioarsenic species. This process suggests that contamination of currently pristine aquifers with arsenic can occur up to over 1.5 times faster than previously thought, leading to a deterioration of urgently needed water resources.

Nearly 3 billion people depend on groundwater as a drinking water resource¹. The quality of groundwater, including concentrations of toxic chemicals within aquifers, is largely modulated by redox processes. The reductive dissolution of iron (Fe) oxides is widely considered among the most important redox transformations in these aquifers because it is assumed to control dissolved concentrations of several toxic metals^{2,3} and metalloids including arsenic (As)^{4–7}. Naturally occurring As contamination affects an

estimated 94–220 million people across the world⁸, largely by consumption of untreated groundwater from millions of private wells sourced in anoxic aquifers⁴. In the present study, we challenge the notion that aquifers currently low in As are safeguarded against contamination as long as Fe reduction does not occur. We present evidence that limited sulfate reduction instead is sufficient to facilitate groundwater contamination with As up to over 50% faster than expected.

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Numerous laboratory experiments have considered microbial Fe reduction coupled to organic carbon oxidation as the main cause of As repartitioning from sediments into groundwaters^{5,9}. Loss of Fe mineral substrates for As adsorption and competition for adsorption sites by phosphate and other ions is usually invoked to explain observed patterns of As release⁴. However, evidence of causation rather than mere correlation of Fe reduction and As release is surprisingly limited^{5,9,10}. This explanation for As release implies that enough Fe reduction must occur to reduce a meaningful fraction of the available Fe(III) oxides, therefore requiring a sufficiently large pool or supply of labile organic carbon to sustain microbial Fe reduction. Studies have also investigated the role of sulfur redox reactions in regulating As mobility, and the emerging role of thioarsenic species^{11–13}, but most of the responsibility for As contamination in groundwater remains attributed to microbial Fe reduction^{14–16}.

Among the Earth's most important places to examine the control of Fe on As mobility are Pleistocene aquifers deposited >12,000 years ago that are generally low in dissolved As but threatened by the incursion of reducing, high-As groundwater from younger Holocene aquifers^{17–20}. For millions of people relying on As-impacted groundwater in South and Southeast Asia, a particularly cost-effective solution has been to switch from the extraction of groundwater from shallow Holocene-aged ($\leq 12,000$ years) aquifers to using deeper and typically low-As water from Pleistocene aquifers²¹. The Pleistocene aquifers contain oxidized sediments assumed to greatly limit As contamination due to strong adsorption onto Fe (oxyhydr)oxide minerals⁴. Over the past several decades, however, groundwater extraction from Pleistocene aquifers in (mega)cities such as Dhaka and Hanoi has increased by several orders of magnitude, thereby lowering groundwater levels, altering regional groundwater flow patterns and causing the infiltration of high-As water into low-As aquifers^{17,22–24}.

From a global health risk perspective, nowhere is it more important to understand redox processes and transformations than at the interface between high-As Holocene aquifers and low-As Pleistocene aquifers. A complete assessment of the sustainability of groundwater pumping from low-As aquifers requires not only accounting for hydrogeological complexities^{20,24}, but also integrating a broader suite of biogeochemical processes relevant to As mobility. In this Article, we examine the release and transport of As in a unique hybrid column experiment conducted in the field in Bangladesh where Holocene groundwater is pumped through intact columns of freshly cored Pleistocene sediments. Time series of column effluent composition and sediment characterization were interpreted through process-based reactive transport modelling^{25,26}. Unlike previous studies often performed with highly idealized systems, our experiment employed mixed mineral assemblages, ambient microbes and reactive groundwater species that were unaltered from in situ field conditions. Our unique approach therefore combines the advantages of using native and undisturbed aquifer materials with the benefits of better control and characterization provided by column experiments, thereby reducing uncertainties. Surprisingly, the modelled observations indicate that the major control on As mobility is not exerted by adsorption/desorption on Fe minerals undergoing reduction, as typically assumed, but is driven instead by sulfate reduction and the formation of weakly adsorbing thioarsenic species^{27,28}. Accordingly, arsenic complexation with sulfur can act as a switch to expedite contamination in vital low-As aquifers despite the availability of oxidized Fe minerals.

Experimental observations

Anoxic high-As ($4.32\ \mu\text{M}$, $320\ \mu\text{g l}^{-1}$) groundwater from a Holocene aquifer was continually pumped through a column (inner diameter 1.6 cm, length 7.5 cm) filled with freshly cored Pleistocene aquifer sediments²⁹. The sediments consisted of sands importantly containing 2.4% Fe, of which the majority was determined to be the Fe(III) minerals ferrihydrite or nano-goethite³⁰ (Supplementary Table 1) and reflects

the average fraction of ferrihydrite found in Pleistocene sediments elsewhere in South and Southeast Asia³¹. Notably, the reduced non-silicate Fe(II) content was only 4% of total Fe at the beginning of the experiment, based on X-ray absorption spectroscopy (XAS) (Supplementary Table 1). Solid-phase As concentrations were also low, averaging $2.0 \pm 0.6\ \text{mg kg}^{-1}$ (ref. ²⁹). The observed effluent concentrations of major and minor groundwater constituents, such as As, phosphate, sulfate and Fe, varied considerably over time relative to the nearly constant influent concentrations. These variations indicate a pronounced geochemical disequilibrium between the inflowing Holocene groundwater and the Pleistocene sediments (Fig. 1).

Early in the experiment, concentrations of As rose in the effluent from the column (Fig. 1). This was accompanied by <5% reduction of Fe(III) oxides to Fe(II) in the aquifer sediment over the entire course of the experiment (Fig. 2a). The spectroscopic data are consistent with limited and incomplete reduction of Fe oxides indicated by only a partial change in sediment colour from orange to grey²⁹. In contrast, after a brief immediate peak, effluent sulfate concentrations decreased by an order of magnitude relative to the inflow during the beginning of the experiment and remained generally low thereafter (Fig. 1). This decrease in sulfate is attributed to sulfate reduction converting aqueous sulfate to sulfide. Inflow sulfate concentrations are not unusually high for shallow Bangladesh aquifers. Nearly all high-As aquifers of Bangladesh have low sulfate ($<0.2\ \text{mM}$)³². Low sulfate concentrations are usually assumed to preclude sulfur-driven processes from dominating As release and/or transport^{4,32}. Inducing sulfate reduction to remove As has even been suggested for remediation^{33,34}. Arsenic mineralogical characterization confirms that sulfate reduction did occur and indeed trapped some As in the solid phase. Arsenic speciation, measured by As X-ray absorption near edge structure (XANES) spectroscopy (Methods), indicates that the initial unaltered Pleistocene sediment contained only adsorbed forms of arsenate and arsenite. By the end of the experiment, however, >50% of sediment As was found in As sulfide minerals on average (Fig. 2b and Supplementary Table 2). Overall, the increase of As in groundwater exiting the column of Pleistocene sediment was imminent and accompanied by little, if any, increase in Fe reduction and also occurred in spite of trapping of As in the column in the form of sulfide, indicating that Fe reduction played a subordinate role on the observed As behaviour.

For comparison, three additional columns also containing intact freshly cored Pleistocene aquifer sediments were simultaneously run in parallel with the main column described above during our hybrid field-column experiment: (1) a replicate of the column at the same main flow rate, (2) a modified replicate column at a slower (approximately halved) flow rate and (3) a modified replicate column at a faster (approximately doubled) flow rate (Methods and Supplementary Table 3). In total, four columns containing Pleistocene aquifer sediments were collected and run in parallel at three different flow rates (Supplementary Table 3). The disequilibrium between the inflowing Holocene groundwater and Pleistocene sediments across all columns was apparent in the measured effluent concentrations, with similar decreases in sulfate concentrations and corresponding multiple peaks in As concentrations (Supplementary Fig. 1). Across all columns, Fe extended X-ray fine structure (EXAFS) showed that the reduction of Fe(III) oxides to Fe(II) in the aquifer sediment remained similarly minimal at <5% in each column (Supplementary Table 1). Likewise, As XANES revealed the production of As sulfides within all columns at the end of the experiment (Supplementary Table 2).

Model-based analysis of field experiment

We tested several combinations of biogeochemical processes with process-based reactive transport modelling to quantify the complex coupling between Fe and sulfur cycling with the adsorption of As and other solutes. To provide the best constrained system for reactive transport modelling, we focused on model construction and results using

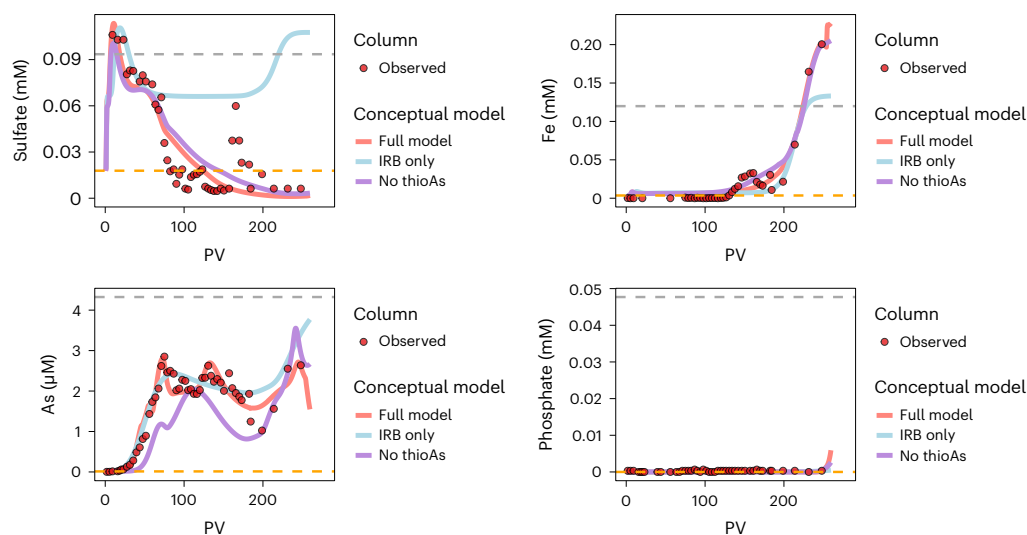


Fig. 1 | Time series of measured effluent groundwater composition (that is, from the column outlet, represented by circles) compared with model fits including all biogeochemical reactions ('full model'), without thioarsenic species ('no thioAs') and IRB only ('IRB only'). The grey horizontal dashed line indicates influent groundwater concentration from the field experiment, while the orange horizontal dashed line indicates representative Pleistocene groundwater concentrations of the column sediment. The fit of the models is marked by agreement of solid lines with observed concentrations denoted

by red points. Arsenic concentrations in the effluent groundwater quickly rise above both the World Health Organization guidelines of $10 \mu\text{g l}^{-1}$ ($0.13 \mu\text{M}$) and Bangladesh maximum contaminant level of $50 \mu\text{g l}^{-1}$ ($0.67 \mu\text{M}$). The brief increase in sulfate at 170 PV is an artefact from a pump failure (Methods). Measured effluent concentrations and matching full model runs for the replicate of this column and the column experiments run at two additional flow rates are available in Supplementary Fig. 1.

experimental observations of the main column with the medium flow rate (1) for clarity and (2) as it was run over the longest number of pore volumes (PV) and performed in duplicate at the same flow rate. The most plausible, and most complete, biogeochemical reaction network (Fig. 3) we identified includes (1) dissimilatory Fe(III) reduction of reactive ferrihydrite coupled to organic carbon oxidation, (2) dissimilatory sulfate reduction coupled to organic carbon oxidation, (3) precipitation of secondary Fe minerals including mackinawite, goethite, magnetite and siderite, (4) precipitation of As sulfides (orpiment), (5) sulfide oxidation coupled to ferrihydrite reduction and additional sulfur disproportionation, (6) formation of thioarsenic species and (7) sorption of arsenate and arsenite alongside other competing ions on major Fe minerals. Each of these steps is thermodynamically viable and, in most cases, has well-established chemical equilibria (Methods).

The complete model and alternative model variants, which lack one or more reactions (Supplementary Table 4), were each subjected to an automated inverse modelling procedure during which parameters were estimated by minimizing the residuals between simulated and observed concentrations. Parameters with a high degree of certainty remained fixed to known values, while uncertain parameters were estimated (Methods and Supplementary Table 7). The evaluation against measured data and a comparison among the investigated model variants was interpreted to determine the most plausible conceptual/numerical model and to identify which processes critically affect As mobility.

The numerical implementation of the complete biogeochemical reaction network illustrated in Fig. 3 includes both Fe and sulfate reduction and most closely reproduced the experimental data (Fig. 1 and Supplementary Fig. 2). The complete biogeochemical reaction network also accurately simulated experimental data from columns operated at the additional flow rates with low normalized root mean squared errors (Supplementary Fig. 1, model evaluation). These flow rates are representative of groundwater velocities under scenarios of increased groundwater pumping by growing (mega)cities in South and Southeast Asia, driving vertical infiltration from shallower Holocene aquifers to deeper Pleistocene aquifers via discontinuities in the

intervening clay layer (for example, in Bangladesh)³⁵ or accelerating lateral infiltration from Holocene aquifers into adjacent Pleistocene aquifers (for example, in Vietnam)^{17,23}. These scenarios, where elevated As contamination has been found^{17,23,35}, represent a growing threat to groundwater quality, especially where fast flow paths may lead to underestimated contamination²⁴ (Discussion).

A model variant based on Fe reduction by iron-reducing bacteria (IRB) only ('IRB only', Fig. 1) did not consume sulfate and failed to match observations. Importantly, the 'IRB only' model cannot describe the production of As sulfide minerals, even after model recalibration to fit the data as closely as possible, as evidenced by a lower composite objective function for the full model (Supplementary Fig. 3 and Supplementary Tables 4 and 8). The 'IRB only' variant couples all organic carbon respiration to dissimilatory Fe reduction by IRB while excluding activity from sulfate-reducing bacteria (SRB), thus preventing all processes requiring sulfide. The required inclusion of sulfate reduction underscores the importance of the sulfur cycle in regulating As concentrations, especially in systems traditionally identified as Fe reducing. The modelling results confirm that Fe reduction has a negligible impact on As transport behaviour during the experiment (Supplementary Fig. 4).

From the full model, the experiment can be separated into three distinct periods: (1) an initial sorption phase lasting 30 PV during which sorption/desorption is the dominating process controlling aqueous concentrations, especially those of As, sulfate, Fe and phosphate (Fig. 1), and exchange reactions control major cations (Na, K, Mg and Ca) (Supplementary Fig. 2), followed by (2) an extended period marked by the onset and persistence of sulfate reduction in which a combination of sorption reactions, redox transformations and mineral reactions affected aqueous compositions and (3) finally, after 200 PV, a period of accelerating Fe(III) reduction.

Multiple impacts of sulfate reduction

After an initial period of 30 PV, during which effluent sulfate briefly peaked above influent sulfate concentrations due to desorption from Fe(III) minerals (Supplementary Fig. 5), dissolved sulfate concentrations declined rapidly by about an order of magnitude from $90 \mu\text{M}$ to

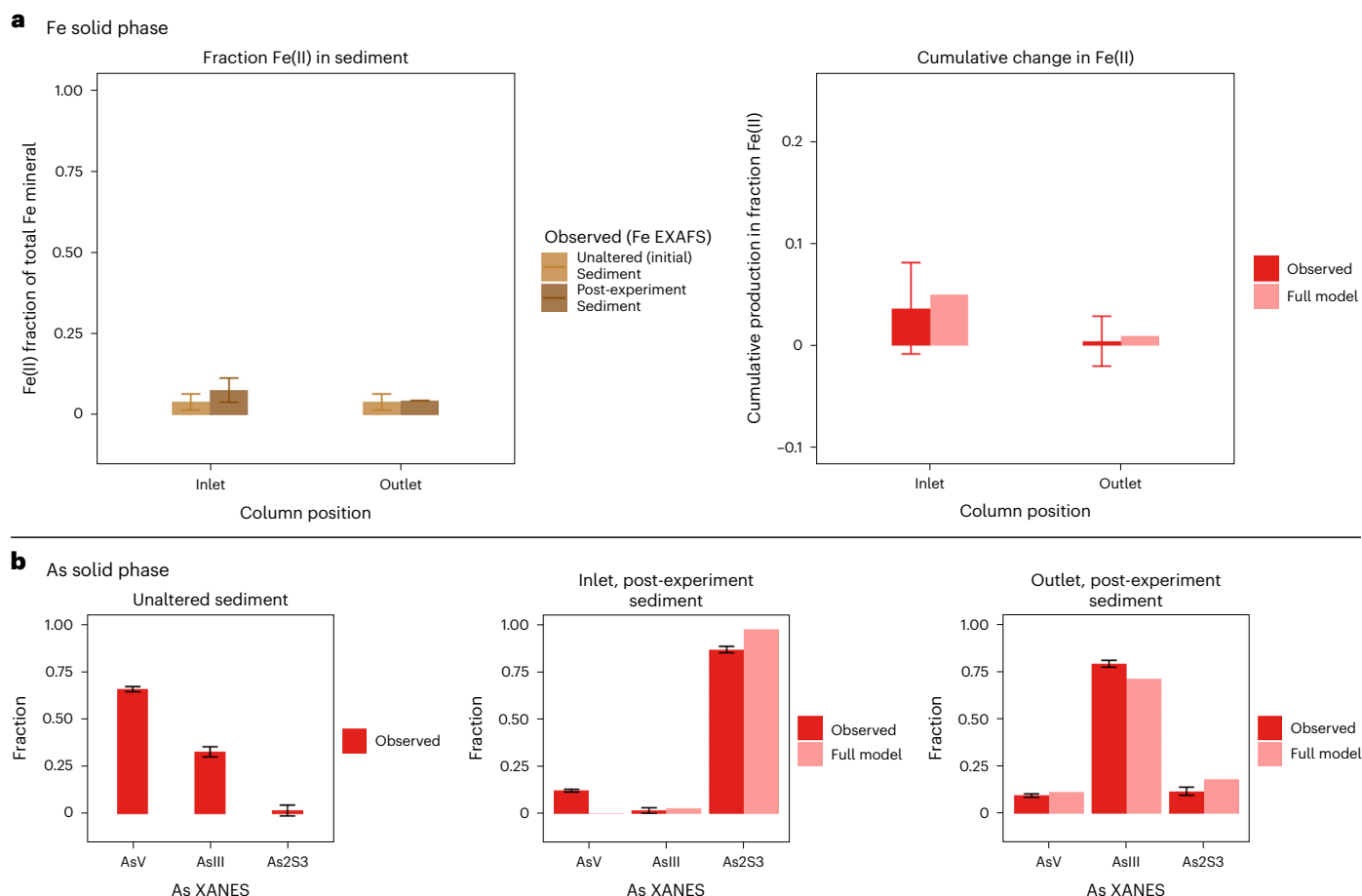


Fig. 2 | Observations of solid-phase Fe and solid-phase As, as measured by XAS and represented as fractions of the total Fe or As, compared with full model results. The unaltered sediment is representative of the initial Pleistocene sediment before the start of the experiment. **a**, The total fraction of non-silicate Fe(II) found in the sediment is small (<10%), both in the unaltered (initial) sediment and in the post-experiment sediment. The cumulative change in non-

silicate fraction Fe(II), with a positive change indicating production of Fe(II), at the end of the experiment is almost negligible. **b**, Arsenic speciation does change between the initial unaltered sediment and the post-experiment sediment, in both the inlet and outlet of the column, with more As sulfide minerals (As_2S_3) post-experiment. The error bar represents the standard error from the linear combination fit of the XAS measurements.

5 μM (Fig. 1). This delayed onset of sulfate consumption is interpreted as a short microbial lag before SRB growth (Methods)^{36,37}. Once initiated, sulfate reduction persisted throughout the remainder of the experiment (Supplementary Fig. 6) with model-inferred SRB activity primarily responsible for organic carbon turnover. While SRB activity is often reported to occur in parallel with Fe reduction by IRB^{38,39}, the employed model-based interpretation allowed for untangling of the contribution of individual processes towards overall redox cycling. Our modelling shows that the rate of sulfate reduction was up to 200 times greater than Fe(III) reduction until near the end of the experiment (around 200 PV), when increased microbial Fe reduction rates (Supplementary Fig. 7) led to corresponding increases in effluent dissolved Fe concentrations and a very minor (<5%) increase in the fraction of Fe(II) minerals (Fig. 2a and Supplementary Fig. 7). Siderite is formed from inflowing Fe(II), combined with some internally produced Fe(II), and the bicarbonate generated during organic carbon oxidation, mostly by SRB (Supplementary Fig. 8). Mackinawite may also form from available Fe(II) along with sulfide produced from sulfate reduction; however, when modelled, mackinawite composes only ~1% of the total Fe at the end of the experiment, agreeing with the mackinawite fraction of <1–3% found by Fe EXAFS across all columns at the end of the experiment (Supplementary Table 1 and Supplementary Fig. 8).

Our model-based interpretation suggests that sulfide produced during sulfate reduction is an important reactant not only for (1) Fe and

As sulfide mineral formation and (2) sulfide oxidation (recycling) within the column, primarily by reducing Fe(III) minerals, but also crucially for (3) aqueous As complexation. Abiotic sulfide oxidation produces zero-valent sulfur as ferrihydrite is reduced to Fe(II) (ref.⁴⁰). Zero-valent sulfur produced from sulfide oxidation can undergo a number of predominantly biological reactions including sulfur disproportionation⁴¹, that is, oxidation to sulfate or reduction to sulfide (Fig. 3). This partial conversion to sulfide and sulfate results in a cryptic sulfur cycle, which refers to potentially extensive but poorly characterized redox cycling within a small standing pool of sulfur^{42,43}.

Spatial and temporal dynamics of As retention and re-mobilization

The model results demonstrate that inflowing As is almost exclusively partitioned onto the Pleistocene sediments via surface complexation of arsenite, the dominant form of As in influent groundwater over the first 30 PV (Fig. 4 and Supplementary Fig. 5). In the model, this behaviour was accurately simulated by invoking surface complexation reactions onto Fe(III) minerals: ferrihydrite and goethite. The sorption capacity of the sediments is linked to the surfaces provided by ferrihydrite and goethite, and thus changes dynamically where and when mineral concentration changes occur. Increasing As effluent levels at ~60 PV coincided with the start of intensive sulfate reduction, after the initial microbial lag period (Fig. 4). Our modelling indicates that dissolved sulfide concentrations

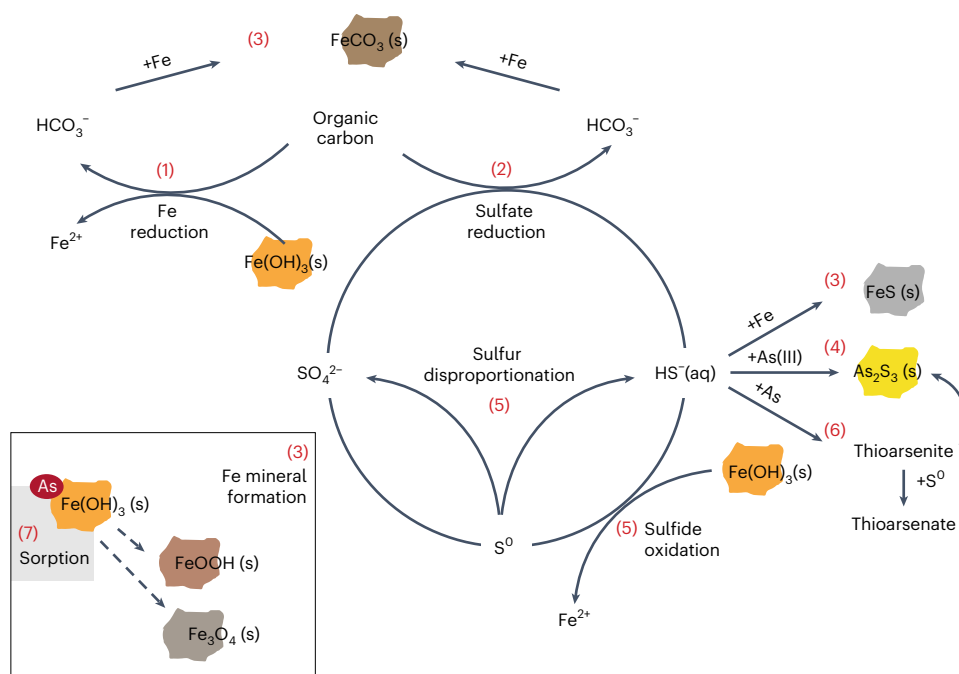


Fig. 3 | Biogeochemical reaction network of major processes related to Fe, As and sulfur cycling included in the full model and corresponding numerical implementation. The included processes are as follows: (1) dissimilatory Fe(III) reduction of reactive ferrihydrite ($\text{Fe}(\text{OH})_3$) coupled to organic carbon oxidation, (2) dissimilatory sulfate reduction coupled to organic carbon oxidation, (3) precipitation of secondary Fe minerals including mackinawite (FeS), goethite

(FeOOH), magnetite (Fe_3O_4) and siderite (FeCO_3), (4) precipitation of As sulfides (for example As_2S_3), (5) sulfide oxidation coupled to ferrihydrite reduction and additional sulfur disproportionation, (6) formation of thioarsenic species and subsequent transformations and (7) sorption of arsenate and arsenite alongside other competing ions on major Fe minerals.

facilitate formation of abundant thioarsenic species, which accumulate in solution because they are poorly retained through adsorption^{27,28}. Dissolved sulfide was also partially consumed to create sulfide minerals that are retained in the sediment (Supplementary Fig. 9). Following sulfate reduction, retention of As in the solid phase is dominated by As sulfide minerals, consistent with As XANES observations (Fig. 2b and Supplementary Table 2). Arsenic sorption on produced mackinawite was also considered in the As XANES fits, but as mackinawite made up a very small fraction of the total Fe (~1%) in both measurements and model results, As sorption on produced mackinawite was minimal in the total As budget and was not simulated in the model (Methods).

Desorption of As into the aqueous phase occurs despite considerable residual surface sites being available for As retention—it is not caused by phosphate, which is often assumed to compete with As for sorption sites^{44,45}. While phosphate indeed increasingly occupies sorption sites, low effluent phosphate concentrations for nearly the entirety of the experiment implies that sorption sites are not saturated and unrelated to increases in effluent As, despite high influent phosphate concentrations. However, desorption of the majority of As on Fe minerals begins to occur at ~60 PV, in sync with sulfate reduction and thioarsenic complexation (Supplementary Fig. 9). Sorption sites also retain influent and internally produced dissolved Fe (Fig. 1 and Supplementary Fig. 5), eventually minimally transforming ferrihydrite to magnetite⁴⁶ (Supplementary Figs. 5 and 8). Although in situ magnetite formation has the potential to sequester As through sorption and incorporation into its structure^{47,48}, the amount of magnetite formed is minimal (Supplementary Fig. 8) with no noticeable change in Fe EXAFS from the control (Supplementary Table 1) and thus is unlikely to affect As in solution.

Thioarsenic species and mobility

While the formation of thioarsenic species is increasingly recognized as a contributor to As mobility^{11,13,49}, due to their notably lower affinity for

sorption on Fe minerals^{27,28}, the mechanism of thioarsenic formation as an overall driver and exacerbator of As contamination has not yet been directly quantified against the commonly-attributed mechanism of iron reduction by IRB. Thioarsenic species are difficult to properly preserve and measure^{50–52}, and thus are often not measured or are overlooked, including during our field experiment. Comparative simulations based on a conceptual model that excluded thioarsenic species formation ('no thioAs', Fig. 1), however, failed to capture complete As solubilization and the solid As speciation, even after model recalibration (Fig. 1 and Supplementary Fig. 3), and thus thioarsenic formation is needed to explain the experimental results (Supplementary Tables 4 and 8).

The formation of mobile thioarsenic species inferred from the model accounts for up to 95% of As in the effluent (Supplementary Fig. 9). The modest sulfide activity may even enhance thioarsenic formation as sulfide activity must be within a narrow range for thioarsenic species to be stable⁵³, achieved by a combination of sulfate reduction, sulfide mineral dissolution–precipitation and coupled redox cycling. Thioarsenic species have been found in groundwater with high soluble Fe levels⁵⁰, despite previous assumptions about competition for sulfide with dissolved Fe. Specifically, thioarsenic species can form from (1) the dissolution of As sulfide minerals²⁸ or (2) reactions between As and sulfur species^{54,55} (Methods). Since As sulfide minerals were not found in the initial unaltered Pleistocene sediments but were found in greater fractions at the conclusion of the experiment (Fig. 2b and Supplementary Table 2), initial thioarsenic species probably formed from reactions in solution between dissolved As and sulfur. However, future thioarsenic release from dissolution of As sulfide minerals could occur, especially when aqueous compositions change. Although there is some debate about the equilibria between thioarsenates and thioarsenites^{56–60}, thioarsenic speciation is important in regulating As mobility and is included to the extent available (Methods, Supplementary Information Text and Supplementary Figs 10–12); the full model includes both thioarsenite and thioarsenate and all presently known thioarsenic transformations

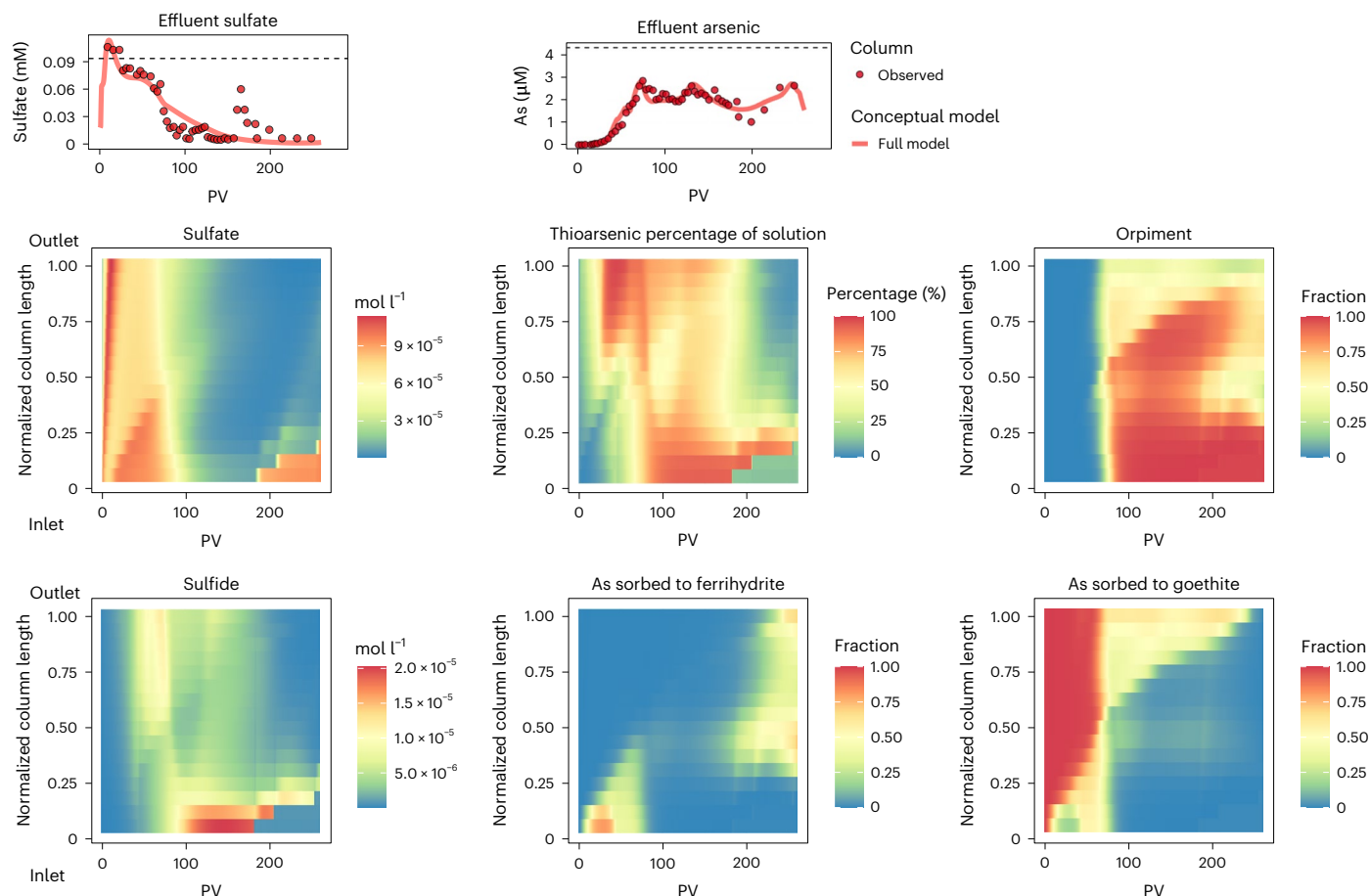


Fig. 4 | In space (normalized column length on y axis) and time (PV, on x axis), decrease in sulfate concentrations leads to production of sulfide and the heterogeneous formation of thioarsenic species. The inlet and outlet of the column are labelled accordingly on the y axis. Model-derived solid As speciation (orpiment, As sorbed to ferrihydrite and As sorbed to goethite) are displayed in fraction As over the total As (solid and solution) in the column. The fate of As

within the column can be described mainly by sorption to ferrihydrite or goethite during the initial ~60 PV of the experiment, after which the fate of As within the column is dominated by sequestration into arsenic sulfides (generalized by orpiment). The time series at the top are effluent time series of sulfate and As from the outlet of the column (as in Fig. 1).

for aqueous species. Overall, the fundamental inclusion of thioarsenic is essential for explaining the observed data (Fig. 1).

Discussion

Our experiment and the resulting model are probably representative of the hydrological migration of high-As groundwater from Holocene aquifers into uncontaminated Pleistocene aquifers, as recently documented in both Bangladesh³⁵ and Vietnam¹⁷. The three flow rates in our experiment were run at porewater velocities of 40 cm day⁻¹, 81 cm day⁻¹ and 155 cm day⁻¹, translating to 146 m year⁻¹, 296 m year⁻¹ and 566 m year⁻¹ (Supplementary Table 3). The corresponding Darcy fluxes are 0.5 cm h⁻¹, 1.0 cm h⁻¹ and 2 cm h⁻¹, respectively (Supplementary Table 3). These flow rates reflect increasing groundwater flow rates in present and projected future pumping scenarios, not only at our field site in Araihaaz, located at the edge of the depression cone created by large-scale pumping in the nearby megacity of Dhaka, Bangladesh^{20,24,35,61}, but also broadly across other deltas in South and Southeast Asia^{17,18,62}. As a comparison, previous estimates in well-calibrated regional groundwater flow models across the Bengal Basin of unperturbed flow in predevelopment times (that is, pre-groundwater pumping) were estimated to be 8 m year⁻¹ on average and increased by five times under pumping scenarios to ~40 m year⁻¹ for the model year 2002 (that is, a snapshot from two decades ago)⁶³. Since then, groundwater flow models of the Dhaka region, with recently updated

pumping rates and better incorporation of heterogeneity, have found even faster flow paths than expected in the Dhaka region²⁴. Due to preferential flow, more recent estimates showed a ten times faster travel time to deeper low-As aquifers that correspond to our experimental flow rates²⁴; contamination of these low-As Pleistocene aquifers, similar to those cored for our experiment, could occur within a decade instead of a century²⁴. Likewise, at our field site in Araihaaz, vertical recharge rates from shallow Holocene aquifers to deeper Pleistocene aquifers are also expected to increase as the depression cone around Dhaka expands⁶¹. There, vertical recharge through discontinuities in the clay layer between the aquifers, that is, through geological windows, and subsequent lateral transport have been associated with elevated As concentrations in the Pleistocene aquifer³⁵. Finally, the Darcy fluxes used within the experiment also correspond to those estimated of 20 cm day⁻¹ (~0.8 cm h⁻¹) around Dhaka⁶⁴. Thus, while certainly fast compared with undisturbed groundwater flow rates, our experimental flow rates correspond to groundwater flow rates in the intensified pumping scenarios, such as those around the megacity of Dhaka.

These scenarios of increased flow rate and infiltration of high-As water into Pleistocene aquifers are also observed in hydrogeological conditions that occur elsewhere, for example in aquifers near Hanoi, Vietnam^{17,23}. There, groundwater velocities were shown to reach between 38 m/year and 48 m/year, as estimated in 2011, while being expected to further increase at a rate of about 2 m year⁻¹ (refs. ^{17,23}) due

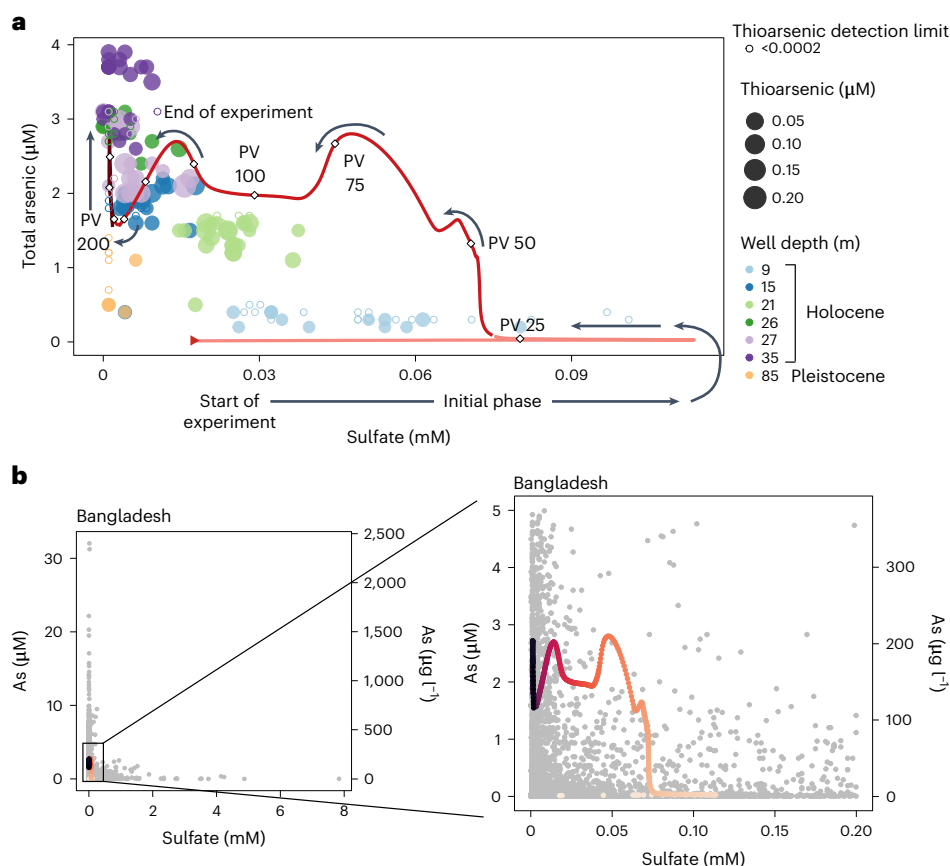


Fig. 5 | The end of our experiment coincides with the points of reported higher concentrations of monothioarsenate measured from groundwater in an aquifer setting in the field. a, The red line indicates the experiment over time: the evolution of the modelled effluent concentrations from our column experiment of sulfate (x axis) and total As (y axis), compared against the points representing measured concentrations of sulfate and total As from natural groundwaters at a nearby field site (compiled from an external study, Planer-Friedrich et al.¹²). This line describes temporal evolution of our experiment marked every 25 PV by the white diamonds; if concentrations were measured only at the beginning and end, the processes responsible for the increase in As would not be apparent. The size of the circle points is representative of the

concentration of thioarsenic (monothioarsenate) measured at the nearby site; thioarsenic concentrations below detection limit are indicated by an unfilled circle. The column effluent concentrations evolve from low sulfate and low As similar to the nearby field site's Pleistocene groundwater (orange points), to sulfate and As concentrations similar to the nearby Holocene aquifer by the end of the experiment. The reported monothioarsenate concentration may also be a minimum concentration. **b,** To present a potential alternative interpretation of typical sulfate versus As comparisons, the experiment eventually matches with low sulfate and high As concentrations found across Bangladesh (from compiled references, see Supplementary Table 9).

to increased groundwater pumping. Similarly, in a region just outside of the Hanoi cone of depression in the Red River Delta, porewater velocities in the boundary between early Holocene and Pleistocene aquifers were found to be up to 0.3 m day^{-1} (110 m year^{-1}) where more 'natural' vertical infiltration of high-As groundwater from Holocene aquifer to the Pleistocene aquifer were also implicated to drive As contamination⁶². Thus, the groundwater flow rates in our experiment are around the same order of magnitude of present and future scenarios, which are likely to be exacerbated under increased water demands, if not already. The consistency of the agreement of modelling results with observation across varying flow velocities (Supplementary Fig. 1, model evaluation) provides confidence that the experiment and modelled reaction network adequately incorporates the flow alongside the appropriate minerals, aqueous solutions and microbial processes.

Our experiment and the resulting model are also representative of the biogeochemical reactions that probably occur in As-affected aquifers in many parts of Asia, as corroborated by geochemical measurements from the field. External measurements of thioarsenic concentrations in groundwater from a nearby field site in Bangladesh as compiled from Planer-Friedrich et al.¹², with similar hydrochemical and hydrogeological characteristics and sediment ages, substantiate the

evolution of groundwater As in our model and the generalization that thioarsenic species are responsible for increased As mobility in this setting. This nearby site, only about 40 km away¹², contains a shallow Holocene and deeper Pleistocene aquifer structure⁶⁵. This aquifer structure is typical and widespread across Bangladesh, and accordingly carries the risk of high-As Holocene waters infiltrating into the deeper low-As Pleistocene aquifer as simulated in our experiment^{20,22,24,61}.

The evolution of our experimental effluent composition starts near the field site's Pleistocene aquifer concentrations (low As, low sulfate and low or undetectable sulfide) and the end of experiment coincides with aqueous As and sulfate concentrations near the field site's Holocene aquifer, where increased monothioarsenate concentrations in natural groundwater are measured (Fig. 5a). The similarities between the column effluent concentration changes and the field measurements suggest that (1) Pleistocene sediments subjected to hydrological disturbances at different times, that is, evidenced by the temporal evolution from the column experiment, can have wildly different As concentrations (temporal differences) and (2) perturbed Pleistocene sediments sampled at different locations along a flow path can also have different As concentrations (spatial differences). From modelling results of our hybrid field-column experiment, we

find the initial effluent As concentrations are primarily composed of arsenite, but soon after transforms to thioarsenic species (Fig. 4). Thus, ultimately, low-As Pleistocene aquifers that are infiltrated by high-As Holocene groundwater may evolve to have concentrations of sulfate and As akin to that of high-As Holocene groundwater and the measured presence of thioarsenic species.

Additionally, these field measurements have measured only monothioarsenate species¹², which indicates that thioarsenic species are indeed present, but also presents an incomplete description of thioarsenic speciation and a potential minimum of thioarsenic concentrations. The lack of other thioarsenic species is attributed to the complexity of measuring thioarsenic species in the field, due to changes in speciation caused by preservation methods or sample decomposition. For example, using acidification as a sample stabilization technique converts higher thiolated species of As, if present, to arsenite^{50,66}. In these field measurements from Bangladesh¹², the use of acidification for these samples restricted the detection of species to monothioarsenate only, as higher thiolated species would be converted to arsenite¹². Given this, if higher thiolated species were present, the concentration of measured thioarsenic species (monothioarsenate) represents a minimum concentration of highly mobile As in the field. The model suggests that other thioarsenic species may also be important (Supplementary Information Text). Ideally, experimental measurements of thiolated As species would have been done in our column experiment to confirm this result; however, this was not possible because of the nature of the experiment conducted in the field (manually operated fraction collector, large number of concurrent column experiments, small volumes of effluent available and acid additions to the vials to prevent metal precipitation). However, given that perturbations of Pleistocene aquifers are destined to accelerate under most future pumping scenarios²⁴, the mechanisms unravelled here would suggest that As will be more readily mobilized across larger areas than previously recognized (Supplementary Fig. 13). Future investigations should prioritize confirmation of these results, specifically with preservation methods and analytical confirmation of thioarsenates^{50,51}.

The full impact of sulfur cycling on modulating As mobility has been overlooked due to two common field observations that are often presented to exclude sulfate reduction as an influential process in groundwater systems: (1) low sulfate and (2) low, often undetectable sulfide concentrations^{12,32}; see high-As aquifers of Bangladesh in Fig. 5b. The common assumption that such low sulfate levels prevent active sulfur cycling is often used to imply that insufficient sulfide is produced to induce sulfide mineral formation or otherwise affect As mobilization¹² despite the presence of As sulfide minerals and high groundwater As found in low-sulfate, low-sulfide groundwater⁶⁷. Even when sulfate reduction has been acknowledged, previous research has attributed As release into solution to extensive reduction of Fe substrates by sulfide^{15,49,68,69}. However, this study provides clear evidence supporting active sulfate reduction without the buildup of noticeable sulfide, under sustained, low sulfate concentrations that ultimately affect As transport in these environments. Conditions evolve over the course of the experiment from low-As, higher-sulfate waters in deeper wells to high-As, low-sulfate waters found in typically shallower wells across Bangladesh and beyond (Supplementary Fig. 14). The evolution of thioarsenic species in this experiment can explain the inverse relationship between As and S (Supplementary Fig. 14). Thus, active sulfate reduction is probably affecting As mobility even in environments with comparatively low sulfate concentrations.

Arsenic in these experiments was mobilized rapidly despite only modest changes in overall Fe oxidation state; thus, despite the great presence of oxidized Fe minerals, indispensable low-As aquifers may be jeopardized by arsenic contamination faster than expected. This work suggests that Fe reduction is not responsible for the expedited observed (partial) As breakthrough, by neither IRB nor sulfide oxidation. Our work shows that the complexation of As in solution via

sulfate reduction and the formation of thioarsenic species are a key contributor to rapid increases in aqueous As concentrations. This conclusion challenges previously established conceptual models for As solubilization and transport. Since iron in sediments is reasonably abundant (often 1% w/w), it was previously assumed that a large quantity of organic carbon was necessary to substantiate Fe reduction, and the associated loss of sorption sites, needed to mobilize As (ref. 70). In contrast, our results indicate that only small quantities of reactive organic carbon are required to enhance As mobility or facilitate As mobilization at hot spots of As release—just enough to induce thioarsenic formation from sulfate reduction. Similarly, formation of thioarsenic species helps explain the release of As in systems containing recalcitrant crystalline Fe (oxyhydr)oxides that resist reduction due to insufficient microbial energy yield. Given the variety of aqueous systems where thioarsenic species are being increasingly found, from expected environments with excess-S such as hot springs⁵², and even peatlands^{71,72} to the more surprising and S-limited environments of paddy fields⁵¹ and organic carbon-limited aquifers^{12,49}, the biogeochemical reactions modelled here are likely to be a key mechanism affecting As mobilization and mobility across a broad range of environments. These perturbed Pleistocene environments just may be where the aforementioned processes are most distinguishable, where water resources are imminently threatened and where this process may have the largest impact on human health.

Methods

Field site

The field site was located in Araihaazar, Bangladesh (90.6352°, 23.8542°)²⁹ where large-scale As monitoring⁷³ and public health impacts of As (ref. 74) have been investigated. Concerns of high-As Holocene groundwater infiltration into low-As Pleistocene aquifers have arisen across South and Southeast Asia^{17,18,20,23,24,35,61}, including in Bangladesh with large-scale pumping by the megacity Dhaka^{20,24,35,61}.

Field-based/hybrid column experiment

Fresh Pleistocene sediments were cored for columns at the field site in Araihaazar²⁹; the intact centre of the core was recovered under nitrogen in a glove chamber to immediately prepare the columns. The columns had a length of ~7.5 cm and an inner diameter of 1.6 cm (Supplementary Table 3) and were kept anoxic; the experiment was run directly in the field. Anoxic groundwater was continuously pumped from a high-As well screened at 18–20 m depth, a typical depth for peak As concentrations^{4,73}, into a custom-made pyramidal storage bag (Supplementary Table 5). This bag had flexible walls designed to prevent both gas exchange and oxidation, and a pyramidal shape to allow simultaneous filling and prevention of entrained air by expelling bubbles through a valve in the apex of the pyramid²⁹.

The columns were operated at three different flow rates (pore-water velocities of 155 cm day⁻¹, 81 cm day⁻¹ and 40 cm day⁻¹) (ref. 29), with the rate controlled by peristaltic pumps connected to the storage bag by a manifold (Supplementary Table 3). All columns were run for as long as possible, leading to the varied time duration (PV) for each column. While the columns were run in parallel and set up identically except for the flow rate, the sediment core sections used for each column were not homogenized to preserve the intact sediment structure and thus, experimental results were affected by inherent sediment heterogeneity. Despite this, the sediment compositions of each column at the end of the experiment were similar (Supplementary Tables 1 and 2). The porosity of the sediments was ~0.3, based on a Br tracer test²⁹ (Supplementary Table 3). Effluent solutions were collected regularly via a fraction collector and acidified with trace metal grade HCl for trace metal analysis or kept unacidified for anion analysis²⁹. Daily and duplicate sampling of influent groundwater was performed throughout the experiment, before entry into the columns, to ensure that influent composition was constant and unaltered by oxidation²⁹. Limitations of

conducting a column experiment in such a remote setting briefly led to pump failure. The stoppage of the pumps was due to a generator problem powering the peristaltic pumps as there was no electricity in the field. This occurred a little after 170 PV in both the main column and its replicate, as seen by the spike in sulfate concentrations (Fig. 1), which could potentially indicate sulfide oxidation to sulfate, during which Fe(II) and Mn concentrations briefly decrease, perhaps also due to minor oxidation. This pump failure was accounted for during model calibration. However, the benefits of using real groundwater, intact freshly cored sediments and preserving the natural microbial community greatly outweighed the drawbacks of conducting this experiment in the field.

Experimental analyses

Aqueous data. Acidified groundwater and column effluent samples were analysed by high-resolution inductively coupled plasma mass spectrometry (ICP–MS)²⁹. Elemental concentrations of As, Fe, S, P, Mn, Na, K, Ca, Mg, Ba and Sr were measured, with a reported detection limit of $0.1 \mu\text{g l}^{-1}$; comparison with laboratory standards were within 10% (ref. ²⁹). Unacidified samples for anions (Br , Cl and SO_4^{2-}) were measured by ion chromatography with a Dionex Integrion HPIC System and an AS-18 column with a detection limit of 0.5 ppm. SO_4^{2-} concentrations from the IC generally matched the S concentrations from the ICP–MS²⁹; thus, ICP–MS concentrations were used for the better detection limit. Thioarsenic concentrations were not measured nor preserved for at the time of the experiment.

Solid-phase data. XAS was performed at Stanford Synchrotron Radiation Lightsource on beamlines 4-1 and 11-2 to measure iron EXAFS and As X-ray absorption near-edge structure (XANES). The spectra were collected with a 32- or 100-element Ge detector in fluorescence mode²⁹. For Fe EXAFS, a Mn filter was used with an Fe foil in series for calibration. For As XANES, a Ge filter was used with an Au foil in series for calibration. The samples contained low As concentrations, but the edge could still be distinguished. No oxidation occurred during data collection. Fe EXAFS spectra were normalized in Larch⁷⁵; As XANES spectra were normalized in SIXPack⁷⁶ (normalized spectra available in Supplementary Information). Normalized Fe EXAFS data were fit by linear combination fitting using Larch⁷⁷; the top nine standards out of 11 Fe mineral standards (mackinawite, pyrite, siderite, goethite, haematite, magnetite, biotite, augite, hornblende, nontronite and ferrihydrite) were used for each sample's fit (Supplementary Table 1). The top 9 standards were chosen because using all 11 standards led to increased errors and higher reduced chi-squares, with fits close to 0 for the remaining two standards but with all other fits remaining the same⁷⁷. The fraction of non-silicate Fe(II) was calculated from the Fe(II) fraction in each non-silicate mineral standard. The normalized As XANES spectra were fit in SIXPack⁷⁶ by linear combination fitting using three main standards: arsenate sorbed on ferrihydrite, arsenite sorbed on ferrihydrite and As_2S_3 (orpiment) (Supplementary Table 2). Importantly, we consider As_2S_3 as representative of the redox state of As sulfides as a broad category and not directly indicative of a pure As_2S_3 /orpiment mineral phase. FeAs was another standard tested in combination with those three standards, but was not needed to explain any fits. Arsenic sorption on FeS/FeS₂ was also tested as a standard, in combination with the main three standards, to determine whether the proportion of the As_2S_3 fit would be shifted. The average reduced chi-square was similar between the fits including As sorption on FeS/FeS₂ (reduced chi-square of 0.0071) and excluding As sorption on FeS/FeS₂ (reduced chi-square of 0.0098). However, the average error was smaller for the fits excluding As sorption on FeS/FeS₂ (error of 0.016 ± 0.007 excluding versus 0.031 ± 0.021 including As sorption on FeS/FeS₂). Since FeS was measured to be <1% by Fe EXAFS at the experiment's end, attributing the As proportion to As sulfides seemed more likely. Solid-phase organic carbon content was not measured in this experiment.

Solid-phase concentrations of Fe and As from sediment core cuttings were measured by X-ray fluorescence²⁹.

Modelling approaches

A 1D flow model was set-up in MODFLOW⁷⁸ to represent the experimental column (Supplementary Table 3). Reactive transport modelling was performed using PHT3D⁷⁹, which couples PHREEQC-2 (ref. ⁸⁰) to MT3DMS⁸¹. Pre-processing for MODFLOW/PHT3D was performed with the GUI Open Reactive Transport interface 3D (ORTi3D). The model simulation time was set to 25 days for the medium flow rate column to capture the full experimental period. The model was modified for each column at the other flow rates and their corresponding total simulation times: to 40 cm day^{-1} (simulated for 15 days) for the slow flow column and 155 cm day^{-1} (for 10 days) for the fast flow column.

A range of conceptual models and their numerical implementations were evaluated against aqueous and solid observation data (Figs. 1 and 2 and Supplementary Fig. 2). Each model variant was calibrated via PEST++, or particle swarm optimization, to estimate the uncertain or adjustable model parameters^{82,83} (Supplementary Table 7) that minimize the residual between simulation and observation results, while all other parameter values remained consistent with a modified standard PHREEQC database. This inverse modelling procedure provides a hypothesis testing mechanism to evaluate the plausibility of each conceptual model considered (Supplementary Table 4) and, importantly, is performed for each conceptual model variant for comparison of optimized models.

Biogeochemical reaction network

Each variant of the tested conceptual hydrogeochemical models consisted of

- aqueous components, aqueous complexes, minerals, ion exchangers and surface complexes defined in the standard PHREEQC database with addition of relevant entities from literature values
- addition of kinetically controlled reaction processes that play a role in As-affected environments^{84,85}
- modification of the standard PHREEQC database such as redox-decoupling of Fe(III) and Fe(II), As(V) and As(III), and sulfur species (sulfide, sulfate and zero-valent sulfur)

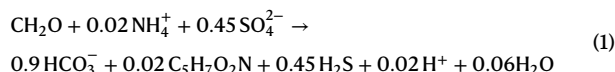
Initial mineralogy. The main minerals considered to regulate As mobilization in affected aquifers are Fe minerals^{4,45}. The Fe EXAFS measurements of the unaltered initial Pleistocene aquifer sediments²⁹ showed that the prevailing Fe minerals were ferrihydrite and goethite before the experiment started (Supplementary Table 1).

Exchange reactions. Exchanger sites were included to account for the cation exchange capacity provided by aquifer sediments. Exchange reactions were considered for Na^+ , K^+ , NH_4^+ , Ca^{2+} , Mg^{2+} , Mn^{2+} and Fe^{2+} (Supplementary Table 6). Ion exchange coefficients were adopted from the original PHREEQC database, but allowed to vary as part of the inversion process around the initial values as part of the model calibration (see note in Supplementary Table 7).

Surface complexation reactions. Arsenic sorption to mineral surfaces, especially to Fe oxides, is a key factor in controlling As mobility in aquifers, and varies strongly as a function of solution and solid-phase composition^{4,45}. Sorption is represented through surface complexation models (SCMs) for ferrihydrite and goethite (Supplementary Table 6). The SCM accounts for the variations in As sorption affinity resulting from spatially and temporally variable solution and mineral compositions, and for the impact of competitive sorption. Importantly, the sorption site density used by the SCM is dynamically linked to the respective concentrations of ferrihydrite and goethite to account for

the spatio-temporal changes in ferrihydrite and goethite concentrations during the experiment. The initial site density was set to be lower than the site densities often applied in conjunction with the SCM proposed by Dzombak and Morel⁸⁶ since our study used natural sediments rather than freshly synthesized iron minerals^{84,85}. For ferrihydrite, which composes the majority of Pleistocene sediments here (Supplementary Table 1), the SCM of Stollenwerk et al.⁸⁷ specifically developed for orange Pleistocene sediments in Bangladesh was used. This model (Supplementary Table 6) has equilibrium constants for the adsorption of arsenate, arsenite, phosphate, bicarbonate, silica, OH⁻/H⁺ and major cations (iron, calcium, magnesium and manganese). Furthermore, the SCM was extended to include surface complexes with Fe(II) and sulfate, including S-Fe(II) ternary surface complexes^{88–90}. Surface complexation constants from Dzombak and Morel⁸⁶ and Dixit and Hering⁴⁵ were used for goethite, for the same anions and cations as ferrihydrite. Sorption on all other Fe minerals (siderite, magnetite, mackinawite and pyrite) was not considered because the total percentage of all other Fe minerals in the entire column was comparatively insignificant (Supplementary Fig. 8). For the surface complexation calculations, the double diffuse layer option was invoked.

Microbial growth and decay. In the model, dissimilatory microbial sulfate reduction was coupled to the microbially mediated oxidation of sediment organic carbon (CH₂O) by SRB^{36,37}:

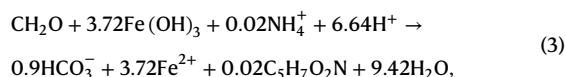


where C₅H₇O₂N represents microbial biomass of SRB⁹¹. The role of SRB and their microbial growth and decay kinetics were explicitly simulated³⁶. The growth rate r_{SRB} of SRB, and rate of sulfate reduction, is

$$r_{\text{SRB}} = \nu_{\text{max SRB}} \times I_{\text{bio}} \times \frac{C_{\text{OrgC}}}{K_{\text{OrgC}} + C_{\text{OrgC}}} \times \frac{C_{\text{SO}_4}}{K_{\text{SO}_4} + C_{\text{SO}_4}} \times \frac{C_{\text{Nitr}}}{K_{\text{Nitr}} + C_{\text{Nitr}}} \times X_{\text{SRB}} \quad (2)$$

where $\nu_{\text{max SRB}}$ is the maximum growth rate of SRB, I_{bio} is a microbial biomass inhibition term, X_{SRB} is the SRB biomass concentration, C is the concentration and K is the half-saturation constant for organic carbon (OrgC), sulfate (SO₄) or nitrogen (Nitr), respectively. The applied half-saturation constants (in mol l⁻¹) were: $K_{\text{OrgC}} = 1 \times 10^{-5}$, $K_{\text{SO}_4} = 5 \times 10^{-5}$ and $K_{\text{Nitr}} = 1 \times 10^{-7}$, based on literature values³⁶. $\nu_{\text{max SRB}}$ and I_{bio} are based on literature values, but are also parameters in inverse modelling with PEST. Sediment organic carbon was not measured in this experiment and thus was included as a parameter in PEST.

Probably occurring in parallel^{38,39}, the reduction of ferrihydrite by dissimilatory IRB³⁶:



where C₅H₇O₂N represents microbial biomass of IRB⁹¹, was also kinetically simulated. Reduction of ferrihydrite to aqueous Fe(II) was coupled to oxidation of sediment organic carbon to bicarbonate by growth of IRB, with the growth rate r_{IRB} as

$$r_{\text{IRB}} = \nu_{\text{max IRB}} \times I_{\text{bio}} \times \frac{C_{\text{OrgC}}}{K_{\text{OrgC}} + C_{\text{OrgC}}} \times \frac{C_{\text{Fh}}}{K_{\text{Fh}} + C_{\text{Fh}}} \times \frac{C_{\text{Nitr}}}{K_{\text{Nitr}} + C_{\text{Nitr}}} \times X_{\text{IRB}} \quad (4)$$

where $\nu_{\text{max IRB}}$ is the maximum growth rate of IRB, I_{bio} is a microbial biomass inhibition term³⁶, C is concentration and K is a half-saturation constant for the respective species of organic carbon (OrgC), ferrihydrite (Fh) and nitrogen (Nitr). The half-saturation constants

(mol l⁻¹) are: $K_{\text{OrgC}} = 1 \times 10^{-5}$, $K_{\text{Nitr}} = 1 \times 10^{-7}$ and here, $K_{\text{Fh}} = 0.05$. X_{IRB} is the concentration of the IRB biomass. I_{bio} , the microbial biomass inhibition term³⁶, is

$$I_{\text{bio}} = \frac{X_{\text{max}} - X_{\text{SRB, or IRB}}}{X_{\text{max}}} \quad (5)$$

where X_{max} is the maximum microbial population concentration and X is the microbial population concentration of SRB or IRB respectively for the respective growth rate equation⁹²; this term is a surrogate model used to consider the potential diffusional limitations of biogeochemical turnover of the microbial community with increasing biofilm thickness. Modelling of biofilm, however, does not necessarily imply that the biomass is spatially continuous.

The rate of organic matter degradation is defined as the rate of ferrihydrite reduction by IRB plus the rate of sulfate reduction by SRB:

$$r_{\text{OrgC}} = r_{\text{SRB}} + r_{\text{IRB}} \quad (6)$$

As in studies where iron and sulfate reduction may occur simultaneously^{42,93,94}, we consider a thermodynamic potential factor term to ensure that microbial reactions are thermodynamically favourable before allowing them to proceed kinetically:

$$F_T = 1 - \exp\left(\frac{\Delta G + m\Delta G_p}{\chi RT}\right) \quad (7)$$

where F_T is the thermodynamic potential factor; an F_T between 0 and 1 indicates a forward drive for respiration, while a 0 or negative value indicates the reverse reaction (no drive for respiration)⁹⁴ (Supplementary Fig. 6). Gibbs free energy, ΔG , was calculated for both sulfate reduction and Fe reduction, respectively. m , the adenosine triphosphate (ATP) number, and χ , the average stoichiometric number, was set at $m = 1$ and $\chi = 6$ for sulfate reduction and $m = 5/4$ and $\chi = 8$ for Fe(III) reduction^{93,94}. ΔG_p , the energy converted to ATP by the microbes, was set to 45 kJ per mol ATP⁹³. For Fe, a representative ΔG corresponding to the dominant mineralogy of ferrihydrite (Supplementary Table 1) was used⁹⁵. R is the universal gas constant; temperature T was set to 298 K.

For SRB and IRB microbial communities respectively, the rate of decay³⁶ is

$$r_{\text{decay}} = \nu_{\text{decay}} \times X_{\text{SRB, or IRB}} \times I_{\text{dec_inhib}} \quad (8)$$

where ν_{decay} is the rate constant of decay (respectively for SRB and IRB), $X_{\text{SRB, or IRB}}$ is the concentration of SRB or IRB biomass, and $I_{\text{dec_inhib}}$ is an inhibition term, defined as $(X_{\text{SRB, or IRB}} - X_{\text{min}})/X_{\text{SRB, or IRB}}$, that prevents the microbial concentration to drop below a user-defined threshold value of X_{min} .

Fe mineral transformation and formation. Rate laws derived from transition state theory⁹⁶ were implemented for the precipitation/dissolution rates of the Fe oxides, where the reaction rate is related to the departure from equilibrium:

$$\text{Rate} = k(1 - \text{SR}^p)^q \quad (9)$$

k is a rate constant, SR is the mineral saturation ratio, and p and q are dimensionless fitting parameters that were assumed to equal 1, as commonly employed⁹⁷. The mineral saturation ratio (SR) is defined as the ratio between the ion activity product and the solubility product (K_{sp}). Concentrations of aqueous Fe(II), from ferrihydrite reduction and influent groundwater, can lead to Fe(II) sorption on solid Fe mineral surfaces and catalyse Fe mineral transformation^{46,98}, such as magnetite formation from ferrihydrite.

The rate of magnetite formation is:

$$r_{\text{magnetite}} = k_{\text{mag}} \times C_{\text{Fe(II)}} \times \frac{C_{\text{Fe(II) sites on Fh}}}{C_{\text{total sites on Fh}}} \times (1 - \text{SR}_{\text{magnetite}}) \quad (10)$$

where k_{mag} is the rate coefficient, $\text{SR}_{\text{magnetite}}$ is the saturation ratio, and $C_{\text{Fe(II)}}$, $C_{\text{Fe(II) sites on Fh}}$ and $C_{\text{total sites on Fh}}$ represent concentration of Fe(II), Fe(II) sites on ferrihydrite (Fh) and total sites on Fh, respectively.

Formation of known secondary minerals^{46,99}, such as neo-formed goethite and siderite, are described by equation (9), where rate of goethite formation is:

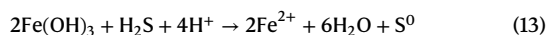
$$r_{\text{goethite}} = k_{\text{goethite}} \times (1 - \text{SR}_{\text{goethite}}) \quad (11)$$

Siderite formation from (1) influent Fe(II) or Fe(II) produced from reduction, and (2) bicarbonate from organic carbon mineralization by SRB and IRB, is described by the rate:

$$r_{\text{siderite}} = k_{\text{siderite}} \times (1 - \text{SR}_{\text{siderite}}) \quad (12)$$

Relevant sulfur processes. Various processes sequestering sulfide, as reported from other As-impacted and sulfate-reducing environments^{11,15,33,53,68}, were included in the reaction network: (1) sulfide oxidation coupled to ferrihydrite reduction (chemically), (2) mackinawite (FeS) formation, (3) orpiment (As₂S₃) formation and (4) aqueous thioarsenic formation.

Abiotic oxidation of sulfide to zero-valent sulfur, coupled to ferrihydrite reduction,



was modelled according to the rate equation by Poulton et al.⁴⁰:

$$r_{\text{sulfide ox.}} = k_{\text{sulfide ox.}} \times C_{\text{sulfide}}^{0.5} \quad (14)$$

where $r_{\text{sulfide ox.}}$ is the rate of sulfide oxidation, $k_{\text{sulfide ox.}}$ is the rate constant and C_{sulfide} is the concentration of sulfide.

The presence of Fe(II) and S may also lead to the formation of mackinawite (FeS) or a general As sulfide mineral as represented by As mineral orpiment (As₂S₃), as identified by As XANES data (Supplementary Table 2). Mackinawite formation was considered as:

$$r_{\text{mackinawite}} = k_{\text{mackinawite}} \times (1 - \text{SR}_{\text{mackinawite}}) \quad (15)$$

and orpiment formation as:

$$r_{\text{orpiment}} = k_{\text{orpiment}} \times (1 - \text{SR}_{\text{orpiment}}) \quad (16)$$

where r is the rate of formation, k is the respective rate constant and SR is saturation ratio for each mineral.

Formation of zero-valent sulfur following sulfide oxidation can lead to sulfur disproportionation (a 'cryptic sulfur cycle'), which increasingly is shown to be important in Fe-S environments^{41,100} due to reproduction of sulfate. While so far most studies considering cryptic sulfur cycling have focused on marine sediments and settings^{100,101}, there is increasing evidence of cryptic sulfur cycling occurring in terrestrial environments^{42,102}. Following a simplified stoichiometry (and not accounting for intermediate S species for simplicity)¹⁰⁰, sulfur disproportionation is represented as:

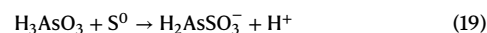


Assuming the availability of zero-valent sulfur is the rate limiting step, the kinetics are approximated for sulfur disproportionation as microbially driven⁴¹ by:

$$r_{\text{sulfur disprop.}} = k_{\text{sulfur disprop.}} \times C_{\text{S}^0} \times \frac{C_{\text{S}^0}}{1e^{-8} + C_{\text{S}^0}} \quad (18)$$

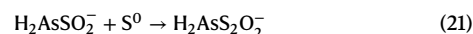
where $r_{\text{sulfur disprop.}}$ is the rate of sulfur disproportionation, $k_{\text{sulfur disprop.}}$ is a rate constant and C_{S^0} is the concentration of zero-valent sulfur. However, the extent of this cryptic sulfur cycle, including potential to drive overall Fe reduction via sulfide oxidation, is difficult to evaluate on the basis of (1) the limited extent of Fe reduction during the experiment, and (2) because measuring zero-valent sulfur concentrations was overlooked during this field-based experiment, as it was thought not to be important in addition to challenges in partitioning small effluent volumes with different preservation methods for all aqueous geochemical measurements in the field. However, future work should prioritize measuring zero-valent sulfur, including stabilizing samples in field settings. Furthermore, C_{S^0} is not simply the concentration of zero-valent sulfur (α - or β -C₈), but includes zero-valent sulfur in polysulfides, which might be more reactive than insoluble zero-valent sulfur, but should also be determined.

Thioarsenic formation. On the basis of the observations from comparable sulfate reducing environments^{11,13,49}, we account for the potential presence of thioarsenite and thioarsenate complexes. To consider these complexes in the numerical model, we adopted thermodynamic data from Helz and Tossell⁵⁶, as modified by Vlassopoulos et al.⁵⁷, for an improved fit to thioarsenite species. Although the thermodynamic equilibrium constants have been noted to be preliminary, previous discrepancies between measured thioarsenic speciation and the equilibrium constants have been attributed to disequilibrium, that is, slow reaction kinetics. To account for additional proposed reactions involving the formation of thioarsenic species, we investigated multiple conceptual models that account for the formation of thioarsenates by zero-valent sulfur in kinetically controlled redox reactions^{54,55}. Although in the literature the kinetics have not yet been well defined for these proposed reactions, we include the formation of the commonly found species monothioarsenate (MTA), as found nearby the site of the column experiment¹², and also dithioarsenate (DTA), via reactions with zero-valent sulfur to capture the potential multiple pathways of formation in simple first-order rate equations:



$$r_{\text{MTA}} = k_{\text{MTA}} \times C_{\text{arsenite}} \quad (20)$$

and



$$r_{\text{DTA}} = k_{\text{DTA}} \times C_{\text{monothioarsenate}} \quad (22)$$

where k is the respective rate constant and C_{arsenite} and $C_{\text{monothioarsenate}}$ are the concentrations of arsenite and monothioarsenite, respectively. Understanding speciation of thioarsenic species is indeed important (Supplementary Information Text, thioarsenic speciation) for understanding the mobility of As in groundwater, and thus, to the extent available in the present literature, we have included speciation (Supplementary Figs. 10–12). However, since verifying the speciation was beyond the scope of the current experimental and modelling study, we have abbreviated the emphasis on speciation. Rather, our modelling tests the impact of the complete presence or absence of thioarsenic species. Importantly, the models show that consideration of thioarsenic species versus no thioarsenic species is essential for explaining the measured As breakthrough data (Fig. 1).

Initial and boundary conditions

The influent groundwater composition into the columns was very consistent²⁹; averaged concentrations were thus used to define a constant

influent water composition over the entire simulation period (Supplementary Table 5 and Fig. 1). To define the initial occupancy of the ion exchanger and surface sites, a 'background solution' composition was used on the basis of the groundwater composition measured near the field site from Zheng et al.¹⁰³ (Supplementary Table 5); as these concentrations were not measured at the time of the experiment, they were included as parameters in PEST (Supplementary Table 7). The initial concentrations of ferrihydrite and goethite in the model were defined based on sediment Fe concentration measured by X-ray fluorescence²⁹, multiplied by the mean fraction of ferrihydrite and goethite from Fe EXAFS data in unaltered (pre-experiment) sediment core sections (Supplementary Table 1).

Model calibration procedure

The reactive transport model was calibrated using PEST++, a parallelized version of PEST that includes an implementation of particle swarm optimization⁸³. The calibration dataset included both temporally varying effluent concentrations and solid-phase data. Considered solutes included Ca, Fe(II), K, Mg, Mn, Na, P, As and sulfate. Ca, Mg, Mn and Na data were used to constrain the cation exchange capacity of the sediments. Arsenic XANES solid-phase data determined for inlet and outlet ends of the columns were included as additional observations. The number of total observations used for the calibration of the medium flow rate column, column B, was 439 (433 aqueous observations, 6 solid-phase observations). For the slow column (column G), there was a total of 339 observations (333 aqueous, 6 solid) used for calibration; for the fast column (column E), there was a total of 453 observations (447 aqueous, 6 solid). Observation weights were assigned on the basis of the inverse of relative measurement noise and the number of observations belonging to each observation type⁸⁴, while balancing the contributions from the varying observation types (for example, the low aqueous concentrations of phosphate). Greater observation weighting can also place more emphasis on the corresponding parameter(s). To emphasize fitting of aqueous As and sulfate observations through their relevant parameters, aqueous As and sulfate observations were given greater weighting⁸⁴. The parameters were estimated by minimizing an objective function comprising the weighted sum of squared residuals between observations and the model output; however, the observation weights during the pump failure were reduced to 0 to not bias the objective function. The values for the final composite objective function for each conceptual model are in Supplementary Table 4; the objective function values for each aqueous observation group for each conceptual model are in Supplementary Table 8. The parameters estimated by the model calibration procedure are in Supplementary Table 7. See also the model evaluation section and the data RMSE.xlsx in Supplementary Information.

Model uncertainty

Model uncertainty analysis was performed with the function GENLINPRED, available within PEST, to provide a formal analysis of model parameter uncertainty via linearized Bayesian methods. Values of identifiability, relative error variance reduction and relative uncertainty variance reduction were produced for each calibrated parameter. The values of each respectively range between 0 and 1, where 0 indicates low identifiability or variance reduction while 1 indicates high identifiability or variance reduction, as constrained by observation data (Supplementary Fig. 15). Many parameters have high relative uncertainty variance reduction near 1 (Supplementary Table 7). The few observations that have low relative uncertainty variance reduction closer to 0, and also low composite scaled sensitivity, are related to surface complexation log *K* values for ferrihydrite and are probably correlated with other related parameters of surface complexation log *K* values for the same ions that have high relative uncertainty variance reduction.

Data compilation of groundwater in Bangladesh

Concentrations of groundwater As and relevant parameters (Fig. 5b) were compiled from literature (Supplementary Table 9). All values were cleaned and merged with consistent units into a database with the statistical program R³¹. Where necessary, samples below detection limit concentrations were flagged and half the detection limit was reported.

Reporting summary

Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

Data availability

The column transport parameters and geochemical solute data for the influent groundwater and all column effluent used for modeling have been previously published and can be found in Mozumder et al.²⁹. The solid-phase data for the unaltered sediment can also be found in Mozumder et al.²⁹. The mineralogy data at the end of the experiment for the Pleistocene columns are in Supplementary Tables 1 and 2. The normalized XAS spectra and standards used for both Fe EXAFS and As XANES are also available in the additional Supplementary Information files. The data compilation of groundwater in Bangladesh is a subset of data publicly available at <https://doi.org/10.7916/d8-zenj-yx36>.

Code availability

ORTI3D was used for the initial model construction and manual model development and calibration phase (<https://orti3d.ensegid.fr/>). ORTI3D is an open source software that is a graphical user interface for the public domain codes MODFLOW⁷⁸, MT3DMS⁸¹ and PHT3D⁷⁹. MODFLOW is available from the United States Geological Survey (USGS) at <https://www.usgs.gov/software/software-modflow>, and PHT3D is available from www.phtd.org. PEST++ for model calibration is also publicly available (<https://github.com/usgs/pestpp>). Finally, the input data files for the reactive transport model are publicly available at <https://doi.org/10.7916/945c-9w77>.

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Acknowledgements

The experimental study was supported by the US National Institute of Environmental Health Sciences (NIEHS) grants ES0101349, ES009089 (B.C.B. and A.v.G.) and P42ES033719 (B.C.B. and H.P.), US National Science Foundation (NSF) grant 1521356 (B.C.B. and A.v.G.) and US Department of Energy (DOE) grant EE0009596 (B.C.B. and H.P.). The computational modelling work was supported by an NSF Graduate Research Opportunities Worldwide (GROW) grant awarded to A.A.N. to conduct research in Western Australia. A.A.N. was also funded by an NSF Graduate Research Fellowship (NSF grant 1644869). Use of the Stanford Synchrotron Radiation Lightsource, SLAC National Accelerator Laboratory, is supported by the US Department of Energy, Office of Science, Office of Basic Energy Sciences under contract

no. DE-AC02-76SF00515. Computing resources, including high-performance computing, were provided by CSIRO Australia. The data for part of this work were compiled as a part of the Characterizing Global Variability in Groundwater Arsenic Working Group supported by the John Wesley Powell Center for Analysis and Synthesis, funded by the US Geological Survey. We also thank M. Selim, M. Atikul Islam, E. Shoenfelt Troein, T. Ellis, B. Mailloux and I. Choudhury for their contributions to the original experimental work. We also thank M. Stahl and D. Postma for their useful discussions.

Author contributions

A.A.N., H.P. and B.C.B. conceived the reactive transport modelling work based off the column experiment work conceived by M.R.H.M., A.v.G. and B.C.B. The field work and column experiment work were performed by M.R.H.M., B.C.B. and K.M.A.; M.R.H.M. measured and provided data from the column experiment work. A.A.N. and H.P. performed the flow and reactive transport modelling. A.A.N., H.P., J.J. and B.C.B. contributed to the conceptual models underlying the numerical models. A.A.N. performed data curation, data visualization, analysis and model calibration of the reactive transport modelling results. H.P., A.S. and J.J. also contributed to the methodology, software/computing resources, model calibration and validation of the reactive transport modelling. A.S. also performed model sensitivity analysis. A.A.N. provided funding acquisition for the reactive transport modelling work; B.C.B. and A.v.G. provided funding acquisition for the column experiment and field work. A.A.N., H.P. and B.C.B. wrote and prepared the original manuscript, and all authors contributed to the review and editing of the manuscript.

Competing interests

The authors declare no competing interests.

Additional information

Supplementary information The online version contains supplementary material available at <https://doi.org/10.1038/s44221-022-00022-z>.

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Peer review information *Nature Water* thanks Rasmus Jakobsen, Britta Planer-Friedrich and the other, anonymous, reviewer(s) for their contribution to the peer review of this work.

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Reporting Summary

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Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a Confirmed

- ☐ ☒ The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement
- ☐ ☒ A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
- ☐ ☒ The statistical test(s) used AND whether they are one- or two-sided
Only common tests should be described solely by name; describe more complex techniques in the Methods section.
- ☐ ☒ A description of all covariates tested
- ☐ ☒ A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
- ☐ ☒ A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
- ☐ ☒ For null hypothesis testing, the test statistic (e.g. F , t , r) with confidence intervals, effect sizes, degrees of freedom and P value noted
Give P values as exact values whenever suitable.
- ☒ ☐ For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
- ☒ ☐ For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
- ☐ ☒ Estimates of effect sizes (e.g. Cohen's d , Pearson's r), indicating how they were calculated

Our web collection on [statistics for biologists](#) contains articles on many of the points above.

Software and code

Policy information about [availability of computer code](#)

Data collection

ORTI3D was used for the initial model construction and manual model development and calibration phase (<https://orti3d.ensegid.fr/>). ORTI3D is an open source software that is a graphical user interface (GUI) for the public domain codes MODFLOW, MT3DMS and PHT3D. MODFLOW is available from the United States Geological Survey (USGS) at <https://www.usgs.gov/software/software-modflow>, and PHT3D is available from www.phtd.org. PEST++ for model calibration is also publicly available (<https://github.com/usgs/pestpp>). Concentrations of groundwater As and relevant aqueous parameters, such as sulfate, and depth of groundwater in Bangladesh (as shown in Fig. 5b) were compiled from published literature values (Table S9). All values were cleaned and merged with consistent units into a database with the statistical program R. Where necessary, samples below known detection limit concentrations were flagged and a value of half the detection limit was reported.

Data analysis

Data analysis and visualization were performed in R, version 4.0.5.

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio [guidelines for submitting code & software](#) for further information.

Data

Policy information about [availability of data](#)

All manuscripts must include a [data availability statement](#). This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our [policy](#)

The column transport parameters and geochemical solute data for the influent groundwater and all column effluent used for modeling have been previously published and can be found in Mozumder et al. 2020. The solid phase data for the unaltered sediment can also be found in Mozumder et al. 2020. The mineralogy data at the end of the experiment for the Pleistocene columns are in Table S1 and Table S2. The normalized XAS spectra and standards used for both Fe EXAFS and As XANES are also available in the additional SI files. The data compilation of groundwater in Bangladesh is a subset of data publicly available at <https://doi.org/10.7916/d8-zenj-yx36>.

Human research participants

Policy information about [studies involving human research participants and Sex and Gender in Research](#).

Reporting on sex and gender	N/A
Population characteristics	N/A
Recruitment	N/A
Ethics oversight	N/A

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☐ Life sciences ☐ Behavioural & social sciences ☒ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Ecological, evolutionary & environmental sciences study design

All studies must disclose on these points even when the disclosure is negative.

Study description	Anoxic high-As groundwater from an aquifer was continually pumped through columns filled with freshly-cored Pleistocene aquifer sediments at three different flow rates.
Research sample	Fresh Pleistocene sediments were cored for columns at the field site in Bangladesh and the center portion of the core was recovered under nitrogen in a glove chamber to prepare the columns. Increasing concerns of intrusion of Holocene groundwater into typically low-As Pleistocene aquifers have arisen across South and Southeast Asia, but especially in Bangladesh where large-scale pumping by the mega-city Dhaka with the threat of high-As groundwater intrusion into low-As Pleistocene aquifers. The columns were kept anoxic and immediately prepped, and the experiment was run directly in the field. Effluent solutions were collected regularly via a fraction collector, where sample volume was noted and the solutions were preserved accordingly; samples were acidified with trace metal grade HCl for trace metal analysis or kept unacidified for anion analysis.
Sampling strategy	In total, four columns containing Pleistocene aquifer sediments were collected and run in parallel at three different flow rates (Table S3). The columns were: (1) a column with an additional replicate of the column at the same main flow rate, (2) a modified replicate column at a slower (approximately-halved) flow rate, and (3) a modified replicate column at a faster (approximately-doubled) flow rate (see methods, Table S3).
Data collection	Effluent solutions were collected regularly via a fraction collector, where sample volume was noted and the solutions were preserved accordingly; samples were acidified with trace metal grade HCl for trace metal analysis or kept unacidified for anion analysis. Daily and duplicate sampling of freshly pumped and influent groundwater was also performed, before entry into the columns to ensure that influent composition was constant and unaltered by oxidation. Acidified samples collected of the influent groundwater and all column effluent over time were analyzed by high resolution inductively-coupled plasma mass spectrometry (ICP-MS). X-ray absorption spectroscopy (XAS) was performed at Stanford Synchrotron Radiation Lightsource on beamlines 4-1 and 11-2 to measure iron extended X-ray fine structure (EXAFS) and As X-ray absorption near-edge structure (XANES) for the sediment samples at the end of the experiment.
Timing and spatial scale	The columns were simultaneously run in parallel and set up in the same manner except for adjusting the flow rate. The Pleistocene

Timing and spatial scale	sediment columns were operated at three different flow rates (porewater velocities of 155 cm/day, 81 cm/day, and 40 cm/day), with the flow rate being controlled by peristaltic pumps connected to the custom-made storage bag by a manifold (Table S3). Accordingly, all columns were run for as long as possible in the field setting, leading to the varied time lengths (pore volumes) obtained for each column experiment. The prepared columns had a length of ~7.5 centimeters and an inner diameter of 1.6 centimeters (Table S3). Anoxic groundwater was continuously pumped from a shallow aquifer with high concentrations of As from a well screened at a depth of 18-20 meters, typical of the depth of peak As concentrations (in high-As Holocene aquifers).
Data exclusions	Limitations of conducting a hybrid column experiment in a more remote setting such as at our field site in Bangladesh briefly led to pump failure. The stoppage of the pumps was due to a generator problem powering the peristaltic pumps as there was no electricity in the field location; this occurred at a little after 170 pore volumes in both the main column and its replicate at the same flow rate, as seen by the spike in sulfate concentrations in Fig. 1. This pump failure was later accounted for during model calibration. However, the benefits of using real groundwater, intact and freshly cored aquifer sediments, and preserving the natural microbial community greatly outweighed the drawbacks of conducting this representative column experiment in the field setting.
Reproducibility	The definition and evaluation of various biogeochemical reaction network implementations was at the core of this study. Each variant of the tested conceptual hydrogeochemical models. The metrics used for model evaluation include root mean squared error (RMSE) and normalized RMSE (NRMSE) between model results and experimental observations, for each observation group. The RMSE and NRMSE were calculated for the (i) full model across all columns run in the experiment, including at varying flow rates (Table S3), and (ii) across all conceptual models tested on the main medium flow rate column (Table S4). The RMSE data are located in the additional SI file RMSE.xlsx; see tab within Excel sheet for model descriptions. The composite objective function was also computed during model optimization for the different conceptual models that were calibrated on the main medium flow rate column, including the overall composite objective function (Table S4) and the contributions to objective function per observation group (Table S8; also see methods).
Randomization	Not relevant as our study was a mechanistic experiment and subsequent modeling.
Blinding	Not relevant.
Did the study involve field work?	☒ Yes ☐ No

Field work, collection and transport

Field conditions	Pleistocene sediments were collected from a field site located in Araihasar, Bangladesh (90.6352°, 23.8542°), east of Dhaka but within the Dhaka groundwater cone of depression, in a rural setting in Bangladesh.
Location	Fresh Pleistocene sediments were cored for columns at the field site in Araihasar, Bangladesh (90.6352°, 23.8542°). This site is representative of many sites across South and Southeast Asia, with respect to the depositional history of deltaic sediments, where older Pleistocene aquifer sediments typically contain lower groundwater As and younger Holocene aquifer sediments typically contain high groundwater As. Anoxic groundwater was continuously pumped from a shallow aquifer with high concentrations of As from a well screened at a depth of 18-20 meters, typical of the depth of peak As concentrations (in high-As Holocene aquifers), into a custom-made pyramidal storage bag that was continuously filled with fresh groundwater (high-As influent groundwater in Table S5).
Access & import/export	We worked with a wide range of Bangladeshi scientists, government officers and local people to access the sites used in the study. These interactions include the graduate students, field assistants and professors of Dhaka University, the local and national governmental officers involved in public health decision making related to arsenic in water, and the land owners at each site. In many cases, these people have been authors on studies that resulted from this work. For this manuscript, which involves modeling those data rather than field data directly, the lead professor, Kazi Matin Ahmed, from Dhaka University, has contributed and is an author. Analyses made in support of this research were conducted at the Stanford Synchrotron Radiation Laboratory. Those geological samples are exempt from import restrictions applying to soil but were brought from the field to the US using soil import permits issued to our laboratory. Collaborative research between the US and Australia involved data and intellectual exchange but no sensitive information.
Disturbance	No disturbance was caused by this study.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☒	☐ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☒	☐ Animals and other organisms
☒	☐ Clinical data
☒	☐ Dual use research of concern

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging