



Article Unraveling the Coupled Dynamics between DOM Transformation and Arsenic Mobilization in Aquifer Systems during Microbial Sulfate Reduction: Evidence from Sediment Incubation Experiment

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Abstract: Geogenic arsenic (As)-rich groundwater poses a significant environmental challenge worldwide, yet our understanding of the interplay between dissolved organic matter (DOM) transformation and arsenic mobilization during microbial sulfate reduction remains limited. This study involved microcosm experiments using As-rich aquifer sediments from the Singe Tsangpo River basin (STR) and Jianghan Plain (JHP), respectively. The findings revealed that microbial sulfate reduction remarkably increased arsenic mobilization in both STR and JHP sediments compared to that in unamended sediments. Moreover, the mobilization of As during microbial sulfate reduction coincided with increases in the fluorescence intensity of two humic-like substances, C2 and C3 (R = 0.87/0.87 and R = 0.73/0.66 in the STR and JHP sediments, respectively; p < 0.05), suggesting competitive desorption between DOM and As during incubation. Moreover, the transformations in the DOM molecular characteristics showed significant increases in CHOS molecular and low-O/C-value molecular intensities corresponding to the enhancement of microbial sulfate reduction and the possible occurrence of methanogenesis processes, which suggests a substantial bioproduction contribution to DOM components that is conducive to As mobilization during the microbial sulfate reduction. The present results thus provide new insights into the co-evolution between As mobilization and DOM transformations in alluvial aquifer systems under strong microbial sulfate reduction conditions.

Keywords: dissolved organic matter; arsenic; microbial sulfate reduction; aquifer sediment; incubation experiment

1. Introduction

Arsenic (As) is a ubiquitous toxic element that has extensively existed as $H_3As(III)O_3$ or $H_2As(V)O_4^-$ in neutral aqueous environments. Long-term exposure to As can result in severe adverse health effects, including cancers [1]. The elevated groundwater As concentrations from southern and southeastern Asia have historically posed a terrible threat to the safe drinking water supply for millions of people [2], and thus, the World Health Organization (WHO) has established a safe guideline of 10 µg/L for As in drinking water. Recent studies have underscored the widespread occurrence of geogenic As enrichment (exceeding the WHO guideline of 10 µg/L) in the Tibetan Plateau [3–5], which is also the source region for the downstream drainage basins in southern and southeastern Asia. Since revealing the biogeochemical processes regulating the mobilization of As from aquifer sediments is a critical step in preventing the health risk for drinking water utilization, it



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Copyright: © 2024 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). is urgent to study the mechanisms accounting for elevated As concentrations in aquifer systems from the Tibetan Plateau.

Microbially mediated coupled Fe-S redox cycling with concurrent transformations in As speciation is the most pivotal biogeochemical reaction regime accounting for the fate of As in aquifer systems. It is well-known that the microbially mediated iron reductive dissolution could be an important process either in releasing As into the aqueous phase or incorporating As into solid phases [6,7], wherein the accumulation of aqueous and adsorbed Fe(II) could facilitate the formation of secondary iron minerals (e.g., siderite, magnetite, goethite, and lepidocrocite) with a strong retention capacity for aqueous As [8]. Notably, there is a growing consensus that bacterial sulfate reduction (BSR) plays an important role in determining the transport and fate of arsenic in groundwater systems because the BSR can facilitate As mobilization through either the abiotic reduction of As-bearing iron (hydro)oxides or adsorbed arsenate via the potent reductant HS⁻ [9]. Conversely, the precipitation of iron sulfide minerals (e.g., mackinawite and pyrite) and arsenic sulfide minerals (e.g., realgar or orpiment) can result in the partitioning of As into the solid phase and a subsequent decrease in aqueous As concentrations [10,11]. Recent studies also highlighted the transformation of H₃AsO₃ and H_xAsO₄^{3-x} into thiolated and methylated As, leading to variations in their mobility in groundwater systems [12]. In addition, microbially mediated arsenate reduction also contributes to As mobilization, either through the reduction of dissolved arsenate or adsorbed arsenate to enhance its mobility [13]. Although the mechanisms of As mobilization have been well-established in the Quaternary aquifer systems in relatively low-lying plains regions [14], the knowledge remains far from complete regarding how these biogeochemical processes regulate As mobilization from the aquifer systems in the alpine and cold Tibetan Plateau.

In organic-matter-rich aquifer systems, dissolved organic matter (DOM) plays an essential role in fueling versatile microbial metabolic activities [15], and these microbially mediated transformations in minerals and aqueous chemical components could subsequently regulate the groundwater As concentrations. Recent studies highlighted several critical aspects involved with DOM functioning and As mobilization in groundwater systems: (1) Bioavailable DOM could fuel the reductive dissolution of arsenic-bearing iron (hydr)oxides or the microbial sulfate reduction, subsequently influencing the mobilization or incorporation of arsenic [16]; (2) Humic components in the DOM could form ternary complexes with Fe and As, enhancing the stability of As in the aqueous phase [17]; (3) Some particular humic components could serve as electron shuttles due to their redox-active quinone moieties, expediting the biogeochemical processes accounting for As mobilization in groundwater [18]; (4) Humic substances could also compete for active surface sites with As on iron (hydr)oxides and facilitate the As release [19]; (5) Diverse DOM could regulate the ecological niches of various microbial compositions, resulting in the redox zonation that subsequently affects the spatial distribution of As in aquifer systems [20]. Numerous studies have recently emphasized the significant functions of DOM in facilitating arsenic mobilization in aquifer systems from the central Yangtze River Basin [21], Yellow River Basin [22,23], and Pearl River Delta [24]. However, there is a knowledge gap regarding how DOM transforms under microbial sulfate reduction conditions in aquifer systems and interacts with As mobilization from aquifer sediments.

To fulfill these questions, the present study has collected alluvial aquifer sediments from the Singe Tsangpo River basin (STR) in the westernmost part of the Tibetan Plateau, where our previous studies have revealed significant geogenic As enrichment in multiple environments (e.g., surface water, river sediments, and groundwater [25]). Microcosm experiments with STR aquifer sediments were conducted in comparison with the As-rich aquifer sediments from the Jianghan Plain under microbial sulfate reduction conditions. The primary objectives of this research are as follows: (1) Elucidate the underlying mechanisms controlling As mobilization under bacterial sulfate reduction conditions in the STR; (2) Compare the differences in As mobilization between the STR alluvial aquifers (under a relatively arid and cold sedimental environment) and JHP alluvial aquifers (under a relatively humid and warm sedimental environment); (3) Unveil the critical transformations in dissolved organic matter (DOM) components corresponding to As mobilization in aquifer sediments under microbial sulfate reduction.

2. Materials and Methods

2.1. Study Area and Sample Collection

The Singe Tsangpo River basin (STR) is located in the westernmost part of the Tibetan Plateau ($79^{\circ}42'$ E to $81^{\circ}48'$ E and $31^{\circ}6'$ N to $32^{\circ}50'$ N, Figure 1); it also includes the upper course of the Indus River within Pakistan. The climatic and hydrogeological conditions have been extensively characterized in previous studies [3,5,25]. Briefly, the STR was characterized as an alpine subarctic and arid climate, with an average elevation of approximately 4800 m.a.s.l. and an annual precipitation/temperature of 5.5 mm/0 °C. The alluvial aquifers in the STR are predominantly located in the lower course of the river, and the aquifer sediments are primarily composed of sand and gravel with minimal clay content with sediment depths varying from tens to hundreds of meters below the ground surface (b.g.s). In contrast, the Jianghan Plain (Figure 1) shows a remarkably different subtropical monsoonal climate, characterized by an annual precipitation of 1269 mm and an annual temperature of around 16 °C. The Quaternary aquifer systems in the Jianghan Plain can be categorized into two groups: (1) the Holocene unconfined aquifer, comprising Holocene alluvial and lacustrine deposits (clay, silt, and fine sand) at depths ranging from 3 to 15 m b.g.s; and (2) the late- and middle-Pleistocene confined aquifer, consisting of alluvial sediments (medium-coarse sand and gravel with clay lenses) at depths from 15 to 80 m b.g.s.



Figure 1. Geographical location of sediment sampling sites in the Singe Tsangpo River basin and the Jianghan Plain. (a) The geographic locations of sediment sampling sites. (b) The vertical section of shallow alluvial aquifers from the STR. (c) The aquifer medium structure of JHP.

According to our previous investigation [5], the average groundwater level of shallow alluvial aquifer systems from the STR are around 4353.6 m, and the groundwater flow is controlled by the regional topography. In contrast, the average groundwater levels in the shallow alluvial aquifer from the JHP are around 21.5 m [26]. Two As-rich sediment samples from the alluvial aquifers were chosen for the microcosm incubation experiments (Table 1). The sediment samples were collected from approximately 25 m below ground surface (b.g.s.) in the aquifers of the Jianghan Plain (113°16′0.59″ E, 30°16′1.41″ N, Yaohe village) and from approximately 80 m (b.g.s) in the alluvial aquifer of the Singe Tsangpo River basin (80°9′19.08″ E, 32°22′33.69″ N, Geji County). These samples were collected during the borehole construction process. Once the sediment cores were brought out to the surface, sediment samples were collected immediately, and only the inner sections of the cores were sampled to maintain their original condition. The sediment samples were then placed in 50 mL sterile centrifuge tubes, sealed with a sealing film, stored at approximately 4 °C, and transported to the laboratory for further analysis and microcosm experiments.

Table 1. Initial geochemical compositions of the sediments utilized in the incubation experiments, and the average concentration of major chemical compositions in groundwater from the Singe Tsangpo River basin [5] and Jianghan Plain [27].

Sediment	STR (Depth = 72 m)	JHP (Depth = 20 m)	Groundwater_Mean	STR (n = 13)	JHP (n = 186)
Na ₂ O (%)	2.2	1.8	T (°C)	7.7	20
MgO (%)	1.3	3	pН	8.4	7
Al ₂ O ₃ (%)	15.9	16.2	Eh (mV)	215.5	-136
SiO ₂ (%)	61	67.7	EC (µs/cm)	322.2	818.1
P ₂ O ₅ (%)	0.2	0.3	K (mg/L)	3.3	4
K ₂ O (%)	4.5	2.4	Na (mg/L)	24.5	17
CaO (%)	10.7	1.4	Ca (mg/L)	40.7	92
Fe ₂ O ₃ (%)	3.4	5.1	Mg (mg/L)	9	29
S (%)	0.019	0.0096	Fe (mg/L)	0.03	1
Ti (mg/kg)	2761.9	4885	As $(\mu g/L)$	32.2	532
V (mg/kg)	59.2	97	DOC (mg/L)	-	6
Cr (mg/kg)	39.2	61	HCO_3^- (mg/L)	157.8	550
Mn (mg/kg)	486.3	616	Cl^{-} (mg/L)	11.2	9
As (mg/kg)	59.3	108	NO_3^{-} (mg/L)	9.3	2
Rb (mg/kg)	164.8	97	SO_4^{2-} (mg/L)	38	4
Sr (mg/kg)	342.3	190			

Note(s): -: No data.

2.2. Microcosm Setup

The microcosm experiments were set into three groups in order to delineate the different scenarios of As mobilization from aquifer sediments, enhanced sulfate-reduction conditions, and inhibited sulfate-reduction conditions. The first group underwent anaerobic incubation without any amendment (natural conditions), denoted as JHP_NA and STR_NA, respectively. In the second group, anaerobic incubation occurred with the introduction of 10 mM lactate and 20 mM sulfate, denoted as JHP_CS and STR_CS (enhanced sulfatereduction condition). The third group underwent anaerobic incubation with the introduction of 10 mM lactate and 10 mM molybdate, denoted as JHP_CM and STR_CM (inhibited sulfate-reduction condition). And each group comprised 100 mL of artificial groundwater mixed with 10 g of aquifer sediments sourced from the Jianghan Plain (JHP) and the Singe Tsangpo River basin (STR), respectively. The ionic compositions and pH conditions of the artificial groundwater were set up according to the actual groundwater chemistry in situ. For the Jianghan Plain, the artificial groundwater consisted of CaCl₂ (2.3 mmol/L), MgCl₂ (0.6 mmol/L), and PIPES buffer (5 mmol/L), with a pH condition of 6.95. In contrast, for the Singe Tsangpo River, the artificial groundwater comprised CaCl₂ (0.75 mmol/L), Na₂SO₄ (0.39 mmol/L), and PIPES buffer (5 mmol/L), with a pH of 8.3. The microcosm groups

were incubated for 95 days at 18 °C conditions, and sediment–liquid mixing samples were collected in an anaerobic glove box under a nitrogen atmosphere at various time points: day 0, day 7, day 14, day 21, day 28, day 35, day 54, and day 95. There were two parallel samples in each of the three experimental groups. The data points in all figures in this paper depict the mean values of the measured parameters across duplicated treatments, with error bars indicating standard deviations. A total of 96 samples were collected for the analysis of As, Fe, microbial functional gene abundance, and DOM fluorescent components. Additionally, DOM molecular samples for FT-ICR-MS analysis were exclusively collected from STR/JHP_NA and STR/JHP_CS on both day 0 and day 95, resulting in a total of 6 samples (note: NA and CS shared identical organic matter compositions on day 0; hence, the determination was not repeated). The detailed configuration of the microcosm experiments along with information preservation is provided in the Supplementary Information (SI).

At each sampling time, 10 mL of supernatant was collected by syringe and filtered through a 0.45 μ m filter. Subsamples were then taken for the analysis of total arsenic concentrations, arsenic species, sulfate concentration, and excitation–emission matrix (EEM) samples. The remaining slurries were centrifuged and collected for the dissolved organic matter (DOM) analysis by FT-ICR-MS. For the total arsenic and sulfate analysis, the samples were immediately preserved at 4 °C after collection, while the samples of total arsenic were preserved with concentrated HCl. For the arsenic species analysis, 0.2 mmol/L of EDTA solution was added to preserve. For the Fe(II) analysis, the total Fe(II) (dissolved and adsorbed Fe(II)) was extracted by adding 1 mol/L HCl in an equal volume for 24 h. For the FT-ICR-MS analysis of DOM, the samples were acidified to pH < 2 using H₃PO₄. The remaining residue was thoroughly mixed and placed into a centrifuge tube under anoxic conditions. The solid samples were collected through centrifugation, and the remaining solids were stored at -80 °C for subsequent sedimental DNA extraction.

2.3. Chemical Analysis

The concentration of total arsenic (As_{total}) was quantified using inductively coupled plasma mass spectrometry (ICP-MS) with a detection limit of 0.1 μ g/L. Sulfate concentration was determined through ion chromatography (ICS-600, Thermo Fisher, Waltham, MA, USA) with a detection limit of 0.01 mg/L. The concentrations of As species, including As(III) and As(V), were determined using high-performance liquid chromatography–inductively coupled mass spectrometry (Prin-Cen ELSpe-2 HPLC, Prin-Cen Scientific, Guangzhou, China; NexION 300X ICP-MS, Perkin Elmer, Waltham, MA, USA) with a detection limit of 0.05 μ g/L. The concentration of total Fe(II) (Fe(II)_{total}, extracted from the water–sediment mixture by 1 mol/L HCl for 24 h, was determined by inductively coupled plasma optical emission spectrometry (ICP-OES) with an analytical precision of $\pm 3.0\%$ and a detection limit of 0.01 mg/L.

2.4. Quantitative Polymerase Chain Reaction (qPCR) Analysis of the arsM, arrA, and dsrB Genes

In order to delineate the variations in the quantities of functional microbial compositions involved with sulfate reduction and the As speciation transformation during the incubation, qPCR was employed for analysis of the gene abundance of dissimilatory sulfite reductase gene *dsrB*, dissimilatory arsenate reductase gene *arrA*, and As(III)-methyltransferase *arsM*. The primer sets used for different functional genes were as follows: As1F (5'-CGAAGTTCGTCCCGATHACNTGG-3')/As1R (5'-GGGGTGCGGTCYTTNARYTC-3') for the *arrA*, arsM-F1 (5'-TCYCTCGGCTGCGGCAAYCCVAC-3')/arsM-R2 (5'-CGWCC GCCWGGCTTWAGYACCCG-3') for the *arsM*, and DSRp2060F (5'-CAACATCGTYCAYA CCCAGGG-3')/DSR4R (5'-GTGTAGCAGTTACCGCA-3') for the *dsrB*.

The qPCR was performed using an real-time PCR machine (LightCycler480 II-384, Roche, Basel, Switzerland). The amplification efficiencies (E) and R² values for arsM, arrA, and dsrB were as follows: arsM: E = 91.99%, R² = 0.9988; arrA: E = 90.94%, R² = 0.9991; and dsrB: E = 91.99%, R² = 0.9985. The amplification protocol for the three functional genes involved an initial step of 95 °C for 5 min, followed by 40 cycles of 30 s at 94 °C and 30 s at

60 °C. Each reaction volume was 10.8 μ L, consisting of 10 μ L SYBR Green Real-time PCR Master Mix (ABI, Los Angeles, CA, USA), 0.4 μ L specific primer F (10 μ mol/L), and 0.4 μ L specific primer R (10 μ mol/L).

2.5. Characterization of DOM Fluorescence and Molecular Compositions

Three-dimensional fluorescence spectral scanning was conducted using a fluorescence spectrophotometer (F-4700, Hitachi, Hitachi City, Japan). Parallel Factor Analysis (PARAFAC) was employed to deconvolute the overlapped fluorophore signatures into distinct peaks. Preprocessing of excitation–emission matrix (EEM) data involved the elimination of primary Raman scattering, secondary Raman scattering, blanking, and internal filtering effects as well as the normalization of Raman intensity following the methodology outlined by Murphy et al. [28]. The construction of PARAFAC models was executed using MATLAB R2018a (MathWorks, Natick, MA, USA) and the drEEM Toolbox 1.0 [29]. A comprehensive dataset was generated by amalgamating samples obtained at various time points during the microcosm experiment, accompanied by the calculation of Fmax, FI, BIX, and HIX indices for each sample.

Furthermore, the dissolved organic matter (DOM) samples derived from the solid–water mixture of sediments before incubation, sediments after 95 days of incubation under bacterial sulfate reduction conditions, and sediments without amendment underwent analysis via Fourier Transform Ion Cyclotron Resonance Mass Spectrometry (FT-ICR MS), with SPE solidphase extraction and test analysis details provided in the Supplementary Information (SI). Mass spectrometry data were calibrated using known CHO compounds in DOM as internal standards. After calibration, molecular formula matching was performed according to the following parameters: S/N > 4 with no limitations on the number of atoms of C, H, and O elements and with the number of atoms of N ranging from 0 to 4 and the number of atoms of S ranging from 0 to 2, resulting in possible molecular formula results. For the screening method of molecular formula data, we selected molecular formulas with H/C values between 0 and 2.5 and O/C values between 0 and 1.2; if an m/z value (observed m/z) corresponds to multiple possible molecular formulas, the molecular formula with the smallest number of heteroatom atoms (NSP count and smallest) is retained; if there are non-unique molecular formulas, the molecular formula with the smallest error value is retained. The analysis focuses on mass spectrometry peaks within an m/z range of 200–800 Da.

3. Results

3.1. Geochemical Compositions of Sediments and Major Constituents of Groundwater

The initial geochemical compositions and As contents in the aquifer sediments utilized in the present study are shown in Table 1. Additionally, we have also collected ground-water geochemical data from our previous studies [5,27,30] for comparing the different geochemical environments in the Singe Tsangpo River (STR) and JHP basins, respectively.

Both the aquifer sediments utilized in the microcosm incubation experiments showed much higher As contents compared to global average sediment As concentrations (typical range: 5–10 mg/kg [31]), with an As content of 59.3 mg/kg in the STR and 108 mg/kg in the JHP, respectively. Moreover, both sediments exhibited significant iron oxide contents (as indicated by the Fe₂O₃ of 3.4% in the STR and of 5.1% in the JHP, respectively). Furthermore, sediments from the JHP showed significantly higher contents of metal concentrations such as Ti, V, Cr, and Mn compared to sediments from the STR, whereas sediments from the STR showed higher S content.

The groundwater geochemistry also showed a significant difference between the STR and JHP. The groundwater temperature of the STR was relatively lower than that of the JHP (7.7 °C vs. 20 °C). Moreover, the groundwater generally showed weak alkaline and oxidizing conditions in the STR (mean pH 8.4, mean Eh 215.5 mV), while a relatively lower mean pH of 7 and mean Eh value of -136 mV were observed from the groundwater in the JHP. The As concentrations in the groundwater of the STR and JHP showed average concentrations of 32.2 µg/L and 532 µg/L, respectively, both exceeding the WHO guideline

of 10 μ g/L. In addition, groundwater from the JHP showed higher Fe concentrations and lower SO₄²⁻ concentrations compared to those from the STR (Table 1).

3.2. Variations in the Astotal, Fe(II)total, and Sulfate Concentrations

The mobilization of As in STR sediments showed different patterns across different experimental groups during the incubation period (Figure 2). In the STR_NA group, a transient release of As_{total} into the aqueous phase occurred from day 7 to day 28, reaching a maximum concentration of 2.4 μ mol/L. Conversely, the introduction of sulfate in the STR_CS group did not result in a significant mobilization of As_{total} compared to the STR_NA in the initial 28 days of incubation. However, it initiated pronounced As_{total} mobilization from day 54 to day 95, reaching a maximum of 6.2 μ mol/L, accompanied by a sulfate consumption of 15 mmol/L. Furthermore, the introduction of molybdate in the STR_CM group resulted in a robust competitive desorption of solid-phase As, leading to substantial As_{total} mobilization with a maximum concentrations in Fe(II)_{total} concentrations showed similar trends across the STR_CS, STR_NA, and STR_CM groups, indicating substantial Fe(II) production followed by a gradual decrease over the incubation period. Additionally, the extent of Fe(II) production in different groups followed the sequence: STR_NA > STR_CS > STR_CM (Figure 2).



Figure 2. Variations in As_{total} , SO_4^{2-} , and $Fe(II)_{total}$ concentrations from different microcosm groups during the incubation period.

The sediment samples from JHP showed various patterns of As mobilization across different experimental groups during the incubation period (Figure 2). Within the JHP_NA group, As_{total} gradually released into the aqueous phase between day 14 and day 54, reaching a maximum concentration of 3.1 μ mol/L, followed by a retention of released As_{total} towards the end of incubation. In the JHP_CS group, a robust mobilization of As_{total} occurred from day 7, reaching a maximum concentration of 6.1 μ mol/L. Subsequently, the As_{total} concentration showed an attenuation process but re-increased by day 95, with a maximum As_{total} release of 7.4 μ mol/L and a final sulfate consumption of 15 mmol/L. Conversely, the introduction of molybdate in the JHP_CM group resulted in strong As mobilization, while the As_{total} concentrations in Fe(II)_{total} concentrations showed similar patterns among the JHP_CS, JHP_NA, and JHP_CM groups, indicating significant production of Fe(II) and a gradual decrease throughout the incubation period. Additionally, the extent of Fe(II) production also followed a sequence: JHP_NA > JHP_CS > JHP_CM (Figure 2).

3.3. Variations in the Aqueous As Species

In the STR_NA group, As(V) was the predominant As species in the transiently released As, constituting more than 95% of the total As (Figure 3). Within the STR_CS group, As(III) was the predominant As species in the aqueous As, suggesting the occurrence of As reduction during the incubation corresponding to the As mobilization. An interesting observation in the STR_CM group revealed a prevalence of As(V) speciation in the released aqueous As (Figure 3), indicating a pronounced desorption of As(V) during the incubation with a maximum concentration of 10.0 μ mol/L.



Figure 3. Variations in the concentrations of As(III) and As(V) species from different microcosm groups during the incubation period.

In the JHP_NA, the gradual mobilization of total As concentrations also corresponded with the increase in As(III) concentrations throughout the incubation, suggesting the intense occurrence of arsenate reduction during the incubation (Figure 3). Additionally, a more pronounced increase in As(III) concentrations was observed during the incubation within the JHP_CS group (Figure 3), indicating that the introduction of sulfate facilitated the As reduction. Notably, the introduction of molybdate led to the desorption of As(V) in the

3.4. Variations in the dsrB, arrA, and arsM Gene Abundance

JHP_CM group with a maximum of 6.9 µmol/L.

In the STR sediments, the abundance of the *arsM* gene showed a noteworthy increase on days 54 and 95, with the STR_CM group displaying the highest *arsM* abundance of 2.1×10^4 copies/g (Figure 4). Conversely, the abundance of the *arrA* gene significantly increased on day 14 in the STR_CS group, reaching its peak abundance of 3.5×10^3 copies/g on day 95 (Figure 4). Furthermore, substantial increases in *dsrB* gene abundance were observed in both the STR_CS and STR_NA groups, reaching maxima of 3.0×10^5 copies/g and 4.3×10^3 copies/g, respectively (Figure 4). The overall scenarios indicated corresponding increases in the *arsM*, *arrA*, and *dsrB* gene abundances from day 54 to day 95 in the STR_CS group.



Figure 4. The variations in *dsrB*, *arsM*, and *arrA* gene abundance during the incubation.

In the sediments from the JHP, the abundance of the *arsM* gene showed a significant increase on days 54 and 95, with the JHP_CS group displaying the highest *arsM* gene abundance, reaching 7.3×10^4 copies/g (Figure 4). Moreover, the abundance of the *arrA* gene showed a gradual ascent across the JHP_CS, JHP_CM, and JHP_NA groups, reaching its peak in the JHP_CS group on day 54, with a maximum of 1.1×10^4 copies/g (Figure 4). Additionally, a substantial increase in *dsrB* gene abundance was observed in both the JHP_CS and JHP_NA groups, peaking at 1.7×10^6 copies/g and 4.2×10^3 copies/g, respectively, on day 54 (Figure 4). The overall scenarios also showed corresponding increases in the *arsM*, *arrA*, and *dsrB* gene abundances from day 54 to day 95 in the JHP_NA group.

3.5. Variations in the DOM Fluorescence Components

Three distinct components (C1, C2, and C3, Figure S1) were discerned in the dissolved organic matter (DOM) through the PARAFAC model. C1, characterized by an Ex/Em peak at 225/335 nm and 275/335 nm, was identified as a tryptophan-like component, indicating a simple, low-molecular-weight component prevalent in anthropogenically affected watersheds [32]. C2 exhibited an Ex/Em peak at 325/405 nm, classifying it as a humic-like component commonly found in agricultural environments [33]. The Ex/Em peak at 445/495 nm identified C3 as another humic-like component, previously observed in landfill environments [34]. The longer wavelength of the humic-like C3 compared to other components is likely associated with a larger molecular size and a more condensed DOM structure, indicating a relatively more refractory nature [35].

Fluorescence intensities exhibited diverse patterns in the JHP and STR systems (Figure 5). The STR_ZR and STR_CS groups prominently featured the C1 composition, with a slight decrease noted in the late incubation period. Conversely, the C3 composition displayed a progressive increase throughout the incubation, spiking sharply in the STR_CS group towards the conclusion of the incubation period. In the STR_CM group, an initial transient increase in C1 was observed, giving way to the predominance of C2 and C3 compositions during the incubation.

In the JHP_ZR group, the DOM compositions were characterized by a predominant presence of the C1 composition, exhibiting a gradual increase during the initial 28 days of incubation. Concurrently, the C2 and C3 compositions also exhibited a progressive rise throughout the incubation period. Within the JHP_CS group, all three DOM compositions displayed incremental intensity increases throughout the incubations, with the C2 composition emerging as the predominant component after the incubation. Meanwhile, in the JHP_CM group, an initial transient surge in C1 was noted in the early stages of incubation, giving way to the predominance of C2 and C3 compositions throughout the incubation.

The fluorescence characteristics of the DOM revealed distinct microbial involvement in the incubation systems, as indicated by key DOM indexes (FI > 1.9, BIX > 1.0, and HIX < 1.5). Notably, in the STR_CS and JHP_CS groups, the BIX and HIX indexes exhibited a substantial negative correlation (R = -0.91 and -0.81, p < 0.01). This correlation underscores the substantial influence of microbial metabolic activity as a primary driver for variations in DOM compositions.



Figure 5. The variations in the fluorescence intensities of C1, C2, and C3 during the incubation.

3.6. Variations in the Molecular Characteristics of DOM

FT-ICR MS analysis revealed 3107 molecular formulas in the STR sediment before incubation, and 5916 and 4122 molecular formulas were identified from the STR_NA and STR_CS groups at the end of incubation, respectively (Table S1). Similarly, 1337 molecular formulas were identified from the JHP sediments before incubation, with 1956 and 3728 molecular formulas identified from the JHP_NA and JHP_CS groups at the end of incubation, respectively (Table S1).

The intensification of sulfate reduction conditions in the JHP_CS group facilitated an increase in lignin-like molecular abundance and significantly elevated the levels of lipidand protein-like molecules (Figure 6). In contrast, the JHP_NA group exhibited a weaker increase in the abundance of lignin- and lipid-like molecules and displayed a decrease in protein molecules. Additionally, sulfate reduction enhancement contributed to increases in the protein- and lignin-like molecular abundances in the STR_CS group. Meanwhile, the STR_NA group showed a much more substantial increase in the lignin- and lipid-like molecular abundance. Notably, the *I_bioprod* index exhibited a significant increase in both the JHP_CS and STR_CS groups (3.55 vs. 6.59 and 3.30 vs. 5.96, respectively), indicating heightened bioavailability of the dis-



solved organic matter (DOM). In contrast, the JHP_NA and STR_NA groups demonstrated a lower *I_bioprod* index compared to the JHP_CS and STR_CS groups (Table S2).

Figure 6. The van Krevelen diagram of DOM molecular distribution (**a**) and the variations in the relative abundance of DOM identified as different organic components and molecular compositions (**b**).

4. Discussion

4.1. Microbial Sulfate Reduction Facilitates As Mobilization

The present results showed a transient mobilization of arsenic from the STR sediments under natural conditions (STR_NA, Figure 2), while a significant enhancement in the As mobilization was observed in the STR sediments under microbial sulfate reduction conditions (STR_CS, Figure 2). Within the STR_NA group, the transient As release during the incubation could be attributable to the reductive dissolution of iron (hydro)oxides and the concurrent desorption of $A_{S}(V)$ [6,36]). This scenario was supported by the substantial increase in Fe(II)_{total} (Figure 2) and As(V) concentrations (Figure 3). Furthermore, the subsequent attenuation of released As could be ascribed to the As partitioning into secondary iron mineral phases (e.g., magnetite) under predominant bacterial iron reduction conditions, since the continuous accumulation of aqueous Fe(II) could catalyze the formation of magnetite or other secondary iron mineral phases [7]. Such scenarios could be supported by two lines of evidence in the STR_NA: (1) The limited sulfate consumption during the increase in As concentrations suggested that microbial iron reduction could be predominant in the systems (Figure 2); (2) The Fe(II)total production was more pronounced in comparison to that from the STR_CS group (Figure 2). In contrast, the strong As mobilization in the late incubation stage from the STR_CS group could be attributed to enhanced dissimilatory arsenate reduction under enhanced sulfate reduction conditions [9,37]. This scenario could be evidenced by the intense increases in the arrA gene abundance and As(III) concentrations (Figures 3 and 4). Notably, we also observed the attenuation of As in the STR_CS group, which is consistent with observations in previous studies under bacterial sulfate reduction [38]. Such scenarios could be attributed to the formation of iron sulfide minerals, resulting in the As sequestration during the incubation [39]. Additionally, although the introduction of molybdate inhibited the microbial sulfate reduction in the

STR_CM group, it triggered strong mobilization of As(V) in turn (Figure 3), which could be ascribed to the competitive desorption of molybdate with As for the active surface sites on iron (hydro)oxides [40].

Similarly, a transient mobilization of As followed by the sequestration of As into the solid phase under natural conditions was also observed in the JHP sediments, which was consistent with previous studies in the Jianghan Plain showing the limited As concentration in the shallow, unconfined aquifer under prevailing microbial iron reduction conditions [27]. This scenario was also supported by our previous incubation study of JHP sediments in which the incorporation of released As into secondary iron mineral phases could occur under mimicking natural incubation conditions [41]. Additionally, the enhanced As mobilization in JHP sediment under microbial sulfate reduction was also consistent with prior observations that the concurrent dissimilatory arsenate reduction and the formation of thiolated As species could enhance the As mobilization from aquifer sediments under bacterial sulfate reduction [37]. Notably, the JHP sediments showed significantly stronger extents of As mobilization in comparison with that from the STR sediments. Such scenarios could be attributable to the different sediment nature characteristics between those two different aquifer systems, which could supported by two lines of evidence: (1) The much higher dsrB and arrA gene abundances (Figure 4) and the predominant species of arsenic in the NA group suggested possibly a stronger microbial metabolic potential of the microbial sulfate/arsenate reduction community in the JHP sediments in comparison to that from the STR sediments [42,43]; (2) The sequential extraction results also supported the different distribution of As fractions between the JHP and STR sediments, where the JHP sediments showed a higher adsorbed As fraction (21.5 mg/kg vs. 10.3 mg/kg) and a lower pyrite-associated As fraction (1.2 mg/kg vs. 2.8 mg/kg) compared to those of the STR sediments (Table S4). Therefore, it was reasonable to speculate that the more intense occurrence of microbially mediated sulfate and arsenate reduction could result in a stronger As mobilization of adsorbed As fraction in the JHP sediments in comparison to that from the STR sediments.

4.2. Co-Evolution between DOM Transformation and As Mobilization under Microbial Sulfate Reduction

The observation of increases in the fluorescence intensities of protein-like C1 and humic-like C2/C3 components in both the JHP_CS and STR_CS groups (Figure 5) suggested that the microbial sulfate reduction significantly enhanced the transformation of fluorescence DOM components during the incubation, which could possibly be ascribed to the strong microbial metabolic activities and the accumulation of microbial biomass under microbial sulfate reduction [44]. Such a scenario was also supported by the strong increase in the BIX indexes (Figure S3) during the late incubation stage within the JHP_CS and STR_CS groups, since the BIX is associated with the concentration of the β fluorophore as an indicator of an autochthonous DOM origin [45]. Moreover, it was interesting to observe negative correlations between the HIX and BIX indexes in both the STR_CS (R = -0.91, p < 0.01) and JHP_CS (R = -0.81, p < 0.01) groups, indicating opposing trends between the enhancement of bioproduction and humification extents in the sediments during the incubation. This scenario suggests the competitive production of small-molecular bio-produced DOM and complex high-molecular DOM under bacterial sulfate reduction conditions [45].

The substantial microbial contributions to DOM transformation under microbial sulfate reduction conditions were further identified by FT-ICR-MS results, where the elevated *I_bioprod* index (Table S2) denoted an increase in the abundance of microbially produced DOM molecules in both the STR_CS and JHP_CS groups [46]. This scenario was also supported by the increase in abundance of lipid- and protein-like molecules during incubation in STR_CS/JHP_CS (Figure 6), which were classified as newly formed small molecules with high bioavailability [46]. Intriguingly, the intensification of bacterial sulfate reduction conditions also influenced the degradation pathways of DOM in the aquifer sediments, as evidenced by the increase in CHOS molecular abundance with low

O/C values in STR_CS/JHP_CS compared to sediments under conditions mimicking the natural ones (Figure 7). This pattern aligns with the thermodynamic ladder during microbial utilization with different electron acceptors [47]: our prior observations indicated that microbial methanogenesis predominates in the late stage of aquifer sediments under bacterial sulfate reduction conditions [37], which could lead to an increase in low-O/Cvalue DOM molecular abundance (<0.5) due to the high CO_2 :CH₄ production ratio [15]. In contrast, the sediment without amendment showed a high abundance of N-containing DOM molecules, which could be attributed to the release of sediment-bound organics under predominant iron reduction conditions with a preferential release of low-O/C-value (<0.67) DOM molecules [48]. Additionally, the abundance of CHOS formulas showed a significant increase under enhanced bacterial sulfate reduction conditions in both STR_CS/JHP_CS, contrary to a recent study suggesting minimal incorporation of S(-II) into organic matter during abiotic sulfidation of ferrihydrite [49]. Given that CHOS molecules were primarily identified as bio-produced DOM molecules through CIA calculation, it is reasonable to speculate that bacterial sulfate reduction significantly promotes the degradation of DOM into small-molecular DOM and the transformation into organic S species, thereby enhancing DOM bioavailability.



Figure 7. The variations in the molecular compositions before and after incubation in the JHP_NA/STR_NA and JHP_CS/STR_CS groups.

Notably, the correlations between fluorescence intensities of C2/C3 and As concentrations in both the STR_CS and JHP_CS groups (R = 0.87/0.87 and R = 0.73/0.66, p < 0.05, respectively) highlighted the influence of DOM transformation on As mobilization under bacterial sulfate reduction conditions. Since C2 and C3 components were identified as humic-like components, the observed co-evolution between DOM and As could be attributed to the dual role of humic-like DOM during the As mobilization, where the humic-like DOM components could contribute to both the microbially mediated reductive dissolution of As-bearing iron (hydro)oxides and the formation of As-DOM or As-Fe-DOM complexes to facilitate As mobilization [21,50]. This scenario is partially different from the findings of co-increases between As concentrations and terrigenous humic-like components in in situ monitoring of As-rich groundwater [21,23]. Thus, the current study emphasizes the role of endogenous humic-like DOM components as a significant factor contributing to As mobilization under bacterial sulfate reduction conditions.

4.3. Environmental Implications

The present study highlighted that bacterial sulfate reduction conditions significantly enhanced arsenic mobilization in aquifer sediments from the STR. Previous research emphasized the importance of adsorption/desorption processes in controlling As distribution in sediments from riverine systems or reservoirs in the STR [51,52]. Additionally, the recharge of As-rich surface water or geothermal water serves as a substantial source of As-rich groundwater in the STR [3,5]. The current findings underscore the critical role of endogenous As mobilization from aquifer sediments under prevailing anaerobic conditions with sufficient sulfate abundance as a significant source of As in the Quaternary aquifer systems in the STR. These new insights provide strong implications for the cautious utilization of deep anaerobic groundwater in the STR as a local drinking water source, which could pose potential health risks for local residents.

The present study also suggests that bacterial sulfate reduction conditions significantly enhance the bioavailability and terrigenous signature of DOM. This enhancement is attributed to an increase in bio-produced DOM molecules and elevated levels of recalcitrant lignin-like DOM molecules. While previous studies have suggested that sediment organic matters could influence diverse microbial metabolic activities and, subsequently, change the DOM characteristics [23], a comprehensive understanding of DOM transformation in arsenic-rich aquifer sediments under bacterial sulfate reduction remains incomplete. This study underscores the pivotal role of bacterial sulfate reduction in DOM transformation within in situ aquifer systems, leading to the increase in CHOS abundance in DOM at the molecular scale (Figure 7). These processes could provide highly bioavailable DOM molecules and stimulate associated microbial activities, while the mobilization of humic-like components during the sediment incubation could also enhance the As mobilization concurrently. Our findings align partially with previous observations in arsenic-contaminated aquifer systems [21], particularly highlighting the presence of endogenous humic-like DOM components that could be conducive to As mobilization under microbial sulfate reduction conditions. Moreover, the observed DOM transformation and predominant components after prolonged incubation align with earlier findings in groundwater from the Niyaqu catchment [53] and a permafrost zone on the Tibetan Plateau [54]. Therefore, the present novel insights could provide evidence for the biogeochemical processes controlling DOM transformation in alpine and cold aquifer systems, and they can also improve our understanding of co-evolution between DOM transformation and As mobilization in As-rich aquifer systems worldwide.

5. Conclusions

In the present study, the co-evolution between the DOM transformation and As mobilization under microbial sulfate reduction was investigated by microcosm experiments with As-rich aquifer sediments from the Singe Tsangpo River basin and the Jianghan Plain. The results highlighted that the microbial sulfate reduction condition could significantly facilitate the arsenic mobilization from both STR and JHP sediments by enhancing the reductive dissolution of (hydro)oxides and the dissimilatory arsenate reduction. In contrast, the As mobilization was limited under the mimicking natural conditions due to the sequestration of As by the formation of secondary iron oxides under predominant iron reduction conditions. Moreover, the results from 3D fluorescence spectra have identified two humic-like DOM components and one protein-like DOM component, wherein the strong microbial sulfate reduction has notably enhanced the fluorescence intensities of a humic-like DOM component during the incubation of both STR and JHP sediments. Moreover, the FT-ICR-MS results have successfully revealed that the microbial sulfate reduction has significantly facilitated the contribution of bioproduction and enhanced the terrigenous signatures within the DOM compositions as well as resulted in a notable increase in the CHOS molecular abundance in comparison with that from the sediment systems without amendment. In addition, the significant correlations between the humic-like DOM components and As in both STR and JHP sediments under BSR conditions provided new evidence

that DOM transformation is conducive to As mobilization, possibly through the competitive adsorption and the formation of ternary complex species. Therefore, the present study provided novel insights into the co-evolution between As mobilization and DOM transformation under microbial sulfate reduction conditions in aquifer systems, which is also applicable in similar aquifers worldwide. Moreover, the strong As mobilization from aquifer sediments from the western Tibetan Plateau also highlighted the careful utilization of deep anaerobic groundwater during water resource management.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/w16091266/s1.

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