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Microbially Mediated Release of As from Mekong Delta Peat Sediments

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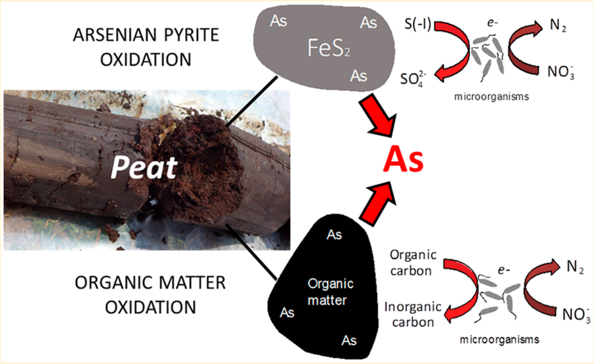
Ⓢ *Supporting Information*

ABSTRACT: Peat layers within alluvial sediments are considered effective arsenic (As) sinks under reducing conditions due to the binding of As(III) to thiol groups in natural organic matter (NOM) and the formation of As-bearing sulfide phases. However, their possible role as sources of As for anoxic groundwaters remains unexplored. Here, we perform laboratory experiments to provide evidence for the role of a sediment peat layer in releasing As. Our results show that the peat layer, deposited about 8,000 years ago in a paleomangrove environment in the nascent Mekong Delta, could be a source of As to porewater under reducing conditions. X-ray absorption spectroscopy (XAS) analysis of the peat confirmed that As was bound to NOM thiol groups and incorporated into pyrite. Nitrate was detected in peat layer porewater, and flow-through and batch experiments evidenced the release of As from NOM and pyrite in the presence of nitrate. Based on poisoning experiments, we propose that the microbially mediated oxidation of arsenic-rich pyrite and organic matter coupled to nitrate reduction releases arsenic from this peat. Although peat layers have been proposed as As sinks in earlier studies, we show here their potential to release depositional- and/or diagenetically-accumulated As.



■ INTRODUCTION

Aquifer contamination by geogenic arsenic (As) is a severe environmental issue in south and southeast (SE) Asia, as it results in the chronic exposure of ~80 million people to As.[1−4] It is reported that Himalayan weathering and the associated transport of As-bearing materials via the major river systems of the region[5] results in the accumulation of As in the sediments of flood plains and deltas. During and after burial and the establishment of anoxic conditions, As is released from naturally As-rich sediments into the porewater and transported to the groundwater.[6,7] Owing to the magnitude of this problem, numerous investigations have focused on the study of the source and mechanism of release of As from aquifer sediments.[1−4] The most accepted model states that the influx of organic carbon (from natural and anthropogenic sources) results in the microbial reductive dissolution of iron (oxy)hydroxides, releasing adsorbed As(V),[1,2,4,8,9] followed by its reduction to As(III).[10,11] Although this mechanism of Fe(III) reduction is thought to be responsible for As release in some deltaic areas,[4] it may not be the dominant mechanism in reduced sediment layers where As is not associated with Fe(III) (oxy)hydroxides.[12] In those areas, other solid phases are likely implicated in As sequestration, and possibly mechanisms other than Fe(III) and As(V) reduction are responsible for As release.

Natural organic matter (NOM), along with iron and sulfur, plays a significant role in controlling the mobility and fate of As

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in the environment.[13] Buried NOM, in the form of peat layers, is common in delta environments, resulting from the development of mangrove flats during the last sea level high stand.[12,14] Peat can act as an As sink in surface peatlands,[15,16] where the enrichment of As in peaty sediments has been attributed to the existence of sulfur-containing functional groups.[15,16] In particular, NOM of marine or estuarine origin exhibits a strong affinity for As.[15] Additionally, the formation of As-rich pyrite (arsenian pyrite)[17−19] or realgar (αAs_4S_4)[16] is observed in anoxic sediments and peat layers and occurs through microbially mediated processes.[17,18] In the Mekong Delta, the development of mangrove forests during marine transgressions[20] has promoted Fe(III) and sulfate reduction and, consequently, pyrite precipitation along with NOM accumulation. The resulting peat layer pyrite includes As atomic concentrations of 0.06−0.4%.[12] Despite the undeniable role of peat as an As sink during its formation, correlations between the concentrations of dissolved As and dissolved organic carbon (DOC) in groundwaters in Bangladesh have suggested that buried peat deposits and dispersed organic matter can also act as a source of As thousands of years after peat deposition.[21] Other studies have implicated peat layers as sources of dissolved organic matter (DOM),[22−25] which could fuel the reduction of Fe(III) (oxy)hydroxides and thus the release of As and its mobilization from sediment to groundwater. According to these studies, the distribution of buried peat deposits rather than the distribution of As in aquifer sediments is correlated with the severity and distribution of As contamination.[23] Furthermore, DOM can be involved in other processes that may also influence As mobility, such as, complexation of As by dissolved humic substances, competitive sorption, and electron shuttling reactions mediated by humics.[26−31]

To date, direct evidence of peat layers as a source of arsenic is lacking, and the underlying potential mechanism of release under reducing conditions is unknown. Therefore, our main goals were (a) to evaluate the role of peat layers as sources of As in sediments from the Mekong Delta in Vietnam and (b) to understand the potential mechanism(s) of As release.

■ MATERIALS AND METHODS

Solid Sampling. The sampling area is located in the Mekong Delta in Vietnam near the Cambodian border in an area where heavy groundwater contamination is reported[32] (Figure S1). The site is in the An Giang Province, in a rice paddy 200 m away from the Bassac River, one of the main branches of the Mekong River. The sediments used in this study were from three sediment cores (QT-C3, QT-C5, and QT-C7) collected in January 2015, October 2016, and December 2017 from the same or immediately adjacent locations to those studied in Wang et al.[12] and exhibited similar lithological and chemical compositions as a function of depth. Sediments from the peat layer, at 16 m depth, were sampled in liners, which were previously cleaned with diluted bleach and 70% ethanol and rinsed several times with DI water to avoid microbial contamination of the sediment. Immediately after collection, the sediment samples were placed in an Ar-filled field anoxic chamber sealed in double MYLAR bags and refrigerated at 4 °C. Sediment sample analysis and handling procedures are similar to those reported by Wang et al.[12] and are described in the Supporting Information.

Porewater Extraction and Analysis. A peat sample from QT-C5 was placed in a cell (squeezer) under anoxic conditions to obtain the porewater at a pressure of 1.5 MPa[33] by the squeezing technique, which involves the expulsion of interstitial fluid from saturated material[34] by using a hydraulic press. Inside an anaerobic chamber, porewater was filtered through a 0.2 μm pore membrane filter and distributed in various subsamples. A water sample for major and trace element analysis was acidified with 5 M HNO_3, a sample for dissolved Fe(II) determination was acidified with HCl, adjusting its pH to less than 1, a sample for As speciation was immediately treated with acetic acid and EDTA, a sample for sulfide was preserved with Zn acetate, and the sample for anion analyses was not acidified. All the water samples were stored in the dark at 4 °C until analyzed. Major and trace elements were determined by inductively coupled plasma-optical emission spectrometry (ICP-OES), except Na and K that were analyzed by atomic absorption spectrometry. Anions were measured by ion chromatography. Aqueous Fe(II) and sulfide concentrations were determined spectrophotometrically. DOC (dissolved organic carbon) was analyzed with a TOC-VCSH analyzer, and arsenic speciation (As(III) and As(V)) was determined by HPLC/ICPMS (high performance liquid chromatography−inductively coupled plasma mass spectrometry).

More information about the squeezing technique and analysis is presented in the Supporting Information.

Flow-through Leaching Experiments. A total of 20 flow-through experiments were carried out (see Table S1). These experiments were performed using unstirred flow-through reactors (ca. 20 mL in volume) under anaerobic conditions at room temperature in a Coy anaerobic chamber (5% H_2/95% N_2). Sediments were ground in an agate mortar, homogenized under anoxic conditions, and placed as a suspension in the reactors. In four reactors, the sediments were pretreated with sodium azide in order to inhibit microbial activity. Influent solutions were either artificial porewater (APW) or artificial groundwater (AGW) (Table S1). The composition of APW (pH = 7.5; 1.25 mM Ca^{2+}, 3.35 mM Mg^{2+}, 45.5 mM Na^+, 3.5 mM NH_4^+, 57.6 mM Cl^-, 3.8 mM mM HCO_3^-, 0.03 mM NO_3^-, 0.5 mM acetate) is similar to that of the peat layer porewater given in Table S2, and AGW (pH = 8.1; 1.5 mM Ca^{2+}, 0.8 mM Mg^{2+}, 0.05 mM K^+, 5 mM Na^+, 1.3 mM NH_4^+, 6.5 mM Cl^-, 4 mM HCO_3^-, 0.5 mM NO_2^-, 1 mM acetate) is representative of the composition of the aquifers of the study area. The artificial input solutions were purged with N_2 to remove dissolved oxygen, autoclaved, and filtered with a 0.22 μm PES filter prior to use. A 0.22 μm PES filter was also placed at the effluent end of the reactor to prevent sediment loss. Effluent water was collected at various experimental times, every 3−4 days, to track the evolution of its chemical composition. The water samples were distributed in different subsamples and preserved appropriately according to the type of analysis and stored at 4 °C in the dark until analysis. After 600 h (25 days), at the end of the experiments, solids were collected from the reactors, dried in a desiccator under vacuum in the anaerobic chamber, and stored in sealed anoxic vials until analysis. The BET surface area of the sediment from each of the sediment layers considered was measured using 7-point N_2 adsorption isotherms with a Quantachrome Nova 4000e surface area analyzer, and the obtained values were used to normalize the amount of As released from the experiments.

Nitrate-Amended Batch Experiments. To probe the mechanism of As release from the peat layer, 5 g of moist peat

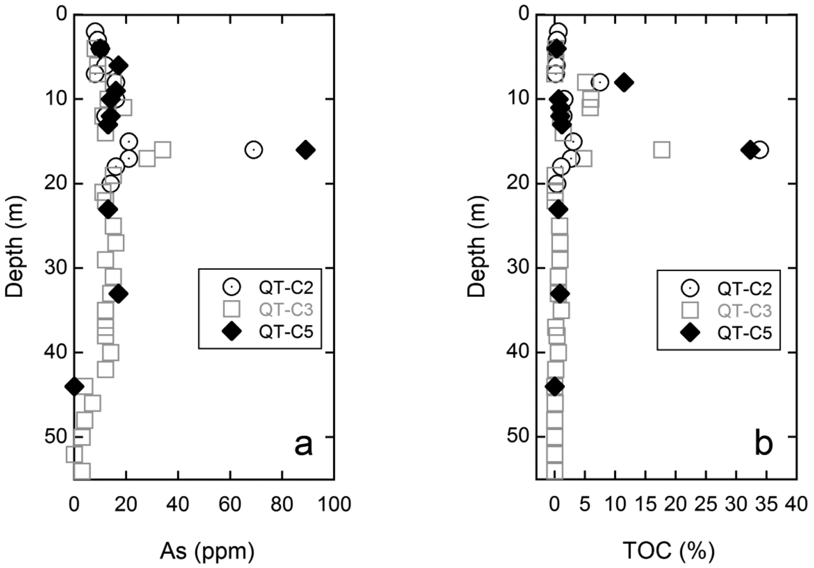

Figure 1. Panels (a) and (b) show the arsenic and total organic carbon content profiles in the solid phase of cores QT-C2, QT-C3, and QT-C5, respectively.

(corresponding to 1.5 g dry weight) and 25 mL of artificial porewater (APW-B with pH of 7.5 and composition: 1.25 mM Ca^{2+}, 3.35 mM Mg^{2+}, 45.5 mM Na^+, 3.5 mM NH_4^+, 57.6 mM Cl^-, 3.8 mM HCO_3^-, 0.5 mM acetate) amended with varying nitrate concentrations (0, 0.05, 0.5, 1, 2.2, 3.4 mM) were placed in acid-washed 30 mL glass serum bottles sealed with butyl rubber stoppers and aluminum crimps and flushed with N_2/CO_2 (80/20%). The bottles were shaken (140 rpm) at 30 °C for 40 days in the dark in triplicate. Additional experiments were carried out with sediments poisoned with 10 mL of sodium azide (final concentration = 10 mM) to inhibit microbial activity. Furthermore, additional shorter experiments (11 days) were performed to detect nitrate formation in the sediments. Prior to the experiments, all the glassware and butyl rubber stoppers were autoclaved, and the solutions were purged with N_2 to remove dissolved oxygen. After the experiment, the bottles were opened inside a Coy anaerobic chamber (Coy, Ann Arbor, MI), and the supernatant was filtered through 0.22 μm PES filter, preserved appropriately according to the type of analysis, and stored at 4 °C.

Water Chemistry Analysis. Concentrations of major elements in solution from laboratory experiments were measured by a multitype inductively coupled plasma (ICP) emission spectrometer. Arsenic and iron were analyzed by high resolution inductively coupled plasma mass spectrometry (HR-ICPMS). Sulfide, ferrous iron, and nitrite concentrations were determined using a UV−spectrophotometer. Arsenic speciation (As(III) and As(V)) was determined by HPLC/ICPMS (high performance liquid chromatography−inductively coupled plasma mass spectrometry) and DOC using a TOC-VCPH analyzer. More details can be found in the Supporting Information.

X-ray Absorption Spectroscopy (XAS). Bulk As, Fe, and S K-edge X-ray absorption near edge structure (XANES) and extended X-ray absorption fine structure (EXAFS) spectra of selected sediment samples from the QT-C3 core and the postleaching peat sample at 16 m depth were collected in fluorescence detection mode at beamlines (BL) 11-2 and 4-3 at the Stanford Synchrotron Radiation Lightsource (SSRL, California, USA), respectively. Fe K-edge XAS measurements were performed in fluorescence and transmission mode at BL 4-1 at SSRL and at BL XAFS Elettra sincrotrone (Trieste, Italy). The contributions of Fe and As species were quantified using linear combination fitting (LCF) analysis of the EXAFS, and also XANES in the case of As, data according to procedures previously reported in Wang et al.[12] The S species were quantified using LCF analysis on XANES spectra. Details about experimental setup, measurement conditions, and data processing and analysis are presented in the Supporting Information.

■ RESULTS AND DISCUSSION

Sediment Characterization. A full description (chemical composition, mineralogy, hydraulic conductivity, water content, As and Fe speciation) of the sediment cores QT-C2 and QT-C3, which were used in this study, was previously provided by Wang et al.[12] This previous study identified five distinct sediment types (I to V) as a function of depth in the QT sediment cores, whereas an additional deeper one was identified here (the sandy aquifer, type VI) (Figure S2). Sediment As content and total organic C (TOC) were measured as a function of depth for cores QT-C2, QT-C3, and QT-C5 (Figure 1). For all three cores, the depth-dependent concentration trends are similar. The distribution of As and TOC in the sediments showed that the highest As concentration is associated with the layer with the highest organic carbon content (the peat layer[12]) located at 16 m depth and the lowest concentration of As with sandy aquifer sediments (>42 m depth). Enrichment of As in the organic matter-rich layers was previously observed in other wetland systems and peaty sediments[15,16,35−38] and was attributed to the presence of sulfur-bearing functional groups.[15,16]

According to LCF of XANES and EXAFS data (Figure 2a, Figure S3, Table S3), arsenic speciation in sediment type III (12 m) is dominated by As in arsenian pyrite and S-bound As(III), together with contributions from O-bound As species (both trivalent and pentavalent) (Figure 2). In S-poor sediments type V (22 and 35 m), arsenian pyrite is absent, whereas S-bound As(III) and O-bound arsenite and arsenate are dominant (Figure 2a), confirming the report by Wang et

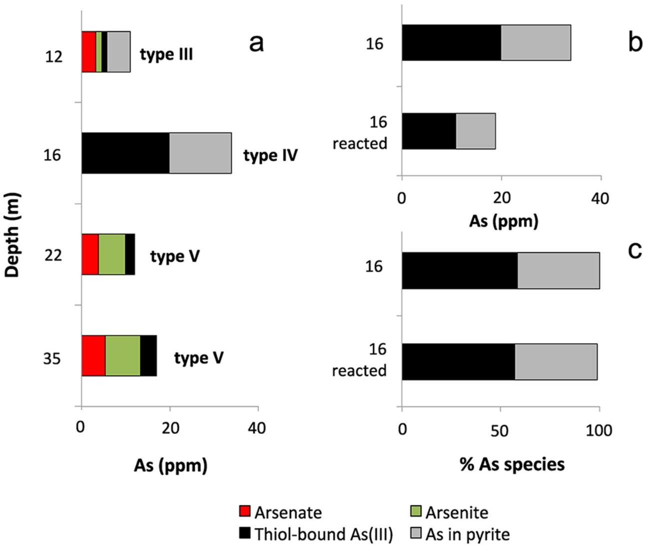

Figure 2. (a) Arsenic content in ppm by speciation of sediment types III, IV, and V from core QT-C3 calculated by multiplying As concentration in sediment samples by the fraction of As as each of the species obtained by LCF of As K-edge EXAFS data; comparison of the concentration of As obtained by XRF in the peat layer before and after the leaching experiment (with AGW) in ppm (b) and in percentage of As species (c).

al.[12] In the peat layer (type IV, 16 m), As in arsenian pyrite and S-bound As(III) are the only detected species, without contributions from O-bound As species, and thus, As speciation is distinct from that in the surrounding sediment layers (Figure 2a).

Chemistry of the Peat Layer Porewater. The composition of the peat sediment porewater (obtained by squeezing) is reported in Table S2. The pH value of the porewater is circumneutral (pH value = 7.3), with sodium as the dominant cation (with a concentration of 44.6 mM) and chloride the predominant anion (56.4 mM). Peat porewater is brackish as a result of the estuarine depositional environment of the peat.[12] Sulfate, nitrate, and ferrous iron are also present in the porewaters but at substantially lower concentrations: 90, 30, and 3.1 μM, respectively (Table S2), while ammonium is present at 3.4 mM. The arsenic concentration is high (0.84 ± 0.04 μM) and is predominantly present as As(III).

Arsenic Release in the Flow-through Leaching Experiments. To probe the potential mobility of arsenic from the sediment under anoxic conditions, flow-through experiments were performed in which the different sediment layers were exposed to a continuous flow of APW or AGW. The results show that there are large differences in the total amount of As released over 600 h, depending on the sediment type (Figure 3a, Table S1). All the sediment layers release As, as observed in earlier studies from shallow sediments in other regions of SE Asia.[11,7] However, the peat layer releases the most As by far, followed by the sandy aquifer sediment when normalized to surface area and arsenic concentration in the sediment layer (Figure 3b). The latter could be due to the reduction of Fe(III) oxides that are present in the aquifer sediments (Figure S4, Table S4) and the release of arsenic sorbed onto their surfaces. As was released from the peat layer (16 m) in experiments performed with either AGW or APW (Figure 3a). This fact suggests that, although NOM may have sequestered As during the formation of these organic matter rich layers,[16] peat may become a source of As after its formation. In addition, the differences observed in the amount of arsenic released between the experiments performed with untreated peat sediments and those azide-treated indicate that the arsenic release is at least partially mediated microbially (Figure S5, Table S1).

To investigate the potential change in solid phase As speciation during the flow-through experiments, As speciation in the peat layer (type IV, 16 m) was also probed at the end of the experiment conducted with AGW (Figures 2b and 2c, Figure S3, Table S3). The results of LCF analysis of XANES and EXAFS spectra were consistent and showed that As in the post flow-through peat is present as arsenian pyrite (~42%) and as As(III) bound to NOM through thiol groups (~58%) (Figure 2c). Hence, the fractions of the two As species after the flow-through experiment were very similar to those in the original peat (Figure 2c, Table S5), suggesting that comparable

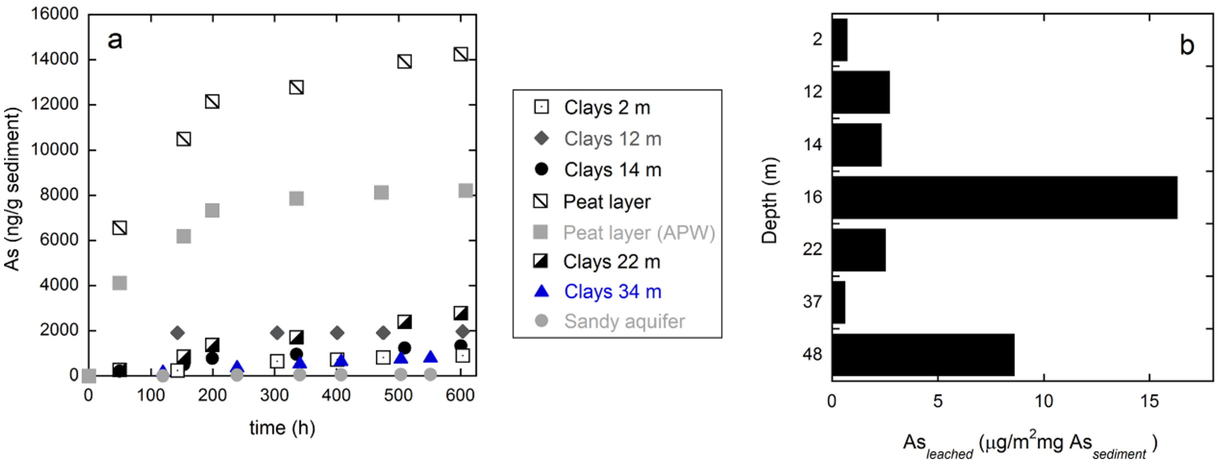

Figure 3. Arsenic released after 600 h in the anoxic flow-through experiments from QT core sediments at different depths versus time (a) and depth (b). All the experiments were conducted with artificial water AGW, except for the peat layer (gray squares in part a) for which the tests were also performed with the artificial porewater (APW). The different types of sediments defined by Wang et al.[11] were used for the experiments (2 m depth sediments (Type I), 14 m depth sediments (Type II), 12 m depth sediments (Type III), 16 m depth (Type IV), 22 and 35 m depth sediments (Type V), and the sandy aquifer sediments (Type VI)).

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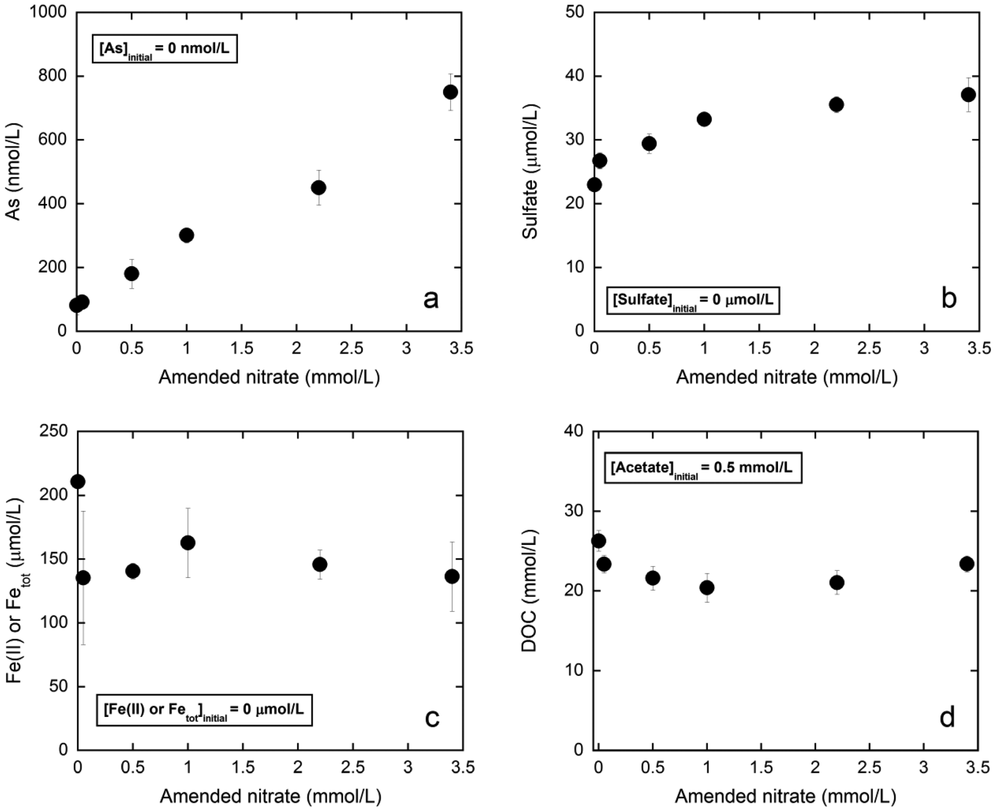

Figure 4. Concentration of As (a), sulfate (b), Fe(II) or Fe total (c), and DOC (d) at the end of the batch experiments (after 40 days) versus the nitrate concentrations amended to APW-B (0, 0.05, 0.5, 1, 2.2, and 3.4 mM).

amounts of arsenic were released from NOM and arsenian pyrite. The difference in arsenic concentration in the peat layer before and after the flow-through experiment (as measured by XRF) also corresponds to the amount of As released to solution (as measured in the effluent) (Table S5) and indicates that about 40% of As was released into the effluent water from the peat layer (Table S5 and Figure 2b). According to XAS results, the amounts of As released from NOM and arsenian pyrite were thus ~8 and ~6 ppm, respectively, from initial amounts of ~20 and ~14 ppm. The flow-through experiments allow us to assess the amount of As that can be potentially released from the peat layer, and this amount is expected to be much higher than in the field peat layer.

Arsenic speciation in the peat porewater, as well as the effluent of flow-through experiments performed with the peat layer, reveals that most of the As is in its reduced As(III) form (Table S2). Due to the high affinity of As(III) for sulfide/sulfur,[39] the formation of thioarsenic species has been reported in sulfidic solutions[40,41] and in previous studies of the dissolution of As−S- or As−Fe−S-minerals.[42] The formation of mobile thioarsenic species could play an important role for arsenic mobility and its environmental impact.[43,44] While we did not test their formation during the experiments, this possibility cannot be discounted and will require future work. Colloid formation could also exert an influence on As mobility. However, it is unlikely here because the As concentration in effluent samples from peat layer leaching did not change after filtration through 0.22 μm or 10 kDa pore size filters (data not shown). In contrast, As binding to DOC-thiol groups could be also envisaged. An important question arising from our observation is the mechanism of As release from NOM and FeS_2.

Mechanism of As Release from the Peat. The peat sediment flow-through experiments were performed either with AGW or APW, which respectively contain nitrite or nitrate as the only electron acceptor. From the literature, we expect that DOM and pyrite could be oxidized with the reduction of nitrate (only biologically) or nitrite (both biologically and abiotically).[45,46] In particular, denitrification coupled to anaerobic pyrite oxidation has been identified in natural groundwater systems, including in the presence of organic carbon.[47−51] The coupled decrease in nitrate and increase in sulfate concentrations was reported in groundwaters and was attributed to microbial chemolithotrophic denitrification linked to pyrite oxidation,[52−55] even at low nitrate concentrations.[56,57]

The flow-through experiments performed with AGW or APW (containing nitrite or nitrate as the only electron acceptor, respectively) evidenced As release from the peat layer. Thus, we hypothesized that nitrate reduction coupled to NOM and/or arsenian pyrite oxidation in the porewater may have caused As release to the water during the experiments. Indeed, although present at low concentration in the *in situ* porewater (~30 μM), nitrate is the most relevant potential electron acceptor for arsenian pyrite and NOM oxidation in peat layer porewater (Table S2).

To test our hypothesis, we carried out batch experiments in which the peat sediment was amended with increasing concentrations of nitrate (APW-B with nitrate amendments ranging from 0 to 3.4 mM). In these batch experiments, the concentration of As released increased almost linearly with the amended nitrate concentration (Figure 4a), which suggested a role for nitrate in the release of As from the peat.

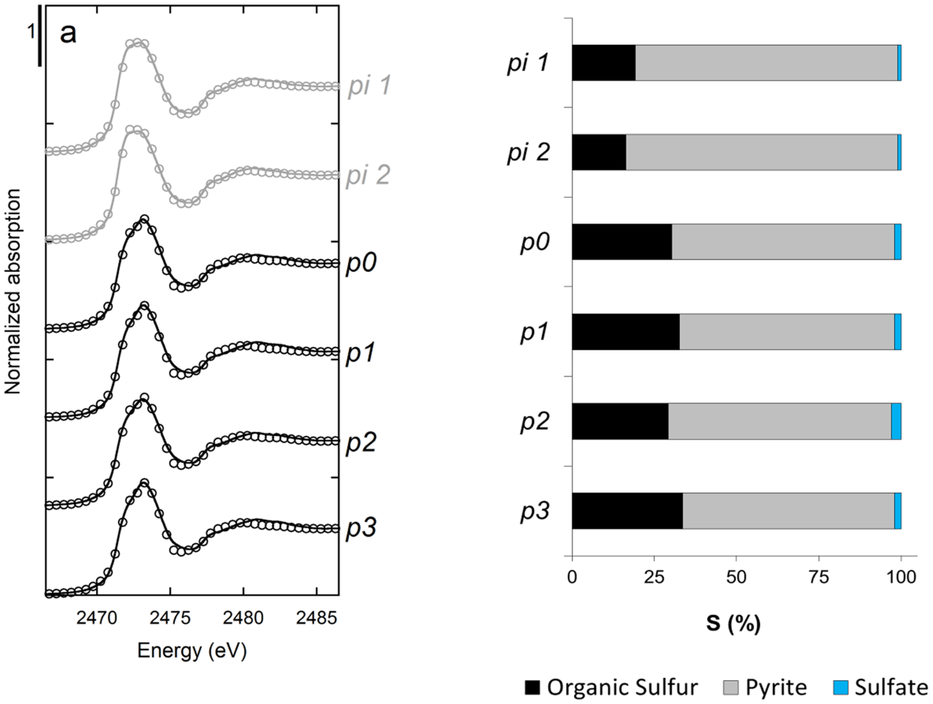

Figure 5. (a) S K-edge XANES data for peat sediment samples and (b) the results of linear combination fit (LCF) before the reaction of initial peat unreacted subsamples (pi1 and pi2) and after batch reaction with APW-B amended with 0, 1, 2.2, and 3.4 mM of nitrate (corresponding to p0, p1, p2, and p3) for 40 days.

In addition, we observed that the proportion of pyrite in the solid phase S speciation decreased (Figure 5), supporting concomitant pyrite oxidation and nitrate reduction. Indeed, using an LCF approach applied to the S K-edge XANES spectra, pyrite (FeS_2) and organic sulfur (S-cysteine) were identified as the major S-bearing species involved in the system, even if the occurrence of S^0 could not be strictly excluded. After 40 days of reaction, the fraction of organic sulfur increases at the expense of pyrite (Figure 5, Table S6). However, these S K-edge XANES results also show that the amount of pyrite depletion is similar (11−18%, Table S6) regardless of the initial nitrate concentration, including in the batch experiment performed with unamended APW-B synthetic groundwater (p0 in Figure 5), which contained neither nitrate nor any other electron acceptor. These results suggest that pyrite is oxidized by nitrate but that the nitrate concentration is not the limiting factor for this process and point to a putative endogenous source of nitrate.

The sulfate concentration at the end of each incubation also increased with the nitrate concentration amended but in a nonlinear fashion (Figure 4b). This increase of sulfate with initial nitrate concentration could be interpreted as being due to pyrite oxidation to sulfate. However, the total production of aqueous and solid sulfate is far too low to account for the amount of pyrite that has been removed from the samples, as estimated from S K-edge XANES analysis (Table S7). Moreover, the amount of nitrate amended does not account for the pyrite oxidized. In fact, at the maximum concentration of amended nitrate, the stoichiometry of pyrite oxidation to sulfate by nitrate dictates that approximately 30.4 μmol of pyrite would be oxidized (Table S7). In contrast, based on the S K-edge XANES, we estimate that between 59 and 75 μmol are oxidized. A possible explanation for the low sulfate concentration could be its consumption via sulfate reduction. Alternatively, this discrepancy could be due to the oxidation of pyrite to S^0 instead to sulfate. In that case, the S mass balance would be closer to being satisfied, with a predicted maximum of 121.4 μmol of pyrite oxidized if all nitrate amended is consumed by this process (Table S7). Considering the fraction of pyrite removed by oxidation during the experiments (a range of 11−18%), S^0 would represent only 7.6−12.4% of the total S, which is very close to the XANES detection limit (~10%), and hence may not be detectable by S XANES. We conclude that pyrite is oxidized either to sulfate and/or to S^0 with nitrate as an electron acceptor and As is released in the process.

At the end of these batch experiments, both nitrate and nitrite concentrations were below the detection limit, supporting denitrification as the metabolic process and N_2 or NO_x gases as the ultimate N products. Further, the decrease in DOC concentration with increasing initial nitrate concentration could be due to heterotrophic denitrification (Figure 4d). Overall, we interpret the results of this series of experiments as denitrification via autotrophic (coupled to pyrite oxidation)[58] and/or heterotrophic (coupled to NOM oxidation) routes.[59]

In addition, ammonium concentration decreased from the original 3.5 mM down to 0.6−1.1 mM regardless of the concentration of amended nitrate in the batch experiment performed with APW-B synthetic groundwater (Figure S6e). Ammonium is present in peat porewater and could be assimilated or microbially oxidized (through an unknown mechanism) and may be the source of nitrate.

In this work, we found that As is released via nitrate-dependent oxidation of pyrite and NOM. However, the presence of nitrate in the peat layer is unexpected due to the prevailing reducing conditions and the absence of nitrate in the porewaters of the layers immediately above or below of the peat (data not shown). This suggests that nitrate is being generated within the peat layer rather than coming from diffusion or advection from the surface or surrounding layers. To conclusively demonstrate its presence, we quantified nitrate in a fresh suspension of peat and nitrate-free APW-B (Figure 6). We observed the presence of nitrate (~0.6–18.4 μM) and nitrite in low concentration (~0.6–0.7 μM) in the freshly prepared suspension. A stable concentration of nitrate is observed during the first 3 days followed by a subsequent decrease. Nitrite concentrations are low but constant throughout the experiment (Figure 6). We interpret these data as a combination of the production of nitrate in the peat layer via an unknown mechanism followed by its denitrification.

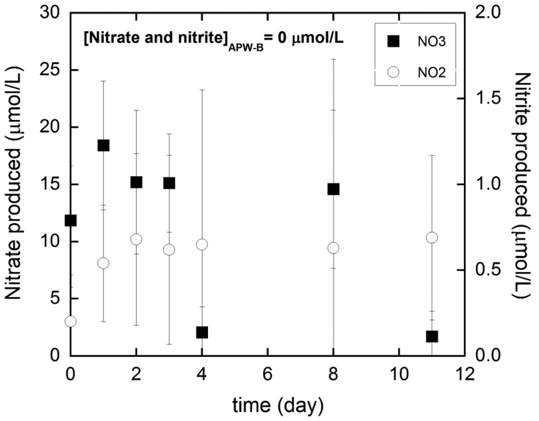

Figure 6. Concentration of nitrate and nitrite produced at different sampling times in a batch experiment performed with the peat layer (sediment type IV) and artificial porewater (APW-B) without added nitrate or nitrite.

We have invoked denitrification as the reductive process coupled to pyrite and NOM oxidation; to ascertain the role of microorganisms in this process, we used sodium azide as a poison. Sodium azide, known to target S and Fe oxidation,[60] had a clear impact on the release of As and the production of sulfate, which were systematically greater in the untreated control (Figure S6a,b). This result supports those of the flow-through experiments (Figure S5) and validates the role of oxidative microbial processes in the release of As from peat. Interestingly, the sodium azide-treated cultures show less consumption of ammonium as compared to treated cultures (Figure S6e), suggesting that azide partially inhibits an ammonium-oxidizing microbial process. Further, higher DOC concentrations were observed in the poisoned experiments compared to the untreated ones, reflecting the greater oxidation of DOC by the microbial community in the untreated systems (Figure S6d). Hence, in the absence of the poison, the pool of biodegradable organic carbon released from NOM in the peat can be consumed by the native microbial community. Sodium azide appears to inhibit many of the oxidative processes in this system, which are consequently attributed to microbial activity: iron and sulfur oxidation, ammonium oxidation, and NOM oxidation.

Thus, overall, the data collected through this study provide convincing evidence of the microbially mediated oxidation of pyrite and NOM with nitrate and the associated release of As from the sediment peat layer and suggest a complex interplay of the C, N, and S biogeochemical cycles.

Environmental Implications. Buried natural organic matter (peat) layers are common in delta environments in SE Asia, resulting from the paleo-development of mangrove flats. Peat layers have been proposed to serve as effective sinks for As under reducing conditions due to the covalent binding of trivalent As to thiol groups of NOM and the formation of As sulfides.[15,16] However, our results provide evidence that peat, deposited about 8,000 years ago in paleomangrove environments of the Mekong Delta,[12] can become an As source in the long term, even under reducing conditions. Here, we show that As is released through microbial processes from the NOM-thiol bound As and arsenian pyrite. These results imply that, in the presence of nitrate, peat can serve as a long-term source of As. Interestingly, nitrate production from the peat was also suggested here, providing a continuous endogenous source of nitrate. Therefore, in locales where sediments are reduced, peat layers could play a crucial role in releasing As. This is likely true for reduced deltaic sediments but potentially also in As-contaminated peatlands and other organic matter rich areas.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.9b02887.

> Methods; Figure S1, location of study area; Figure S2, lithology of QTC-2, QTC-3, and QTC-5 cores and pictures of sediments at various depths; Figure S3, As K-edge XAS data; Figure S4, Fe K-edge EXAFS data and LCF of Fe K edge EXAFS data for QT sediments; Figure S5, arsenic leached in leaching flow-through experiments; Figure S6, comparison of concentrations of As, sulfate, Fe(II) or Fe total, DOC, and NH_4^+; Table S1, experimental conditions and total As leached in flow-through leaching experiments; Table S2, composition of peat layer porewater of QT-C5 core; Table S3, LCF results of XANES and EXAFS data at As K-edge of QTC3 sediments; Table S4, LCF results of EXAFS data at Fe K-edge of QTC sediments; Table S5, amount of arsenic released in flow-through experiments; Table S6, LCF results of XANES data at S K-edge for peat sediment samples; Table S7, mass balance calculation (PDF)

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Notes

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