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Article

Reciprocal Interactions of Abiotic and Biotic Dechlorination of Chloroethenes in Soil

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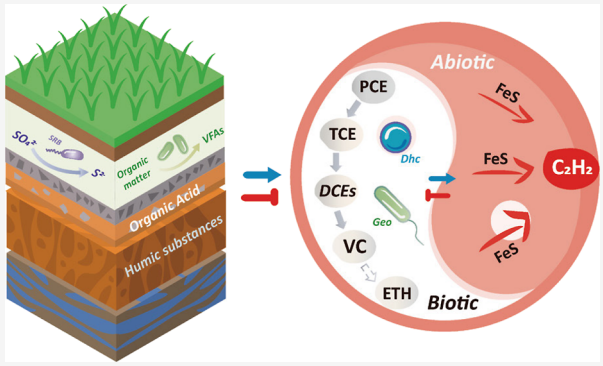
ABSTRACT: Chloroethenes (CEs) as common organic pollutants in soil could be attenuated via abiotic and biotic dechlorination. Nonetheless, information on the key catalyzing matter and their reciprocal interactions remains scarce. In this study, FeS was identified as a major catalyzing matter in soil for the abiotic dechlorination of CEs, and acetylene could be employed as an indicator of the FeS-mediated abiotic CE-dechlorination. Organohalide-respiring bacteria (OHRB)-mediated dechlorination enhanced abiotic CEs-to-acetylene potential by providing dichloroethenes (DCEs) and trichloroethene (TCE) since chlorination extent determined CEs-to-acetylene potential with an order of *trans*-DCE > *cis*-DCE > TCE > tetrachloroethene/PCE. In contrast, FeS was shown to inhibit OHRB-mediated dechlorination, inhibition of which could be alleviated by the addition of soil humic substances. Moreover, sulfate-reducing bacteria and fermenting microorganisms affected FeS-mediated abiotic dechlorination by re-generation of FeS and providing short chain fatty acids, respectively. A new scenario was proposed to elucidate major abiotic and biotic processes and their reciprocal interactions in determining the fate of CEs in soil. Our results may guide the sustainable management of CE-contaminated sites by providing insights into interactions of the abiotic and biotic dechlorination in soil.



KEYWORDS: *abiotic/biotic dechlorination, chloroethenes, acetylene, organohalide-respiring bacteria, Dehalococcoides, Geobacter*

INTRODUCTION

As a sink of various organic contaminants, soil could affect the fate of organic pollutants (e.g., organohalides) and their subsequent transport to other environmental matrices, including groundwater and atmosphere.[1,2] The fate of organic pollutants in soil was synergistically determined by combined biophysiochemical processes, including accumulation and transformation through sorption/desorption, abiotic degradation, and biotransformation, which highly depended on the soil compositions and physiochemical properties as well as soil microbiomes.[3,4] In China, according to the National Soil Contamination Survey Bulletin published in 2014, around 16% of surveyed soil samples had at least one organic pollutant exceeding standard values, particularly the organohalide pollutants.[5] Similarly, an agricultural soil survey reported that volatile organohalides were present in all tested samples. Among them, chloroethenes (CEs) detection frequency ranged from 3.57 to 6.25%, with a concentration range from 0.9 to 3.61 ng/g.[6] Hydrophobic CEs, denser than water, could migrate vertically downward from the surface soil to the saturated zone and be trapped in the soil pores to form dense non-aqueous-phase liquids.[7,8]

Soil as a complex mixture of clays, minerals, organic matter, and many other uncharacterized compositions could be involved in the conversion of various pollutants.[2] Under anaerobic conditions, the natural attenuation of CEs was observed to be mediated by solid-phase organic substances and reduced minerals.[9] For example, ferrous minerals (e.g., mackinawite, green rust, and pyrite) as essential components involving heterogeneous redox reactions were identified to mediate the abiotic dechlorination of trichloroethene (TCE).[10−12] In addition, soil organic matter (SOM) could also affect the fate, mobility, and bioavailability of CEs through adsorption/desorption and other processes.[13] Mechanisms underlying the SOM adsorption of CEs included hydrogen bonding, electrostatic attraction, and pore-filling.[14] Therefore, humic substances (e.g., humic acid and humin) as typical SOM generally function as electron mediators/carriers to improve

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CE attenuation.[15,16] However, the soil organic and mineral compositions, as well as their biophysiochemical properties, could be highly variable, e.g., organic matter (0.8−173 g/kg), water content (0.05−0.6 m^3 m^{-3}), porosity (40−60%), and bulk density (1.11−1.59 kg/m^3),[5] posing challenges in understanding the major players and their specific roles in abiotic attenuation of organohalides. In addition to these abiotic compositions and processes, microorganisms also played critical roles in removing organohalides.[9] For reductive dehalogenation of organohalides, a diverse range of microbial lineages, including obligate and facultative organohalide-respiring bacteria (OHRB), had been isolated and characterized from varied soil environments to couple their cell growth with halogen removal from organohalides.[17−20] The OHRB-mediated dehalogenation process was identified to play important roles in attenuation of organohalides in a wide range of soil environments, particularly in the dechlorination of CEs.[21,22] Notably, in the OHRB-mediated dechlorination of CEs, other microorganisms, including *Methanosarcina* and *Desulfovibrio*, could form metabolic networks with OHRB by providing electron donors and carbon sources.[23] In addition, *Desulfovibrio* as sulfate-reducing bacteria (SRB) was recently identified to maintain low redox conditions for the OHRB by producing soluble microbial products and consequently facilitated TCE dechlorination.[24] Moreover, SRB-derived FeS and pyrite were recently identified to mediate the abiotic dechlorination of organohalides, suggesting reciprocal interactions of the abiotic and biotic processes.[25−27] Overall, the natural attenuation of CEs could be a combined biogeochemical process involving various abiotic and biotic processes in complex soil environments.[28] Also, insights into these abiotic and biotic processes enabled efficient remediation of CE pollution at contaminated sites. However, in the complex soil environments, information on the functional components involved in the abiotic and biotic attenuation of CEs, as well as their reciprocal interactions, remained scarce.

Therefore, this study aimed to elucidate the complex network relationships among varied minerals, microorganisms, and organic substances in the attenuation of CEs. First, we conducted a comprehensive evaluation of the dechlorination activity and major catalyzing matter in selected typical soil samples. Then, PCE dechlorination was investigated in three dechlorinating cultures (i.e., pure cultures of *Dehalococcoides mccartyi* CG1 and *Geobacter lovleyi* LYY, and microcosm SZ1) upon amendment of FeS and humic acid. Finally, a scenario was proposed to describe the reciprocal interactions between abiotic and biotic PCE dechlorination processes. These findings could provide novel insights into the attenuation of CEs at contaminated sites.

■ MATERIALS AND METHODS

Chemicals and Soil Samples. All chemicals were purchased at highest purity available from Sinopharm (Shanghai, China). Minerals (i.e., Magnetite or Fe_3O_4, pyrite or FeS_2, and mackinawite or FeS) and humic substances (humic acid or HA; and fulvic acid or FA) were supplied by Aladdin (Shanghai, China). Humin was kindly provided by Prof. Dongdong Zhang (Zhejiang University), which was prepared with estuary sediment (31° 10′ N, 122° 50′ E) as described.[29] Five different soil samples were collected from varied sites in China, i.e., black soil (northeast China, 51°43′51.726″ N, 126°42′53.686″ E), brown soil (middle China, 30°28′14.343″ N, 116°50′21.984″ E), laterite soil (southern China, 23°1′47.604″ N, 113°25′16.68″ E), paddy soil (southern China, 23°9′50.5242″ N, 113°21′40.608″ E), and river sediment (southern China, 23°4′20.244″ N, 113°23′47.965″ E). To exclude the involvement of anaerobic OHRB in reductive dechlorination of PCE and minimize potential impact derived from other soil pretreatment methods, the soil samples were air-dried under aerobic conditions for 30 days. Subsequently, the samples were crushed and sieved through a 0.2 mm mesh screen. The physicochemical characterization of these soil samples was conducted as described previously.[30] Absence of OHRB in these soil samples was confirmed with 16S rRNA gene amplicon sequencing analysis.

PCE Dechlorination in Soil and Identification of the Key Dechlorination-Catalyzing Matter. To test abiotic PCE dechlorination activity in the soil samples, 20 g of pretreated sample was added to 50 mL autoclaved serum bottles, with amendment of 8.5 mL of oxygen-free water as described.[30] The soil water content was identified to be 30.0%, calculated as water/(water + soil) (w/w). The serum bottles were sealed with black butyl rubber septa (Geo-Microbial Technologies, Ochelata, OK, U.S.A.) and secured with aluminum crimp caps. SiO_2 was used as soil control. The above-described experiments were performed under anaerobic conditions. All bottles were amended with 1 mM PCE as an electron acceptor. To identify the predominant dechlorinating matter in these soil samples, different iron minerals (i.e., Fe_3O_4, FeS_2, and FeS) and humic substances (i.e., humic acid, fulvic acid, and humin) were added to 25 mL of oxygen-free water, separately, with a final concentration of 250 g/L. Oxygen-free water was used as a blank control. This high concentration facilitated the rapid identification of the active soil components involved in PCE dechlorination. All dechlorinating bottles were amended with 1 mM PCE. Unless otherwise clarified, the above experimental sets were set up in triplicate and incubated at 35 °C in the dark without shaking.

Reciprocal Interactions between FeS and OHRB. To explore the reciprocal interactions between the major abiotic and biotic dechlorinating matter and their affecting factors in soil, FeS-OHRB combined batch experiments were set up in 100 mL serum bottles under anaerobic conditions. 1 mM PCE was added as an electron acceptor. Two pure cultures (i.e., *D. mccartyi* CG1, *G. lovleyi* LYY) and one PCE/PCB-dechlorinating mixed culture (SZ1) were employed in this study.[19] The defined bicarbonate-buffered mineral medium for these cultures was prepared as described.[31,32] Briefly, the medium was supplemented with 10 mM lactate for the microcosm SZ1 and with 10 mM acetate as a carbon source and 5×10^4 Pa hydrogen gas as an electron donor for the pure cultures. L-cysteine (24 ppm) and $Na_2S{\cdot}9H_2O$ (48 ppm) were provided as reductants for redox potential control, and resazurin (5 ppm) was added as a redox indicator. Medium pH was maintained at around 7.2 by adding N_2/CO_2 (80:20, vol/vol) mixture gas (18 psi). FeS minerals with gradient concentrations (i.e., 2.5, 25, and 250 g/L) were added to the OHRB-containing cultures (i.e., *D. mccartyi* CG1, *G. lovleyi* LYY, and SZ1). The biotic control bottles inoculated with OHRB cultures and abiotic control bottles with gradient concentrations of FeS minerals (i.e., 2.5, 25, and 250 g/L) were also established. Purified FeS was employed in these experiments.

Roles of Humic Substances in PCE Dechlorination. LYY-HA-FeS experimental sets were established in 100 mL serum bottles to investigate the impact of humic substances on

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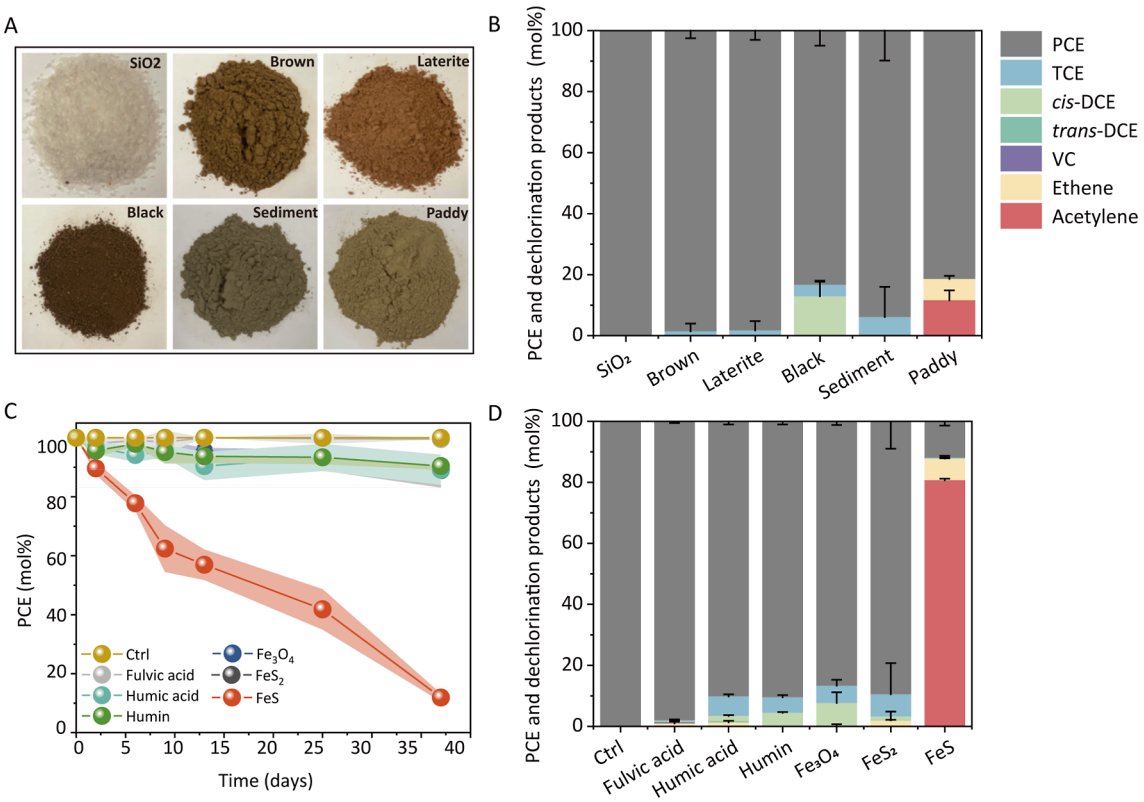

Figure 1. Dechlorination of PCE in soil samples: (A) five different soil/sediment samples and SiO_2 as a control. (B) Abiotic dechlorination of PCE in soil samples. (C) PCE dechlorination efficiency and (D) profile of dechlorination product(s) by humic substances and minerals as major soil compositions. Error bars indicate SDs of triplicate experiments.

PCE degradation. Specifically, FeS (25 g/L) was pre-incubated with humic acid (i.e., 0.25, 1, and 10 g/L) for 2 weeks at 35 °C in the dark with shaking (150 rpm/min). Then, *G. lovleyi* LYY was added to the bottles. At the same time, two control experimental sets were set up: (i) LYY-HA, cultures amended with LYY and gradient concentrations (i.e., 0.25, 1, and 10 g/L) of HA and (ii) FeS-HA, abiotic bottles amended with 25 g/L FeS and gradient concentrations (i.e., 0.25, 1, and 10 g/L) of HA. Purified FeS was employed in these experiments.

Analytical Techniques. CEs and their dechlorination products were analyzed with an Agilent 7890B gas chromatograph equipped with a flame ionization detector (Agilent, Wilmington, DE, USA) and a DB-5 capillary column (30 m × 0.32 mm × 0.25 μm, Agilent J &W Scientific, Folsom, CA, USA) as described.[33] The temperature program was initially held at 45 °C for 1 min, increased at 30 °C/min to 150 °C, and held for 2 min. Agilent OpenLab was used for further analysis.

DNA Extraction, PCR Amplification and Sequencing, and qPCR. Microbial cells were harvested by centrifugation (15 min, 10,000*g*, 4 °C) with 1 mL samples. The FastDNA Spin Kit for Soil (MP Biomedicals, Carlsbad, CA, USA) was used to extract genomic DNA according to the manufacturer's instructions. To extract DNA from samples with FeS, an additional processing step is needed. After centrifugation, samples were re-suspended in EDTA (0.25 M) and incubated at 55 °C for 5 min before removing the supernatant by centrifugation. The EDTA washing step was repeated twice before proceeding with DNA extraction using a kit. The DNA extraction failure of CG1 may be due to its sensitivity to FeS and biomass loss during pretreatment. The 16S rRNA gene was amplified with the U515F forward primer and U909R primer as described.[31] Illumina sequencing (Illumina, San Diego, CA, USA) service was provided by Magigene (Shenzhen, China). The qPCR enumerations of *Dehalococcoides* (Dhc) and *Geobacter* (Geo) cells were performed with a QuantiTect SYBR Green PCR kit as described previously.[34]

Sequencing Data Deposition. Raw Illumina Miseq sequencing reads were deposited in the European Nucleotide Archive at EMBL-EBI under accession number PRJEB62147.

■ RESULTS

Abiotic PCE Dechlorination in Soil and Identification of Key Dechlorinating Matter. To test abiotic PCE dechlorination activity in varied sources of soil, 5 different soil samples (Figure 1A) were collected from varied geographic sites in China, i.e., black soil, brown soil, laterite soil, sediment soil, and paddy soil. To exclude the involvement of anaerobic OHRB in the reductive dechlorination of PCE and minimize potential impact derived from other soil pretreatment methods, the soil samples were air-dried under aerobic conditions for 30 days. Results showed different extents of PCE dechlorination after 80 days of incubation (Figure 1B), i.e., 1.5, 1.8, 16.7, 6.1, and 18.6% PCE dechlorination in the brown soil, laterite soil, black soil, sediment soil, and paddy soil, respectively. Interestingly, in contrast to PCE dechlorination to TCE and/or *cis*-dichloroethene (*cis*-DCE) in other four soil samples, both ethene and acetylene were observed as major dechlorination products in the paddy soil (Figure 1B).

The dechlorination extent and product composition in these soil samples could be affected by the soil minerals and organic matter by providing dechlorinating matter and electron

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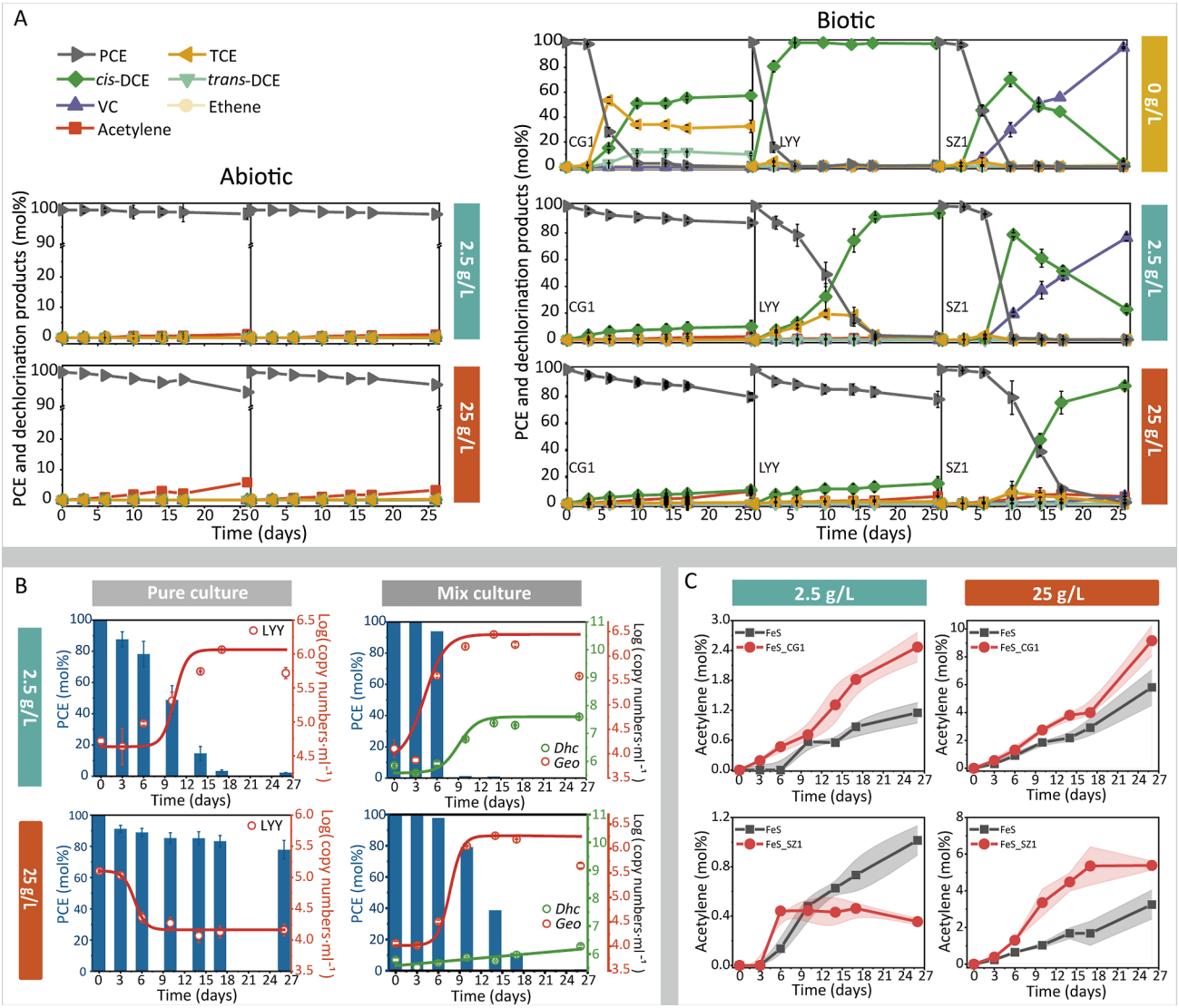

Figure 2. Abiotic and/or biotic dechlorination of PCE: (A) PCE dechlorination in the biotic and/or abiotic experiments with two pure cultures *Dehalococcoides mccartyi* CG1 (CG1) and *Geobacter lovleyi* LYY (LYY) and a mixed culture SZ1 containing both *Dehalococcoides* (Dhc) and *Geobacter* (Geo). (B) Cell growth of OHRB in the biotic- and abiotic-combined experiments with the pure culture LYY and the mixed culture SZ1. (C) Acetylene content of the biotic- and abiotic-combined experiments. Purified FeS was employed in the abiotic and combined experiments. Error bars indicate SDs of triplicate experiments.

donors/shuttles, respectively.[10,35,36] To identify the critical dechlorinating matter in the soil, different minerals and organic matter were selected as major soil components to test their dechlorination activities, magnetite (Fe_3O_4), pyrite (FeS_2), and mackinawite (FeS) as representative soil iron minerals, and humic acid (HA), fulvic acid (FA), and humin as representative soil organic matter. Like the above-described observations in soil samples, varied extents of PCE dechlorination were observed in the batch experiments (Figure 1C), i.e., 2.0, 9.9, 9.6, 13.4, 10.6, and 88.1% PCE dechlorination in FA, HA, humin, Fe_3O_4, FeS_2, and FeS, respectively. Notably, acetylene as the complete dechlorination product was only observed in FeS-amended experimental sets with the most extensive dechlorination extent compared to other experimental sets (Figure 1D). The FeS-mediated PCE-to-acetylene dechlorination may rationalize the acetylene production in paddy soil samples since FeS can be generated in paddy soil during the flooded phase.[37] Compared to paddy soil, higher PCE-to-acetylene dechlorination activity in FeS experimental sets could be due to the comparatively less amount of FeS in paddy soil. Experimental tests on the PCE dechlorination efficiency with different concentrations of FeS showed a significant positive correlation between PCE-to-acetylene dechlorination activity and the FeS concentration (Figure S1A). In addition to acetylene, ethene was also observed as a major dechlorination product in the FeS bottles, which could be possibly due to the impurity of the mackinawite mineral used in this study. To confirm the assumption, the mackinawite mineral was ground into powder under an obligate anaerobic condition, and then a magnet was employed to remove the magnetized matter from the mackinawite mineral powder. Surprisingly, results showed higher PCE-to-acetylene dechlorination activity without ethene generation with the purified mackinawite mineral powder (Figure S1B) relative to the unpurified mackinawite mineral, which could be mediated by reductive β-elimination.[38,39] Therefore, these results confirmed that ethene production in both paddy soil and mackinawite mineral experiments should

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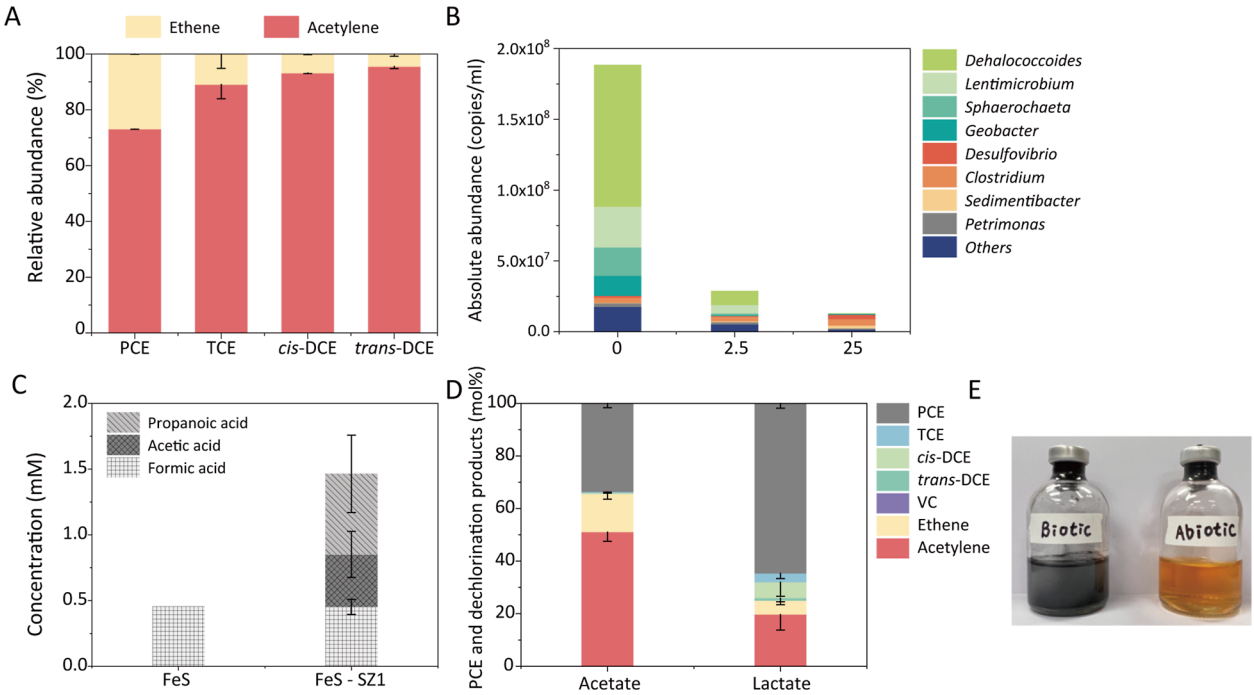

Figure 3. Factors affecting generation of acetylene: (A) 250 g/L FeS-mediated generation of acetylene from different CEs. (B) Microbial community compositions at the genus level in mixed culture SZ1 amended with gradient concentrations (0, 2.5, 25 g/L) of FeS. (C) VFA concentrations in 25 g/L FeS-amended SZ1 and abiotic control; FeS-mediated abiotic PCE dechlorination products in medium amended with lactate or acetate. (E) Generation of FeS in *Desulfovibrio vulgaris* Hildenborough fed with lactate, sulfate, and $Fe^{3+}$and its abiotic control. Error bars indicate SDs of triplicate experiments.

be due to their impurity, and acetylene could be employed as an indicator of FeS-mediated abiotic dechlorination of CEs.

Reciprocal Influence between Abiotic and Biotic Dechlorination of CEs. In natural soil environments, both abiotic and biotic dechlorination processes may synergistically determine the fate of organohalides.[40−43] To test the reciprocal influence between the FeS-based abiotic dechlorination and OHRB-mediated biotic dechlorination activities, batch experiments were set up with two pure cultures (i.e., *Dehalococcoides mccartyi* CG1 and *G. lovleyi* LYY) and a PCE/PCB-dechlorinating mixed culture SZ1 containing two dechlorinating populations, i.e., *Dehalococcoides* (Dhc) and *Geobacter* (Geo).[19] Results showed different tolerances of the *D. mccartyi* CG1 (CG1) and *G. lovleyi* LYY (LYY) in their cultures amended with 2.5 g/L FeS, i.e., 11.0 and 96.2% PCE dechlorination in FeS-amended CG1 and LYY, respectively, in contrast to 100.0 and 99.3% in their control cultures without FeS addition (Figure 2A). Notably, amendment of 25 g/L FeS largely inhibited PCE-to-DCE dechlorination in both CG1 (11.13% PCE dechlorination) and LYY (16.82% PCE dechlorination). In the 25 g/L FeS-amended CG1 culture, 9.2% PCE dechlorination could be contributed by FeS-mediated abiotic dechlorination based on the acetylene generation (Figure 2A,C). In contrast, microcosm SZ1 had the highest tolerance to the inhibitive effect of FeS amendment, e.g., 99.1% PCE dechlorination in cultures amended with 25 g/L FeS. Nonetheless, the amendment of 25 g/L FeS obviously prolonged the lag phase of microcosm SZ1 and changed the predominant dechlorination product from vinyl chloride (VC) to *cis*-DCE. Furthermore, 0.4 and 5.4% of PCE dechlorination in the 2.5 and 25 g/L FeS-amended experiments, respectively, could be attributed to FeS-mediated abiotic dechlorination based on acetylene generation.

To monitor the impact of FeS on OHRB's cell growth, qPCR was employed to test the temporal changes in cell concentrations of OHRB in the pure culture LYY and microcosm SZ1. No data were derived from the pure culture CG1 amended with 2.5 or 25 g/L FeS due to repeated failed DNA extraction. Results showed that strain LYY grew in 2.5 g/L FeS-amended cultures but perished in 25 g/L FeS-amended cultures (Figure 2B), being in line with changes in their PCE dechlorination activities. In contrast, the two dechlorinating populations (i.e., Dhc and Geo) in microcosm SZ1 had different growth patterns in cultures amended with 2.5 and 25 g/L FeS. For the *Dehalococcoides* being capable of dechlorinating CEs to VC and ethene, 25 g/L FeS completely inhibited its cell growth (Figure 2B), corroborating the observation of no generation of VC and ethene. It is noteworthy that microcosm SZ1 exhibited enhanced tolerance to FeS, allowing the growth of both *Geobacter* and *Dehalococcoides* in cultures amended with 25 and 2.5 g/L of FeS, respectively. The improved tolerance could be attributed to the formation of flocs or biofilm, which prevents direct exposure of *Geobacter* and *Dehalococcoides* to FeS. Interestingly, percentages of acetylene in both PCE-to-DCE dechlorinating pure cultures and microcosms were higher relative to their abiotic controls (Figure 2C). By contrast, lower percentages of acetylene were observed in the PCE-to-VC cultures compared to their abiotic controls. The possible mechanism underlying the changes in acetylene's percentages might be the higher efficiency of acetylene's generation from DCEs compared to PCE, TCE, and VC.

Mechanisms Underlying the Reciprocal Influence between Abiotic and Biotic Dechlorination. To gain mechanistic insight into changes in acetylene generation in the FeS-amended cultures, three sets of experiments were

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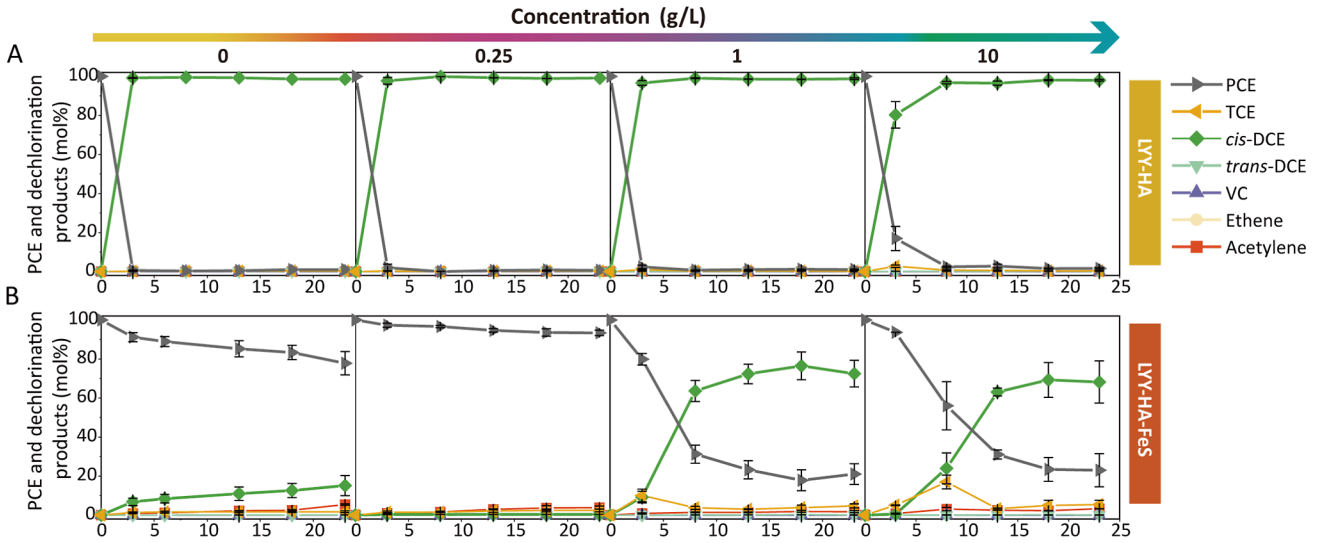

Figure 4. Impact of HA on biotic/abiotic-combined dechlorination: (A) impact of gradient concentrations (i.e., 0, 0.25, 1 and 10 g/L) of HA on biotic dechlorination of PCE in pure culture LYY. (B) Impact of gradient concentrations of HA on biotic/abiotic-combined dechlorination of PCE in pure culture LYY amended with 25 g/L FeS. Error bars indicate SDs of triplicate experiments.

established under different conditions: (1) FeS-mediated abiotic dechlorination of different CEs, i.e., PCE, TCE, *cis*-DCE, and *trans*-DCE; (2) impact of acetogens on FeS-mediated abiotic dechlorination; (3) impact of humic substances on abiotic/biotic-combined dechlorination. In the first experimental set, the higher ratio of acetylene was observed to be generated from DCEs, relative to abiotic dechlorination of PCE and TCE, with an order of *trans*-DCE > *cis*-DCE > TCE > PCE (Figure 3A). Therefore, OHRB-mediated PCE-to-DCE dechlorination could facilitate FeS-mediated generation of acetylene by providing DCEs and/or TCE. In the second experimental set, 16S rRNA gene amplicon sequencing was employed to test community composition changes in mixed culture SZ1 amended with gradient concentrations (i.e., 0, 2.5, and 25 g/L) of FeS (Figure 3B). Based on qPCR quantification, the cell number of *Dehalococcoides* in SZ1 cultures amended with 2.5 and 25 g/L FeS decreased from 9.8×10^7 copies/mL (biotic control) to 9.76×10^6 copies/mL and 7.03×10^4 copies/mL, respectively. Similarly, abundance of *Geobacter*, a facultative OHRB, decreased from 1.38×10^7 copies/mL (biotic control) to 3.88×10^5 copies/mL (2.5 g/L FeS) and 4.4×10^5 copies/mL (25 g/L FeS; Figure 3B). Remarkably, the relative abundance of fermenting populations (*Clostridium*, *Sedimentibacter*, and *Desulfovibro*) in mixed culture SZ1 amended with 25 g/L FeS was 2.3 times higher than that in the biotic controls without FeS amendment (Figure 3B). Accordingly, higher concentrations of volatile fatty acids (VFAs) were detected in the 25 g/L FeS-amended cultures (Figure 3C). To further confirm the impact of VFAs on FeS-mediated abiotic dechlorination, FeS-mediated abiotic dechlorination experiments were set up with lactate/acetate to analyze the PCE-to-acetylene generation (Figure 3D). Surprisingly, a much higher percentage of acetylene was observed in bottles amended with acetate (51.0%) relative to that in bottles amended with lactate (19.7%) (Figure 3D). Therefore, acetogenic populations could promote FeS-mediated abiotic dechlorination by providing acetate. Moreover, the enrichment of *Desulfovibro*, an SRB capable of reducing SO_4^{2-} and Fe^{3+}, in culture SZ1 might facilitate abiotic PCE-to-acetylene dechlorination via regeneration of FeS. This phenomenon has been confirmed in batch experiments with *Desulfovibrio vulgaris* Hildenborough (DvH) fed with lactate, sulfate, and Fe^{3+} (Figure 3E).

In natural soil environments, humic substances could coexist with OHRB and FeS and consequently affect the abiotic- and biotic-dechlorination.[4,44] As illustrated in Figure 2, LYY exhibited dechlorination activities in experimental sets amended with 2.5 or 25 g/L FeS, indicating its tolerance to the side impact of FeS. To explore the specific impact of humic substances, three experimental sets were set up with the pure culture of strain LYY and gradient concentrations (0−10 g/L) of HA: (1) LYY-HA, culture LYY amended with HA; (2) LYY-HA-FeS, culture LYY amended with 25 g/L FeS and gradient concentrations of HA; (3) HA and HA-FeS were abiotic controls without inoculation of LYY. In the LYY-HA, HA was shown to have negligible impact on the LYY-mediated PCE-to-DCE dechlorination (Figure 4A), in which HA-mediated abiotic dechlorination was minimal, e.g., only 1.2% PCE dechlorination in abiotic controls amended with 10 g/L HA (Fig. S2). In contrast, HA could significantly ($p = 0.003$) mitigate the inhibitive impact of FeS on LYY-mediated biotic dechlorination (Figure 4B). For example, PCE dechlorination efficiency increased from 22.2 to 79.0 and 77.0% in LYY-HA-FeS cultures amended with 0, 1, and 10 g/L HA, respectively. The promotion of PCE dechlorination in the LYY-HA-FeS amended with HA could be due to HA's protection of LYY from direct contact with FeS, and the impact of HA on FeS-mediated abiotic dechlorination could be negligible (Fig. S2).

■ DISCUSSION

In natural soil, CEs at contaminated sites could be attenuated through both abiotic and biotic processes, which could be synergistically determined by the soil organic/inorganic- and microbial-compositions, including humic substances, minerals, and OHRB.[16,45−52] Previous studies have extensively investigated the attenuation of CEs by a specific humic substance or an individual mineral[53−59] as well as the impact of organic/inorganic matter on microbial reductive dechlorination or organohalide respiration of OHRB.[41,60−63] Nonetheless, in the complex soil environments, detailed information on the major

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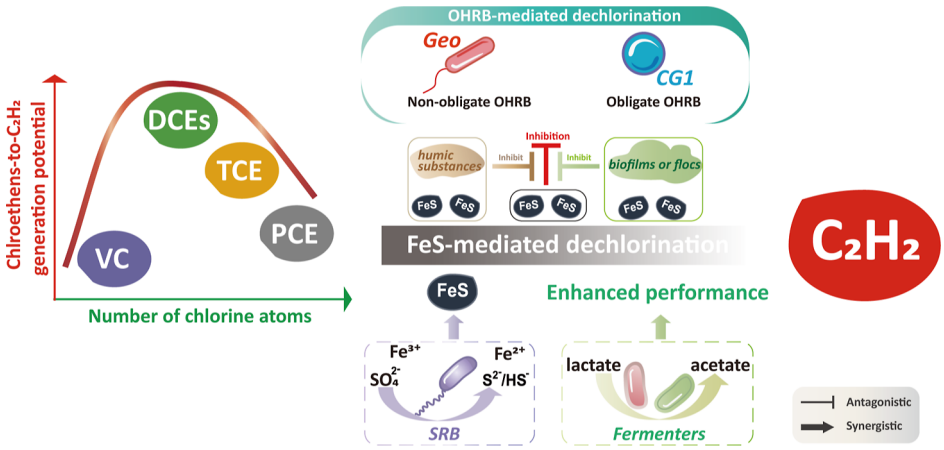

Figure 5. Scenario describing abiotic/biotic-combined dechlorination of CEs in soil.

bio/abio-players and their contribution, as well as their complicated reciprocal-influence and interactions, remains unknown. In this study, multiple lines of experimental evidence helped to identify the FeS as a major catalyzing matter in abiotic dechlorination of CEs in soil. Other organic matter, minerals, and microorganisms formed complicated interactions and reciprocal impacts with the OHRB and FeS, which collectively determined the fate of CEs in soil. Based on the experimental evidence, a scenario was proposed in this study to describe the abiotic/biotic-combined dechlorination of CEs in soil (Figure 5). The FeS-mediated abiotic and OHRB-mediated biotic dechlorination of CEs could affect and be affected by other soil environmental processes (e.g., sulfate reduction and acidogenic fermentation) and matter (humic substances and biofilm/flocs): (1) FeS could mediate chlorine removal from CEs with acetylene as a unique dechlorination product, and acetylene ratio generated from the abiotic dechlorination depended on the specific CEs with an order of *trans*-DCE > *cis*-DCE > TCE > PCE; (2) OHRB-mediated dechlorination could be inhibited by FeS, but at the same time, OHRB affected FeS-mediated abiotic dechlorination by providing different dechlorination products; (3) SRB might enhance FeS-mediated abiotic dechlorination by regeneration of FeS; (4) acidogenic fermenters could affect CEs-to-acetylene dechlorination by providing varied VFAs; (5) organic matter and biofilm/flocs might alleviate FeS-derived inhibition on OHRB by preventing direct contact between FeS and OHRB's cells. Therefore, the overall abiotic/biotic-combined dechlorination of CEs in soil could be a holistic net result from the above-mentioned processes and matter.

Pertaining to the FeS-mediated abiotic dechlorination of PCE, contradicting experimental observations were reported in previous studies. Butler and Hayes first reported that synthetic FeS powder (with a surface area of 0.05 m^2/g) dechlorinated PCE to acetylene as a major product, and TCE and *cis*-DCE as minor products, without generation of ethene.[38] In contrast, both acetylene and ethene were detected as major dechlorination products from FeS-mediated abiotic PCE dechlorination in other studies.[39,55,64] Our study, notably, clarified that acetylene should be the only complete dechlorination product of the FeS-mediated abiotic dechlorination of PCE. Also, ethene could be generated together with acetylene when PCE was dechlorinated by the unpurified FeS. In this study, unpurified FeS in both mackinawite and paddy soil could mediate PCE dechlorination with acetylene and ethene as dechlorination products, being consistent with previous reports that abiotic dechlorination of CEs generated acetylene through β-elimination and ethene through hydrogenolysis as a competing process.[65] Nucleophiles (e.g., sulfide and polysulfide) were identified to be capable of promoting the β-elimination reactions.[66] For example, combining experimental evidence and density functional theory calculations, Xu and colleagues discovered that sulfur addition to nanoscale zero-valent iron (nZVI) could lower electron transfer resistance and consequently enhance TCE removal through the β-elimination process to generate acetylene.[67] Though further hydrogenation reaction from acetylene to ethene was proposed based on mathematical simulation models, solid evidence at theoretical and experimental levels remains scarce.[68] Nonetheless, the dissociation and chemoselective transfer of H to acetylene were identified to be readily achieved at Fe(III)-O sites,[69] being consistent with our observation of ethene generation from unpurified FeS. The mechanism underlying the presence of ethene in FeS-mediated abiotic dechlorination of PCE was further corroborated by previous experimental studies with magnetite and synthesized green rust (Fe(II)/(III) hydroxides).[70,71]

Interestingly, this study found that OHRB could promote FeS-mediated PCE-to-acetylene dechlorination by providing less-chlorinated ethenes (e.g., TCE and DCEs). Though there could be competition between β-H and β-Cl elimination in FeS-mediated dechlorination of CEs, the low pK_aH (the aqueous pK_a of the conjugate acid) of the chlorines was recently identified to favor the β-Cl elimination.[72] Therefore, β-Cl elimination was preferentially enabled in the abiotic dechlorination of TCE and DCEs. DCEs with two chlorines were converted to acetylene through dichlorine elimination. In contrast, PCE and TCE were converted to chloroacetylene and then to acetylene via hydrogenolysis. These properties rationalized the varied CEs-to-acetylene potentials observed in this study. Moreover, for the stereoelectronic selectivity, *trans*-elimination of the leaving group could be favored over *cis*-elimination, and the best geometry for the E2 transition state should be anti-coplanar.[73] Thus, the acetylene generation potential from the dechlorination of CEs followed the order of *trans*-DCE > *cis*-DCE > TCE > PCE, being largely consistent with the order observed in Fe(0)-mediated dechlorination, i.e., the order of β-elimination percentage of *trans*-DCE (99%) > TCE (97%) > *cis*-DCE (94%) > PCE (87%).[74]

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The inhibitive effect of FeS on OHRB was observed in this study, particularly the significant inhibition of isolated *Dehalococcoides* by 2.5 g/L FeS being comparable to its concentrations in soil.[21,75] Previous studies had shown that soil clays being rich in ferrous minerals (e.g., pyrite and pyrrhotite, $Fe_{1-x}S$, x = 0−0.2) exhibited bacteriostatic properties under reducing conditions.[76,77] The soluble Fe^{2+}-derived hydroxyl radical ($^{\bullet}OH$) could attack cell membrane and intracellular proteins.[77] Moreover, acetylene could also inhibit organohalide respiration of OHRB by downregulating genes encoding ATP synthase, biosynthesis, and Hym hydrogenase.[78,79] Nonetheless, these FeS-derived inhibitive effects on OHRB could be alleviated by humic substances and biofilm formation, which could be employed to improve OHRB-mediated bioremediation of CE-contaminated sites.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.est.3c04262.

Material and methods for DvH cultivation; qPCR and 16S rRNA gene amplicon sequencing analysis; acetylene detection method; FeS-mediated PCE dechlorination at varying concentrations and in diverse mediums (PDF)

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Notes

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