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Anaerobic Transformation of DDT Related to Iron(III) Reduction and Microbial Community Structure in Paddy Soils

Manjia Chen,[#] Fang Cao,[#] Fangbai Li,* Chengshuai Liu,* Hui Tong, Weijian Wu, and Min Hu

Guangdong Key Laboratory of Agricultural Environment Pollution Integrated Control, Guangdong Institute of Eco-Environmental and Soil Sciences, Guangzhou 510650, P.R. China

Supporting Information

ABSTRACT: We studied the mechanisms of microbial transformation in functional bacteria on 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane (DDT) in two different field soils, Haiyan (HY) and Chenghai (CH). The results showed that microbial activities had a steady dechlorination effect on DDT and its metabolites (DDx). Adding lactate or glucose as carbon sources increased the amount of *Desulfuromonas*, *Sedimentibacter*, and *Clostridium* bacteria, which led to an increase in adsorbed Fe(II) and resulted in increased DDT transformation rates. The electron shuttle of anthraquinone-2,6-disulfonic disodium salt resulted in an increase in the negative potential of soil by mediating the electron transfer from the bacteria to the DDT. Moreover, the DDT-degrading bacteria in the CH soil were more abundant than those in the HY soil, which led to higher DDT transformation rates in the CH soil. The most stable compound of DDx was 1,1-dichloro-2,2-bis(*p*-chloro-phenyl)ethane, which also was the major dechlorination metabolite of DDT, and 1-chloro-2,2-bis-(*p*-chlorophenyl)ethane and 4,4′-dichlorobenzo-phenone were found to be the terminal metabolites in the anaerobic soils.

KEYWORDS: *DDT, reductive dechlorination, cyclic voltammograms, microbial community, lactate, anthraquinone-2,6-disulfonate*

INTRODUCTION

1,1,1-Trichloro-2,2-bis(*p*-chlorophenyl)ethane (DDT) is a persistent organic pollutant that has been widely used for controlling arthropod disease vectors and agricultural pests.[1] Although its use has long been prohibited because of its high toxicity, DDT continues to be detected in the soil, water, and air in many countries.[2] Although the use of DDT as a pesticide has been legally banned in China since 1983,[3] it continues to be produced as a raw material and processed for dicofol production.[4] 1,1-Dichloro-2,2-bis(*p*-chlorophenyl)ethylene (DDE) and 1,1-dichloro-2,2-bis(*p*-chloro-phenyl)ethane (DDD) are the two major metabolites of DDT that occur naturally in contaminated soils together with their parent compound.[5] Exposure to DDT, DDD, and DDE (collectively designated hereafter as DDx) may cause health problems, including liver, kidney, blood, and nervous system damage.[6,7] Moreover, DDx are confirmed human carcinogen compounds.[8,9] Therefore, it is crucial to understand the transformation mechanisms through which they may be metabolized in the environment.[10]

The removal of DDx from contaminated soils has become an environmental priority because soil is the final destination of these pollutants. DDx can also slowly dissipate in soils by abiotic processes. In soil, particularly in iron-rich soil, ferrous iron or Fe(II) plays an important role in the reductive transformation of chlorinated compounds.[11,12] With the participation of soil microorganisms, chlorinated compounds may undergo faster transformation because of both the reductive ability of the microorganisms and the biogenic Fe(II) formed by these microorganisms.[13] For example, iron-reducing bacteria in soils can reduce iron minerals into biogenic Fe(II) and thereby enhance the dechlorination rate of DDx under anoxic conditions.[14]

Soil microorganisms such as bacteria and fungi can degrade DDx directly in both pure culture media and in natural soils. For example, strains of *Alcaligenes eutrophus* A5,[15] *Serratia marcescens* DT-1P,[16] *Pseudomonas fluorescens*,[17] and fungi[4,18] have been confirmed as active for DDT transformation with DDD as the transformation product. Kamanavalli and Ninnekar[19] isolated a strain of *Pseudomonas* sp. from DDx-contaminated soil, which exerted an efficient degradation effect on DDT through a 4-chlorobenzoic acid as one of the metabolites. Consequently, microorganisms with the potential to degrade these compounds could be present in soil.[20] Therefore, bioremediation that emphasizes the activities of bacteria and biogenetic Fe(II) has been proposed as an optimal method to remove chlorinated pollutants in contaminated soils.[9,21]

The biogeochemical cycles of major and trace elements usually have important effects on the formation of different Fe(II) species.[22] Humic substances (HS) are among the major organic components in anoxic soil, are redox-active, and can be utilized as nutrients by microorganisms. Furthermore, HS are reported to accelerate the microbial reduction of Fe(III) minerals by acting as electron shuttles between cell and Fe(III) minerals,[23] which results in increased generation of biogenic Fe(II). For example, anthraquinone-2,6-disulfonic disodium salt (AQDS), an analogue of HS compounds, can transfer electrons to iron minerals in the degradation of chlorinated compounds as terminal electron acceptors.[24,25] However, only the latter Fe(II)-generating phenomenon and amount were reported in

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these previous studies; any connection to the transformation of target pollutants has rarely been studied. Furthermore, the key role for pollutant transformation lies in the microorganisms that utilize DDT as a terminal electron acceptor; this role is relevant to the overall environmental degradation rate. Therefore, the mechanism by which microbial communities change during electron transport under anaerobic conditions in iron-rich soils must be further investigated.

This research systematically studied the generation of adsorbed Fe(II) and the change of microbial communities during transformation of DDT in iron-rich paddy soils and aimed to learn about the mechanism behind the microbial transformation of DDT in soils. Experiments were conducted to perform the following tasks: (i) to investigate the kinetics of DDT biodegradation by microorganisms in paddy soils from South China; (ii) to study biogenic Fe(II) generation during transformation of DDT; (iii) to examine the effects of exogenous carbon sources (glucose or lactate) and an electron mediator (AQDS) on the microorganisms that transform DDT; and (iv) to determine the microbial community structure during transformation under anaerobic conditions in soil microcosm settings.

MATERIALS AND METHODS

Soil Samples. The two paddy soils used in the present study were collected from the surface horizon (0−10 cm) of paddy fields in Guangdong Province, China. One sampling site was located at Haiyan Town, Taishan City (HY), and the other was located at Chenghai Town, Shantou City (CH). The basic physicochemical properties of the soils were analyzed, and the results are listed in Table 1. Using standard analytical methods, no DDT was detected in the sampled soils. The soil samples were air-dried and were then passed through a 2 mm sieve. The sieved soils were stored at 4 °C before analyses and experiments. Soils for controlled experiments were sterilized by irradiation with γ rays before use with a ^{60}Co source and a dose of 50 kGy.

Table 1. Selected Properties for the Two Soils Used

soil	pH_{KCl}	TN[a] (%)	TP[b] (%)	K_2O (%)	OC[c] (%)	Fe_2O_3 (g/kg)
HY	6.64	0.11	0.07	2.36	2.11	60.64
CH	6.09	0.15	0.04	2.28	2.64	26.57

[a]TN: total nitrogen. [b]TP: total phosphorus. [c]OC: organic carbon.

Chemicals. DDE (99%) and DDD (99.3%) were purchased from Supelco (USA). DDT (100%), DDMU [1-chloro-2,2-bis-(*p*-chlorophenyl)ethane, 100%], DBP (4,4′-dichlorobenzo-phenone, 100%), decachlorobiphenyl (1,1′-biphenyl,2,2′,3,3′,4,4′,5,5′,6,6′-deca-chloro, PCB209, 100%), and 4,4′-dichlorobiphenyl (4,4′-dichloro-1,1′-biphenyl, PCB15, 100%) were purchased from Accustandard (USA). High-performance liquid chromatography-grade acetone and *n*-hexane (Acros Organics, USA) were used without further purification. AQDS (97+% purity) and PIPES (1,4-piperazinediethanesulfonic acid, 98+% purity) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Other chemicals of an analytical grade were obtained from Guangzhou Chemical Co., China. All solutions were prepared in deoxygenated ultrapure water (18 MΩ cm, Easy Pure'II RF/UV, USA). Anhydrous sodium sulfate was purified by drying at 450 °C for 4.5 h.

DDT Transformation in Paddy Soils by Batch Experiments. The batch experiments were conducted in 100 mL serum bottles with Teflon-coated butyl rubber stoppers and crimp seals. Prior to use, all materials, including serum bottles, butyl rubber stoppers, pipet tips, and solutions were sterilized in an autoclave at 121 °C for 20 min. The reactors contained 2.0 g of soil in dry weight and 20 mL of PIPES buffer (50 mM) solution to keep the pH value at 7.0. The following six batch experiments were conducted in this study: (1) sterile soil (SC), (2) soil (CK), (3) soil + glucose (SG), (4) soil + glucose + AQDS (SGA), (5) soil + lactate (SL), and (6) soil + lactate + AQDS (SLA). Glucose, lactate, and AQDS were added to achieve concentrations of 10 mM, 10 mM, and 100 μM, respectively. Subsequently, 50 μL of acetone-based stock solution of DDT, DDD, or DDE were added to each serum bottle, and all obtained initial concentrations of 5 mg/L. This concentration was normalized to 50 ng/kg of soil when mixed with 2.0 g of soil in the study, which is in the range of residue concentrations of DDT in paddy soils in China.[26] After being purged with O_2-free N_2 gas (99.99%) for 30 min, the mixtures in the reactors were sealed with Teflon-coated butyl rubber stoppers and crimp seals. All reactors were prepared in triplicate and incubated in a BACTRON Anaerobic/Environmental Chamber II (SHELLAB, Sheldon Manufacturing Inc.) at 30 °C in the dark. At specific reaction time intervals, the reactor bottles were taken out for analyses. Initially, the sampling intervals were designated at 7, 14, and 28 days. In addition, supplementary batch experiments with additional sampling intervals designated were conducted to study the transformation of DDx with more accurate processes.

Analytical Procedures. To measure dissolved Fe(II), the culture samples were centrifuged at 1000*g*; following this, the supernatant was filtered through a 0.22 μm syringe filter, and the filtrate was measured using the 1,10-phenanthroline colorimetric method at 510 nm on a UV−vis spectrophotometer (TU-1810PC, Beijing Purkinje General Instruments, China).[27] The HCl-extractable Fe(II) in the reaction suspension was extracted using 0.5 M HCl for 1.5 h; after the suspension was centrifuged, the supernatant was decanted for Fe(II) analysis using the same colorimetric method. The difference between the total and dissolved Fe(II) was defined as adsorbed Fe(II).[28]

Cyclic voltammetry (CV), a method used to test the redox potential of the Fe(II)/Fe(III) couple in the reactor, was conducted in a conventional three-electrode electrochemical cell using a CHI 660C potentiostat. A glass carbon electrode was used as the working electrode, with a saturated calomel electrode (SCE) and Pt wire as the reference and counter electrode, respectively.[14] The concentration of DDT and its metabolites in the soil suspension were determined using ultrasonic extraction.[24] The incubated samples were extracted three times with 20 mL of *n*-hexane and were then dried through anhydrous sodium sulfate, collected in a 100 mL flat-bottom flask, and subsequently concentrated to approximately 1 mL on a vacuum rotary evaporator (almost to dryness). More *n*-hexane was added to redissolve the DDT and its metabolites and to augment the volume to 1 mL prior to determination. All samples were analyzed with a ThermoFisher trace gas chromatograph equipped with a ThermoFisher DSQ mass selective detector and Trace TR-5MS silica-fused capillary column (ThermoFisher Scientific, USA, 30 m × 0.25 mm × 0.25 μm).[24] External standards of DDT, DDD, and DDE were prepared in hexane and in a standard curve-fit linear line for determination. The reported concentrations are the average of triplicate measurements. The triplicate concentrations are consistent with the relative percentage differences, which are typically less than 15%.

DNA Extraction, Polymerase Chain Reaction (PCR) Amplification, Terminal Restriction Fragment Length Polymorphism (T-RFLP) Analysis, and Clone Libraries. DNA from soil enrichment culture samples was collected by centrifugation (7378 g/min for 10 min at 4 °C) and then extracted using the PowerSoil DNA kit (MO BIO Laboratories, USA) by following the manufacturer's directions. DNA was eluted with 100 μL of sterile, nucleotide-free water. DNA concentrations were determined with the Qubit 2.0 Fluorometer (Invitrogen, USA). ExTaq DNA polymerase (TaKaRa BIO INC.), a primer pair 27F (5′-AGA GTT TGA TCM TGG CTC AG-3′), and 1492R (5′-AGA GTT TGA TCM TGG CTC AG-3′) were used for amplification of the 16S rRNA gene fragments. The 27F primer was labeled at the 5′ end with 6-carboxy-fluorescein phosphoramidite. For T-RFLP, PCR was performed in triplicate, with the products purified using the E.Z.N.A. Gel Extraction kit (OMEGA bio-Tek, USA). Aliquots of the purified PCR products were digested with the restriction enzymes *MspI* (TaKaRa BIO INC.) at 37 °C for 3 h. Terminal restriction fragments (T-RFs) were separated on a 3730xl

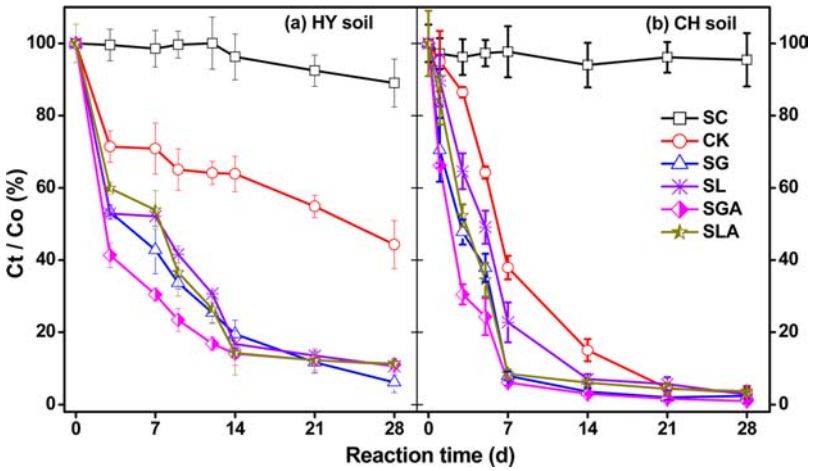

Figure 1. Kinetics of DDT transformation under different treatments in (a) HY and (b) CH soils. (SC) Sterile soil + PIPES, (CK) soil + PIPES, (SG) soil + PIPES + glucose, (SL) soil + PIPES + lactate, (SGA): soil + PIPES + glucose + AQDS, and (SLA) soil + PIPES + lactate + AQDS.

Table 2. Treatment Methods, First-Order Rate Constants (k), and Half-Lives ($t_{1/2}$) of DDT Transformation in HY and CH Soils[a]

		DDT transformation kinetics parameter					
		HY soil			CH soil		
treatments	treatment methods	k (day^{-1})	R^2	$t_{1/2}$ (day)	k (day^{-1})	R^2	$t_{1/2}$ (day)
SC	sterile soil + PIPES						
CK	soil + PIPES	0.032 ± 0.003	0.941	21.66	0.120 ± 0.012	0.939	5.78
SG	soil + PIPES + Glucose	0.106 ± 0.004	0.99	6.54	0.163 ± 0.015	0.942	4.25
SL	soil + PIPES + Lactate	0.093 ± 0.006	0.967	7.45	0.132 ± 0.010	0.961	5.25
SGA	soil + PIPES + glucose + AQDS	0.105 ± 0.012	0.91	6.60	0.188 ± 0.012	0.974	3.69
SLA	soil + PIPES + lactate + AQDS	0.096 ± 0.008	0.955	7.22	0.142 ± 0.013	0.938	4.88

[a]The initial DDT concentration was 14 mM, the PIPE buffer concentration was 50 mM, the glucose and lactate concentrations were both 10 mM, and the AQDS concentration was 0.1 mM. (SC) Sterile soil + PIPES, (CK) soil + PIPES, (SG) soil + PIPES + glucose, (SL) soil + PIPES + lactate, (SGA) soil + PIPES + glucose + AQDS, (SLA) soil + PIPES + lactate + AQDS.

Genetic analyzer (Applied Biosystems, USA). The data were analyzed using the Peak Scanner software V1.0 (Applied Biosystems, USA). Peaks with T-RFs comprised between 50 and 550 bp. The relative abundance of individual T-RFs was calculated as the percentage of the total peak area in a given T-RFLP profile. Only T-RFs with a relative abundance >1% were included in the analysis.

For cloning and sequencing purposes, the 16S rRNA was amplified in triplicate as described above but with an unlabeled primer. Purified PCR products were cloned into vector pCR 2.1-TOPO (Invitrogen, USA) and then transformed into *Escherichia coli* DH5α cells by following the manufacturer's instructions. Selected clones were grown in 0.8 mL of a Luria–Bertani medium amended with 50 μg/mL of ampicillin. Following harvesting, plasmid DNA was purified from cell pellets using a 96-well alkaline lysis procedure.[29] The DNA was sequenced using an ABI 3730xl sequencer.

Sequence comparisons were performed using the stand-alone version of BLAST against the Silva SSU data sets with a threshold of e-value <10^{-20}. Subsequently, the taxonomy of 16S rRNA sequences was determined by parsing with the results of BLAST. The sequences were deposited to GenBank with the accession numbers JX473589–JX473653.

Statistical Analyses. A statistical analysis of the experimental data was performed using the SPSS statistical software. Differences were determined by one-way analyses of variance (ANOVA) on ranks followed by Fisher's least-significant-difference test (LSD). MspI T-RF profiles of 16S rDNA amplified directly from the soil samples of different batches were used to construct the principal component analysis (PCA).[30] First, relative percentage abundance (Ap) of each T-RF was calculated as follows: $Ap = ni/N \times 100$, where ni represents the peak area of one distinct T-RF and N is the sum of all peak areas in a given T-RFLP pattern. Following this, the T-RFs from all the treatments were applied to the principal component analysis. The calculated Ap by following the T-RFs' sizes ranging from 127 to 531 bp of each T-RF was expressed two-dimensionally on the plot.

RESULTS

Anaerobic Transformation of DDT in the Paddy Soils. The results of the kinetic study of DDT transformation in the HY and CH soils under different treatments are shown in Figure 1, panels a and b, respectively. In this study, the DDT degradation data fit the pseudo-first-order kinetics, and the values of the degradation rate constants (k) and the relative coefficient (R^2) within the reaction time of 28 days are listed in Table 2. The transformation ratios of DDT in both sterilized soils (the SC treatment) were similarly small, with values of approximately 11% at the 28 day reaction time. The transformation ratios were too low to fall into the relative standard deviations within triplicate sets of analyzed samples (up to a maximum of 15%); this may be the result of the uneven distribution of contaminants in the soil. Furthermore, no DDT metabolites were detected in the treatment with the sterilized soil for either soil. These results suggested that abiotic processes have no significant contribution to the removal of DDT in the paddy soils. By contrast, DDT transformation ratios of 56% and 97% were obtained in the experiments with the nonsterile HY and CH soils (the CK treatment) with constants of 0.032 and 0.12 day^{-1} and half-lives of 21.7 and

5.78 days, respectively. This finding indicated that the soil microorganisms might be highly active in DDT transformation, particularly in the CH soil.[21] Additionally, the presence of carbon sources such as glucose and lactate further enhanced DDT transformation. As shown in Figure 1, the constant k values for DDT transformation ratios in the SG and SL treatments increased to 0.105 and 0.093 day^{-1} for the HY soil and to 0.163 and 0.132 day^{-1} for the CH soil, respectively. A significant difference was observed in the average degradation rate k values between CK and SG or SL (p = 0.041 and p = 0.019, respectively) (Figure 2). DDT is rarely used as a carbon

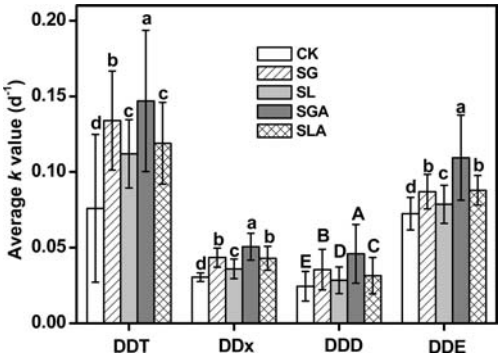

Figure 2. Results of the variance analysis and the trend of the average k values of DDT, DDx, DDD, and DDE transformations classified under different treatments. The average value within the same treatment that has different lowercase letters (i.e., a or b) and capital letters (i.e., A or B) are significantly different at $p < 0.05$ and $p < 0.01$, whereas the average value within the same treatment that has identical lowercase letters is not significantly different at p = 0.05. The definitions of the acronyms used are those in Figure 1.

source for microbial metabolism and is typically biodegraded cometabolically.[31] Therefore, the addition of alternative carbon sources such as glucose and lactate as nutrients would stimulate the growth of bacteria that are performing the DDT dechlorination and therefore accelerate the transformation process. Moreover, approximately 99% and 96.3% of DDT were dechlorinated after a 28-day incubation in the CH soil and in the SGA and SLA treatments, respectively. The k values for DDT transformation in the SGA and SLA treatments increased to 0.188 and 0.142 day^{-1} in the CH soil, respectively, with half-lives of 3.69 and 4.88 day, respectively. Similar results were found in the HY soil. This finding suggests that AQDS had a positive effect on the transformation rate of DDT and may be utilized as a carbon source for microorganisms in the CH soil.[32] In both soils, the values of k for the different treatments were ranked as follows: SGA > SG > SLA > SL > CK.

Formation of Metabolites during DDT Transformation. Concomitant with DDT transformation, its metabolite, DDD, was produced in all treatments except for those in the sterilized soil. The concentration variations of produced DDD are presented in Figure 3, panels a and b for the HY and CH soils, respectively. DDD is the primary transformation product of DDT that that has undergone reductive dechlorination in both the HY and CH soil systems under anoxic conditions. The overall reductive abilities of the reaction systems for the removal of DDx (including DDT, DDD, and DDE in the reaction systems) were also evaluated during the DDT transformation processes. The total concentrations of DDx in both the HY and CH soils were measured (Figure 3, panels c and d, respectively), and the corresponding pseudo-first-order

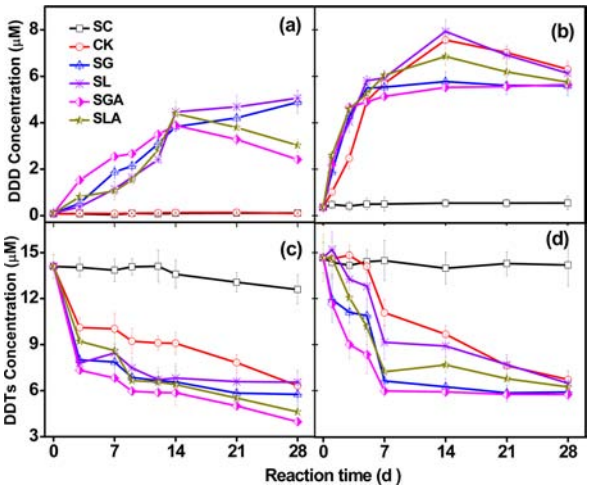

Figure 3. DDD formation in (a) HY and (b) CH soils during DDT transformation as in Figure 1 and total DDx (the sum of DDT, DDD, and DDE) transformation in (c) HY and (d) CH soils during DDT transformation. The definitions of the acronyms used are those in Figure 1.

rate constants (k) and half-lives were calculated (Table 3). The overall removal rate of DDx was 55% with a k value of 0.032 day^{-1} and a half-life of 21.8 days with the CK treatment in the HY soil and a reaction time of 28 days. The presence of glucose and lactate slightly enhanced the removal rate of DDx, with k values of 0.044 and 0.040 day^{-1} and half-lives of 16.0 and 17.7, respectively. A significant difference was observed in the average k value of the degradation rates between CK and SG or SL ($p < 0.01$ and p = 0.011, respectively) (Figure 2). With the addition of AQDS, the half-lives of DDx further decreased to 12.5 and 14.3 days with glucose and lactate, respectively. As shown in Figure 2, a significant difference was also found in the average k value between the SG and SGA treatments. Similar results were found between SL and SLA treatments. These results indicated that the addition of AQDS might enhance DDx transformation in paddy soils. The removal of DDx in soils under different treatments followed a trend similar for DDT that was discussed above, i.e., SGA > SG > SLA > SL > CK (Figure 2).

The transformation pathway of DDT in anaerobic soil systems was studied over a reaction period of 1 year. All the possible transformation metabolites of DDT (DDx) under the SL treatment in the HY soil after reaction with anaerobic incubation for 1 year were investigated using gas chromatography/mass spectrometry (Figure S1). No DDT was detected after the longer reaction time of 1 year; all the DDT was presumed to have transformed. A dominant peak of DDD was detected, which suggested that DDD was the primary dechlorinating product of DDT. In addition to DDD, trace amounts of other DDx, such as DDMU and DBP, were detected in the chromatogram. DDMU and DBP are the terminal metabolites in anaerobic DDT biodegradation.[20] The detection of these two products suggested the complete biodegradation of a portion of the DDT. By contrast, dechlorination of only one chloride functional group occurred in most of the DDT.

Anaerobic Transformation of DDD and DDE in the Paddy Soils. The transformations of DDD and DDE, which were expected to be the main DDT transformation metabolites, in both the HY and CH soils under different treatments were

Table 3. First-Order Kinetics Constants (k) and Half-Lives ($t_{1/2}$) for Removal of Total DDx (the Sum of DDT, DDD, and DDE) in HY and CH Soils during DDT Transformation[a]

	total DDx transformation kinetics parameter					
	HY soil			CH soil		
treatments	k (day^{-1})	R^2	$t_{1/2}$ (days)	k (day^{-1})	R^2	$t_{1/2}$ (days)
CK	0.032 ± 0.003	0.932	21.8	0.029 ± 0.002	0.973	24.0
SG	0.044 ± 0.007	0.838	16.0	0.043 ± 0.007	0.842	16.4
SL	0.040 ± 0.007	0.797	17.7	0.032 ± 0.003	0.945	21.8
SGA	0.056 ± 0.007	0.877	12.5	0.046 ± 0.009	0.773	15.6
SLA	0.049 ± 0.005	0.918	14.3	0.037 ± 0.005	0.862	19.0

[a]Reaction conditions and treatment methods are those in Table 2.

Table 4. First-Order Kinetics Constants (k) and Half-Lives ($t_{1/2}$) of DDD and DDE Transformation in HY and CH Soils[a]

	transformation kinetics parameter					
	HY soil			CH soil		
treatments	k (day^{-1})	R^2	$t_{1/2}$ (days)	k (day^{-1})	R^2	$t_{1/2}$ (day)
			DDD			
CK	0.016 ± 0.003	0.914	43.3	0.033 ± 0.004	0.832	21.0
SG	0.024 ± 0.004	0.875	28.9	0.047 ± 0.006	0.88	14.8
SL	0.021 ± 0.003	0.913	33.0	0.036 ± 0.004	0.834	19.3
SGA	0.029 ± 0.005	0.793	23.9	0.063 ± 0.007	0.888	11.0
SLA	0.021 ± 0.004	0.803	33.0	0.042 ± 0.003	0.75	16.5
			DDE			
CK	0.064 ± 0.007	0.998	10.8	0.081 ± 0.005	0.997	8.6
SG	0.078 ± 0.006	0.992	8.89	0.096 ± 0.007	0.973	7.22
SL	0.069 ± 0.008	0.97	10.1	0.089 ± 0.005	0.997	7.79
SGA	0.085 ± 0.007	0.934	8.15	0.134 ± 0.011	0.993	5.17
SLA	0.082 ± 0.009	0.982	8.45	0.094 ± 0.007	0.98	7.37

[a]The initial concentrations of DDD and DDE were 15.6 mM, the PIPE buffer concentration was 50 mM, the glucose and lactate concentrations were both 10 mM, and the AQDS concentration was 0.1 mM. The treatment conditions are those in Table 2.

studied, and the corresponding pseudo-first-order rate constants (k) and half-lives were calculated (Table 4). Highly significant differences were observed in the average k values of DDD transformation among the CK, SG, SL, SGA, and SLA treatments (all $p < 0.01$) (Figure 2). Additional carbon sources (glucose or lactate) had a significant effect on the degradation of DDE. The transformation efficiencies of DDD and DDE under the different treatments both in the HY and CH soils followed a trend similar to the DDT discussed above, i.e., SGA > SG > SLA > SL > CK. Among the three DDT compounds, DDD experienced the lowest transformation rate. DDD was also the most stable in the soil systems after an incubation reaction period of 28 d. These findings are consistent with the results of previous reports.[14,24] Although the three aliphatic chlorines in molecular DDT may be reductively dechlorinated, DDD, the compound with one chlorine atom detached from DDT, is the major metabolite of DDT transformation in most investigations.[11,33] The long-term incubation experiment results (Figure S1, Supporting Information) also showed complete DDT transformation, with DDD as the main metabolite under the SL treatment in the HY soil reaction system.

Fe(II) Generation during DDT Transformation. The paddy soils used in this study are iron-rich soils collected from South China with a high content of iron oxides (Table 1). Adsorbed Fe(II) species on mineral surfaces are critical to accelerating the reductive process of organic pollutants;[11,34] thus, 0.5 M HCl-extractable Fe(II) has been shown to be effective in extracting produced Fe(II), including adsorbed and dissolved forms.[28,35] The concentrations of dissolved Fe(II) and 0.5 M HCl-extractable Fe(II) are shown in Figures S2 and S3, respectively. In general, the amount of dissolved Fe(II) was well below that of 0.5 M HCl-extractable Fe(II); the differences between these amounts indicate the amount of adsorbed Fe(II),[28] which are shown in Figure 4.

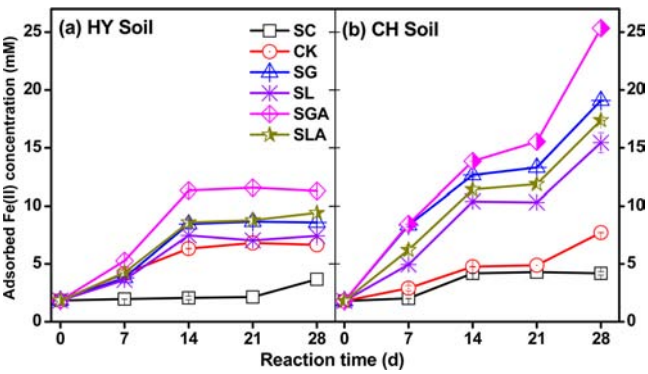

Figure 4. The generation of adsorbed Fe(II) during DDT transformation in (a) HY and (b) CH soils as in Figure 1. The definitions of the acronyms used are those in Figure 1.

The concentrations of adsorbed Fe(II) under different treatments were in accordance with DDT transformation efficiencies. The highest concentrations of adsorbed Fe(II) were generated in the SGA treatment with the presence of glucose and AQDS in both soils. In sterile soils, approximately 3.5 mM adsorbed Fe(II) was generated, which was substantially

Table 5. Dominant T-RF Lengths and Relative Abundance (%) of HY and CH Soils after 7 Days of DDT Incubation[a]

	T-RF relative abundance (%)											
	HY soil					CH soil						
T-RF length (bp)	CK	SG	SL	SGA	SLA	CK	SG	SL	SGA	SLA	predicted genus association	accession no.
127	11.32	1.97	14.74		32.97						*Desulfuromonas*	AF357914
143		14.19		15.43	1.53	20.40	5.70	2.46		3.69	*Sedimentibacter*	AF349757
156	1.69	20.73	7.10	24.99	18.44	18.03	20.08	80.38	44.88	80.85	*Sedimentibacter*	AY221992
163	38.84	5.22	2.60	1.90	17.31	10.54		6.73		5.38	*Sedimentibacter*	AY197417
217		11.87				4.10					*Oxobacter*	AJ229181
280		3.38			5.76	12.46	3.58				*Sedimentibacter*	AY221992
453				17.15							*Lachnospiraceae*	AB377176
485	17.29	3.14	70.26		14.28	5.96					*Lachnospiraceae*	AM501858
502	9.85	12.49		5.29		6.33	1.63				*Clostridium*	GQ356959
521		14.92		35.23	5.99	4.07	69.00	8.43	52.12	8.90	*Clostridium*	FM178806

[a]The reaction and treatment conditions are those in Table 2.

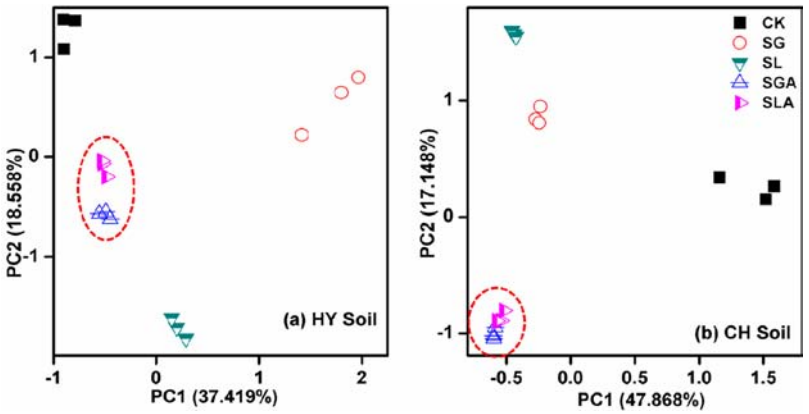

Figure 5. Principal component analysis of T-RFs from the T-RFLP analysis of the 16S rRNA gene from a microbial community representing the different treatments of DDT transformation in (a) HY and (b) CH soils after incubation for 7 days. The definitions of the acronyms used are those in Figure 1.

lower than that found in the other treatments. The content of iron oxide in the HY soil was higher than that of the CH soil. However, the concentrations of adsorbed Fe(II) in the CH soil systems were higher than those found in the HY soil systems in all treatments except CK. These findings are in accordance with the above results showing that DDT obtained higher transformation rates in the CH soil systems than in the HY soil systems under identical treatments. These results also indicated that the iron minerals in the CH soil may be more reducible or that there are microorganisms that are more active for iron reduction in the CH soil.

In the CK treatment with only nonsterile soils, no significant difference was observed in the concentration of adsorbed Fe(II) between the HY soil and the CH soil after an incubation period of 28 days (Figure S4). However, the k value for DDT transformation under the CK treatment in the CH soil was much higher than that in the HY soil. In addition to the content of adsorbed Fe(II) on mineral surfaces, other factors in the soils — such as indigenous microflora — with a dechlorination function acted as dechlorinating agents for DDT transformation in anaerobic soil systems.

Electrochemical Behavior of Adsorbed Fe(II) During DDT Transformation. The dechlorination of DDx in the soil is the result of an electron transfer from an electron donor to DDx in which the reductive potentials control the rates of DDT transformation. Soil reaction systems under different treatments were analyzed by CV scan (Figure S5) to determine the redox potentials of Fe(II)/Fe(III) couples in soils. No obvious redox peaks appeared in the voltammograms of the Fe species in the sterile HY soils (Figure S5a). A pair of redox peaks from 0.2 to 0.5 V was found in the HY soil under all the treatments, which was confirmed to be associated with the redox electrochemistry of manganese dioxides.[36] For the CK treatment in the HY soil, redox peaks at −0.4 V resulted from Fe species that appeared after incubation for 14 days (Figure S5b). When carbon sources were present in the system (SG and SL treatments), the anodic peak of Fe(II) and the cathodic peak of Fe(III) appeared after incubation for 7 days with the generation of adsorbed Fe(II) species. The anodic peak of Fe(II) in the CH soil exhibits a more negative shift compared to that in the HY soil, which suggests a higher reduction potential in the CH soil systems, which may cause higher DDT transformation rates. Further negative shifts of anodic peaks were obtained when additional AQDS was presented (SGA and SLA treatments). The CV obtained in reaction systems with AQDS exhibited another pair of redox peaks in the range of 0.18−0.25 V, which were considered to be the result of the electron donating ability of AQDS.[37]

T-RFLP of the 16S rRNA Gene. The 16S rRNA genes in the soil reaction systems were amplified in all treatment conditions after incubation for 7 days to investigate the reductive abilities of the bacterial community diversity in iron reduction and direct dechlorination of DDx in HY and CH soils. The diversity of the amplified sequences was analyzed by

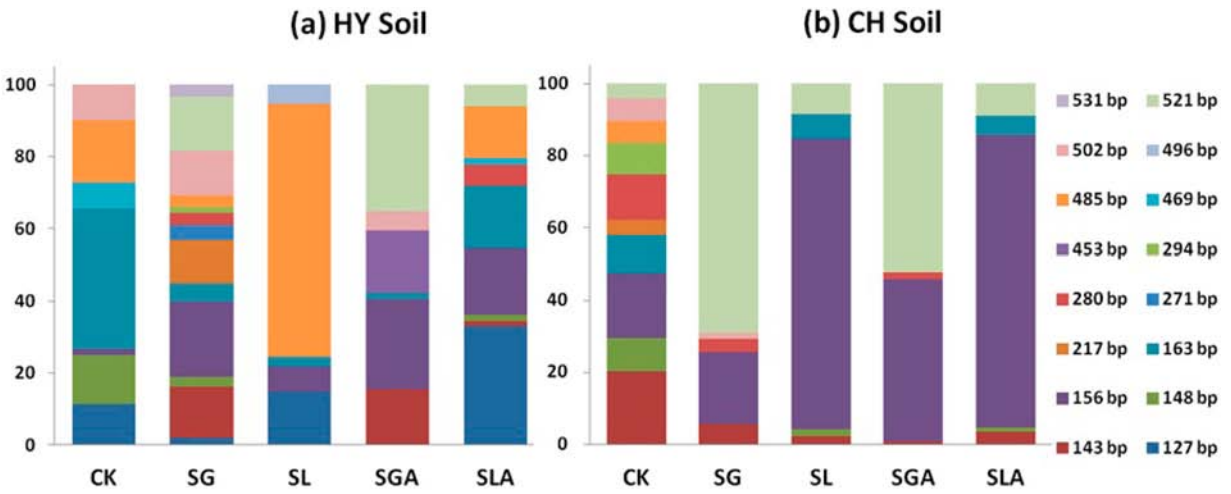

Figure 6. Relative abundances of selected 16S rRNA T-RFs from the different treatments of DDT transformation in (a) HY and (b) CH soils after incubation for 7 days. T-RF sizes are given in base pairs, and the relative abundance of T-RFs is given as a percentage of the total peak area. The definitions of the acronyms used are those in Figure 1.

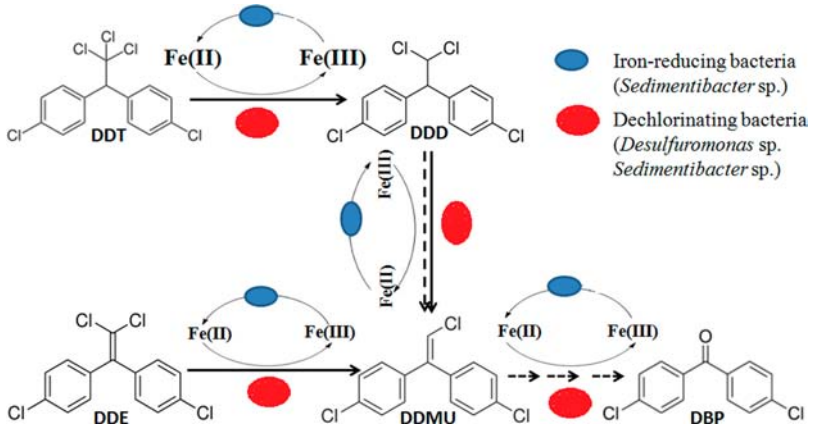

Figure 7. Schematic diagram of the reductive transformation of DDT in the paddy soils.

T-RFLP following enzyme digestion, providing the major taxa present semiquantitatively (Table 5). In the CK treatment, the bacterial community structure in these two soil systems displayed a great diversity in which *Sedimentibacter* sp. was the most abundant species. The addition of glucose, lactate, and AQDS exerted a significant effect on the bacterial community structure in the HY and CH soils, as shown by the principal component analysis (PCA)[30] of T-RFs (Figure 5). The PCA results of the structural diversity show that component 1 separated the SG treatment from the other treatments, which indicates that the glucose addition clearly changed the general microbial community in both soils. As shown in Figure 5, all treatments differed significantly from one another except for the treatment of the AQDS addition. Although there were differences in bacterial composition between the two soils studied, *Sedimentibacter* sp. and *Clostridium* sp. were the predominant bacteria functioning for DDT transformation under all treatments in the two soils.

T-RFs contributing to observed shifts in the community structure are presented in Figure 6, and the corresponding dominant T-RF lengths and relative amounts are provided in Table 5. T-RF 156 represents bacteria that were ubiquitous and found in all the treatments in the two soils. The lactate addition treatment significantly increased the relative amount of T-RF 156 in the CH soil. The 143, 156, and 280 bp end fragments were identified to represent *Sedimentibacter* sp., which has a great degree of similarity to dechlorinating bacteria.[38,39] T-RFs 127 and 163 were identified to represent *Desulfuromonas* sp. and *Sedimentibacter* sp., which have a high degree of similarity to dechlorinating and sulfate-reducing bacteria with iron-reducing ability, respectively.[40,41] T-RFs 217 and 485 were identified to represent bacteria that thrive in DDT-polluted soil under anoxic conditions. [42] T-RFs 453 and 502 were identified to represent bacteria that are highly similar to methanogenic bacteria. T-RF 521 was identified to represent *Clostridium* sp., which has a high degree of similarity to dechlorinating bacteria.[43] As presented in Table 5, the sum abundance of T-RFs that represent bacteria with dechlorinating and iron-reducing functions in the presence of glucose or lactate and with the electron shuttle of AQDS was significantly higher than that in the CK treatment in both soils, which was responsible for the enhanced transformation rate of DDx.

DISCUSSION

DDT is a persistent pesticide that has been widely applied in agricultural activities. The residue of this pesticide and its possible metabolites are still found in various soils. Although adsorbed Fe(II) species have a reductive ability to dechlorinate certain chlorinated compounds — such as pentachlorophenol

(PCP)[12] — the abiotic transformation of DDx in soils is ultimately slow because it is somewhat recalcitrant. As shown in Figure 1, DDT underwent very low transformation in abiotic reaction in sterilized paddy soils. However, with soil microorganisms, DDT obtains efficient transformation rates resulting from microbial processes. When the soil was supplemented with carbon sources that nurtured the growth and metabolism of soil bacteria, DDT was dechlorinated more efficiently by the increased amount of microorganisms with dechlorinating capability. The transformation rates of DDT were significantly enhanced, and DDT could be reduced in a short time with half-lives of only 4−8 days under certain treatments.

HS may act as a redox mediator and play an important role in the anaerobic respiration of microorganisms in accelerating electron transfer.[44] AQDS, an analogue of an HS compound, can shuttle electrons from microorganisms to soil iron oxides or chlorinated compounds directly,[26] which further enhances DDx transformation (Figures 1 and 3). DDD is the main microbial transformation metabolite of DDT even after undergoing a long incubation reaction of one year in the anaerobic soil system. In the two paddy soils that we studied, we obtained low proportions of the biodegradable terminal DDT metabolites, DBP and DDMU,[20] with two or three chlorines dechlorinated, respectively, on the straight chains of the molecular DDT. As a result of its more recalcitrant properties, DDD is the main metabolite of DDT microbial transformation in the paddy soils. The anaerobic dechlorination abilities of the soil systems are identical for total DDx. Moreover, the removal of DDx in the paddy soils under different treatments followed similar trends as DDT transformation. Notably, DDD is the most recalcitrant substance to be degraded among these three compounds of DDx.

The schematic diagram of the dechlorination transformation of DDT in the paddy soils is presented in Figure 7. The reductive dechlorination of DDT in the anaerobic soil systems was caused by the process in which DDT acted as the terminal electron acceptors by accepting electrons from either the bacteria or the adsorbed Fe(II).[44] When there is neither an exogenous additional carbon source nor an electron mediator in an anaerobic soil reaction system, bacteria may utilize some of the soil's indigenous organic carbon as the energy source. However, under such circumstances, electrons transfer to iron oxides for adsorbed Fe(II) generation and reductive dechlorination of DDx occurs at a decreased rate and in a relatively lower amount. Alternatively, DDx may act as the electron acceptor directly from the bacteria for dechlorination. As indicated by the adsorbed Fe(II) generation (Figure 4) and redox potential study (Figure S5), lactate and glucose that are added to the soils provide carbon sources for the growth and increase of the iron-reducing and dechlorinating bacteria. In addition, more electrons were also transferred in the reaction systems with the addition of lactate and glucose, which led to a more negative potential for iron reduction and DDT reductive dechlorination.

With more negative potential formed in the reaction systems, the instantaneous electron transfer may not be as efficient for transformation of DDx. AQDS was introduced into the soil systems to mediate the electron transfer and possibly obtain a more efficient electron utilization and higher transformation rates of DDx.[26] In the paddy soils, the added AQDS may act as transitional electron acceptors from the active bacteria by transferring the electrons to the iron oxides or target DDx pollutants, thereby increasing the efficiency of transformation. The CV results show that a further negative shift of anodic peaks was obtained, which indicates the negative potential of the reaction system. In the presence of AQDS, a greater amount of adsorbed Fe(II) was generated and higher microbial DDT transformation rates were obtained.

The key role for the transformation of soil pesticide residues was played by the soil microorganisms acting in either the microbial reduction of iron for adsorbed Fe(II) generation or the directly reductive dechlorination of DDx. However, as reported previously,[7] pesticide degradation *in situ* is usually achieved by a consortium of functions played by different species of bacteria instead of by one species. The microbial-mediated transformation of DDx was strongly dependent on the species and on the abundance of active microbial communities. With respect to the two paddy soils studied here, the transformation rate of DDx was higher in the CH soil than in the HY soil in the CK treatment without an additional carbon source. This result may be attributed to the higher abundance of indigenous bacteria functioning in the transformation of DDx in the CH soil, as indicated by the obtained T-RFLP results.

Carbon sources can be utilized as nutrients for bacterial growth. Thus, adding carbon sources to the soils enhanced the bacterial activities by both stimulating the bacteria and increasing the amount of the indigenous active microbial population.[9] As shown in Figure 1a, the additional glucose or lactate significantly accelerated DDT transformation in the two soils. This finding may be attributed to the increased abundance of dechlorinating bacteria resulting from an increased amount of correlated functional bacteria of *Desulfuromonas* sp., *Sedimentibacter* sp., and *Clostridium* sp. (Figure 6 and Table 5). Different carbon sources created a variety of enhancement effects for the bacteria, which consequently enlarged the extent of DDT transformation. Although both lactate and glucose enhanced the dechlorinating activity of the microbial communities in all treatments in the two soils, the addition of glucose led to a greater increase in the transformation rates of DDT. This result indicated that glucose was the more appropriate carbon source for the dechlorinating and iron-reducing bacteria in the paddy soils.

In conclusion, DDT experienced steady microbial dechlorination processes in the paddy soils in our study. Supplemented carbon sources — lactate or glucose — stimulated the growth and abundance of the soil bacteria with dechlorinating and iron-reducing functions. As a result, more Fe(II) was adsorbed, and the transformation rates of DDT increased. AQDS acted as an electron shuttle and further enhanced the transformation rates of DDT by accelerating the electron transfer speed. The results of this work clarified the anaerobic dechlorination mechanisms of chlorinated compounds in soils and provided a potential application for the dechlorination and detoxification of residue-chlorinated pesticides in soils. The main functional strains of bacteria in the paddy soils were determined based on their transformation efficiencies of DDT. However, the isolation and characteristics of bacteria species and functional genes remain to be investigated further to clarify the functional mechanisms of the bacteria.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Figures S1−S5. This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author

*Phone: +86-20-87024721. Fax: +86-20-87024123. E-mail: cefbli@soil.gd.cn (F.B.L.); csliu@soil.gd.cn (C.S.L.)

Author Contributions

#M.J.C. and F.C. contributed equally to this work.

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Notes

The authors declare no competing financial interest.

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