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Dynamics of the microbial community and Fe(III)-reducing and dechlorinating microorganisms in response to pentachlorophenol transformation in paddy soil

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HIGHLIGHTS

- Microbial community is the driving force behind the transformation of PCP.
- *Veillonellaceae* and *Clostridium sensu stricto* were dominant groups in PCP degradation.
- Microbial community structures shift during PCP degradation.
- Iron-reducing and dechlorinating bacteria play important roles in PCP degradation.
- Lactate and AQDS impact the changes in microbial communities and key microorganisms.

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ABSTRACT

Soil microorganisms play crucial roles in the fates of pollutants, and understanding the behaviour of these microorganisms is critical for the bioremediation of PCP-contaminated soil. However, shifts remain unclear in the community structure and Fe(III)-reducing and dechlorinating microorganisms during PCP transformation processes, especially during the stages from the lag to the dechlorination phase and from the dechlorination to the stationary phase. Here, a set of lab-scale experiments was performed to investigate the microbial community dynamics accompanying PCP transformation in paddy soil. 19 μM of PCP was biotransformed completely in 10 days for all treatments. T-RFLP analysis of the microbial community confirmed that *Veillonellaceae* and *Clostridium sensu stricto* were the dominant groups during PCP transformation, and the structures of the microbial communities changed due to the degree of biotransformation and the addition of lactate and AQDS. However, similar temporal dynamics of the microbial communities were obtained among all treatments. Furthermore, as revealed by quantitative PCR, the dynamics of Fe(III)-reducing and dechlorinating microorganisms, including *Geobacter* sp., *Shewanella* sp., and *Dehalobacter* sp., were consistent with the transformation kinetics of PCP, suggesting the critical roles played by these microorganisms in PCP transformation. These findings are valuable for making predictions of and proposing methods for the microbial detoxification of residual organochlorine pesticides in paddy soil.

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1. Introduction

Pentachlorophenol (PCP) is an important persistent pollutant and has been widely used as a pesticide, herbicide and wood preservative [1]. Although the purchase and use of PCP has not been available to the general public for a long time, PCP can still be detected in surface water, sediments, aquatic organisms, surface soil and food, as well as in human milk due to its molecular stability and sorption properties [1,2], and this compound remains a critical environmental concern because of its accumulation and negative impact on human health [3,4]. As a result, the transformation of PCP in the environment has been the focus of attention for a considerable time [2].

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Biodegradation is the main process for the transformation of organic pollutants, and soil microorganisms play important roles in the degradation of organic pollutants in contaminated soil [5,6]. PCP can be biodegraded by a series of aerobic and anaerobic microorganisms under a variety of conditions in soil, sediments and sludge [2,7–9]. Under anaerobic conditions, *Desulfitobacterium* sp., which is reported to degrade PCP to 3-chlorophenol [7], is the most known and characterized PCP-degrading bacterium in soil [9]. However, isolated and cultivated microorganisms only represent a small percentage of PCP degraders in soil, and the soil microorganisms that function to degrade PCP are uncharted to a large extent [10,11]. Although PCP is too toxic to most soil microorganisms, it can be degraded metabolically or co-metabolically by various microbial specialists in real soil environments [9]. To further understand the simultaneous behaviour of bacteria and PCP transformation, the soil microbial communities and specific bacteria associated with the biodegradation processes of PCP must be investigated. Yoshida et al. [8] found that an enriched microbial community from paddy soil was able to dechlorinate PCP completely to phenol, and *Clostridium* was the predominant functional species in this microbial community. Cea et al. [12] found that PCP degradation was associated with changes in microbial communities in Andisol and members of the bacterial phylum Proteobacteria (Xanthomonadaceae, Burkholderiaceae and Enterobacteriaceae) were the dominant bacteria during the biodegradation of PCP. Furthermore, our previous studies found that the *Veillonellaceae* and *Peptococcaceae* family members, which contain many Fe(III)-reducing species and dehalogenating bacterial genera, respectively, were predominant groups in the transformation of PCP in paddy soil [13]. Fe(III)-reducing bacteria, such as *Geobacter* sp. and Shewanella sp., are abundant in paddy soil and can reduce Fe(III) to Fe(II) [13,14]. The generated Fe(II) species can act as reactive reductants in the biodegradation of organochlorine pesticides in soil environments [15,16]. Dechlorinating bacteria, such as *Dehalobacter* sp. and *Desulfitobacterium* sp., can reduce organochlorine pesticides directly [17,18]. Hence, the Fe(III)-reducing and dechlorinating populations can function for PCP transformation indirectly or directly. The aforementioned reports suggested soil microbial communities and specific bacteria with potential dechlorinating abilities, such as Fe(III)-reducing and dechlorinating bacteria, play important roles in PCP transformation. Therefore, elucidating the dynamics of microorganisms or microbial consortia responsible for PCP dechlorination is necessary for predicting the efficiency of PCP biotransformation.

In addition, our previous studies demonstrated that the kinetics of PCP transformation in soil were generally sigmoidal, consisting of an initial lag followed by a relatively rapid transformation and then a stationary plateau with no further change [19,20]. Previous studies indicated that changes in microbial communities resulted from bacterial growth or death [21], and indigenous microorganisms that respond to ambient conditions that limit their growth and dechlorination activity are important for the biodegradation of organochlorine pollutants [22]. Although Mahmood et al. [21] found that the indigenous microbial community in pristine grassland soil could degrade PCP and that there were differences in microbial communities throughout the incubation period. However, relatively little is known about the microbial community dynamics during PCP transformation in paddy soil, especially during the important stages from the lag phase to the dechlorination phase and from the dechlorination phase to the stationary phase.

In this investigation, we extend our previous study [19], in which the sigmoidal and logistic kinetic of PCP transformation and the microbial communities were determined to investigate the dynamics of indigenous microbial communities, especially those communities at the stages of the lag phase to the dechlorination phase and from the dechlorination phase to the stationary phase during PCP transformation in paddy soil. Priority was placed on elucidating the indigenous microbial communities responsible for the anaerobically reductive transformation of PCP in paddy soil by focusing on (i) the soil microbial community response during the lag and dechlorination phase of PCP transformation and thereby the transformation kinetics of PCP and (ii) the dynamics of specific bacterial genera with potential dechlorinating abilities during the PCP transformation processes.

2. Materials and methods

2.1. Soil sampling

The soil was a red-yellow clay soil collected from the A horizon (0–15 cm, the leached layer) in a paddy field located at Henghe Town (23°17′46.74″N, 114°5′38.88″E), Huizhou City, Guangdong Province, China. The sampling site was located where rice had been planted for at least 15 years. After being passed through 2-mm mesh sieve and air dried, the subsamples were refrigerated at 4 °C before use. At the time of sample collection, no residual PCP was detected in the soil. The basic physicochemical properties of the soil were analysed by the method described previously [23], and the results were as follows: pH (5.56), organic matter (23.90 g kg^{-1}), Fe_2O_3 (13.18 g kg^{-1}), complex-Fe (0.45 g kg^{-1}) and amorphous-Fe (6.03 g kg^{-1}). Soil for the control experiment was sterilized by 50 kGy gamma-irradiation before use.

2.2. Chemicals

PCP (98%), AQDS (97%) and 1,4-piperazinediethanesulfonic acid (PIPES, 98%) were purchased from Sigma-Aldrich (USA). Methanol, hexane and dichloromethane (HPLC grade) were obtained from Acroas (Belgium). Other analytical grade chemicals were obtained from Guangzhou Chemical Co., China. All solutions were prepared with deoxygenated ultrapure water (18.2 MΩ cm, Easy Pure' II RF/UV, USA).

2.3. Batch experiments of PCP transformation in the soil

In all experiments, 20.2 mL serum bottles with silicone-lined septa and aluminium sealing caps were used as the reactors, which contained 0.5 g of soil (dry weight) and 10 mL of PIPES (30 mM) buffer solution to keep the pH value at 7.0 ± 0.1. The following experiments with six treatments were conducted (Table 1): (1) sterile soil + PIPES + PCP (SC1); (2) sterile soil + PIPES + lactate + PCP (SC2); (3) sterile soil + PIPES + lactate + AQDS + PCP (SC3); (4) soil + PIPES + PCP (SP); (5) soil + PIPES + lactate + PCP (SPL); and (6) soil + PIPES + lactate + AQDS + PCP (SPLA). PIPES, lactate, AQDS, and PCP were added to achieve concentrations of 30 mM, 10 mM, 200 μM, and 19 μM, respectively. After all of the designated regents were added, the bottles were purged with N_2 (99.99%) for 20 min, and the gas of volume in the bottle headspace was approximately 10.2 mL. After mixing uniformly, the serum bottles were placed in an anaerobic chamber and were incubated at (30 ± 1) °C. In the anaerobic chamber, the anaerobic condition was maintained by continuously purging a mix gas of 96% N_2 and 4% H_2 with O_2 at <1 pmv by continual atmospheric circulation over a Pd catalyst. Individual bottles were sacrificed at specific time intervals and three operationally defined components were sampled.

2.4. Analyses of PCP and its transformation products

PCP concentrations were determined by high-performance liquid chromatography (HPLC), and the PCP transformation products were identified by gas chromatograph-mass spectrometry

Table 1
Treatment methods and kinetic parameters of the PCP transformation experiments under different conditions. The initial concentrations of PCP, PIPES, lactate and AQDS were 19 μM, 30 mM, 10 mM and 0.2 mM, respectively.

Treatment	Conditions						$k\ (d^{-1})$	A	μ_{max}	$t_{1/2}(d)$
	soil (g)	sterilization	PIPES (mM)	Lactate (mM)	AQDS (μM)	PCP (μM)				
SC1	0.5	yes	30			19	/	/	/	/
SC2	0.5	yes	30	10		19	/	/	/	/
SC3	0.5	yes	30	10	200	19	/	/	/	/
SP	0.5	no	30			19	0.480 ± 0.045	100%	0.120 ± 0.005^{C}	8.34
SPL	0.5	no	30	10		19	0.536 ± 0.017	100%	0.134 ± 0.004^{B}	7.46
SPLA	0.5	no	30	10	200	19	0.589 ± 0.022	100%	0.147 ± 0.005^{A}	6.79

A, B and C within the same column indicate significant differences (P < 0.01).

(GC–MS). The HPLC and GC–MS analytical procedures were the same as previous described [19].

2.5. DNA extractions and amplification

Total DNA was isolated from 0.25 g of reacted soil (in triplicate) obtained from the enrichment cultures after centrifugation at 16,162*g* for 10 min. DNA was extracted with the Power Soil DNA kit (MO BIO Laboratories, USA) according to the manufacturer's recommendations. The primers used for amplification of the bacterial 16S rRNA gene were 27F labelled with 6-FAM on the 5′ end and 1492R. Amplification was performed using a C1000 Touch^{TM} Thermal Cycler (Bio-Rad, USA), as previously described [19].

2.6. Terminal-restriction fragment length polymorphism (T-RFLP) analysis and clone library

The fluorescently labelled amplicons of the 16S rRNA genes were purified using the E.Z.N.A. Gel Extraction kit (OMEGA bio-Tek, USA) according to the manufacturer's recommendations. Four cutter restriction enzymes (*Msp*I, *Alu*I, *Hae*III and *Taq*I) were tested and *Alu*I was selected for providing the best size distribution of the terminal restriction fragments (T-RFs). The amplicons of the 16S rRNA gene were therefore obtained using *Alu*I restriction enzyme (New England Biolabs, USA) for 3 h at 37 °C. The digested PCR products were resolved by electrophoresis using an ABI 3730xl sequencer (Applied Biosystems, USA). GS-500 Rox was loaded as an internal size standard in each lane. The fragment size, peak area and height were calculated using Gene Scan software. Only fragments with an intensity greater than a baseline threshold (50 fluorescent units) were recorded to reduce data "noise". The relative abundance of individual T-RFs was calculated and indicated as the percentage of the total peak area in a given T-RFLP profile. The relative percentage abundance (Ap) of each T-RF was calculated as follows:

$$Ap = n_i/N \times 100 \quad (1)$$

where n_i represents the peak area of one distinct T-RF and N is the sum of all peak areas in a given T-RFLP pattern. Those Ap < 5% for each fragment were excluded from further analysis. Each measurement was performed in quadruplicate.

The purified 16S rRNA gene amplicons for clone library construction were ligated into pGEM®-T Easy vector (Promega, USA), and the resulting plasmids were inserted in *Escherichia coli* Dh5α competent cells (Takara, China). Transformed cells were plated on Luria–Bertani (LB) agar plates containing 50 μg/L ampicillin and were then incubated overnight at 37 °C. Selected clones were randomly selected and grown overnight in LB medium. Clones were screened for inserts with PCR primers M13F and M13R and were sequenced with an ABI 3730xl sequencer (Applied Biosystems). The sequences were retrieved from the Ribosomal Database Project-II using the SEQUENCE MATCH tool [24]. The sequences were submitted to the GenBank database under accession numbers KF228175 to KF228253.

The produced T-RFs to represent the bacterial community were verified by clone libraries analysis using the method described previously [25]. Briefly, virtual restriction enzyme digests of the 16S rRNA gene amplicons and the sequences from the clone libraries were performed using the web-based Restriction Enzyme Mapping Application (REMA) [26]. The resulting predicted T-RF fragment lengths were compared manually to the respective observed T-RF database taking into account the abundance of clone sequences, T-RF peak area, and the proximity of predicted or observed T-RFs. All predicted and observed T-RF matches were within 2 bp from each other.

2.7. Quantitative PCR

Quantitative PCR (qPCR) was used to determine the concentrations of typical dechlorinating and Fe(III)-reducing taxa in all cultures using specific primers (Table 2) with the MyiQ^{TM}2 Two-Color Real-Time PCR Detection System (Bio-Rad, USA). Reaction mixtures (final volume of 25 μL) contained 12.5 μL SYBR Green Mix (Bio-Rad, USA), pairs of primers (100 nM of each) and 1 μL template DNA. The PCR conditions were the same as described previously [18,27,28]. The PCR efficiency ranged between 90% and 99% with R^2 values > 0.99. Each measurement was performed in triplicate. A melting curve analysis was performed at 1 °C/30 s from 55 to 95 °C. Calibration curves were obtained using serial dilutions of plasmids carrying a single, cloned target gene. Copy numbers were determined based on standard curves prepared and analysed following the protocol of Whelan et al. [29].

2.8. Statistical analyses

Statistical analyses of the experimental data were performed using the SPSS 20.0 statistical software. Differences were determined by one-way analysis of variance (ANOVA) on ranks followed by Fisher's least-significant-difference (LSD) test. The *Alu*I-generated T-RF profiles of the 16S rRNA amplified directly from the soil samples of different treatments were used to construct the hierarchical cluster and multidimensional scaling (MDS) analysis as previous study [30]. Hierarchical cluster analysis was used to analyse the similarities of bacterial communities between samples. On the MDS plots, relative changes in community structure were visualized and interpreted as the distances between the points. Communities with high similarity aggregate and communities with low similarity were distributed separately and far apart.

Table 2
Primers used to quantify the 16S rRNA genes of the total bacterial populations and specific bacterial genera with potential dechlorinating abilities using a qPCR approach.

Primer Name	Target gene	Annealing Temperature	Sequence	References
Dhc 793F	*Dehalococcoides* sp. 16S rRNA	55 °C	GGGAGTATCGACCCTCTCTG	[28]
Dhc 946R	*Dehalococcoides* sp. 16S rRNA	55 °C	CGTTYCCCTTTCRGTTCACT	[28]
Dhb 477F	*Dehalobacter* sp. 16S rRNA	55 °C	GATTGACGGTACCTAACGAGG	[28]
Dhb 647R	*Dehalobacter* sp. 16S rRNA	55 °C	TACAGTTTCCAATGCTTTACGG	[28]
Dsb 406F	*Desulfibacteria* sp. 16S rRNA	58 °C	GTACGACGAAGGCCTTCGGGT	[28]
Dsb 619R	*Desulfibacteria* sp. 16S rRNA	58 °C	CCCAGGGTTGAGCCCTAGGT	[28]
Geo 564F	*Geobacter* sp. 16S rRNA	51 °C	CAAGTCGTACGAGAAACATATC	[27]
Geo 840R	*Geobacter* sp. 16S rRNA	51 °C	GAAGAGGATCGTCTTTCCACGA	[27]
She 120F	*Shewanella* sp. 16S rRNA	60 °C	GCCTAGGGATCTGCCCAGTCG	[27]
She 220R	*Shewanella* sp. 16S rRNA	60 °C	CTAGGTTCATCCAATCGCG	[27]
Eub 338F	Bacteria	55 °C	ACTCCTACGGGAGGCAG	[50]
Eub 518R	Bacteria	55 °C	ATTACCGCGGCTGCTGG	[50]

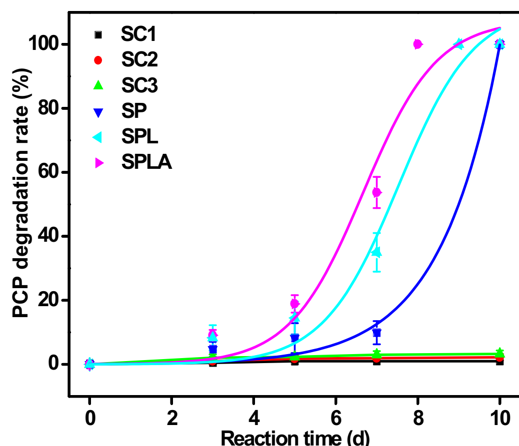

Fig. 1. The kinetics of pentachlorophenol (PCP) transformation by an enrichment culture. Reaction conditions and treatment methods are the same as in Table 1.

3. Results and discussion

3.1. Anaerobic transformation of PCP in the paddy soil

The results of the PCP transformation kinetics in paddy soil under different treatments are shown in Fig. 1. The kinetics of the PCP removal rates were generally sigmoidal and a logistic kinetic model was used to interpret the residue concentration changes of extractable PCP residue data through nonlinear curve fitting as follows [19,20,31]:

$$(C_0 - C_t)/C_0 = A/(1 + Be^{-kt}) \quad (2)$$

where A is the maximum transformation capacity of PCP, B is the regression coefficient, k is the rate constant transformation of PCP, and C_0 and C_t are the concentrations of PCP at reaction times 0 and t, respectively. The maximum reaction rate of PCP transformation, $\mu_{\max}$ (d^{-1}), was obtained from 0.25 Ak. The rate constants (k), half-lives, and $\mu_{\max}$, for PCP transformation in different treatments were calculated and are listed in Table 1. In treatments with sterile soil (SC1, SC2 and SC3), no obvious PCP transformation was observed after reaction for 10 days. In the treatments with non-sterile soil (SP), a significant transformation of PCP was obtained after a lag of 3 days and was transformed completely in 10 days with $\mu_{\max}$ value of 0.120 d^{-1} and half-life of 8.34 days. The differentiated transformation rates of PCP between the treatments with sterile and non-sterile soil suggested that the accelerated PCP transformation rates in the non-sterile soil were attributed to the activities of indigenous bacteria in the paddy soil [32]. PCP has also been reported to be degraded by indigenous microorganisms in grassland soil [21] and paddy soil [8], although with lower transformation rates than obtained in the present study, which may result from the differences of soil types, microorganism types, and incubation conditions.

Compared with that of the SP treatment, the PCP transformation rate with lactate (SPL treatment) was further enhanced ($P < 0.05$) (Table 1), in which PCP was transformed completely within 9 days with the $\mu_{\max}$ value increased to 0.134 d^{-1} and half-life shortened to 7.46 days. Lactate acts as a carbon source in the biological processes of PCP degradation [17,33]. Furthermore, lactate can be decomposed to H_2, acetate, and propionate by soil indigenous microorganisms, and the produced H_2 can act as an electron donor for the dechlorination of PCP and its partially halogenated aromatic derivatives [17]. Therefore, the addition of lactate would stimulate the growth of bacteria that are responsible for the transformation of PCP. Additionally, the addition of AQDS further enhanced the PCP transformation rate, with the $\mu_{\max}$ value for PCP transformation increased to 0.147 d^{-1} and the half-life shortened to 6.79 days in the SPLA treatment. A significant difference was observed in the average max reaction rate of PCP transformation (μ_{max}) between SPLA and SPL/SP ($P < 0.01$ and $P < 0.01$, respectively) (Table 1). These findings suggested that AQDS has an effect on the biotransformation of PCP, which is consistent with that for other chlorinated compounds, such as trichloroethene and perchlorate [19,34–36]. Our previous study demonstrated that the addition of AQDS significantly increased the transformation rate of DDT by enhancing the abundance of iron-reducing and dechlorinating bacteria in paddy soil under anaerobic conditions [37]. AQDS was reported to serve as an electron donor in the microbial reductive dechlorination of TCE to *cis*-DCE [34]. More importantly, AQDS has been shown to act as an effective redox mediator for microbial and chemical reductive dechlorination [34,35,38]. As a result, it can be concluded that the addition of AQDS enhanced the transformation rate of PCP by accelerating the rates of electron transfer and stimulating activities of the bacteria with potential dechlorinating abilities.

During the PCP transformation, the dechlorinated metabolites, including 2,3,5,6-tetrachlorophenol (TeCP), 2,3,5-trichlorophenol (TCP), 3,5-dichlorophenol (DCP) and 3-chlorophenol (CP) with one to four chlorines dechlorinated, respectively, were produced in all treatments except for those with the sterilized soil. The variations in the produced metabolite concentrations during PCP transformation are presented in Fig. 2. The two further dechlorinated products of 3,5-DCP and 3-CP were produced only after reaction for 28 days. The addition of lactate and AQDS affected the dechlorination extents of PCP. This result suggested that the PCP transformation processes were reductive dechlorination reactions under the anaerobic conditions in this paddy soil.

3.2. T-RFLP profiles of the dominant bacteria during the PCP transformation

Based on the results and discussion in the preceding sections, indigenous microorganisms are of great importance for the

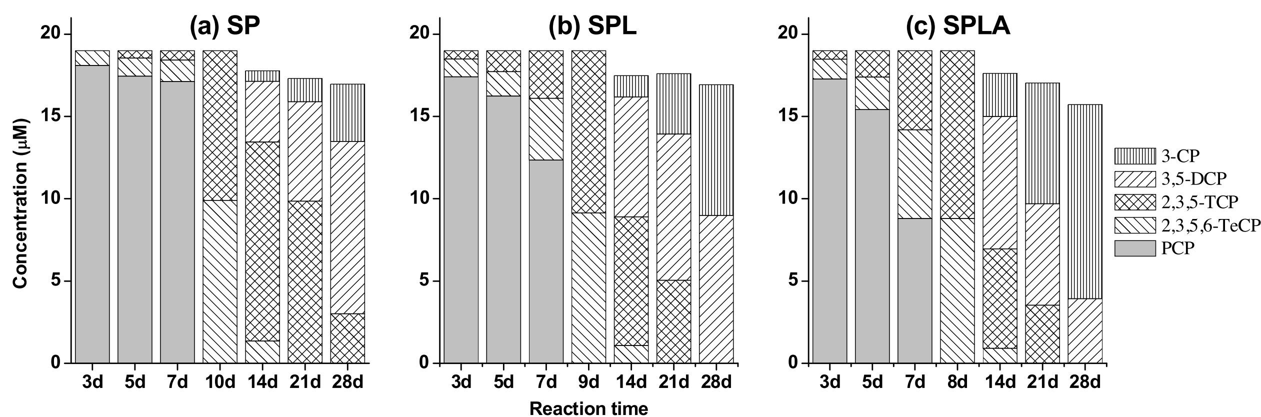

Fig. 2. Anaerobic transformation of PCP and the concentration of intermediate products in (a) SP, (b) SPL, and (c) SPLA treatments. Reaction conditions and treatment methods are the same as in Table 1.

Table 3
Predicted genus associations for the dominant terminal restriction fragment (T-RF) lengths based on bacterial 16S rRNA gene sequences.

T-RF size	T-RF relative abundance (%)													predicted genus association	accession no.	similarity
	Soil	SP				SPL				SPLA						
	(0 d)	3 d	7 d	14 d	28 d	3 d	7 d	14 d	28 d	3 d	7 d	14 d	28 d			
70	3.58	3.32	4.55	5.92	10.29	5.53	11.76	9.15	5.90	5.92	12.47	10.31	10.11	*Sedimentibacter*	AY766466	98%
144	0.00	0.00	0.00	3.80	13.22	0.00	0.00	6.26	9.03	0.00	0.00	5.28	10.93	unclassified_*Veillonellaceae*	DQ431898	99%
215	0.00	0.00	0.00	0.00	0.00	17.11	1.37	3.92	6.62	19.04	1.32	3.50	4.95	unclassified_*Veillonellaceae*	FJ269098	97%
230	43.15	35.05	16.00	9.82	8.80	17.58	10.36	8.38	6.88	15.88	8.82	6.53	4.46	*Clostridium sensustricto*	Y15985	98%
234	5.16	3.61	9.54	6.92	8.12	4.45	13.89	12.09	10.39	6.39	17.18	16.60	15.39	*Sedimentibacter*	AF349757	98%
236	3.06	2.86	3.02	3.31	6.99	7.66	7.56	9.01	10.26	5.24	5.21	10.59	14.34	unclassified_*Veillonellaceae*	DQ833299	99%
244	3.63	2.04	2.28	8.31	7.24	3.99	1.99	6.48	2.55	11.99	4.88	6.50	2.33	*Clostridium sensustricto*	AY667266	98%
248	6.15	45.99	50.26	45.23	22.47	33.98	36.35	24.13	20.84	29.09	33.89	12.10	5.95	unclassified_*Veillonellaceae*	FJ269098	99%
282	0.00	1.66	1.86	3.90	6.88	1.81	2.05	7.01	7.61	0.00	1.50	8.32	10.45	unclassified_*Clostridiales*	AB723835	96%

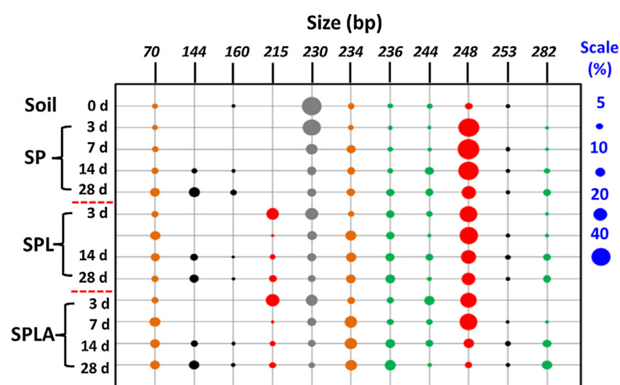

Fig. 3. Distribution and abundance (%) of terminal restriction fragments (T-RFs) from different treatments during anaerobic PCP transformation estimated by T-RFLP. The size of the circles is related to the relative abundance (% of fluorescence intensity of each T-RF). Reaction conditions and treatment methods are the same as in Table 1.

transformation of PCP. Thus, the growth- and stationary stage-related (day 3, 7, 14 and 28) dynamics of microbial communities were investigated in all treatments with the T-RFLP method. A total of 18 distinct T-RFs was identified in all T-RFLP profiles obtained in all treatments. Only those >5% for each fragment were used for further analysis (Fig. 3), and the identities of the taxa are shown in Table 3. Six T-RFs of 70, 230, 234, 236, 244, and 248 bp were detected in all treatments and the most abundant fraction during the incubation process was 248 bp T-RF, which was identified as an iron-reducing bacterium *Veillonellaceae* [39]. A wealth of laboratory studies have shown that biogenic Fe(II) produced by Fe(III)-reducing organisms can degrade organic pollutants [16,40].

The abundance of T-RF 248 bp increased in the growth stage (first 7 days) and decreased in the stationary stage (after incubation for 14 days) in all treatments, and the abundance of T-RF 248 bp in the SPL and SPLA treatments was lower than in the SP treatment (Fig. 3). The abundance trends of T-RF 248 bp were coincident with the transformation kinetics of PCP in all treatments. Furthermore, compared to day 0, the relative abundance of T-RF 248 bp increased obviously in the growth and stationary stages in all treatments (Table 3), which indicated that T-RF 248 bp may be involved in the transformation of PCP. T-RF 70 bp had 98% sequence homology with a *Sedimentibacter* sp., which could assist the genus *Dehalobacter* in reductive dechlorination under anaerobic conditions [41,42]. *Sedimentibacter* sp. is unable to dechlorinate, but plays an important role in the growth and dechlorination of *Dehalobacter* sp. [42]. The abundance of T-RFs 70 bp increased during the incubation process in the SP treatment, which increased during the first 7 days and then decreased after incubation for 14 days in both SPL and SPLA treatments. This change suggested that the abundance of *Dehalobacter* sp. in all treatments changed during the incubation time in response to PCP transformation. T-RF 230 bp was identified as *Clostridium sensu stricto*, which has a high degree of similarity (98%) to polysaccharolytic Clostridia from anoxic rice field soil [43]. Compared to the original soil sample (day 0), the abundance of T-RF 230 bp decreased during the incubation time in all treatments, which indicated that the polysaccharolytic bacteria were rich in the original soil sample in this study, and the abundance decreased after the reaction conditions changed from anoxic to anaerobic. T-RFs 234 and 236 bp were identified to represent Sedimentibacter and unclassified Veillonellaceae, respectively, and shared high similarities with sequences previously obtained from enrichment cultures capable of reductively dechlorinating TCE and vinyl chloride [44,45]. The abundance of T-RFs 234 and 236 bp increased over

the incubation time in all treatments, indicating that the bacteria with direct or indirect dechlorinating abilities were enriched during the PCP transformation in all treatments. T-RF 244 bp was also identified as *Clostridium sensu stricto*, which has a high degree of similarity to dechlorinating bacteria for trichloroethene [45]. T-RF 144 bp was only detected after incubation for 14 days in all treatments and was identified as *Veillonellaceae*. T-RF 215 bp, identified as an iron-reducing *Veillonellaceae* bacterium [39], was found in both the SPL and SPLA treatments, indicating that the addition of lactate and AQDS had stimulated it. The bacterial population with T-RF 282 bp was closely related to the uncultured *Clostridiales*, which was found in the PCP anaerobic mineralization continuous-flow system using only lactate as the external nutrient [17]. The abundance of T-RF 282 bp increased over the incubation time in all treatments, indicating that the bacteria with potential dechlorinating abilities were enriched during the transformation process of PCP. In our previous study [46], we found that the PCP could be reductively dechlorinated or mineralized by indigenous microorganisms in paddy soil and the addition of lactate could enhance the complete mineralization. These results indicated that one of the possible ways of PCP transformation in this study was reductive dechlorination and anaerobic mineralization.

Based on the above results, the bacteria with direct or indirect dechlorinating abilities, such as the bacteria with T-RFs 234, 236 and 282 bp, were enriched during PCP transformation (in the first 28 days of incubation), which also indicated that the reductive dechlorination processes occurred during the whole incubation time, consistent with the production of intermediates (Fig. 2).

In addition, iron reduction plays an important role in the reductive dechlorination of organochlorine pesticides in soil [15]. Soil iron-reducing and dechlorinating community structures can both accelerate the reductive dechlorination of organochlorine pesticides in paddy soil [15]. PCP has been shown to be readily dechlorinated under anaerobic conditions by the native dechlorinating bacteria directly as well as the biogenic Fe(II) produced by an iron-reducing community [16,19,47]. Therefore, the unclassified *Veillonellaceae* and *Clostridium sensu stricto*, which were identified as the iron-reducing and dechlorinating bacteria, respectively, are hypothesized to be the dominant species in all cultures during PCP transformation and play critical roles in the biotransformation of PCP in paddy soil.

3.2. Dynamics of microbial communities during PCP transformation

Fig. 3 shows that the abundance of some T-RFs changed, some fragments appeared and some disappeared, in different treatments throughout the entire 28-day incubation, which indicated that the changes in the T-RFLP profiles were due to the different treatments and the temporal dynamics of the bacterial community. For all treatments and time points, four major groups were obtained in the T-RFLP pattern cluster analysis (Fig. 4a). At the initial time of the transformation stage (first 3 days), cluster I (SP-3d) and cluster II (SPL-3d and SPLA-3d) were formed. This response indicated that lactate had an effect on the microbial community, which is consistent with a previous report [48] of a shift in the microbial community structure when adding lactate as an electron donor for TCE dechlorination. Cluster III was composed of samples in the intermediate time of the transformation stage (SP-7d, SP-14d, SPL-7d and SPLA-7d) and cluster IV contained most samples from all treatments after incubation for 14 days (SP-28d, SPL-14d, SPL-28d,SPLA-14d and SPLA-28d). No significant difference of microbial community structure between SPL and SPLA treatments at the same incubation time was observed (Fig. 4a) due to the obvious effect of lactate. Furthermore, Freeborn et al. [48] reported that lactate had a greater effect on the community structure in TCE-dechlorinating consortia than other electron donors, all of which indicates that lactate plays an important role in the changes of the microbial community structure during the dechlorination process.

An MDS plot presenting the microbial community structure dynamics was also constructed using T-RFLP profiles and the results were consistent with the cluster analysis (Fig. 4b). In the first 7 days of incubation, both treatments with lactate (SPL and SPLA) exhibited a noticeable and regular separation from the treatment without lactate (SP treatment), suggesting that the rapid changes from the lag to the dechlorination phase of the PCP transformation resulted from adding lactate and AQDS to the soil. More importantly, the Dimension 2 values of each treatment increased significantly from the initial point, and then the Dimension 1 values decreased along with the incubation time. These results indicated that the dynamics of the bacterial communities in all treatments tended to be uniform at the final stage.

Based on the results of the cluster and MDS analyses, the addition of lactate and AQDS affected the microbial community structures from the lag phase to the dechlorination phase of PCP degradation, whereas no significant shifts in the microbial community at the stationary stage (after incubation 28 days) were observed. According to the reports by Mahmood et al. [21], PCP degradation was associated with significant changes in the microbial community structure in pristine grassland soil. The microbial community, therefore, appears to be the driving force behind PCP transformation. The addition of lactate and AQDS affected the bacterial communities, which consequently led to higher observed PCP transformation rates.

3.3. Changes in the abundance of bacteria responsible for PCP transformation

Although the results of T-RFLP demonstrated that iron-reducing and dechlorinating bacteria were the major species in all treatments during the PCP transformation, special Fe(III)-reducing and dechlorinating bacteria, such as *Geobacter* sp., *Shewanella* sp., and *Dehalobacter* sp. were not detected directly by the T-RFLP or clone library. To further examine the changes in the abundance of Fe(III)-reducing and dechlorinating bacteria, qPCR was applied to quantify the copy numbers of specific 16S rRNA sequences associated with *Dehalococcoides* sp., *Dehalobacter* sp., *Desulfitobacterium* sp., *Geobacter* sp. and *Shewanella* sp., and the total bacterial populations in cultures. *Dehalococcoides* sp. and *Desulfitobacterium* sp. were not detected in this study. The copy numbers of total bacteria, *Geobacter* sp., *Shewanella* sp., and *Dehalobacter* sp. are shown in Fig. 5. The copy numbers of total bacteria increased during the first 14 days of incubation and then decreased in all treatments. The levels of the total bacteria in the treatments with lactate addition (SPL and SPLA) were significantly higher than that of the SP treatments, which indicates that lactate and AQDS enhanced the growth of microorganism by acting as carbon sources [17,34]. *Geobacter* sp. and *Shewanella* sp. in the δ and γ subclass of *Proteobacteria* are the most intensively and extensively investigated iron-reducing bacteria of the identified strains [40]. The abundance of *Geobacter* sp. increased during the first 7 days of incubation and then decreased, whereas the abundance of *Shewanella* sp. increased for 14 days of incubation and then decreased. The variations in the abundance of the *Geobacter* sp. and *Shewanella* sp. revealed similar trends with the transformation kinetics of PCP, suggesting that these iron-reducing bacteria may be more closely related to the PCP transformation. The results of the qPCR also showed that the 16S rRNA gene copies of *Geobacter* were greater by up to 2 orders of magnitude than that of *Shewanella* at the same incubation time, which indicates that *Geobacter* sp. were more active than *Shewanella* sp. In addition, the abundance of *Geobacter* sp. and *Shewanella* sp. in the SPLA treatment was significantly higher than that in the SPL and SP treatments

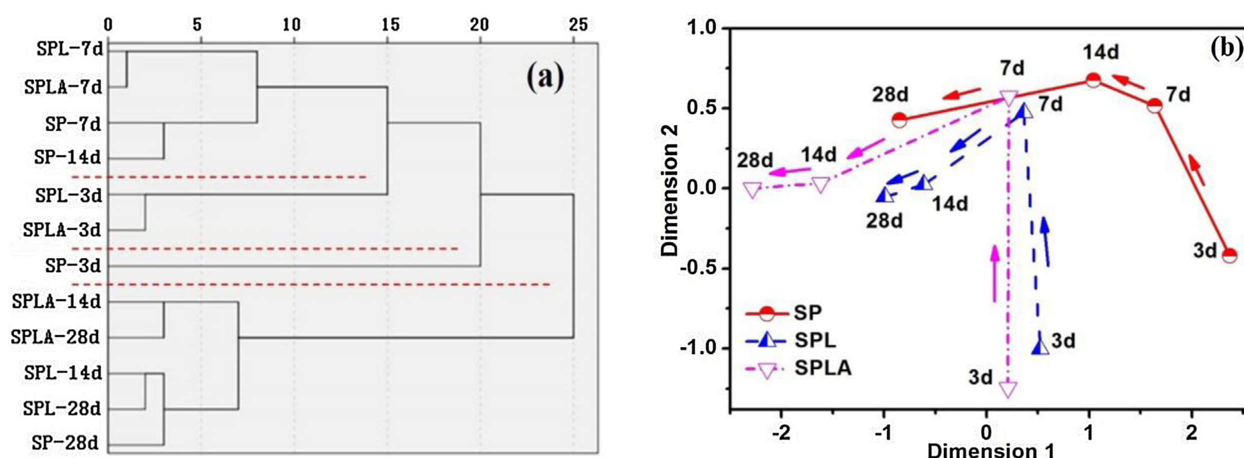

Fig. 4. (a) Hierarchical cluster results of T-RFs' relative abundance percentage for treatments and time-points; (b) MDS plots based on the T-RFLP data from 16S rRNA gene amplicons. Reaction conditions and treatment methods are the same as in Table 1.

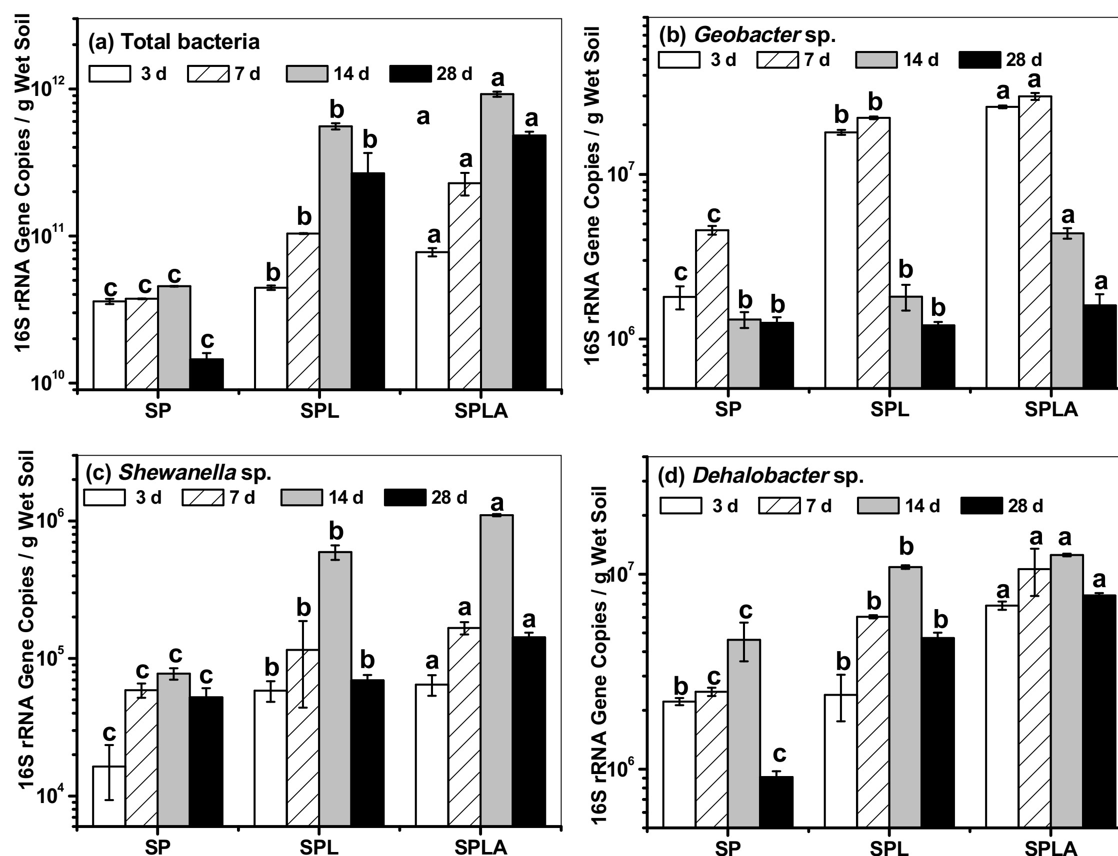

Fig. 5. DNA copy numbers of 16S rRNA genes of (a) total bacteria, (b) *Geobacter* sp., (c) *Shewanella* sp., and (d) *Dehalobacter* sp. during anaerobic PCP transformation in different treatments. The results of Tukey's test for comparison of the means for the treatment effect in the same incubation time were reported as minor letters above each bar.

(with the order of SPLA > SPL > SP) at all time intervals, which confirms that lactate and AQDS drove the growth of *Geobacter* sp. and *Shewanella* sp.

The genus *Dehalobacter* sp. has been extensively investigated as a reductive dehalogenator for chlorinated compounds, such as β-hexachlorocyclohexane, dichlorobenzenes, monochlorobenzene and PCP [17,18,41]. The abundance of *Dehalobacter* sp. in all treatments increased during the first 14 days of incubation and then decreased, similar to the trends in the abundance of T-RF 70 bp revealed by the T-RFLP results. *Sedimentibacter* sp. has been found in cultures for reductive dechlorination, and there was a specific interaction between *Sedimentibacter* sp. and *Dehalobacter* sp. [41,42]. The addition of lactate and AQDS also enhanced the abundance of *Dehalobacter* sp. during PCP transformation. *Dehalobacter* sp. is an obligate organohalide respiring bacteria that strictly depends on halorespiration for growth using hydrogen as its sole electron donor [49]. Lactate can be converted to acetate, CO_2, and H_2 in an energy-driven reaction under anaerobic conditions [17], which suggests that lactate acted as a carbon source for hydrogen production by soil indigenous microorganisms, and the produced

hydrogen acted as an electron donor for the growth of *Dehalobacter* sp. The copy number of *Dehalobacter* sp. slowly increased during the first 7 days of incubation and then increased markedly in the SP treatment, whereas *Dehalobacter* sp. increased markedly only after 3 days in the SPL and SPLA treatments. These results were consistent with the transformation kinetics of PCP in the same treatments, which indicated that the genus of *Dehalobacter* sp. also functioned for PCP transformation.

4. Conclusion

The dynamics of microbial communities and Fe(III)-reducing and dechlorinating microorganisms in response to PCP transformation under anaerobic conditions were systematically investigated in the present study. The indigenous bacterial community could quickly degrade PCP in paddy soil under different conditions. The bacterial populations during PCP transformation in all cultures were mainly composed of members of the *Veillonellaceae* and *Clostridium sensu stricto* groups. Under different incubation conditions, the community structure varied markedly from the lag phase to the dechlorination phase and then tended to be the uniform during the stationary phase. Iron-reducing bacteria and dechlorinating bacteria, including *Geobacter* sp., *Shewanella* sp., and *Dehalobacter* sp., play critical roles in PCP transformation. Lactate and AQDS were confirmed to have significant effects on the bacteria community and Fe(III)-reducing and dechlorinating microorganisms during PCP transformation, which consequently accelerated the transformation rates of PCP in paddy soil.

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