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The effect of electron donors on the dechlorination of pentachlorophenol (PCP) and prokaryotic diversity in paddy soil

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ABSTRACT

Electron donor supplements are commonly used to enhance the degradation of soil organochlorine contaminants. Dynamic processes of pentachlorophenol (PCP) dechlorination induced by adding electron donors are well known, however, their effects on the microbial communities providing these activities are only poorly understood. In this study, the effect of three electron donors (citrate, glucose and lactate) on the rate and extend of PCP dechlorination, and on the prokaryotic (bacterial and archaeal) community structure was investigated. The addition of citrate or lactate increased reductive dechlorinated rates of PCP, whereas the addition of glucose decreased the rates. High-throughput sequencing analysis of microbial communities in the microcosms found that *Pseudomonas*, *Hydrogenophaga*, *Clostridium*, *Dendrosporobacter, Burkholderiaceae*, and *Fervidicella* were the main microorganisms in treatments without electron donor additives, and treatments with citrate or lactate. With the glucose treatment, *Clostridium*, *Fervidicella*, *Sedimentibacter* and *Acetivibrio* were observed to be the main genera. The addition of citrate and lactate enhanced the population of bacteria with a known potential of reductive dechlorination, with the consequence of accelerating the dechlorination of PCP. However, the addition of glucose stimulated the activities of the organisms that can utilize glucose but cannot dechlorinate PCP. These findings indicate the critical role of different electron donors in stimulating different microorganisms and suggest the appropriate selection of electron donors for accelerating the bioremediation of PCP-contaminated soils.

1. Introduction

Pentachlorophenol (PCP) has entered the environment due to its extensive use as a herbicide, insecticide, fungicide, algaecide, disinfectant, and wood preservative over the past decades. Although PCP has been prohibited for purchase and use in China, residual PCP is still found in soils and aquatic sediments due to its persistent properties [1]. This may cause problems for organisms living in such areas and also for human health [2].

Under anaerobic conditions, PCP may serve in soils as electron acceptor (organohalide respiration) [3] and can thereby be chemically or biologically degraded via reductive dechlorination [3–5]. While the abiotically mediated reductive dechlorination process is mainly driven by chemical reductants, such as zero-valent iron particles, usually with low reduction efficiencies [4,5], bacterial reductive dechlorination is considered to be the most economical and efficient way for the remediation of PCP-contaminated soils [3]. Previous studies have reported that PCP could be degraded by a sequence of different bacteria taxa under anaerobic conditions [3,6]. For example, the organohalide-respiring bacterium, *Desulfitobacterium hafniense* strain PCP-1 isolated from PCP-contaminated soil, is capable of dehalogenating PCP into 3-chlorophenol [7]. Also, a number of *Desulfitobacterium* species, such as *Desulfitobacterium hafniense* DCB-2, *Desulfitobacterium hafniense* PCE-S, *Desulfitobacterium frappieri* TCP-A, *Desulfitobacterium dehalogenans* JW/IU-DC1, are capable of transforming PCP by reductive dehalogenation with different dehalogenation spectra under anaerobic condition [8–11]. Several studies demonstrated that some *Dehalococcoides* related strains were able to dechlorinate PCP to chlorophenol via removal of the *ortho* and *para* chlorines in different degradation pathways [12–14]. Bacteria, such as *Clostridium beijerinckii* Z, can reduce Fe(III) to Fe(II) and the latter can act as a reactive reductant for PCP transformation [5]. However, the natural attenuation process of PCP in soil by active Fe (II) species or indigenous microorganisms often requires long periods due to the low microbial population sizes and activities of bacteria with

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dechlorinating abilities resulting in longer half-life values of PCP in soils (> 45 days) [3]. Therefore, stimulating the growth of indigenous soil bacteria with potential PCP-degrading abilities in order to accelerate the biodegradation of PCP is an option for enhancing in situ remediation of soils.

Enhanced natural attenuation (biostimulation) is an efficient technology for soils remediation in which existing bacteria with the desirable abilities are stimulated with the supplements nutrients, electron donors, electron acceptors, or trace minerals in order to dechlorinate/degrade pollutants at contaminating sites [15]. The addition of electron donors is widely used to remediate the organochlorine-contaminated soil and aquifer sediment environments under anaerobic conditions [15,16]. For example, 71% removal of carbon tetrachloride was observed in 40 days in acetate-amended wastewater compared to 10% carbon tetrachloride removal in wastewater not amended with acetate [17]. PCP degradation rates could be enhanced with the addition of lactate, pyruvate, or acetate as electron donors [18]. Addition of glycerol stimulated reductive dechlorination of *cis*-dichloroethene and vinyl chloride at a former trichloroethene contamination site [19]. Therefore, electron donor amendment to soil may be a promising approach to enhance biodegradation of organochlorine contaminants, including PCP, tetrachloride and others [3,6,15,20].

During microbial reductive dechlorination, PCP may act as a terminal electron acceptor in organohalide respiration and H_2 (supplied from organic acids derived from fermentation reactions) or organic acids, e.g. formate or lactate, are used as electron donors. For example, PCP was dechlorinated to 3-chlorophenol and phenol with excess lactate as an electron donor [20]. PCP also could be dechlorinated to 3,5-dichlorophenol by *Dehalococcoides mccartyi* strain JNA in pure culture using citrate as electron donor [13]. Furthermore, growth and dechlorination activities of *Dehalococcoides mccartyi* appeared to be more robust in mixed microbial communities, in which other organisms could provide hydrogen and hydrocarbons (e.g., acetate) as electron and carbon donors, respectively [21]. However, dechlorinating bacteria coexist in communities with other soil microorganisms, where they growth and may compete for electron donors, e.g.with iron-reducing or fermentative bacteria or methanogenic archaea [16].

Previous studies have indicated that following the addition of electron donors, the indigenous microbial communities changed, which had a subsequent effect on their dechlorination abilities in soils [18,22,23]. For example, the addition of crop residues enhanced PCP dissipation by supplying dissolved organic carbon as electron donor, and the microbial communities were modified [22]. A previous study demonstrated that the addition of lactate led to enhanced rates of PCP dechlorination by stimulating the abundance of the bacteria with potential dechlorinating abilities [23]. However, these studies mainly traced the dechlorinating bacteria taxa (using 16S rRNA gene detection) rather than the whole microbial communities and the analyses were based on clone libraries and Sanger sequencing [6,22–24], thus having a low resolution compared to the possibilities provided now by high-throughput DNA sequencing of PCR amplified 16S rRNA gene products.

The objective of this study was to analyze the effects of three different electron donors (citrate, glucose and lactate) on the biodegradation of PCP and soil prokaryotic community structure with an emphasis on analyzing the abundance of bacteria with known potentials for reductive dechlorination of organochlorides including PCP.

2. Materials and methods

2.1. Soil sampling

Soil samples were collected from the A horizon (0–15 cm, the leached layer) in a paddy field located at the Dongpu town (22°15′22.2″N, 112°52′19.92″E), Zhongshan City, Guangdong Province, China. The sampling site had been planted for at least 15 years and where fertilizers and pesticides have been regularly used. After being passed through a 2-mm mesh sieve within one week, the subsamples were refrigerated in an anaerobic bag at 4 °C before use. The basic physicochemical properties of the soil were as follows: pH (5.89), organic matter (74.9 $g\,kg^{-1}$), Fe_2O_3 (81.6 $g\,kg^{-1}$), complex-Fe (1.13 $g\,kg^{-1}$), and amorphous-Fe (8.46 $g\,kg^{-1}$). Soils for control experiments were sterilized by 50-kGy gamma irradiation before use.

2.2. Batch experiments of PCP degradation in the soil

All experiments were carried out in a vinyl anaerobic chamber (96% N_2, 4% H_2, Coy Lab., USA) with O_2 concentrations kept below 1 ppm by continual atmospheric circulation over a Pd catalyst. Microcosms were prepared in triplicate and initiated by combining 0.5 g of soil (dry weight) to 10 ml of 1,4-piperazinediethanesulfonic acid (PIPES, 30 mM) buffer solution for pH 7.0 in 20-ml serum bottles with silicone-lined septa and aluminum sealing caps at 25 ± 1 °C. Electron donors (including citrate, glucose and lactate) and PCP were added to the microcosms and the resulting aqueous-phase concentration were measured to be 10 mM (each electron donor concentration) and 19 μM, respectively. The PCP degradation experiment set-ups are summarized in Table 1, including (1) without electron donor control, CK treatment: soil + PIPES + PCP; (2) with electron donor treatments: soil + PIPES + PCP + citrate/glucose/lactate. To further disclose the role of microorganisms for PCP degradation, four sterile control treatments (sterile soil + PIPES + PCP and sterile soil + PIPES + PCP + citrate/glucose/lactate) were set up to study the contribution of chemical effect on PCP degradation. Prior to use, the water was deoxygenated by sparging with N_2 (99.99%) for 30 min before being transferred into the chamber and then exposed to the anaerobic chamber atmosphere to equilibrate with gas phase for at least 1 h. The stock solutions of PCP, PIPES, citrate, glucose and lactate were prepared inside the anaerobic chamber by dissolving their respective sodium salts in deoxygenated water. Chemical reagents and labware were placed inside the chamber for more than 24 h before use. At specific time intervals, three individual bottles from each treatment were sacrificed for sampling.

PCP concentrations were determined by high-performance liquid chromatography (HPLC) (Waters 1525/2487) with an Xterra C18 reverse-phase column, and the determination of PCP transformation products was performed using a gas chromatograph-mass spectrometry (GS-MS) (Thermo Fisher Trace-DSQ-2000) with electron ionization and Agilent HP-5MS fused silicon capillary column (0.25 mm × 30 m). The

Table 1
Treatment methods and kinetics parameters of pentachlorophenol (PCP) transformation experiments under different conditions. Degradation rates of PCP were obtained with the logistic degradation curve ($(C_0\text{-}C_t)/C_0 = a/(1 + Be^{-kt})$) under different electron donors conditions. a: The maximum degradation fraction; k: The degradation rate constant; μ_{max}: The maximum degradation rate ($\mu_{max} = 0.25ak$).

Treatment	a	k	μ_{max}	R^2
Soil + PIPES + PCP	100%	0.395 ± 0.041	0.111 ± 0.015^B	0.996
Soil + PIPES + PCP + citrate	100%	1.496 ± 0.220	0.394 ± 0.059^A	0.990
Soil + PIPES + PCP + glucose	41.2%	0.136 ± 0.045	0.014 ± 0.004^C	0.895
Soil + PIPES + PCP + lactate	100%	1.811 ± 0.286	0.452 ± 0.034^A	0.990

A, B and C within the same column indicate the significance of difference ($p < 0.01$).

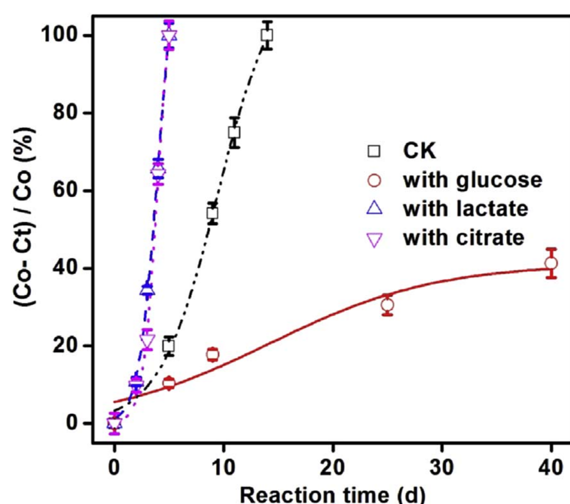

Fig. 1. Pentachlorophenol (PCP) dechlorination processes in all non-sterile treatments with different electron donors, including without electron donor addition (CK), with citrate, glucose and lactate, under anaerobic conditions at 25 °C and pH 7.0.

HPLC and GC-MS analytical procedures were the same as previous described [25].

2.3. DNA extraction, PCR amplification, and high throughput sequencing

Soil samples (0.25 g, wet weight) of the original soil collected and the soil in the four non-sterile treatments after reaction for 15 days were collected separately for molecular analysis. The microbial community of soil sample collected from paddy field was also analyzed as control. Soil DNA samples were extracted separately from each microcosm with the Power Soil DNA kit (MO BIO Laboratories, USA) according to the manufacturer's recommendations. PCR amplification of bacterial and archaeal 16S rRNA genes fragments was performed using the primer set F515 and R806, with a sample-specific 12-bp barcode added to F515 [26]. Each DNA sample was amplified in 30 μl reaction mixtures in triplicate, and replicate amplicons were pooled for purification with a QIAquick Gel Extraction Kit (Qiagen, Chatsworth, CA) and sequenced using an Illumina Miseq (paired-end 250-bp mode) pyrosequencer (Illumina, San Diego, CA).

2.4. Analyses of Illumina MiSeq sequencing data

The 16S rRNA MiSeq sequencing data were processed and analyzed using Mothur [27] and UPARSE [28]. The raw reads with a quality less than 20 at the 3′ end were trimmed and assembled into contigs. Then, the commands “screen.seqs”, “unique.seqs”, “align.seqs”, “filter.seqs”, “pre.cluster”, and “chimera.uchime” in Mothur were used to reduce sequencing error, and identify and remove chimeras. The resulting high-quality sequences were grouped into operational taxonomic units (OTUs) with UPARSE using the “cluster_otus” command [28]. Briefly, this step includes chimera filtering based on models built from more abundant reads, trimming the sequences to a fixed length, optionally discarding singleton reads and then clustering the remaining reads. Sequences with a similarity of 97% were assigned to the same OTU. The taxonomic assignment was determined using the Ribosomal Database Project (RDP, https://rdp.cme.msu.edu/) at an 80% threshold. The relative abundance (%) of individual taxa within each community was estimated by comparing the number of sequences assigned to a specific taxon versus the number of total sequences obtained for that sample.

As the minimum number of quality sequences per community was 25,398 across all 5 samples, calculations of alpha-diversity (including phylotype richness, Faith's phylogenetic diversity [PD], Chao1, Simpson, Shannon and Dominance) and beta-diversity (Bra-Curtis distance) metrics were conducted based on a subset of 25,398 randomly selected sequences per community with 30 iterations. Bray-Curtis-based principal-coordinate analysis (PCoA) was used to find the differences between the original soil sample and the reacted soil samples in the different treatments with electron donors.

2.5. Statistical analyses

Statistical analyses of the experimental data were performed using the SPSS 20.0 statistical software. Differences of the PCP dechlorinated rates, relative abundance of the genera and diversity index between CK treatment and electron donor amended treatment were determined by a one-way analysis of variance (ANOVA) on ranks followed by Fisher's least-significant-difference (LSD) test using parametric method. In all cases, differences were deemed to be significant at $p < 0.05$. The microbial communities from the incubation cultures of different batches were analyzed by principal coordinate analysis (PCoA) with pairwise unweighted UniFrac distance using the QIIME software package.

2.6. DNA sequences deposition

The gene sequences obtained from this study were deposited in the NCBI Sequence Read Archive (SRA) with the project accession number of SRR5429657.

3. Results

3.1. Effect of adding electron donors on PCP dechlorination activities

The kinetics of PCP reductive dechlorination in all treatments under non-sterile and sterile conditions are provided in Fig. 1 and Supplemental Material Fig. A1, respectively. Only 5.5%–7.5% of the added PCP removals were detected after 40 days in all four sterile treatments most likely due to PCP sorption on surface active soil particles [25]. In contrast, the significantly lower PCP concentrations found in all four non-sterile soil incubations indicated the important role of biological PCP dechlorination. The processes of PCP dechlorination in non-sterile conditions can be fitted to a logistic degradation curve, which is usually used for the biodegradation of organic compounds [23]. Based on the logistic model, the maximum dechlorination rate (μ_{max}) and kinetic rate constants (k) of PCP for the four non-sterile treatments were derived and listed in Table 1. In the absence of electron donors (CK treatment), 100% of PCP was degraded within 14 days with a μ_{max} value of 0.111 d^{-1}. The addition of citrate and lactate significantly increased the PCP degradation rate with the μ_{max} values increased to 0.394 d^{-1} and 0.452 d^{-1}, respectively, with PCP degradation rates of 100% achieved in both cases after 5 days. Contrarily, the addition of glucose led to a decreased PCP degradation rate, with a μ_{max} value of 0.014 d^{-1} and only 41.2% of PCP degradation obtained even after reaction for 40 days.

The addition of citrate and lactate affected the dechlorination extents of PCP. The metabolites 2,3,4,5-TeCP and 3,4,5-TCP were detected in all treatments in the first 7 days (Fig. 2). Phenol was detected as the end product of PCP dechlorination in the citrate and lactate treatments after incubation 28 days. However, only 2,3,4,5-TeCP and 3,4,5-TCP were produced after reaction for 40 days in the glucose treatment.

3.2. Generation of active Fe(II) species during PCP dechlorination

The 0.5 M HCl-extracted Fe(II) are critical active species for accelerating the reductive transformation of organic pollutants [5,29]. The concentrations of produced 0.5 M HCl-extracted Fe(II) during the PCP dechlorination under different conditions are shown in Fig. 3 and Supplemental Material Fig. A2. Only 2.0–2.6 mM of HCl-extracted Fe (II) were generated after reaction for 40 days in four sterile treatments

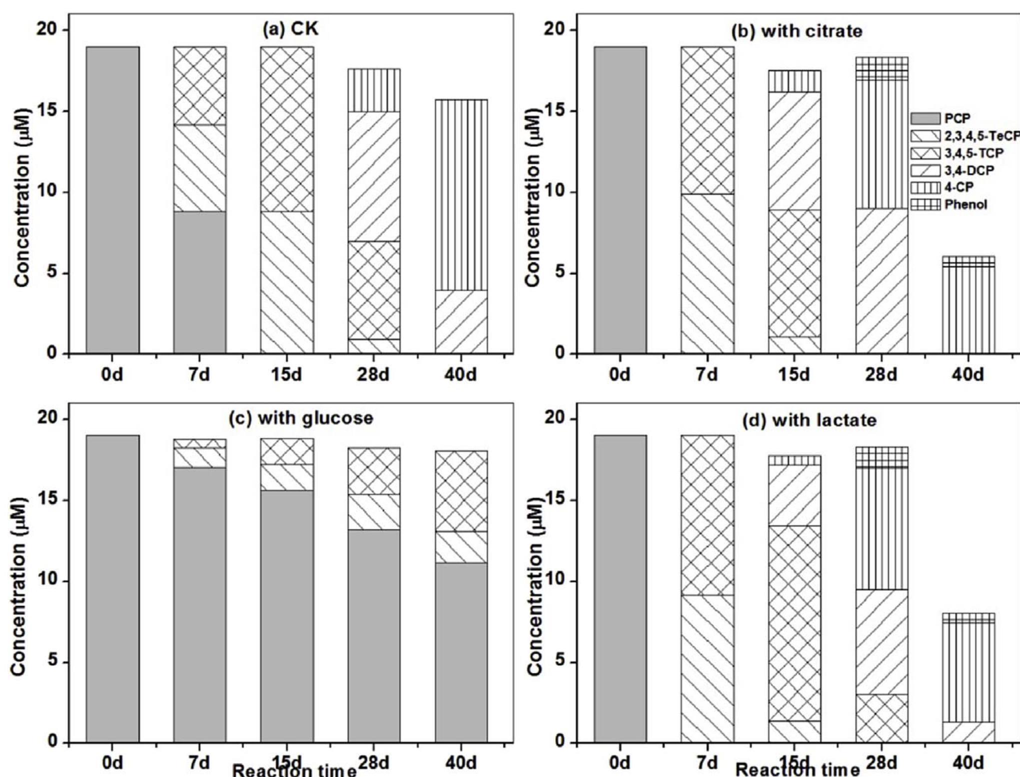

Fig. 2. The concentration of pentachlorophenol (PCP) anaerobic transformation intermediates across reaction time in all treatments, including without electron donor addition (CK), with citrate, glucose and lactate.

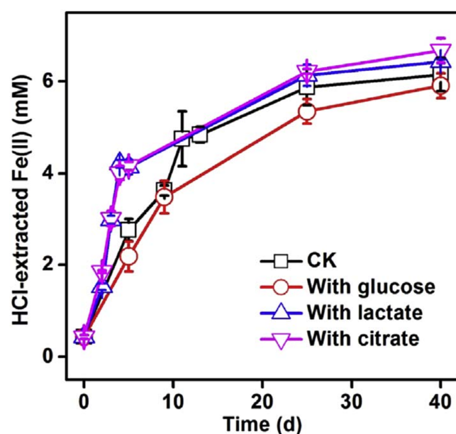

Fig. 3. The generation of 0.5 M HCl-extracted Fe(II) during pentachlorophenol (PCP) dechlorination in all treatments, including without electron donor addition (CK), with citrate, glucose and lactate, under non-sterile condition.

(Supplemental Material Fig. A2). However, the concentrations of HCl-extracted Fe(II) in all four non-sterile treatments increased throughout the reaction, which indicates that most of the Fe(II) species were microbially generated. In addition, as shown in Fig. 3, the number of generated Fe(II) species increased during incubation time in all treatments. Compared to CK treatment (without electron donor addition), the addition of lactate and citrate increased the concentration of Fe(II) species, while decreasing with the addition of glucose. The different concentrations of Fe(II) species with the addition of these three electron donors showed similar trends for altered PCP degradation rates.

3.3. Response of the prokaryotic community structure to PCP amendments

Based on the MiSeq sequencing data, a total of 153,648 high quality sequences were obtained across all analyzed samples, with a range of 25,398 to 42,562 sequences per community (Table 2). The number of unique, classifiable and representative OTUs was 3976 in total. Compared to the treatment without electron donor (CK treatment), lactate addition resulted in a significantly lower alpha diversity index ($p < 0.05$), while glucose addition exhibited a significantly higher alpha diversity ($p < 0.05$). Citrate had no significant effect on the change of alpha diversity index.

Table 2
Summary of 16S rRNA Miseq sequences, operational taxonomic units (OTUs), and microbial diversity estimates. The numbers marked with the treatments refers to the sampling day.

Sample ID	No.of Seqs	No.of OTUs	Observed species	Shannon	Chao1	Simpson
Soil	28,848	1560	1470	6.02	2365	0.903
CK	28,908	1276	1209	6.07	1771	0.921
Citrate	25,398	1262	1256	6.04	1994	0.918
Glucose	27,932	1376	1320	7.04	1986	0.967
Lactate	42,562	1242	1002	5.00	1670	0.845

Bray–Crutis-based PCoA was performed on the sequencing data to assess the differences of microbial communities across all analyzed samples (Fig. 4). PCoA results showed that the soil samples amended PCP clustered together, which significantly distinguished from the original soil (without any treatment) ($p < 0.01$). Interestingly, the treatment with glucose showed a significant difference in microbial community composition from the other three treatments (CK, citrate and lactate) ($p < 0.01$). These results demonstrated that bacterial communities in PCP-amended soils were strongly altered by the addition of glucose, but not so much by citrate or lactate.

3.4. Diversity of prokaryotes at the phylum level

The dominant 16S rRNA genes were assigned to bacteria and

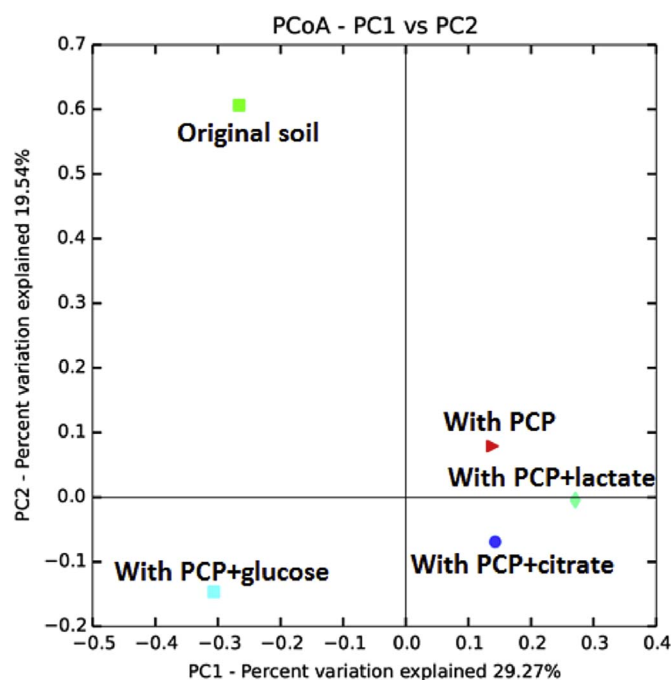

Fig. 4. Principal-coordinate analysis (PCoA) of microbial community composition (using 16S rRNA MiSeq sequencing) based on the Bray-Curtis similarity metrics, showing the differences among the original soil and all PCP-amended microcosms, including without electron donor addition (CK), with citrate, glucose and lactate, after incubation 15 days. The percentages of variation in the microbial communities described by the plotted principal coordinates are indicated on the axes.

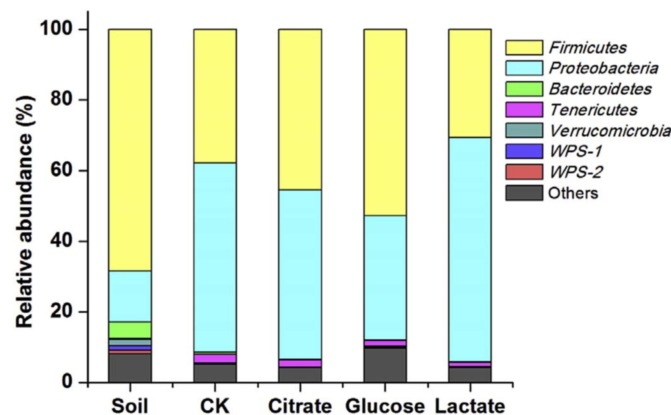

Fig. 5. The relative abundance (%) of dominant microbial taxa in all analyzed samples, including the original soil and all PCP-amended microcosms, including without electron donor addition (CK), with citrate, glucose and lactate, revealed by 16S rRNA MiSeq sequencing. Others include *Acidobacteria*, *Actinobacteria*, *Armatimonadetes*, BRC1, *Candidatus* Saccharibacteria, *Chlamydiae*, *Chlorobi*, *Chloroflexi*, *Cloacimonetes*, *Cyanobacteria*, *Deinococcus-Thermus*, *Elusimicrobia*, *Euryarchaeota*, *Fibrobacteres*, *Fusobacteria*, *Gemmatimonadetes*, *Hydrogenedentes*, *Ignavibacteriae*, *Latescibacteria*, *Nitrospiare*, *Parcubacteria*, *Planctomycetes*, *Spirochaete*, *Synergistetes*, *Thermotogae*, *Verrucomicrobia* and unclassified bacteria (sequences belonging to the Bacteria not classifiable at the phylum level).

archaeal taxa. Their relative abundance and changes in response to the soil amendments with electron donors are shown in Fig. 5. In all 5 analyzed samples, members of *Bacteria* were the predominant prokaryotes and made up 99.9% of the classifiable 16S rRNA sequences, whereas 0.1% of the sequences could be assigned to *Archaea*. Bacterial sequences belonged to 31 phyla, but only 10 were recorded with an abundance over 1%. In all samples, *Firmicutes* and *Proteobacteria* were dominant, comprising 82.9%–94.1% of all sequences, respectively. These results were roughly in accordance with previous reports, which found that bacteria from *Firmicutes* and *Proteobacteria* were the predominant groups in the microbial communities during PCP transformation in soils [24,25]. However, the relative abundance of the main phyla varied greatly among different treatments (Supplemental Material Fig. A3). Compared with the original soil sample (0 day), the addition of PCP resulted in a decrease in the relative abundance of *Firmicutes* and an increase in the relative abundance of *Proteobacteria* even without the addition of any electron donor. Compared to the control without additional electron donors (CK treatment), the addition of citrate and glucose increased the relative abundance of *Firmicutes*, whereas decreased with the addition of lactate. The relative abundance of *Proteobacteria* decreased in the treatment with the addition of citrate and glucose, whereas increased with the addition of lactate. These results indicate that the addition of electron donors had obvious effects on bacterial communities during the PCP degradation in soils.

3.5. Diversity of prokaryotes at the genus level

About 80–93% of the all 16S rRNA gene sequences could be assigned to taxonomically ranks below the phylum level. Among them, for 59% fell into a known genus level. Therefore, the relative abundance of each phylotype was analyzed at the genus level to investigate which bacterial populations were affected by the electron donors during PCP dechlorination (Supplemental Material Table A1).

The dominant genus in the original soil sample was *Symbiobacterium* with a high relative abundance of 56%, which was significantly different from samples with PCP and electron donor added ($p < 0.01$) (Fig. 6, Supplemental Material Table A1). The second most abundant genus in the original soil sample was *Flavisolibacter* with a relative abundance of 3.9%. Clearly, *Hydrogenophaga* (22.9%–35.5%) was the most abundance genus in the CK, citrate and lactate treatments, while *Fervidicella* (9.6%) was the dominant genus in the glucose treatment. With the addition of citrate, *Burkholderia* and *Sedimentibacter* became the second and third most abundance genera, followed by *Dendrosporobacter*, *Azospirillum*, *Clostridium*, *Fervidicella*, *Acetivibrio*. Similar to the

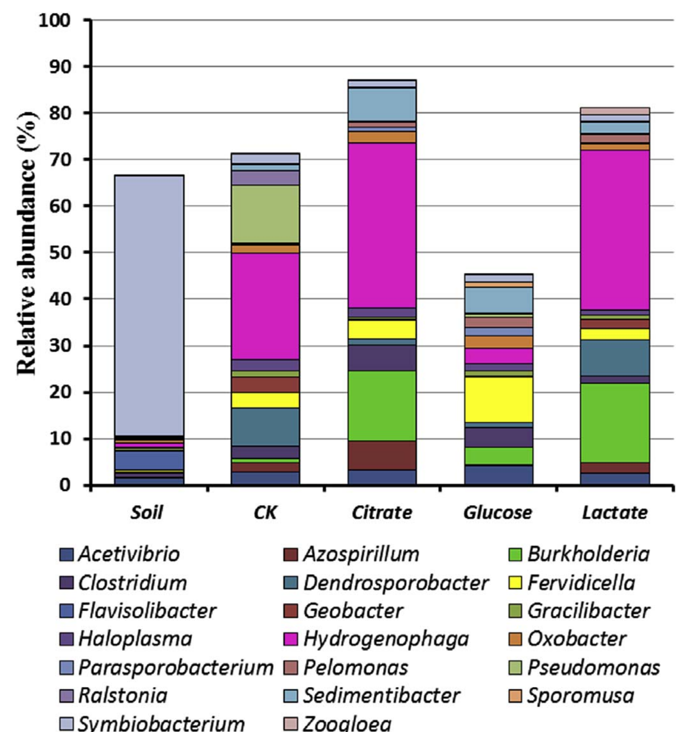

Fig. 6. The relative abundance (%) of microbial genera (> 1%) in the original soil and all PCP-amended microcosms, including without electron donor addition (CK), with citrate, glucose and lactate, revealed by 16S rRNA MiSeq sequencing.

Table 3
The relative abundances of organohalide-respiring bacteria in the original soil and all PCP-amended microcosms, including without electron donor addition (CK), with citrate, glucose and lactate.

	Dehalococcoides	*Dehalobacter*	*Desulfitobacterium*
Soil	0.007 ± 0.001	0.028 ± 0.002	0.052 ± 0.030[d]
CK	/	0.038 ± 0.008	0.277 ± 0.069[b]
Citrate	/	0.037 ± 0.016	0.427 ± 0.084[a]
Glucose	/	0.027 ± 0.001	0.171 ± 0.019[c]
Lactate	0.005 ± 0.008	0.042 ± 0.033	0.415 ± 0.059[a]

a, b, c and d within the same column indicate the significance of difference ($p < 0.05$).

control without additional electron donors (CK treatment) and the treatment with citrate, *Hydrogenophaga* was also found to be predominance in the prokaryotic community with the addition of lactate, and the relative abundance was up to 34.3%. *Burkholderia* and *Dendrosporobacter* were the other two main genera with the relative abundance of 17.0% and 7.8%, respectively. Interestingly, the relative abundance of *Hydrogenophaga* was only at an average value of 3.0% in the glucose treatment, whereas *Fervidicella* (9.6%), *Sedimentibacter* (5.6%), *Clostridium* (4.4%), *Acetivibrio* (4.2%) and *Burkholderia* (3.6%) were the other dominant genera.

High-thought sequencing analysis results showed that organohalide-respiring bacteria, including *Dehalobacter* and *Desulfitobacterium*, were found in all treatments with relative low abundances (< 1%) (Table 3). However, *Dehalococcoides* was only found in the original soil sample and lactate treatment.

4. Discussion

In the present study, the addition of citrate and lactate significantly stimulated the dechlorination rates of PCP in the soil ($p < 0.01$). In contrast, the addition of glucose inhibited the dechlorination capacity of the soil (Fig. 1, Table 1). Previous studies have reported that the supplementation of electron donors into soils could accelerate the degradation of organic pollutants under various conditions [18,20,24]. Citrate has been reported to act a bio-stimulant and increase bacterial degrader cell survival and thus accelerate the degradation of herbicides [30]. In other study, the activities of dechlorinating bacterial community were enhanced after addition of lactate to paddy soil, which further increased PCP degradation rates [24]. Our previous studies indicated that the addition of lactate in paddy soil increased PCP degradation rate through enhancing the abundances of the bacteria with potential dechlorinating abilities, such as such as iron-reduction and dechlorination [23,25]. Hence, we inferred that the addition of citrate and lactate in this study could also stimulate the abundance of bacteria with dechlorinating abilities. Previous report showed that glucose had a stimulatory effect on the anaerobic dechlorination rate of PCP in granular sludge by acting directly as a source of reducing equivalents or as a growth substrate for the dechlorinating organisms [31]. However, some organohalide-respiring organisms, such as *Desulfitobacterium* and *Dehalococcoides*, cannot grow on glucose [9,32].

Symbiobacterium and *Flavisolibacter* were the most abundant genera in the original soil samples. *Symbiobacterium* has been reported to be the dominant bacterium as a symbiont with denitrifiers in rice paddy soil [33]. *Flavisolibacter* has been previously reported as a common microbial community member in paddy soil [34]. The main genera of *Hydrogenophaga*, *Pseudomonas*, *Dendrosporobacte*, *Fervidicella, Geobacter* and *Ralstonia* were detected as the most dominant genera in microcosms where no electron donor was added (CK treatment) (Fig. 6, Supplemental Material Table A1). *Hydrogenophaga* is known as an aerobic dechlorinating bacterium [35], which can grow autotrophically using H_2 as an electron donor [36]. *Pseudomonas* has been reported to be a ubiquitous bacterium with a good potential for bioremediation, and there are many species of the genus *Pseudomonas* with the capability to degrade many chlorophenols, including PCP, trichlorophenol (TCP), dichlorophenol (DCP) and chlorophenol (CP), under aerobic condition and probably also under denitrifying conditions [3,6]. *Dendrosporobacter* can grow chemoautotrophically with H_2/CO_2, and can play an important role in trichloroethene dechlorination as potential corrinoid providers to *Dehalococcoides* [37]. *Fervidicella* has been reported to reduce Fe(III) to Fe(II) by using glucose as carbon sources, and the glucose was fermented to ethanol, acetate, CO_2, and H_2 [34]. *Geobacter* is known as anaerobic tetrachloroethene dechlorinator, but does not dechlorinate chlorophenols [38]. However, *Geobacter* is a well-known dissimilatory iron-reducing bacterium, which can shuttle electron to iron oxides [39]. *Ralstonia* had been described in environments contaminated with phenol and showed polychlorophenol-degrading capacity under aerobic condition [40]. Overall, many dominant OTUs were assigned to bacterial taxa known to be capable of degrading organochlorine compounds and/or reducing iron (Fe(III)) or nitrate.

Compared to the control without additional electron donors (CK treatment), the proliferation of *Burkholderia* was stimulated by the addition of citrate and lactate (Fig. 6, Supplemental Material Table A1). *Burkholderia* has been reported to degrade phenol (the products of PCP dechlorination) in anaerobic paddy soil in our previous study [41]. PCP could be reductively dechlorinated to phenol by organohalide-respiring bacteria and subsequent degradation of phenol by *Burkholderia*. Phenol was detected after incubation for 28 days in the citrate and lactate treatments (Fig. 2). This indicated that the addition of citrate and lactate could enhance growth of *Burkholderia*, which could further degrade the phenol, the metabolite of PCP dechlorination, in this study. *Sedimentibacter*, *Azospirillum* and *Clostridium* were also detected as main genera in the citrate treatment. *Sedimentibacter* is unable to dechlorinate, but promotes growth of organohalide-respiring bacteria via synthesizing cobalamin [42], an essential cofactor of reductive dehalogenases. *Clostridium* has been reported to be an iron-reducing bacterium, which dechlorinate PCP indirectly via iron reduction [5]. Furthermore, *Clostridium* was able to grow on citrate, fermenting it to acetate, ethanol, CO_2, and H_2 [43]. These results suggested that *Clostridium* can be stimulated by the addition of citrate, which could further enhance the potential reductive dechlorination abilities under the conditions prevalent in the microcosms of this study.

The dominant genera in the glucose treatment were different (Fig. 6, Supplemental Material Table A1). The relative abundance of *Hydrogenophaga* in the glucose treatment was clearly lower than that in other treatments (including CK, citrate and lactate), likely because *Hydrogenophaga* can probably not utilize glucose as a growth substrate [44]. *Acetivibrio* is an anaerobic cellulolytic bacteria adapted for growth on glucose, which is metabolized to pyruvate by the Embden-Meyerh of pathway [45].

A range of organohalide-respiring bacteria, including *Dehalobacter*, *Dehalococcoides* and *Desulfitobacterium*, were found in this study. *Dehalobacter* strains are obligate organohalide-respiring bacteria that use hydrogen as energy source and electron donor and acetate as a carbon source [32]. *Dehalococcoides* is a strict anaerobe using only H_2 as the electron donor and chlorophenol as respiratory electron acceptors [14,32]. Although *Dehalobacter* and *Dehalococcoides* were detected in this study, the relative abundances of them were low. *Desulfitobacterium* strains are more widely distributed in anaerobic terrestrial environments, and most of them have been isolated during the course of studies of reductive dechlorination processes [32]. *Desulfitobacterium* strains are able to dechlorinated *ortho*-position PCP rather than the *meta*- or *para*-position, and generally using formate, lactate and pyruvate as electron donors [8–10,32]. The sequence of dechlorination was the same as our found in this study. Furthermore, compare to the original soil, the relative abundance of *Desulfitobacterium* was enriched after PCP amendment (Table 3). The addition of citrate and lactate also enhanced the abundance of *Desulfitobacterium* largely ($p < 0.05$).

Active iron species (Fe(II)) produced by iron-reducing bacteria may enhance the dechlorination of PCP [5,29]. However, the results for the

concentrations of 0.5 M HCl-extracted Fe(II) showed no significant difference among the treatments ($p > 0.05$) (Fig. 3). Bacteria with iron-reducing abilities, such as *Fervidicella*, *Geobacter* and *Clostridium*, were other main genera in this study, especially in the treatment with glucose (Fig. 6, Supplemental Material Table A1). Compared to the control without additional electron donors (CK treatment), the relative abundances of iron-reducing bacteria were significantly enhanced by the addition of glucose ($p < 0.05$). There were no consistent patterns of variation for the concentration of 0.5 M HCl-extracted Fe(II) and PCP dechlorination, indicating no or only a small contribution of active Fe (II) species on PCP degradation. This result also indicated that iron-reducing bacteria were not the main driven force for PCP dechlorination.

Except for the suspected dechlorinating bacteria, the relative abundance of *Sedimentibacter*, *Acetivibrio* and some other bacteria, which have not been reported to have PCP dehalogenation capacity, were also enhanced by the addition of electron donors (Fig. 5, Supplemental Material Table A1). This indicated that the addition of electron donors did not only stimulate the activities of these bacteria but also others, among them possibly yet unknown taxa with a dechlorination activity. Previous study had reported that electron donors, including lactate, methanol, propionate and butyrate, appeared to be suitable electron donors to promote reductive dechlorination of TCE by creating competitive advantage for dechlorinating bacteria [46]. Contrarily, the bacteria without dechlorinating abilities would compete against the bacteria with potential dechlorinating abilities for electron donors in some cases [16]. Both stimulatory and inhibitory effects of electron donors on reductive dechlorination can occur simultaneously in soil environments.

Based on the above results, the bacterial community composition was critical for PCP dechlorination. Therefore, the possible mechanism of accelerated PCP dechlorination with the additions of different electron donors in this study was as followed: the addition of citrate and lactate stimulated the activities of the bacteria with dechlorinating abilities (e.g. *Desulfitobacterium*) by acting as electron donors, which enhanced the rates of PCP dechlorination. However, glucose could not be utilized by some dechlorinating bacteria, like *Desulfitobacterium*. Furthermore, the addition of glucose could stimulate glucose-utilizing microorganisms that compete for electrons or energy with the bacteria with dechlorinating abilities, which subsequently inhibits PCP degradation.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejsobi.2018.01.008.

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