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Microbial community response to the toxic effect of pentachlorophenol in paddy soil amended with an electron donor and shuttle

Manjia Chen [a,b,1], Hui Tong [a,b,1], Jiangtao Qiao [a,b], Yahui Lv [a,b], Qi Jiang [a,b], Yuanxue Gao [a,b], Chengshuai Liu [a,b,c,*]

[a] Guangdong Key Laboratory of Integrated Agro-environmental Pollution Control and Management, Institute of Eco-environmental and Soil Sciences, Guangdong Academy of Science, Guangzhou, 510650, China
[b] National-Regional Joint Engineering Research Center for Soil Pollution Control and Remediation in South China, Guangzhou, 510650, China
[c] State Key Laboratory of Environmental Geochemistry, Institute of Geochemistry, Chinese Academy of Sciences, Guiyang, 550081, China

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ABSTRACT

Understanding the degradation of pentachlorophenol (PCP) by indigenous microorganisms stimulated by an electron donor and shuttle in paddy soil, and the influences of PCP/electron donor/shuttle on the native microbial community are important for biodegradation and ecological and environmental safety. Previous studies focused on the kinetics and the microbial actions of PCP degradation, however, the effects of toxic and antimicrobial PCP and electron donor/shuttle on the microbial community diversity and composition in paddy soil are poorly understood. In this study, the effects of PCP, an electron donor (lactate), and the electron shuttle (anthraquinone-2, 6-disulfonate, AQDS) on the microbial community in paddy soil were investigated. The results showed that the presence of PCP reduced the microbial diversity compared to the control during PCP degradation, while increased the microbial diversity was observed in response to lactate and AQDS. The addition of PCP stimulated the microorganisms involved in PCP dechlorination, including *Clostridium*, *Desulfitobacterium*, *Pandoraea*, and unclassified *Veillonellaceae*, which were dormant in raw soil without PCP stress. In all of the treatments with PCP, the addition of lactate or AQDS enhanced PCP dechlorination by stimulating the growth of functional groups involved in PCP dechlorination and by changing the microbial community during dechlorination process. The microbial community tended to be uniform after complete PCP degradation (28 days). However, when lactate and AQDS were present simultaneously in PCP-contaminated soil, lactate acted as a carbon source or electron donor to promote the activities of microbial community, and AQDS changed the redox potential because of the production of reduced AQDS. These findings enhance our understanding of the effect of PCP and a biostimulation method for PCP biodegradation in soil ecosystems at the microbial community level, and suggest the appropriate selection of an electron donor/shuttle for accelerating the bioremediation of PCP-contaminated soils.

1. Introduction

Pentachlorophenol (PCP) is one of the most toxic and persistent chlorophenols and has been widely used as pesticide, herbicide and wood preservative (Vallecillo et al., 1999). It has been detected in surface waters, sediments, aquatic organisms, surface soils and food, as well as in human milk (Field and Sierra-Alvarez, 2008; Hong et al., 2005). PCP is still a critical environmental concern due to its molecular stability and sorption properties, which may cause problems for organisms in soil and also for human health (Hong et al., 2005). As a result, much attention is paid to remove PCP from the environment (Field and Sierra-Alvarez, 2008).

In water-logged soil environments, microorganisms are the driven force for the nutrient cycle and geochemical dynamics of elements (Xu et al., 2019), and the changes of diversity and distribution of microorganisms would further influence the functional ecosystems and soil quality (Lakshmanan et al., 2014; Siczek et al., 2020). Previous reports have found that biologically mediated degradation is the main transfer

* Corresponding author. Institute of Geochemistry, Chinese Academy of Sciences, Guiyang, 550081, China.
E-mail address: liuchengshuai@vip.gyig.ac.cn (C. Liu).
[1] Manjia Chen and Hui Tong contributed equally to this work.

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pathway of PCP in anaerobic soil, in which PCP acts as an electron acceptor and is then transformed into less toxic compounds (Chen et al., 2016b; Lopez-Echartea et al., 2016). Additionally, the microbial community structure would change to adapt to and engage in the process of PCP degradation under PCP-stress conditions (Li et al., 2019; Siczek et al., 2020; Xu et al., 2019). More important, supplemental nutrients, such as organic acids and humic substances, are usually added to the growth of indigenous soil bacteria with potential PCP-degrading abilities in order to accelerate the biodegradation of PCP (Chen et al., 2018; Li et al., 2019; Tong et al., 2018; Zhang et al., 2015). However, indigenous soil microorganisms are sensitive to exogenous toxic or non-toxic compounds, which could result in the degeneration of soil environment and crop quality (Li et al., 2010; Urrutia et al., 2013). Therefore, understanding the behavior of a native microbial community in paddy soil under PCP stress, organic acids and humic substances is crucial for PCP biodegradation and ecological and environmental safety.

In paddy soil environment, lactate, which is secreted by rice roots, is a byproduct of fermentation or anaerobic respiration (Banti et al., 2013; Rivoal et al., 1991) and can act as an electron donor for anaerobic microbial growth in paddy soil. Previous reports showed that lactate acted as an electron donor for stimulating the microbial activity and further enhanced the PCP degradation (Chen et al., 2018; Cheng et al., 2019; Li et al., 2013; Yoshida et al., 2007). Humic substances (HSs), major organic constituents of soils, can stimulate anaerobic processes by accelerating electron transfer in soils due to their redox-active functions (Van der Zee and Cervantes, 2009). Prior studies found that HSs could act as redox mediator in extracellular electron transfer, so as to enhance the microbial reductive dechlorination of dichloro-diphenyl-tricgloroethane (Cao et al., 2012) and PCP (Pham and Katayama, 2018; Zhang et al., 2015). However, most previous studies focused on the effect of lactate or HSs on the microbial community in the presence of PCP and ignored the shift in the microbial community between treatments with and without an electron donor/shuttle/PCP. For water-logged paddy soil, the coexistence of lactate and HSs might negatively or positively affect the soil microbial communities in the presence and absence of PCP, which also needs further study.

Therefore, this study was aimed to analyze the effects of the electron donor, electron shuttle and/or PCP stress on the microbial community structures in paddy soil under anaerobic conditions, which could further provide the necessary information on the possible in situ bioremediation of PCP-contaminated soils.

2. Materials and methods

2.1. Chemicals and soil samples

PCP (>98%), AQDS (>97%), and 1,4-piperazinediethanesulfonic acid (PIPES, > 98%) were purchased from Sigma-Aldrich (St. Louis, USA). The PCP, and its transformation products, 2,3,4,5-tetrachlorophenol (99.9%) and 3,4,5-trichlorophenol (98.9%), were purchased from AccuStandard (New Haven, USA). The organic reagents for PCP and its byproduct extraction (HPLC grade), including ethanol, methanol, dichloromethane and hexane, were purchased from Acroas (Geel, Belgium). The other analytical grade chemicals were obtained from Guangzhou Chemical Co. (Guangzhou, China). Deionized water for all of the solutions was obtained using ultrapure water system (18 MΩ cm^{-1}, Easy Pure'II RF/UV USA).

The paddy soil sample was collected from Tanzhou town, Zhongshan city, Guangdong Province, China (22° 34.549′ N, 113° 17.162′ E) under water-logged conditions. Sampling sites were selected where rice had been planted for at least 18 years and chemicals (such as fertilizers and pesticides) have been regularly used. To avoid effects of the different content of CPs uptake by vegetation, sampling was carried out where plants with superficial roots are not present. At each sampling point, soils were collected from the surface soil (0–20 cm), in which four subsamples, with 25 × 25 cm surface, were taken and then mixed to obtain a bulk sample. No PCP was detected in the raw soil. The physicochemical properties of the soil sample were as follows: pH of 6.09, total organic carbon (TOC) level of 42.77 g kg^{-1}, cation exchange capacity (CEC) level of 17.32 cmol kg^{-1}, complex-Fe level of 0.33 g kg^{-1}, dithionite-citrate-bicarbonate Fe (DCB-Fe) level of 16.82 g kg^{-1}, amorphous-Fe level of 5.47 g kg^{-1}, and Fe_2O_3 level of 63.0 g kg^{-1}. The method for paddy soil sample collection was described previously (Tong et al., 2018). The soil sample for cultivation was stored at 4 °C before use. Paddy soil samples for DNA analysis were stored at −80 °C in the laboratory. Soil for control treatment was sterilized by γ-irradiation at 50 kGy.

2.2. Experiments set up

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. For incubation cultures, 25 ml glass vial bottles containing 2 g paddy soil (wet weight) and 10 ml PIPES-buffered (10 mM, pH 7.0) medium were flushed with nitrogen gas (99.99%) for 30 min to remove dissolved oxygen and the oxygen in headspaces and were then sealed with silicone-lined septa and aluminum seals. Before the incubation, all bottles and solutions are steam-sterilized at 121 °C for 20 min or were filtrated for sterilization with 0.22-μm filters (Millipore, USA). The lactate as an electron donor, AQDS as an electron shuttle and PCP as an electron acceptor were added at final concentrations of 10 mM, 200 μM, and 19 μM, respectively. Four treatments, without lactate and AQDS (T1): soil + PCP, only lactate (T2): soil + lactate + PCP, the coexistence of lactate and AQDS (T3): soil + lactate + ADDS + PCP, and only AQDS (T4): soil + AQDS + PCP, were set up to investigate the shift of in the microbial community at different incubation times and the relationship between exogenous compounds and functional microbes associated with PCP degradation. Four control treatments were treated without PCP, including T1-CK: soil, T2-CK: soil + lactate, T3-CK: soil + lactate + AQDS and T4-CK: soil + AQDS. The sterile control (sterile soil + PCP) were also applied. All of the experiments were stationary and conducted in triplicate (Table S1).

The incubation bottles were wrapped with aluminium foil to provent PCP photodegradation and were then incubated at 25 °C in the anaerobic chamber. In order to estimate the changes in the microbial community caused by the electron donor, shuttle and PCP, samples were collected at given time intervals and then were stored at −80 °C for DNA analysis.

2.3. Analytical methods for chemical analyses

The active Fe(II), including the dissolved and adsorbed Fe(II), produced by microorganisms, was extracted with 0.5 M HCl for 1.5 h and filtrated through a 0.45-μm filter, followed by measurement with the ferrozine assay on a spectrophotometer at 510 nm (Fredrickson and Gorby, 1996). The methods to determine the concentrations of PCP and its byproducts were described in our previous report (Tong et al., 2018), based on extraction and enrichment, followed by high performance liquid chromatography (HPLC) (Waters Alliance 1527–487, Waters, Milford, USA) and gas chromatography-mass spectrometry (GC-MS) (Trace-DSQ-2000, Thermo Fisher Scientific, Austin, TX, USA) analyses.

The redox potentials in the dechlorination process under different treatments were evaluated by cyclic voltammetry using a CHI 660C potentiostat (Shanghai CH Instrument Company, Shanghai, China) under a nitrogen atmosphere at a scan rate of 50 mV s^{-1}, with a conventional three-electrode electrochemical cell (Tong et al., 2014). All of the voltages are reported based on a saturated calomel electrode (SCE). The samples collected for the CV test were incubated for 14 days in the presence of PCP.

2.4. DNA extraction and microbial community analysis

16S rRNA high-throughput sequencing was used to evaluate the microbial community composition and functional microorganisms. About 0.5 g of soil sample from the incubations was collected to extract genomic DNA using PowerSoil DNA kit (MO BIO Laboratories, USA) following the manufacturer's directions. Sequencing of the 16 S rRNA gene in the V4 region was performed with Illumina fusion primers according to a previous report (F515 (5′-GTGCCAGCMGCCGCGGTAA-3′) and R806 (5′-GGACTACVSGGGTATCTAAT-3′)) (Bates et al., 2011). The concentrations of the polymerase chain reaction (PCR) products after purification were determined using a Qubit 2.0 Fluorometer (Invitrogen, USA), and the products were mixed for high-throughput sequencing (Illumina Miseq platform, PE 250) by Magigene Biotechnology (Guangzhou, China). The bioinformatics analysis of the sequencing data were processed using QIIME 2 (Caporaso et al., 2010) and the details were described in our previous report (Tong et al., 2018). Raw sequencing data obtained in this study were deposited in the NCBI Sequence Read Archive (SRA) under the project accession number PRJNA611460.

2.5. Statistical analysis

Statistical analysis of all of the data was performed using SPSS 20.0 and OriginPro 8.0 software. Differences in the PCP dechlorination rate between the control and treatments with the electron donor and electron shuttle, as well as differences in the relative abundance of dominant microorganisms between treatments with and without lacatate/AQDS/PCP, were evaluated by one-way analysis of variance (ANOVA). Differences were deemed to be statistically significant at $p < 0.05$ or highly significant at $p < 0.01$. The microbial community structures in different treatments at different incubation times were determined by principal coordinate analysis (PCoA) with pairwise unweighted UniFrac distance using the QIIME software package (Lopez-Echartea et al., 2016). The anaerobic dechlorination of PCP in different treatments followed a pseudo first-order exponential decay model (Cai et al., 2013) and was calculated as follows: $C_t = C_0 \exp(-kt)$, where C_0 is the initial concentration of PCP, C_t is the concentration of PCP at time t, t is the incubation time (days) and k is the degradation rate constant of the PCP (day^{-1}). The data from Fig. 1A were used to estimate the dechlorination rates.

3. Results and discussion

3.1. The response of PCP dechlorination to the electron donor and shuttle

The dynamic changes in PCP dechlorination in different treatments are shown in Fig. 1A. In the sterile control, the slight change in the PCP concentration could be ignored due to the adsorption of PCP on soil particles (Tong et al., 2014), indicating that the process of PCP dechlorination was directly or indirectly mediated by soil microorganisms. Compared to the T1 treatment, PCP dechlorination rates were significantly ($p < 0.05$) increased in treatments with lactate (T2 treatment) or AQDS (T4 treatment) (Table S1). In particular, the fastest dechlorination rate of PCP occurred in the treatment with both lactate and AQDS (T3 treatment). Under anaerobic conditions, lactate not only acts as direct electron donor for dehalorespiration, but also provides fermentation products, such as hydrogen, acetate, and ethanol, to support the growth of functional microorganisms and then directly stimulates PCP dechlorination (Yang et al., 2009; Zhang et al., 2015). In addition, the CV results showed that the redox potential shifted toward more negative with lactate and AQDS (T3 treatment) (Fig. S1), suggesting a higher reduction potential in the treatment with lactate and AQDS. This trend could form a strong reduction environment to enhance the dechlorination process (Tong et al., 2014). The addition of an electron donor or shuttle can promote PCP dechlorination in paddy soils, in line with previous reports (Chen et al., 2016a, 2018; Li et al., 2019).

The active Fe(II) concentrations in different treatments are shown in Fig. 1B. The active Fe(II) concentration was significantly increased by the addition of lactate or AQDS compared to T1 treatment ($p < 0.05$). After incubation for 10 days, the concentrations of active Fe(II) in T1, T2, T3, and T4 were 2.1 mM, 2.5 mM, 5.4 mM, and 3.9 mM, respectively (Table S1), increases of 38.1–75.9% in comparison to the sterile treatment. Lactate as an electron donor can be utilized by a wide range of microorganisms, such as dissimilatory iron-reducing bacteria, and further increase the Fe(II) concentration. The presence of AQDS enhances the electron transfer between iron minerals and microorganisms to promote iron reduction (Lovley et al., 1998). More interesting, Fig. 1B showed that the various of Fe(II) concentrations were consistent with the various PCP dechlorination rates in all treatments. Previous studies found that active Fe(II) species could sorb on the surface of soil particles, which could further enhance the chemical dechlorination of PCP (Cao et al., 2012; Chen et al., 2012; Tong et al., 2014). Thus, we can infer that the active Fe(II) produced by indigenous microorganisms played a role in PCP dechlorination in this study. Similar amounts of active Fe(II) in

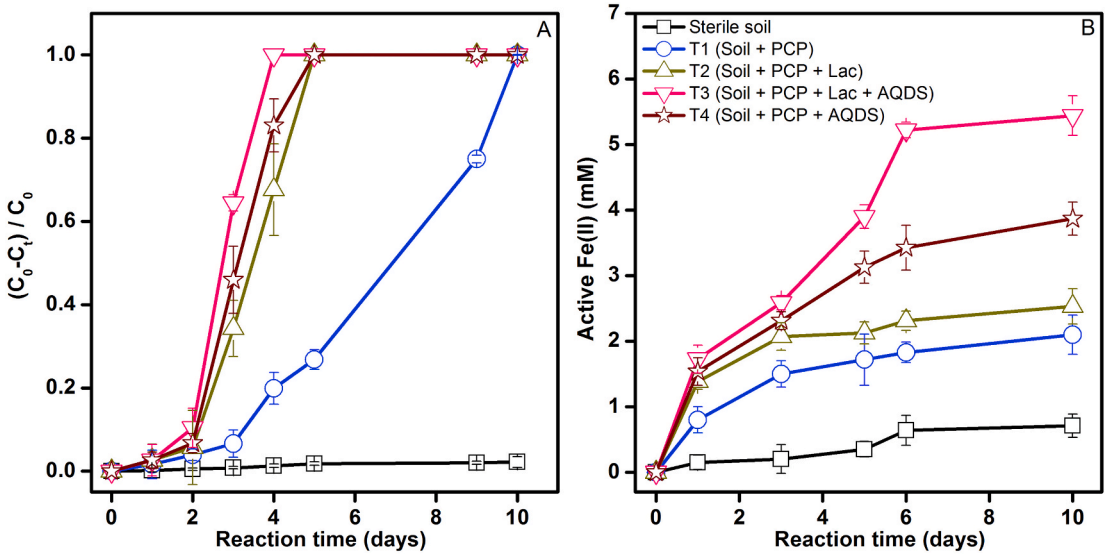

Fig. 1. (A) The dechlorination of PCP and (B) production of active Fe(II) in different treatments. Error bars show the standard errors from triplicate measurements.

the T1 and T2 treatments resulted in large differences in PCP dechlorination rate (Fig. 1). This result suggested that microorganisms play a more important role in PCP dechlorination, although the abiotic and biotic reductive processes coexisted to cooperatively dechlorinate PCP (Chen et al., 2018; Cheng et al., 2019).

To confirm the process dechlorination of PCP, its byproducts were determined using GC-MS (Fig. 2) In all of the treatments, 2,3,4,5-TeCP and 3,4,5-TCP were detected in the first 5 days, when PCP decreased to 0 ppm in the treatments with lactate or AQDS. 3,4-dichlorophenol (3,4-DCP), 4-chlorophenol (4-CP) and phenol were also detected as the end product of PCP dechlorination in the CK treatments after incubation 28 days. These results suggested that the microbially mediated dechlorination process preferred the *ortho* position (Cheng et al., 2019; Susarla et al., 1997). Compared to the treatments without lactate or AQDS (T1), the addition of lactate or AQDS could increase the transformation of 2,3,4,5-TeCP to 3,4,5-TCP. The dechlorination pathway of PCP indicated that the *ortho*-dehalogenase of bacteria in paddy soil could be stimulated by lactate or AQDS, which further enhanced the excretion of dechlorination enzymes (Cheng et al., 2019).

3.2. Response of the microbial community to PCP

Compared with the PCP dechlorination in sterile control, the results from the unsterile treatments suggested that the accelerated PCP dechlorination was attributed to the activities of certain functional microorganisms in soils. The number of microorganisms might be reduced by PCP toxicity and/or increased by co-metabolism (Cheng et al., 2019; Siczek et al., 2020). In the present study, some functional groups that can use PCP as an acceptor could be stimulated to grow under PCP stress. To determine the effect of PCP on the microbial community of paddy soil during PCP dechlorination and after complete PCP degradation, samples were collected after incubation for 2, 7, 14, and 28 days based on the PCP dechlorination rate and previous studies (Chen et al., 2012, 2016b, 2018; Tong et al., 2018). 16 S rRNA high-throughput sequencing was applied to analyze the change in the microbial community in different treatments with or without PCP. In total, 2,760,214 sequences from the 33 samples analyzed were generated by Illumina high-throughput sequencing; sequence frequencies for individual samples ranged from 48,941 to 156,268, and the normalization of the clean sequences was conducted by randomly extracting 48,941 clean sequences from each sample dataset to fairly compare all of the samples at the same sequencing depth, the alpha-diversity is presented in Table S2. The highest OTU richness was found in raw soil. There was significant difference of OTU richness between treatments with PCP and without PCP, which revealed the changes in microbial community diversity, and indicated that PCP had a certain toxic effect on some soil microorganisms. The OTU richness decreased in the first 14 days, and then increased later in the T2-CK, T3-CK and T4-CK treatments, which implied that the presence of lactate and AQDS also affected the OTU richness of the paddy soil.

Based on the data of PCP transformation and byproduct production, the microbial community incubated for 14 days was used to evaluate the effect of PCP on functional groups during dechlorination. The results at the phylum level after incubation for 14 days when the PCP was completely dechlorinated are shown in Fig. S2. Euryarchaeota, Proteobacteria, Firmocutes, Chloroflexi, and Bacteroidetes were the dominant phyla (>1% in at least one treatment) during incubation. In treatment T1, the relative abundances of Firmicutes and Proteobacteria increased with the addition of PCP while that of Bacteroidetes decreased. With the addition of lactate (treatments T2 and T3), changes in the relative abundances at the phylum level in the presence or absence of PCP were similar. In particular, lactate significantly stimulated the growth of Euryarchaeota in the absence of PCP. However, the PCP had a slight impact on the relative abundances of phyla in treatment T4 with AQDS.

Fig. 3A shows the relative abundances of dominant classes, such as *Bacilli*, *Clostridia*, *Alphaproteobacteria*, *Betaproteobacteria*, *Deltaproteobacteria*, *Gammaproteobacteria*, and *Methanobacteria,* in all of the treatments. In previous reports, most anaerobic microorganisms that used chlorophenol as an electron acceptor belonged to the classes *Clostridia*, *Betaproteobacteria*, and *Gammaproteobacteria* (Field and Sierra-Alvarez, 2008; McAllister et al., 1996). Changes in the relative abundances of these three dominant classes were in line with the trends of PCP dechlorination, indicating that these groups could adapt to the stress of PCP and/or be involved in PCP or transformation of its products.

To illustrate the changes in functional groups during PCP dechlorination, the most abundant genera (>1% relative abundance) among all of the treatments after incubation for 2, 7, 14, and 28 days are presented in Fig. S3. The dominant genera in the raw soil samples were *Massilia*, *Brevibacillus*, *Tumebacillus*, *Paenibacillus*, *Clostridium*, *Thauera*, *Methanobacterium*, *Gracilibacter*, which was quite different from the treatments with PCP amended (Fig. S3). *Clostridium*, *Cupriavidus*, *Gracilibacter*, *Oxobacter*, *Pandoraea*, *Thauera*, and unclassified *Veillonellaceae* were the

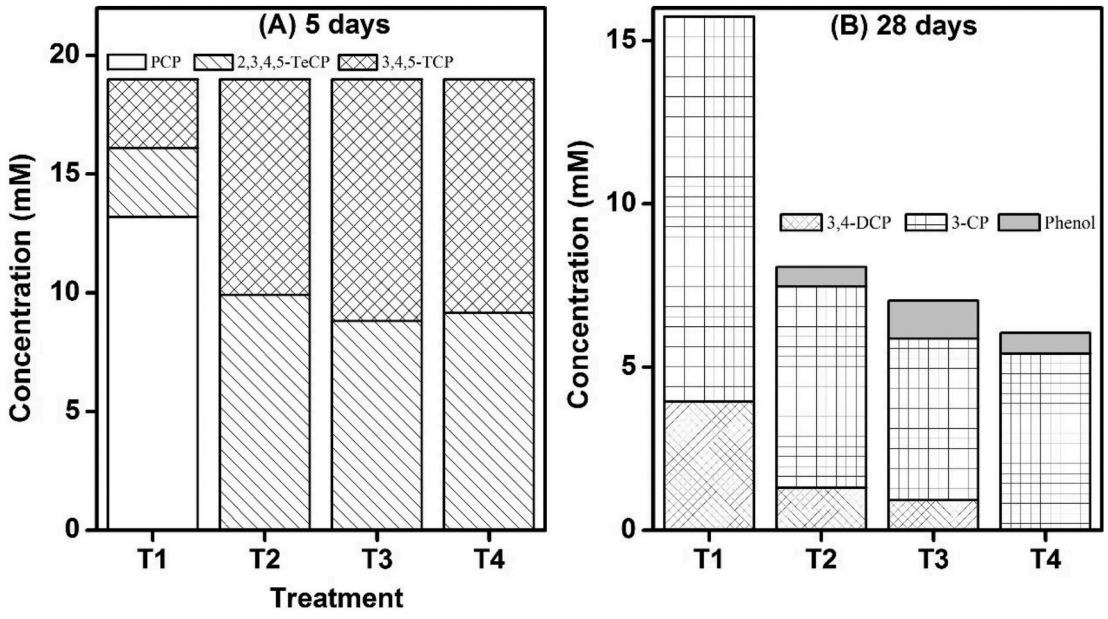

Fig. 2. The concentrations of byproducts during PCP dechlorination after incubation of 5 and 28 days.

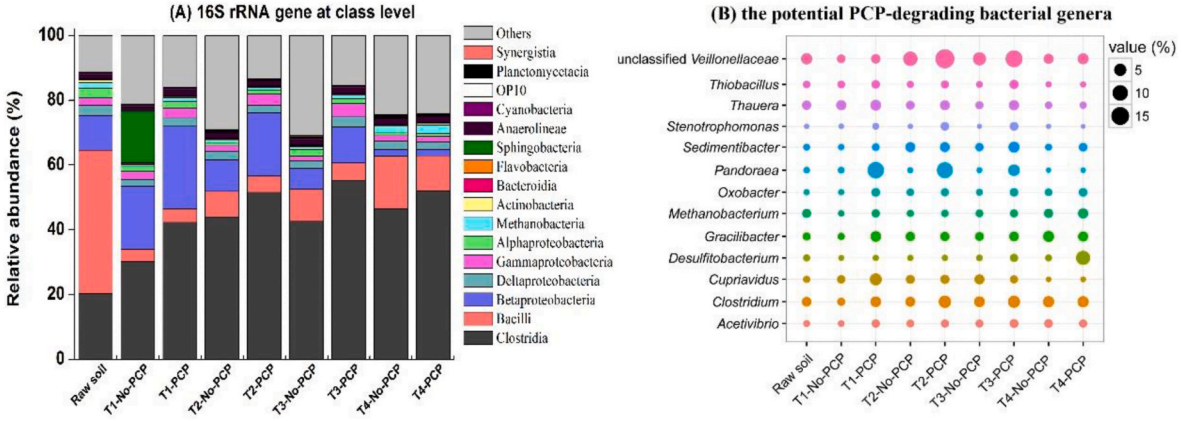

Fig. 3. The microbial community structures of 16 S rRNA gene at class level (A) and the potential PCP-degrading bacterial genera (B) after incubation for 14 days. Others represented the relative abundance lower that 0.5% and unclassified phylum.

dominant genera in the treatments with PCP. After the addition of PCP, the soil microbial community varied greatly from that in treatments without PCP, estimated by the pairwise unweighted UniFrac distance in principal coordinate analysis (Fig. 4). P1 and P2 represented the percentage of variation in the microbial community and mainly explained the difference between the treatments with and without PCP. Whereas these dominant genera were the functional microorganisms involved in PCP dechlorination, the net proportional changes of relative abundances between the treatments with and without PCP were used to estimate their attribution to PCP dechlorination (Fig. S4). The net proportional changes

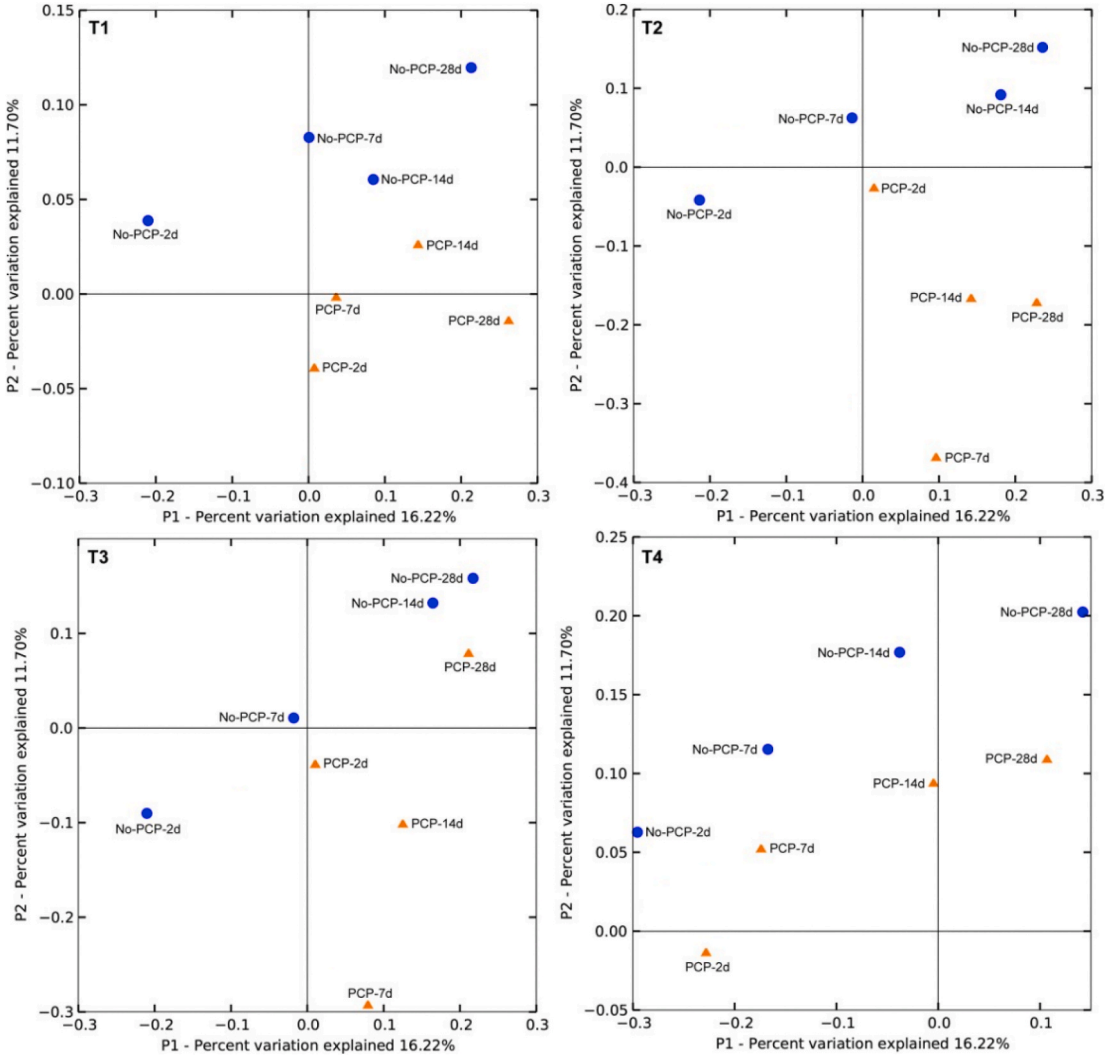

Fig. 4. Principal coordinates analysis (PCoA) of the microbial community in different treatments (T1, T2, T3, and T4) with or without PCP. No-PCP-2d and PCP-2d represented the treatment without PCP for 2 days and the treatment with PCP for 2 days, respectively.

were basically consistent with the dominant genera in the treatments with PCP. Compared to treatment without PCP (T1-No-PCP), the relative abundances of *Pandorae* and *Cupriavidus* increased highly significantly ($p < 0.01$) by 9.39% and 3.48%, respectively, while the relative abundance of *Symbiobacterium* decreased highly significantly ($p < 0.01$) by 5.42%. In the treatments with lactate (T2) or lactate and AQDS (T3), the addition of PCP increased the relative abundances of *Pandora* and *Veillonellaceae* highly significantly ($p < 0.01$) by 3.90–10.75% and 6.52–10.45%, respectively. The toxicity of PCP, however, significantly decreased the relative abundance of *Methanosarcina* by 8.95–10.95% in treatments T2 and T3. Only with the electron shuttle AQDS (T4), did PCP significantly enhance the growth of *Desulfitobacterium* while inhibiting the growth of *Viridibacillus*. The net proportional changes suggested that the microbial communities involved in PCP dechlorination varied depending on the electron donor and shuttle (Chen et al., 2018; Massol-Deyá et al., 2005) and most microbes were able to metabolize under the influence of PCP and overcome its toxicity (Cea et al., 2010; Siczek et al., 2020).

During PCP dechlorination, *Symbiobacterium* was obeserved to decrease significantly in previous reports (Chen et al., 2016b, 2018), indicating that *Symbiobacterium* cannot tolerate PCP contamination. *Methanosarcina* was reported as a dominant microorganism with lactate as electron donor and carbon source (Mladenovska and Ahring, 1997). The decrease in *Methanosarcina* in the present study might be due to the competition for the electron donor between dechlorinating and fermenting microorganisms. Few reports mentioned the roles of *Viridibacillus* in PCP transformation. As such, *Viridibacillus* might be sensitive to stress caused by PCP and AQDS, causing the decrease in relative abundance.

In addition to inhibition, the relative abundances of potential PCP-dechlorinating bacteria would increase under the impact of PCP (Bosso and Cristinzio, 2014; Field and Sierra-Alvarez, 2008). Based on Figs. S2 and S3, a number of potential PCP-dechlorinating bacteria at the genus level, *Acetivibrio*, *Clostridium*, *Cupriavidus*, *Desulfitobacterium*, *Gracilibacter*, *Methanobacterium*, *Oxobacter*, *Pandoraea*, *Thiobacillus*, *Thauera*, *Sedimentibacter*, *Stenotrophomonas*, and unclassified *Veillonellaceae*, were enriched in the treatments with PCP (Fig. 3B). Among these microorganisms, *Clostridium*, *Desulfitobacterium*, and *Methanobacterium* are known as dehalorespiring microorganisms and metabolize PCP as an electron acceptor under anaerobic conditions (Holliger et al., 2004). In addition, previous reports showed that these microorganisms commonly caused the reductive *ortho*-dechlorination of chlorophenols (Castro et al., 1994; Field and Sierra-Alvarez, 2008; Holliger et al., 2004), which is consistent with the pathway of PCP transformation in Fig. 2. Although only three typical dehalorespiring microorganisms were enriched in PCP dechlorination microcosms, the remaining potential PCP-dechlorinating bacteria have been confirmed to have versatile anaerobic respiratory metabolism, such as iron respiration and dehalorespiration (Okeke et al., 2002; Ryan et al., 2009; Teng et al., 2017; van Doesburg et al., 2005). For example, our previous research showed that the dominant microorganism *Veillonellaceae* played a crucial role in disclosing the relationship between PCP dechlorination and iron cycling in paddy soil (Tong et al., 2014). *Thauera* has been report to dechlorinate chlorinated compounds under iron-reducing conditions (Duc, 2019) and to degrade organic pollutants under nitrate-reducing conditions (Fida et al., 2017; Song et al., 2000). The relative abundance of *Pandoraea* increased significantly with the addition of PCP. *Pandoraea* can dechlorinate chlorinated compounds, and also use them as a carbon source to grow under anaerobic conditions (Okeke et al., 2002; Siddique et al., 2002; Yu et al., 2014b). The relative abundances of these microorganisms were enhanced by the presence of PCP compared to the raw soil and the treatments without PCP (Fig. 3B), which indicated that PCP can stimulate the growth of these specific microorganisms (Fig. S3). Additionally, higher rate of PCP dechlorination were detected in the treatment with more these microorganisms, which seemed to confirm that these enriched microorganisms were functional groups in the process of PCP reductive dechlorination (Fig. 1 and S4). Due to the specificity of obligate dechlorinators, these powerful non-typical dehalorespiring microorganisms have been neglected in complex soil environments (Fletcher et al., 2011; Xu et al., 2019). With the versatile metabolism of various electron acceptors and donors, these non-typical dehalorespiring microorganisms can be more likely to adapt the soil environment and biodegrade a wide variety of chlorinated compounds, which might explain the enrichments and fast dechlorination of PCP in the present study. Research needs to pay more attention to the function and metabolic pathways of these non-typical dehalorespiring microorganisms in future.

3.3. The effect of lactate and AQDS on the microbial community in the presence of PCP

It is widely reported that electron donors and shuttles can enhance dechlorination processes by stimulating the functional groups in the microbial community and by changing the redox potential directly or indirectly (Chen et al., 2012; Pham and Katayama, 2018; Xu et al., 2019). These previous studies mostly focused on the typical dechlorinating microorganisms and the changes in physicochemical property between the raw soil (without contamination) and treatments with PCP (Chen et al., 2016b; Pham and Katayama, 2018; Xu et al., 2019; Zhang et al., 2015). However, the effects of electron donors or shuttles on the microbial community structures in the presence of PCP were commonly neglected. In the present study, the microbial community structures exhibited differences among the treatments with or without lactate and AQDS in the presence of PCP (Fig. S5). The PCoA result illustrated that the addition of an electron donor and shuttle had a significant effect on the microbial community in contaminated soil. A similar trend in the microbial community between the treatments with lactate and with lactate and AQDS indicated that the electron donor had a strong contribution to the variation of the microbial community, while the electron shuttle had little impact. However, the microbial community tended to be uniform to some extent after incubation for 28 days when most of chlorophenols were degraded in the treatments with electron donor or shuttle (Chen et al., 2016a, 2016b).

The provision of sufficient electron donors has been applied to guarantee dechlorination in anaerobic paddy soil (Breitenstein et al., 2001; Yoshida et al., 2007). In the present study, lactate, widely distributed in paddy soil due to secretion from rice roots or produced through fermentation under anaerobic conditions (Banti et al., 2013; Rivoal et al., 1991), was used as an electron donor to maintain microbial activity and enhance PCP dechlorination. At end of the incubation, lactate was still detected in lactate-amended treatments (data not shown), suggesting the electron donor was sufficient for the microbial dechlorination process. The dominant genera in lactate treatments were *Acetivibrio*, *Clostridium*, *Cupriavidus*, *Methanosarcina*, unclassified *Veillonellaceae*, *Oxobacter*, *Pandoraea*, *Sedimentibacter*, *Thauera*, and *Thiobacillus* (Fig. S3). In addition, the net proportional changes in the relative abundance of the microbial community between the treatments with and without lactate in the presence of PCP were used to reveal the effect of lactate on the microbial community (Fig. 5A and B). The results showed that incubation of contaminated paddy soil with lactate favored the enrichment of *Methanosarcina*, *Clostridium*, *Sedimentibacter*, and *Veillonellaceae* ($p < 0.05$ or < 0.01). Conversely, other dominant microorganisms were negative or had a near zero net proportion, probably because their growth might rely on pentachlorophenol rather than lactate.

In paddy soil, *Clostridium* was described as functional bacteria for PCP dechlorination under anaerobic conditions (Lévesque et al., 1998; Xu et al., 2014; Yoshida et al., 2007). In addition, *Clostridium* was identified as an iron-reducing bacterium during PCP dechlorination (Xu et al., 2014). Besides *Clostridium*, *Sedimentibacter* and *Veillonellaceae* have been reported to reduce iron in various environments (Li et al., 2011; Liu et al., 2018; Tong et al., 2014; Yu et al., 2014a). The fermentation metabolites produced by *Methanosarcina*, such as propionate and acetate, can

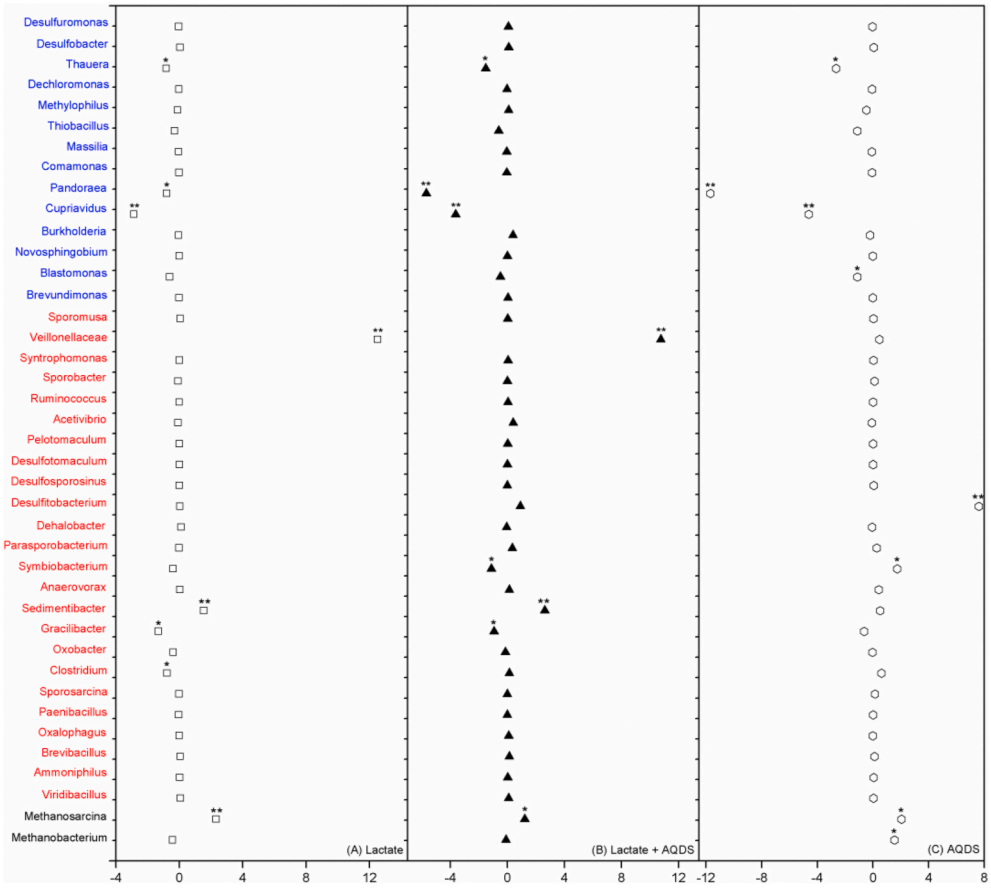

Fig. 5. The effect of (A) lactate, (B) lactate + AQDS, and (C) AQDS on the net proportional changes in relative abundance of microbial community (top 40) at genus level in different treatments (T2, T3, and T4) with PCP, comparison of that in the treatments (T1) with PCP after incubation for 14 days.

stimulate the growth of iron-reducing bacteria (Hori et al., 2010; Lovley et al., 2004; Zellner et al., 1994).

Based on previous reports, the iron-reduction process can produce various active Fe(II) species to enhance the reductive dechlorination of PCP in paddy soil (Li et al., 2010; Xu et al., 2014). These changes in the relative abundance of the microbial community were consistent with the dynamics of PCP dechlorination and iron reduction (Figs. 1 and 5, and S2), indicating the addition of lactate not only directly enhanced the dechlorination process, but it also indirectly stimulated the iron reduction coupling with PCP dechlorination.

The trends of the net proportional changes were similar in the treatments with lactate (T2) and with lactate and AQDS (T3) (Fig. 5), suggesting that the role of lactate in promoting the microbial community might exceed that of AQDS when all of the treatments contain lactate. However, the addition of AQDS made the redox peak shift toward being more negative (Fig. S1), causing a higher reduction potential for dechlorination process (Chen et al., 2012; Zhang et al., 2015), and corresponding to higher PCP dechlorination rates with AQDS (Fig. 1). Treatment with only AQDS also showed the ability to enhance PCP dechlorination, and the favored enrichment was different from that in treatments with lactate (Figs. 1 and 5C). Compared to the control treatment without PCP, the relative abundances of *Methanobacterium* and *Methanosarcina*, *Desulfitobacterium*, and *Symbiobacterium* were significantly increased ($p < 0.05$) in treatment with AQDS (T4 treatment) (Fig. 5C). This difference might be caused by the different exogenous substrate during the dechlorination process. When AQDS acted as mediator in PCP dechlorination by *Desulfitobacterium*, the reduced AQDS could be used as an electron donor for other microorganisms (Zhang et al., 2015). In addition, the byproducts of PCP dechlorination could produce low-molecular-weight organic matter by ring cleavage (Sharma et al., 2009), providing an energy source for *Methanobacterium*, *Methanosarcina*, and *Symbiobacterium* growth. Therefore, AQDS mainly acted as a redox shuttle to enhance PCP dechlorination as well as stimulate the growth of special microorganisms in contaminated paddy soil (Laskar et al., 2019; Zhang et al., 2015).

Based on a combination of the net proportional changes in Fig. 5A and C, lactate and AQDS could affect the composition of functional groups and their relative abundance separately. This is probably because lactate can act as a carbon source for most microorganisms in soil while AQDS could act as an electron donor for specific HSs-respiring microorganisms as well as an electron shuttle for dechlorination and iron reduction processes (Freeborn et al., 2005; Zhang et al., 2015). Fig. 5A and B showed similar changes in the relative abundance of the microbial community, indicating that lactate was the dominant factor that changed the microbial community. Generally, lactate and its fermentative products are preferred by soil microorganisms. Therefore, when lactate and AQDS coexisted in the dechlorination process, the functional group might first oxidize lactate coupled to PCP dechlorination (Yoshida et al., 2007). The effect of AQDS on the microbial community might be inhibited. However, the dechlorination rate was faster in the treatments with lactate and AQDS than in the treatment with lactate, suggesting that AQDS accelerates the dechlorination process due to the function of the electron shuttle. These results provide another perspective in the remediation of PCP-contaminated soil when adding electron donors and shuttles, which needs to consider their basic properties and interactions.

4. Conclusions

In this study, the effects of the electron donor, electron shuttle and/or PCP stress on the microbial community structures in anaerobic paddy soil

were systematically investigated. The results illustrated the negative effect of PCP on the soil microbial diversity and the significant alteration of the soil microbial community due to the toxicity of PCP. The functional groups involved in PCP dechlorination, including dechlorinating and iron reducing microorganisms, such as *Clostridium*, *Desulfitobacterium*, *Pandoraea*, and unclassified *Veillonellaceae*, were stimulated by PCP while some dominant genera in raw soil were inhibited during PCP dechlorination. The addition of lactate or AQDS also changed the microbial community under the stress of PCP. Lactate was used as electron donor or carbon source for the potential dechlorinating bacteria to stimulate the dechlorination rate. AQDS played an important role in stimulating the indigenous special microorganisms for indirect dechlorination in the paddy soil. While both lactate and AQDS presented, AQDS mainly acted as a redox shuttle to accelerate the PCP degradation rate due to its redox property. The results in this study clarified the microbial community response for the stress of PCP, lactate, and AQDS, and provided a potential application for the detoxification of organic chlorinated compounds in soils. However, the isolation and characteristics of bacteria for PCP and the byproducts dechlorination remain to be investigated, which would help to the appropriate selection of exogenous substance for stimulating the bioremediation of PCP-contaminated soils.

Authorship contribution statement

Manjia Chen and Hui Tong participated in performing the experiments, analyzing the results, and writing the paper; Jiangtao Qiao and Yahui Lv participated in analyzing the data, Qi Jiang and Yuanxue Gao performed the experiments, Chengshuai Liu participated in performing, conceiving, and designing the experiments, as well as in improving the paper.

Notes

The authors declare no competing financial interest.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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