Environ Sci Pollut Res (2017) 24:22852–22860
DOI 10.1007/s11356-017-9062-x

Effect of phosphorus addition on the reductive transformation of pentachlorophenol (PCP) and iron reduction with microorganism involvement

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Received: 30 September 2016 / Accepted: 18 April 2017 / Published online: 25 April 2017

Abstract The transformation of phosphorus added to the soil environment has been proven to be influenced by the Fe biochemical process, which thereby may affect the transformation of organic chlorinated contaminants. However, the amount of related literatures regarding this topic is limited. This study aimed to determine the effects of phosphorus addition on pentachlorophenol (PCP) anaerobic transformation, iron reduction, and paddy soil microbial community structure. Results showed that the transformation of phosphorus, iron, and PCP were closely related to the microorganisms. Moreover, phosphorus addition significantly influenced PCP transformation and iron reduction, which promoted and inhibited these processes at low and high concentrations, respectively. Both the maximum reaction rate of PCP transformation and the maximum Fe(II) amount produced were obtained at 1 mmol/L phosphorus concentration. Among the various phosphorus species, dissolved P and NaOH-P considerably changed, whereas only slight changes were observed for the remaining phosphorus species. Microbial community structure analysis demonstrated that adding low concentration of phosphorus promoted the growth of *Clostridium bowmanii, Clostridium hungatei, and Clostridium intestinale* and *Pseudomonas veronii.* By contrast, high-concentration phosphorus inhibited growth of these microorganisms, similar to the curves of PCP transformation and iron reduction. These observations indicated that *Clostridium* and *P. veronii*, especially *Clostridium*, played a vital role in the transformation of related substances in the system. All these findings may serve as a reference for the complicated reactions among the multiple components of soils.

Keywords Pentachlorophenol · Phosphorus addition · Phosphorus species · Fe(III) reduction · Microbial community · Kinetics

Responsible editor: Robert Duran

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Introduction

Pentachlorophenol (PCP), which was extensively used as herbicide, insecticide, fungicide, and wood preservative owing to its antimicrobial property between the 1950s and 1980s, is listed as one of the priority pollutants by the US Environmental Protection Agency due to its high toxicity and persistence. These features of PCP are due to the high chlorine content in its molecular structure (Rosalia et al. 2008). In China, PCP has been widely utilized for the killing of schistosome intermediate host snails for decades (Hong et al. 2005). Although the use of PCP was banned, its widespread existence in the environment, especially in the soil environment is still reported (Rosalia et al. 2008; Yu et al. 2014). Liu et al. found an apparently high PCP level (average concentration 34.77 μg/kg) in the wastewater-irrigated soils in Beijing, China (Liu et al. 2006). Given the substantial adverse effects of PCP to the human body and other animals, its transformation processes have received considerable attention in recent years (Yang et al. 2006; Wang et al. 2012; Yu et al. 2014). Previous studies have concluded that reductive transformation under anaerobic conditions is the crucial mechanism for PCP degradation (Mohn and Tiedje, 1992; Dams

et al. 2007; Sharma et al. 2009). Several dechlorinating bacterial and fungal strains, such as *Sphingomonas chlorophenolica* (Yang et al. 2006), *Acinetobacter* sp. *ISTPCP-3* (Sharma et al. 2009), *Sphingobium chlorophenolicum ATCC 39723* (Dams et al. 2007), have been documented to have PCP dechlorination ability via several subsequent dechlorination processes.

Iron, an element abundantly found on Earth, plays a profound role in environmental biogeochemistry. Iron-bearing minerals are proved to be reactive compositions of the soils (Kappley and Straub. 2005; Liu et al. 2011; Yu et al. 2014). Researchers reported that the iron cycle can be coupled with the carbon cycle, fate of heavy metals, and transformation of nitrogen and organic pollutants (Li et al. 2012; Wang et al. 2012; Yu et al. 2014). Recently, the fact that PCP can be chemically dechlorinated by the high reaction activity of sorbed Fe(II) produced from dissimilatory iron reduction process mediated by dissimilatory iron-reducing bacteria (DIRB), mainly including *Shewanella* and *Geobacter* species, has attracted the attention of researchers (Xu et al. 2014). In fact, PCP transformation in natural soil system is usually the combination of chemical and biological processes, especially in the paddy soils of tropical and subtropical zones, which have high iron contents (Borch et al. 2010).

The availability of phosphorus limits the net primary production of many ecosystems because it is an essential nutritional element to many living things, including microorganisms and plants. Phosphorus contents, fractionation, availability, and cycle in various media have received considerable attention (Cross and Schlesinger 2001; Boke et al. 2015). Its amount in the soils is crucial because the physiological needs of the plants cannot be met and their growth would be limited if the value is too low. Meanwhile, excessive phosphorus can be brought to adjacent aquatic ecosystems through surface runoff, which eventually leads to eutrophication (Sharpley and Menzel 1987; Wang and Zhang 2010; Liang et al. 2010). In fact, soils contain large amount of phosphorus. An estimated 2.5×10^6 t P fertilizer was applied into 55×10^6 M hm^2 farmland in the southern parts of China (Wu et al. 2007). In Korea, the amount of phosphorus (P_2O_5) in the soils has been reported to be in excess of 20 kg ha^{-1} as a result of overfertilization (RDA. 2001). Unfortunately, the amount of phosphorus fertilizer applied to the soil is still on the rise (Chang et al. 2007). Once phosphorus is added to the soil environment, its existing form can change as a result of a series of complicated reactions influenced by various soil environment factors, such as pH, organic matter, soil moisture, soil iron oxides, particle size distribution, sand and clay fractions, and cation exchange capacity (CEC) (Devau et al. 2009; Jalali and Matin 2013; Devra et al. 2014). Of these factors, iron oxides in the soils play an important role in phosphorus transformation. Based on the previous findings, the substantial amount of iron oxides/hydroxides is favorable for phosphate adsorption (Norton et al. 2008). In addition, phosphates that are chemically adsorbed onto the surface of iron oxides/hydroxides can be released upon the occurrence of dissimilatory iron reduction process (Baldwin and Mitchell. 2000). Although considerable number of literatures explored the relationship between phosphorus transformation and iron oxide, little attention has been focused on phosphorus transformation processes and iron species under the circumstance of pollutant coexistence, which is ubiquitous in the actual soil environment. Meanwhile, the soil ecosystem is so complicated that any light changes of the system's conditions can cause a series of chain reactions, including the soil microorganisms. Unfortunately, the details of these processes are ambiguous and further studies are needed to deepen the knowledge on these processes.

The main objective of this research was to investigate the effect and mechanism of phosphorus addition on PCP reductive transformation and iron reduction. The results obtained here would provide a reference for the complicated reactions among nutritional elements, iron cycle, and contaminant transformation.

Materials and methods

Chemicals

PCP; 2,3,4,6-TeCP, 2,4,6-TCP; and 1,4-piperazine diethanesulfonic (PIPES) were purchased from Sigma-Aldrich (St.Louis, MO, USA). Lactic acid, potassium dihydrogen phosphate, and other reagents used in the experiments were obtained from Sinopharm Chemical Reagent Co., Ltd., China. Ultrapure water was utilized in all the study.

Soil sampling

The soil samples used in this study was collected from Jintang Town (114° 10′ 35″ E, 29° 19′ 11″ N) of Chongyang District, Xianning City, Hubei Provinc, China, in October 2013. Basic features of physicochemical properties of the soil samples are listed in Table 1.

Kinetic batch experiment

The reaction vessel consisted of 20-mL resistant bottles with a reaction liquid volume of 15 mL. The carbon source (lactic acid) concentrations were 10 mmol/L, and the PCP concentrations were 0.0188 mmol/L. The source of phosphorus used in the experiment was potassium dihydrogen phosphate. The soil-water ratio was 1:20, and high pure nitrogen gas was introduced into the reaction vessels for 30 min to create an anaerobic environment. Afterward, the bottles were sealed with Teflon-coated butyl rubber stoppers and aluminum caps. All the samples were

Table 1 The basic features of the soil sample's physicochemical properties

CEC (com/kg (+))	Available Si (g/kg)	Organic matter (%)	Total P (mg/kg)	Available N (mg/kg)	Available P (mg/kg)	Available K (mg/kg)	Total Fe (g/kg)	pH	Crystalline Fe (g/kg)	Amorphous Fe (g/kg)
10.9	0.07	3.03	317.5	165	9.82	67.3	36.1	4.94	5.43	3.38

cultured in a biochemical incubator for reaction at 30 °C, and certain samples were removed for analysis at preset reaction intervals. The reaction pH was set to 7 (±0.2), which was controlled using PIPES at concentration of 70 mmol/L. A total of seven treatments were established: (1) sterile soil control (0 mmol/L P), (2) control (0 mmol/L P), (3) 0.5 mmol/L P, (4) 1 mmol/L P, (5) 2.5 mmol/L P, (6) 5 mmol/L P, and (7) 7.5 mmol/L P. Each treatment was repeated three times.

Chemical analysis methods

PCP in the reaction system was firstly extracted with a water/ethanol mixture (50:50 in volume) on a shaking incubator (180 rpm) for 1 h, and then, the soil suspensions were filtered using 0.45-μm syringe filters. PCP concentrations in the filter liquids were measured via high-performance liquid chromatography (HPLC) using an Agilent Technologies 1260 instrument. The mobile phase consisted of a mixture of 1% acetic acid aqueous solution and methanol (*v*/v = 20:80) with a flow rate 1 mL/min, as depicted in our previous report (Wang et al. 2012). The chromatographic column utilized was ZORBAX Eclipse Plus C18 Analytical 4.6 × 150 mm 5-Micron, with 35 °C column temperature and 210 nm detection wave. The intermediates of PCP reductive transformation were identified according to the results of HPLC by using standard substances. The concentrations of different phosphorus species were determined using the method described by Psenner with some modifications (Hupfer et al., 1995). Briefly speaking, dissolved P was extracted through direct suspension centrifugation. Moreover, 1.0 mol/L NH_4Cl was used to extract the labile, loosely bounded, or adsorbed P (NH_4Cl-P), and 0.11 mol/L $NaHCO_3/Na_2S_2O_4$ was used to extract oxidation reduction sensitive state P (BD-P). Furthermore, 0.1 mol/L NaOH and 0.5 mol/L HCl were utilized to extract (hydrated) metal oxide bound P (NaOH-P) and carbonate- or apatite-bound P (HCl-P) respectively. The residual P was calculated using the difference of the total P extracted by 3.5 mol/L HCl after calcination at 450 °C for 3 h with the above five phosphorus species (Franz et al. 2008). The phosphorus contents in all the extracting liquids were determined via the active phosphorus molybdenum blue method (Murphy and Riley 1962). The total Fe(II) was extracted using 0.5 mol/L HCl by shaking at 25 °C for 1.5 h (180 rpm) (termed as HCl-extractable Fe(II)). Both the dissolved and HCl-extractable Fe(II) were determined through 1,10-phenanthroline method at 510 nm using a UV–vis spectrophotometer (Wang et al. 2012).

Microbial community structure analysis

We used the protocol described by Caporaso et al. to determine the diversity and composition of the bacterial communities under different treatments. (Caporaso et al. 2010). PCR amplifications were conducted with the 515f/907r primer set that amplifies the V4 region of the 16S rDNA gene. The primer set was selected because it exhibits few biases and yields accurate phylogenetic and taxonomic information. The reverse primer contains a 6-bp error-correcting barcode unique to each sample. DNA was amplified following the previously described method. (Magoč and Salzberg. 2011). Sequencing was performed on an Illumina HiSeq platform.

Pairs of reads from the original DNA fragments were merged using FLASH, which is a very fast and accurate software tool designed to merge pairs of reads if the original DNA fragments are shorter than twice the length of reads (Edgar. 2013). Sequencing reads was assigned to each sample based on the unique barcode of each sample. Sequences were analyzed with the QIIME software package (Wang et al. 2007).

Results and discussions

The effect of phosphorous concentration on PCP reductive transformation

Fig. 1a presented the kinetics of PCP reductive transformation under different treatments. It can be clearly seen from the results that little PCP transformed for the sterile soil control (0 mmol/L P) during the whole reaction period, which corresponded with low amounts of Fe(II) produced in the system (Fig. 2). After calculation, it was found that PCP reductive transformation under different treatments followed the first-order kinetics, and Fig. 1b depicted the calculated first-order kinetic rate constants (with all the coefficients of $R \geq 0.928$). As can be seen from the results, the phosphorus concentration significantly influenced PCP reductive transformation, by promoting this process at low concentration and inhibiting it at high concentration. When phosphorus concentration changed from 0 to 0.5 mmol/L, the first-order kinetic rate constant increased from 0.0291 to 0.0407/day. The peak

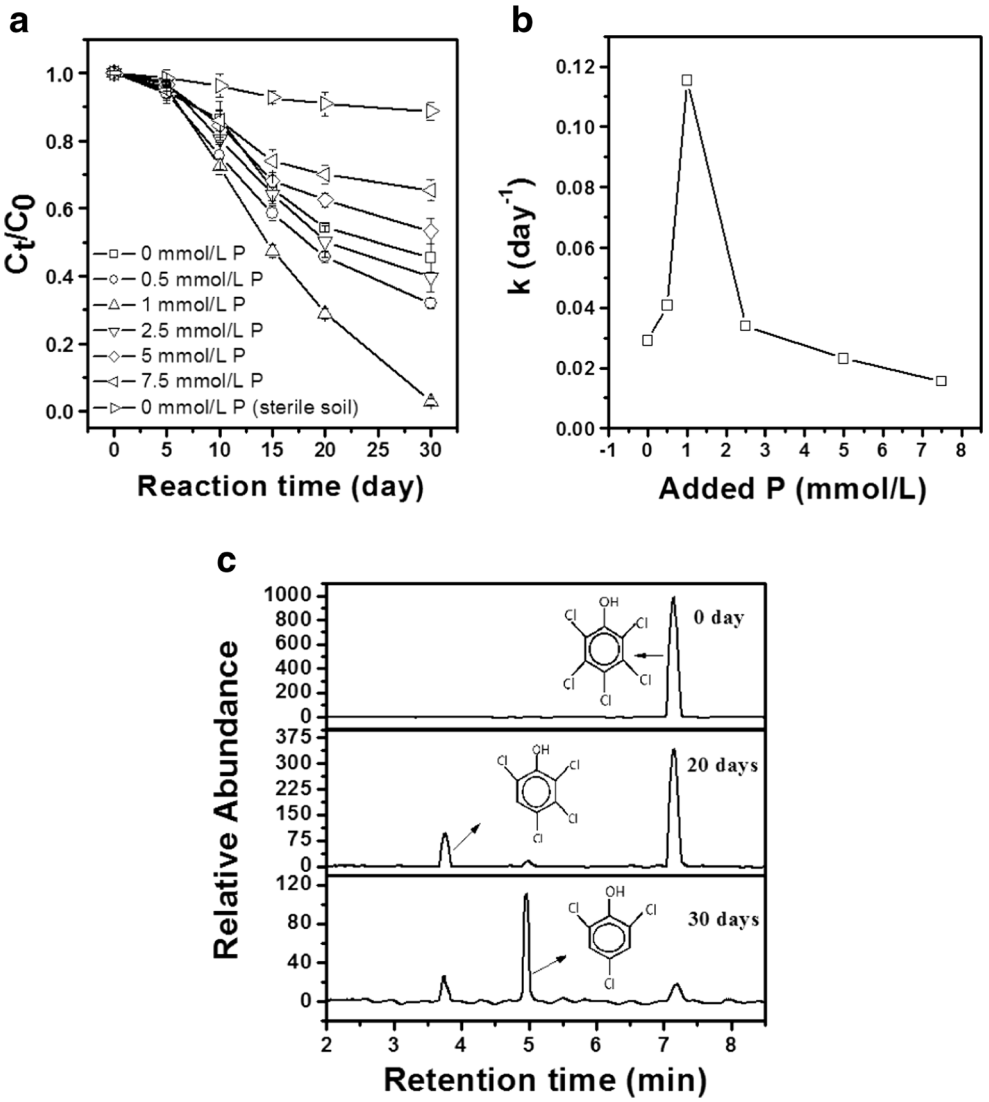

Fig. 1 The kinetics of PCP reductive transformation (**a**), the corresponding first-order kinetic rate constants (**b**), and HPLC results of PCP reductive transformation for 1 mmol/L added phosphorus treatment at 0, 20, and 30 days, respectively (**c**)

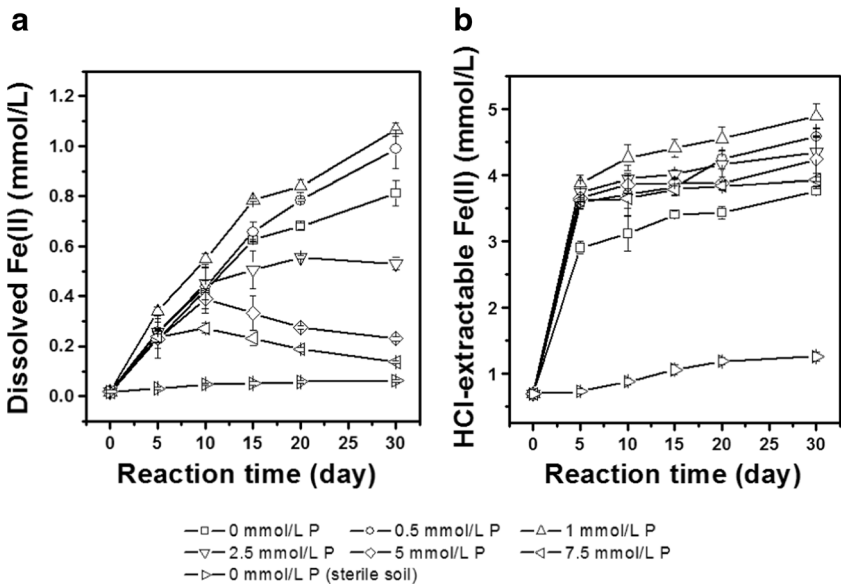

Fig. 2 The changes of Fe species in all the treatments with incubation time

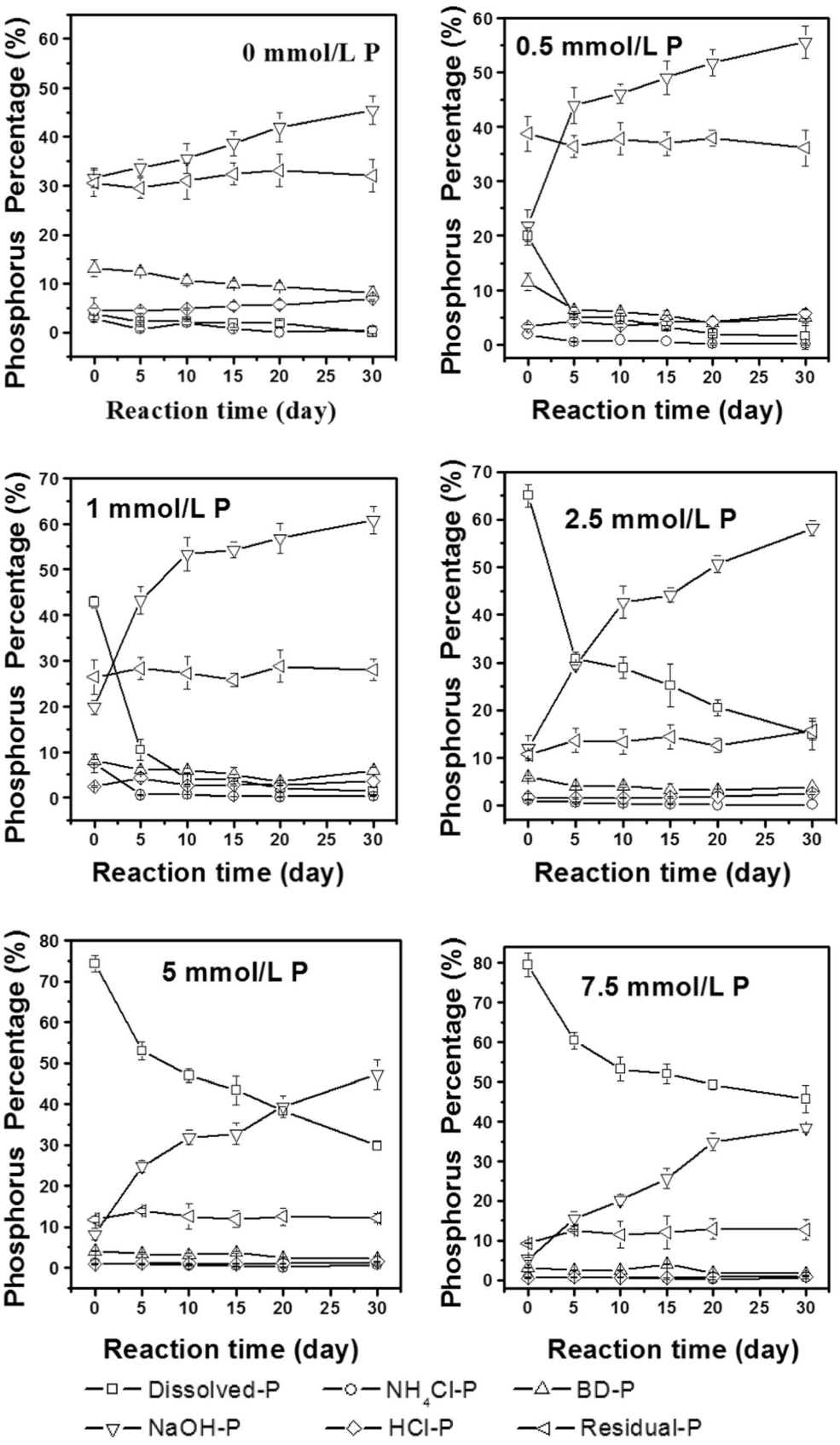

Fig. 3 The changes of different phosphorus species in all the treatments with incubation time

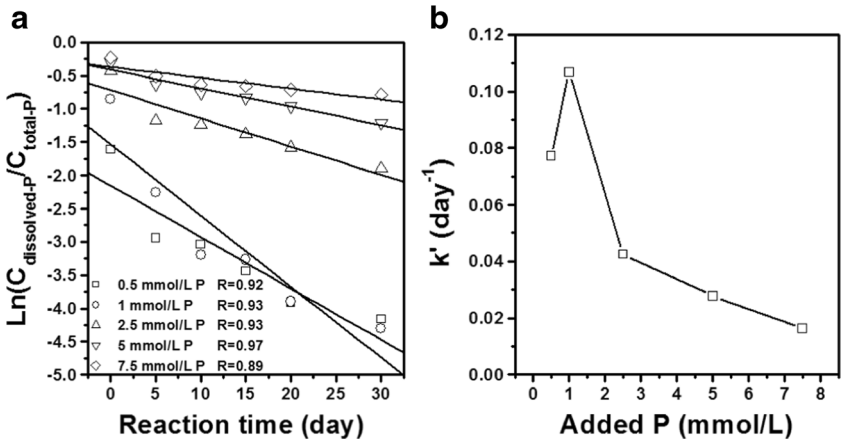

Fig. 4 The fitting of the decrease of dissolved P using the first-order kinetics (**a**) and the corresponding first-order kinetic rate constants (**b**)

value (0.155/day) was reached when phosphorus concentration was 1 mmol/L, being magnified 3.95 times compared with that of 0 mmol/L. However, the first-order kinetic rate constant gradually decreased from 0.155/day to 0.0155/day (7.5 mmol/L P) when phosphorus concentration further increased. These findings indicated that high-concentration phosphorus inhibit the PCP degradation. In addition, two intermediates were identified in the anaerobic transformation of PCP, namely 2,3,4,6-TeCP and 2,4,6-TCP (Fig. 1c) and similar intermediates were also obtained by other researchers (Kennes et al. 1996; Magar et al. 1999; Kamashwaran and Crawford. 2001). From the results, we can see that phosphorus concentration was a crucial factor for the anaerobic transformation of PCP.

Effect of phosphorous concentration on Fe(III) reduction

Fig. 2 shows the dissolved Fe (II) and HCl-extractable Fe (II) changes in the system under different phosphorus concentrations. Based on the results, dissolved Fe(II) and HCl- extractable Fe(II) demonstrated a similar trend to that of the curves of PCP transformation. The addition of 0.5 and 1 mmol/L phosphorus can promote dissolved Fe(II) and HCl-extractable Fe(II) production, whereas the steady increase of phosphorus concentration can gradually decrease the amount of dissolved Fe(II) and HCl-extractable Fe(II). For the control treatment, the quantities of dissolved Fe(II) and HCl-extractable Fe(II) were 0.812 and 3.75 mmol/L, respectively, when the reaction time was 30 days, whereas the corresponding values were 1.07 and 4.88 mmol/L, and 0.137 and 3.93 mmol/L, respectively, for 1 and 7.5 mmol/L added phosphorus treatments.

The transformation of phosphorus species

Fig. 3 demonstrates the dynamic changes of various phosphorus forms in the system under different phosphorus concentrations. As shown in the figure, the dissolved P and NaOH-P in the system substantially changed, whereas no significant change was observed for NH_4Cl-P, BD-P, HCl-P, and residual P with the extension of the reaction time. After calculation, the extent of the decrease of dissolved P and increase of NaOH-P was nearly equal, indicating that P transformation mainly occurred through iron reduction. Given the above results, iron reduction significantly accelerated phosphorus fixation and decreased phosphorus loss potential. In addition, the extent of the percentage increase or decrease of dissolved P and NaOH-P differed from that of phosphorus concentrations. Among them, the addition of 1 mmol/L P resulted in the highest first-order kinetic rate constant increase for dissolved P, which was probably related to largest Fe(II) amount in the 1 mmol/L P treatment (Fig. 4).

Effect of phosphorous concentration on microbial community structure

To investigate the changes in microbial community structure of the system, samples incubated with different treatments for 5, 15, and 30 days were chosen for the analysis of the microbial community structure with the aid of 16S RNA. Figure 5 presented the changes in the relative abundance of the microorganisms for each treatment. Based on the results, four kinds of microorganisms existed in the system besides the unclassified species, including *Clostridium (C. bowmanii, C. hungatei, and C. intestinale), Desulfosporosinus meridiei, Methanobacterium bryantii, Pseudomonas veronii*. Interestingly, *P. veronii* presented an upward relative abundance trend upon treatments with 0.5 and 1 mmol/L P compared with that of the control treatment (0 mmol/L P). However, the values exhibited a downward trend in the following treatments that consisted of high phosphorus concentrations. The relative abundance of the *P. **veronii*** species in the control treatment was 30.52% when the inoculation time was 30 days. In addition, the corresponding values for the 0.5 and

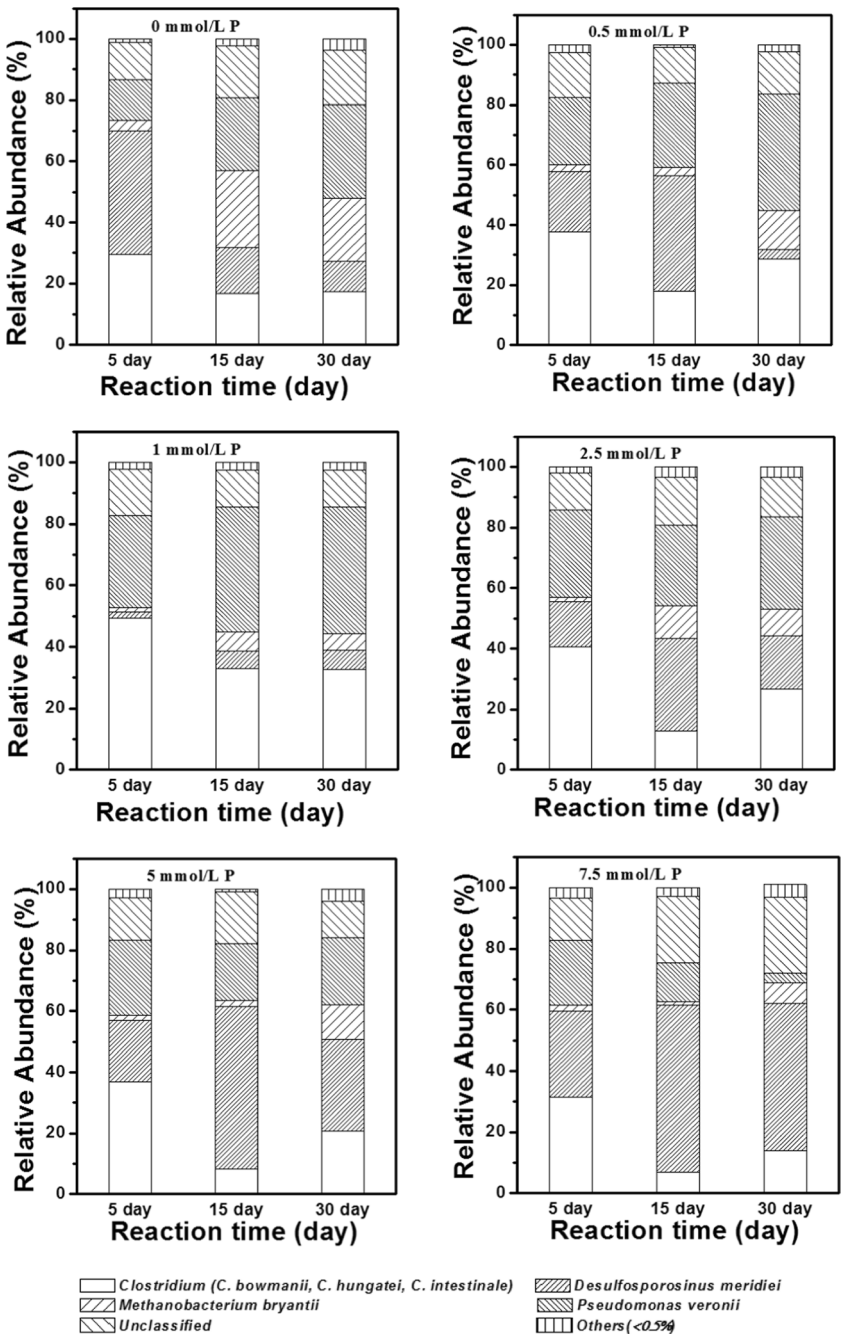

Fig. 5 The changes of the relative abundance of the microorganisms

1 mmol/L P treatments were 38.76 and 41.40%, respectively, whereas the value for the added 7.5 mmol/L P treatment was 3.18%. This finding indicated that the growth of *P.* ***veronii*** was promoted by the addition of low phosphorus concentration and inhibited by high phosphorus concentration. The growth of *Methanobacterium* was inhibited by the added phosphorus treatments compared with the control treatment. This growth inhibition was mainly attributed to the following: (1) the DIRB were more capable of utilizing acetate and H_2 than the methanogens (Lovley and Phillips. 1987) and (2) the methanogenesis can be directly inhibited by ferric ion (Jäckel and Schnell. 2000; Bodegom et al. 2004). In addition, the relative abundance of the *M.* ***bryantii*** in all the added phosphorus treatments was nearly the same, demonstrating that the addition of different phosphorus concentrations exerted a slight effect on the growth of methanogens. Similar to the species of *P. veronii*, the growth of *C. bowmanii, C. hungatei, and C. intestinale* was also affected by phosphorus concentrations, which promoted and inhibited growth at low and high concentrations, respectively. The sum of the relative abundance of *C. bowmanii,* ***C.*** *hungatei****,*** *and C. intestinale,* which were proved to be DIRB of fermentative microorganisms

(Lovley. 1991), reached peak values when 1 mmol/L P was added among the different treatments, with the corresponding values of 49.30, 32.88, and 32.57%, respectively, for the inoculation times of 5, 15, and 30 days. The increased population of *Clostridium* was proven to be linked with significantly acclerated Fe(III) reduction and organochlorine pesticide DDT transformation (Chen et al. 2013). On the contrary, the relative abundance of the species of *D. meridiei* firstly decreased and then increased with the increase of the concentration of the added phosphorus. Based on our results and the previous findings, it can be inferred that the *Clostridium* and *P. veronii*, especially *Clostridium*, played a vital role in the transformation of related substances.

Conclusion

The present research has focused on the effect of phosphorus addition on PCP reductive transformation and Fe(III) reduction. The results obtained showed that phosphorus addition can substantially influence the reductive transformation of PCP and reduction of Fe(III), which promoted and inhibited these processes at low and high concentrations, respectively. Furthermore, the maximum reaction rate of PCP transformation and Fe(III) reduction were achieved when phosphorus concentration was 1 mmol/L. The iron reduction significantly accelerated phosphorus fixation and decreased phosphorus loss potential. Microbial community structure analysis demonstrated that the above processes were closely related to *Clostridium* and *P. veronii*, especially *Clostridium.* The above findings may provide a reference for the complicated reactions among the multiple components existing in the soils.

Acknowledgments This research was financially supported by the National Natural Science Foundations of China (No. 41303090; 21307112). The authors also would thank the financial support from Hubei Key Technology Support Program (No. 2014BHE0030) and Science and Technology Research Project of Hubei Province, China (No. D20154401).

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