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Effects of Corn Straw and Citric Acid on Removal of PAHs in Contaminated Soil Related to Changing of Bacterial Community and Functional Gene Expression

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Abstract
Root exudates can stimulate microbial degradation in rhizosphere, but it is unclear whether the rhizodegradation of polycyclic aromatic hydrocarbons (PAHs) occurs in corn straw-amended soil. Either citric acid or corn straw was added into PAHs-contaminated soil to investigate their effect on the removal of PAHs. Either corn straw (Y) or combined application of corn straw and citric acid (YN100) significantly ($p < 0.05$) enhanced the removal of soil PAHs by 8.43% and 18.62%, respectively. Both Y and YN100 treatments obviously increased the abundance of PAHs degraders and the potential hosts of PAH-ring hydroxylating dioxygenase (PAH-RHDα) genes. Interestingly, the copies of PAH-RHDα Gram-negative bacteria genes under YN100 treatment was significantly ($p < 0.05$) higher than those under Y treatment. The present results indicated that combined application of corn straw and citric acid could efficiently enhance the removal of PAHs in soil, mainly via increasing the relative abundances of PAH-degrading bacteria and the expression of PAH-RHDα genes in contaminated soil.

Keywords PAHs · Corn straw · Citric acid · Biodegradation · PAH-RHDα genes

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous and persistent in the environment, some of which are mutagenic and carcinogenic benzene-ring hydrophobic aromatic pollutants. It has been found that industrial site associated with petroleum refining, gas production, and the processes of coke production industries posed severe threats to surrounding environment and human health (Jia et al. 2017). According to China soil pollution status survey in 2014, 1.4% of the sampled soils were contaminated with PAHs in China (Ministry of Ecology and Environment 2014). Therefore, it is necessary to eliminate PAHs to reduce their negative effects on human health and ecosystem.

Generally, plant roots secrete a myriad of root exudates (i.e. sugar, organic acids, flavonoids, amino and fatty acids, and secondary plant metabolites), which can stimulate microbial growth and increase degradation of contaminants in soils (Jia et al. 2018). Low molecular weight organic acids (LMWOAs), for instance, citric, malic and oxalic were the main components of root exudates (Ling et al. 2009). Compared with other compounds in root exudates, LMWOAs play a key role in degradation of pollutants. Gao et al. (2015) found that LMWOAs could dramatically promote the release of bound PAHs in soil and that citric acid has the largest capacity for releasing PAHs in soil. These may enhance the bioavailability of PAHs in soil and their degradation, which needs further investigation of related mechanisms.

As a national policy in China, crop straw was recommended to return to the fields to reduce air pollution caused by open-field burning, improve and retain the soil

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fertilization due to that crop straw returning could enhance soil macro-aggregation, promote carbon storage, improve soil structure, and increase the richness and diversity of microbial communities (Liu et al. 2019; Zhou et al. 2020). Corn straw returning is increasing, accounting for 30.8% of total crop straw in northeast and north China (Wang et al. 2011; Gu et al. 2015). Previous studies indicated that agricultural wastes returning to soil could improve soil conditions, enhance the bioavailability of PAHs, thereby accelerate PAHs degradation in soils (Sigmund et al. 2017; Wu et al. 2020). Although some studies have reported that carbon substrates or LMWOAs could increase PAHs degradation in soils (Zhang et al. 2017; Huang et al. 2019), there are lacking studies to investigate whether the rhizo-degradation of PAHs occurs in straw-amended soils.

Biodegradation of PAHs by bacteria mainly depends on the activities of enzymes encoded by the degradation-related genes (Zeng et al. 2017). Some dioxygenase genes involved in PAHs metabolism in bacteria have the characteristics of substrate specificity, high conservation and direct correlation with the biodegradation function of PAHs, which are regarded as indicator genes of PAHs metabolism (Baldwin et al. 2003). PAHs dioxygenase is the key enzyme of PAHs degradation, because molecular oxygen is incorporated into aromatic nucleus by multi-component aromatic RHD enzyme system in the initial step of PAHs metabolism (Cébron et al. 2008). So far, most researches aimed to evaluate the change of microbial communities or PAH-ring hydroxylating dioxygenase (PAH-RHDα) genes in PAHs-contaminated soils (Jurelevicius et al. 2011; Kong et al. 2018). However, few studies have attempted to explore the relationship between the association of bacteria and PAH-RHDα genes.

The main aims of the present research were to: (1) investigate whether combined application of corn straw and citric acid could enhance PAHs removal in PAHs-contaminated soil and (2) study the change of the copies of PAH-RHDα genes and bacterial community structures in PAHs-contaminated soil.

Materials and Methods

PAHs-contaminated soil was collected from the surface (0–20) of agriculture soil located in the surrounding of a coal-power plant in Shaan Xi Province of China and the detailed information of the sampling site was described Bao et al. (2020). Soil pH is 8.37 (water: soil = 2.5:1, w/v) and soil organic matter (SOM) is 15.16 g kg^{-1}. The texture of the soil belongs to a sandy loam, which contains 58.76% of silt. Concentration of PAHs in the soil is shown in Table S1. The source and physicochemical properties of corn straw have been previously described by Bao et al (2020).

The indoor simulation included four treatments: soil added with no citric acid or corn straw (CK), 100 mg kg^{-1} citric acid (N100), 5% corn straw (Y) or combined application of 100 mg kg^{-1}citric acid and 5% corn straw (YN100). Citric acid used was analytical purity and obtained from Sinopharm Chemical Reagent Co., Ltd of China. For each treatment, 200 g of air-dried soil was placed in a 480 mL plastic vial. Each treatment included triplicate and incubated at 25 °C in dark. Soil was watered with distilled water to keep 70% water holding capacity. After 28 days of incubation, one part of soil in pot was reserved for determination of soil PAHs and the other part was stored at − 80°C for analysis of soil microbial community structure and functional gene. The methods of DNA extraction and quantification of PAH-RHDα gene and the specific methods for microbial community analysis were described in Supplementary materials.

PAHs in soil was extracted according to USEPA Standard Method 3540C (USEPA 1996) and our previous study (Wang et al. 2020). Briefly, air-dried soil sample (5.0 g) was Soxhlet extracted with 120 mL mixture of dichloromethane (DCM) and acetone (3:1, v/v) for 18 h. The extraction solution was concentrated to 2.0 mL and then through a silica gel column (sub-layer 2.0 g silica gel and top layer 2.0 g anhydrous Na_2SO_4). The column was eluted using 60 mL mixture of DCM and hexane (1:1, v/v). The extracts were concentrated and adjusted to 2.0 mL with methanol. PAHs concentration in the extracts was determined with HPLC-FLD (Shimadzu, LC-20A). The recovery rate of sixteen US EPA priority PAHs was 78.6–134.1%, and the LOD was 0.06–1.40 μg kg^{-1}.

One-way ANOVA and LSD post-hoc comparison tests, linear regression analysis and correlation analysis were conducted by SPSS 23.0. Network analysis based on Spearman's rank correlation coefficients between PAH-RHDα genes and the bacterial communities was drawn by the Gephi (Version 0.9.2) platform.

Results and Discussion

After 28 days of incubation, total concentration of PAHs in soil decreased from 2275 to 1873 μg kg^{-1} under CK (Fig. 1), suggesting the contribution of PAHs degradation by the indigenous microbes (Huang et al. 2019; Li et al. 2019b). There was insignificant difference in the final concentration of PAHs between CK and N100 treatments. Vázquez-Cuevas et al (2020) also found that although citric acid could promote the desorption of ^{14}C-phenanthrene in soil, there is no proof that citric acid have the ability to enhance the removal of ^{14}C-phenanthrene in soils. Compared to CK, Y treatment significantly ($p < 0.05$) increased PAHs removal in soils. Agricultural wastes could improve soil aeration and nutrient levels, as well as provide shelter for soil microorganisms,

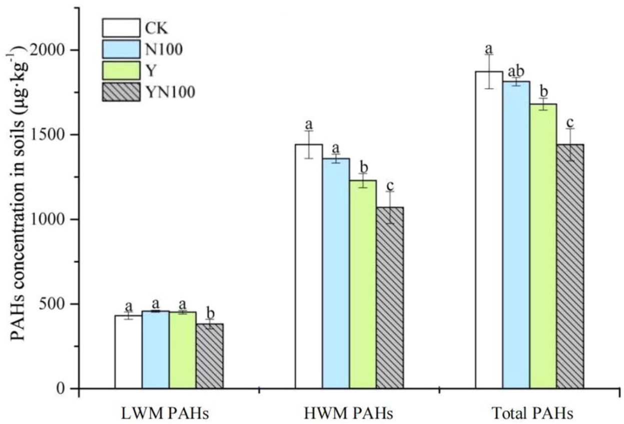

Fig. 1 Concentration of LMW PAHs, HMW PAHs and Total PAHs in PAHs-contaminated soils added with no citric acid or corn straw (CK), 100 mg kg^{-1} citric acid (N100), 5% corn straw (Y) or combined application of 100 mg kg^{-1}citric acid and 5% corn straw (YN100) after 28 days of incubation. Bars marked with different letter are significantly ($p<0.05$) different among different amendment treatments according to least significant difference (LSD) test (mean ± SD, n = 3)

thus improving the activities of microorganism and enhancing the degradation of organic pollutants (Barathi and Vasudevan 2003). Similarly, agricultural wastes returning to field could stimulate the biodegradation of PAHs in contaminated soil (Huang et al. 2019). Compared to the other three treatments, YN100 treatment significantly ($p<0.05$) decreased PAHs concentration in soil after 28 days of incubation. The increase of removal rate of PAHs in soils could be explained from three possible perspectives. Firstly, the addition of citric acid in soil could promote the desorption of PAHs in soil (Zhang et al. 2017). Secondly, soil PAHs were adsorbed on corn straw, reducing PAHs residue in soil. Thirdly, corn straw returning to soil stimulated the growth of degraders related to the removal of PAHs (Figs. S1; S2), thus increased the biodegradation of PAHs. Additionally, the removal rate of high molecular weight (HMW) PAHs was significantly ($p<0.05$) improved under Y and N100 treatments, and increased by 11.98% and 20.95% (Fig. 1). However, the removal rate of low molecular weight (LMW) PAHs was significantly ($p<0.05$) improved only under YN100 treatment. Similarly, Huang et al (2019) concluded that sawdust added to soils resulted in higher removal rate of 5–6 rings PAHs in soils than 2–4 rings PAHs.

The abundance and composition of PAHs degradation related genes can reflect the ability of PAHs degradation in soil (Haleyur et al. 2019). As shown in Fig. 2, both Y and YN100 treatments significantly ($p<0.05$) enhanced the copies of PAH-RHDα GP and GN genes, compared with CK and N100 treatments. Han et al (2017) found that addition of mushroom cultivation substrate waste, cow manure and wheat stalk could significantly enhance the copies of related-PAHs degradation genes.

The copies of PAH-RHDα GP and GN genes under YN100 treatment were higher than those under Y treatment, and significantly ($p<0.05$) level was up for PAH-RHDα GN genes. One possible reason is due to that citric acid increased the bioavailability of PAHs in soil, thus facilitating the biodegradation of PAHs via enhancing expression of PAH-RHD genes. The present study indicated that the combined addition of corn straw and citric acid is one of the effective ways to improve the abundance of PAHs degradation genes in soil. However, the abundance of PAH-RHDα genes under N100 treatment did not change significantly after 28 days incubation. Similarly, Li et al (2019a) found that there was

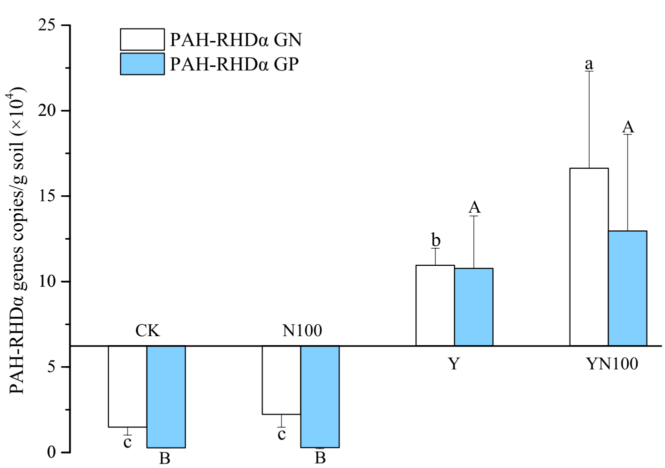

Fig. 2 Variation in abundance of PAH-RHDα genes numbers in PAHs-contaminated soils added with no citric acid or corn straw (CK), 100 mg kg^{-1} citric acid (N100), 5% corn straw (Y) or combined application of 100 mg kg^{-1}citric acid and 5% corn straw (YN100) after 28 days of incubation. Bars marked with different lowercase and uppercase letters indicate significant differences of PAH-RHDα GN genes copies and PAH-RHDα GP genes copies among different amendment treatments, respectively (mean ± SD, n = 3)

a minor effect of root exudates on the change of PAH-RHDα gene in soils. As shown in Table 1, the removal rate of total PAHs in soils had a significant ($p < 0.05$) positive correlation with the copies of PAH-RHD GN or PAH-RHD GP genes. Similarly, previous study found that PAH degradation in soils was related to the copies of PAH-RHDα genes (Li et al. 2019a). In addition, the PAH-RHD GN gene had a positive significant ($p < 0.01$) correlation with PAH-RHD GP gene, indicating that conditions required for the two degrader populations are similar, consistent with the results of Cébron et al (2008).

Principal coordinate analysis (PcoA) was applied to investigate the change of the soil bacterial community. As shown in Fig. S3, The bacterial community under CK was similar with N100 treatment, but different from corn straw treatment (Y and YN100 treatment). Figure S1 showed the changes of bacterial communities at the phylum level under different treatments. The prevailing bacterial phylum with relative abundance more than 1% were *Proteobacteria*, *Acidobacteri*a, *Actinobacteria*, *Planctomycetes*, *Bacteroidetes*, *Chloroflexi*, *Firmicutes*, *Verrucomicrobia, WPS-1* and *Gemmatimonadetes*, which accounted for 97.07%–98.63% of the total bacterial community in soils. As shown in Fig. S4, the removal rate of PAHs was significantly ($p < 0.01$) positive correlation with the abundance of *Proteobacteria*, *Bacteroidetes* and *Firmicutes*. *Firmicutes* and *Bacteroidetes* have been reported to show great potential for PAHs degradation (Zhu et al. 2017; Guo et al. 2020). What's more, the abundance of *Firmicutes* and *Bacteroidetes* under Y and YN100 treatments were much higher than those under CK and N100 treatment, suggesting the higher degradation potential of PAHs under Y and YN100 treatments.

Network analysis was used to analyze the relationship between bacterial community and PAH-RHDα genes and determine the possible hosts of PAH-RHDα genes. As shown in Fig. 3, the potential hosts of PAH-RHDα genes were *Lysobacter*, *Rhizobium*, *Bacillus*, *Devosia*, *Ohtaekwangia*, *Ramlibacter*, *Massilia*, *Steroidobacter*, *Phenylobacterium* and *Microvirga*. It is reported that *Ohtaekwangia*, *Bacillus*, *Lysobacter* and *Rhizobium* had the ability to degrade PAHs (Bao et al. 2020). *Devosia* was abundant in crude oil and might play important roles in the degradation

Table 1 Correlation coefficients (r) among the removal rate of LMW PAHs, HMW PAHs, total PAHs and PAH-RHDα genes

	LWM PAHs	HMW PAHs	Total PAHs	PAH-RHD GN	PAH-RHD GP
LWM PAHs	1.000				
HMW PAHs	0.595*	1.000			
Total PAHs	0.716**	0.987**	1.000		
PAH-RHD GN	0.628*	0.912**	0.917**	1.000	
PAH-RHD GP	0.507	0.853**	0.842**	0.962**	1.000

*Correlation is significant at the 0.05 level (2-tailed)

**Correlation is significant at the 0.01 level (2-tailed)

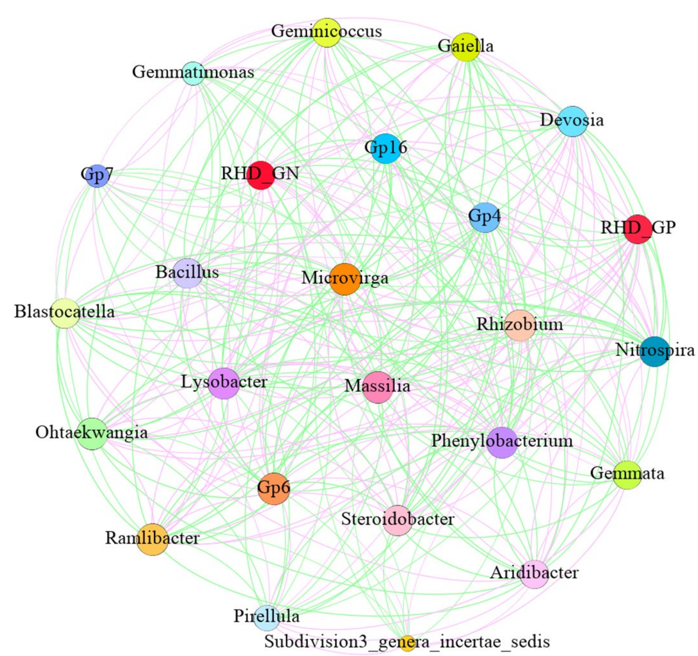

Fig. 3 Network analysis based on the co-occurrence of PAH-RHDα genes and their potential host bacteria. A connection represents a significant positive (purple line) or (green line) correlation ($p < 0.05$) according to Spearman's rank analysis

of asphaltene in soils (Song et al. 2018). In addition, *Massilia*, *Phenylobacterium* and *Steroidobacter* were regarded as key genera for PAHs degradation in soils (Cebron et al. 2015; Li et al. 2019b; Huang et al. 2019).

The changes in the relative abundances of PAHs bacteria at genus level were shown in Fig. S2. Some PAHs degraders were higher under CK and N100 treatments, such as *Lysobacter*, *Ohtaekwangia* and *Steroidobacter*. However, the primary genera under Y and YN100 treatments were *Lysobacter*, *Rhizobium*, *Bacillus* and *Devosia*. The ten genera referred had significantly ($p < 0.05$) correlation with the removal rate of HMW PAHs ($r = 0.725–0.834$) and total PAHs ($r = 0.708–0.835$), respectively (Fig. S4). Compared with CK, the abundance of ten genera related to PAH degradation were significantly ($p < 0.05$) increased in Y and YN100 treatments, indicating that the significant increase biodegradation of total PAHs and HMW PAHs might be related to increase of PAHs-degrading bacteria. However, there was no significantly difference in the abundance of bacterial genera related to PAHs degradation between CK and N100 treatments. Consequently, this may be due to that the removal rate of PAHs was low in soil treated with citric acid.

Combined application of corn straw and citric acid significantly increased the removal of PAHs in contaminated soil, but citric acid alone exhibited a slight contribution to accelerate PAHs removal in soil. The increased removal rate of PAHs under YN100 treatment might be related to the fact that citric acid improved the mobility and solubility of PAHs in soils and that corn straw increased the copy number of PAHs degradation genes and the abundance of PAHs degradation bacteria. Further study investigating the changing of PAHs bioavailability treated with corn straw and citric acid will contribute to a better understanding of the potential mechanisms for the removal of PAHs in PAHs-contaminated soils.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00128-022-03477-8.

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