Enhanced rhizodegradation of polycyclic aromatic hydrocarbons in corn straw-amended soil related to changing of bacterial community and functional gene expression

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Research Article

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Abstract

Root exudates can stimulate microbial degradation in rhizosphere, but it remains unclear whether the rhizodegradation of polycyclic aromatic hydrocarbons (PAHs) occurs in corn straw-amended soil. Hence, in the present study, either citric acid, a common low molecular weight organic acid in the root exudates, or corn straw was added into aged PAHs-contaminated soil to investigate their effectiveness in the biodegradation of PAHs. The present study showed that either corn straw (Y) or combined application of corn straw and citric acid (YN100) significantly ($P < 0.05$) enhanced the degradation of total PAHs in soil after 28 days incubation, which increased by 8.43% and 18.62% compared with control (CK), respectively. High-throughput sequencing suggested that both Y and YN100 treatments led to a shift in bacterial community in soil and increased the abundance of PAHs degraders. Interestingly, the copies of PAHs ring-hydroxylating-dioxygenase (PAH-RHD) Gram-negative bacteria (GN) genes under YN100 treatment was significantly ($P < 0.05$) higher than those under Y treatment in the soil. Network analysis showed that the potential hosts of PAH-RHDα genes were *Lysobacter*, *Rhizobium*, *Bacillus*, *Devosia*, *Ohtaekwangia*, *Ramlibacter*, *Massilia*, *Steroidobacter*, *Phenylobacterium* and *Microvirga*. *Bacillus*, *Lysobacter*, *Rhizobium* and *Ohtaekwangia* and all ten genera obviously increased under Y and YN100 treatments. These results indicate that combined application of corn straw and citric acid increased the PAH-degrading bacteria and PAH-RHDα genes, thus improving the biodegradability of PAHs in the soil. As these results verified, a combined corn straw-rhizosphere approach should be a feasible remediation strategy for PAHs-contaminated soil.

Introduction

Polycyclic aromatic hydrocarbons (PAHs), some of which are mutagenic and carcinogenic benzene-ring hydrophobic aromatic pollutants, are ubiquitous and persistent in the environment (Zhang et al. 2018). It has been found that industrial site associated with petroleum refining, gas production, and the processes of coke production industries posed severe threats to the surrounding environment and human health (Jia et al. 2017). According to China soil pollution status survey in 2014, 1.4 % of soils were polluted by PAHs and PAHs become the second largest soil organic pollutant in China (Ministry of Ecology and Environment 2014). It is now necessary to eliminate them to reduce their negative effects on human health and ecosystem, as well as their spread to other environmental media.

Plant-bacteria interactions, in the process of phytoremediation and microbial remediation, have long been recognized as efficient and economical solution to remedy PAHs-contaminated soils through different mechanisms, especially for the soils where large areas of contamination do not require emergency remediation (Cristaldi et al. 2017; Posada-Baquero et al. 2020). During plant growth, 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 the bioavailability of contaminants from contaminated soils (Guo et al. 2017; Jia et al. 2018). Low molecular weight organic acids (LMWOAs), for instance, citric, malic and oxalic were reportedly the main components in root exudates (Ling et al. 2009). There are reports that compared with other compounds in root exudates, LMWOAs play

a key role in pollutant degradation (Sivaram et al. 2019). Vázquez-Cuevas et al. (2020) concluded that citric acid and malic acid could increase the desorption of phenanthrene in soil. Similarly, Gao et al. (2015) found that LMWOAs could dramatically promote the release of bound PAH residues, and citric acid has the largest release of PAHs in soil. The bioavailability of PAHs in soils could increase by desorption of LMWOAs, therefore, it may cause potential harm to ecosystems and human through specific exposure pathways.

Through this measure the increase of PAHs degraders in rhizosphere soil might enhance the degradation of PAHs desorbed by LMWOAs. As a national climate policy, crop straws were recommended to be returned to the fields to control air pollution caused by open-field burning, improve and retain the soil fertilization due to that crop returning straw to the field could enhance soil macro-aggregation, promote carbon storage, improve soil structure, and increase the richness and diversity of microbial communities (Wang et al. 2018; Liu et al. 2019; Zhou et al. 2020). China has a large agricultural output and straw producing reached ~700 million tons annually (Latifmanesh et al. 2020). Corn straw returning to the soil is increasing every year, accounting for 30.8% in northeast and north China (Wang et al. 2011; Gu et al. 2015). Numerous studies found that carbon substrate returning to soil could improve soil conditions, enhance the bioavailability of PAHs, thereby accelerating PAHs degradation in soils (Hamdi et al. 2007; Li et al. 2012; Wu et al. 2013; Garcia-Delgado et al. 2015; Sigmund et al. 2017; Wu et al. 2020). Although many studies have reported that the effect of carbon substrate or LMWOAs on the PAHs degradation in soils, there are only limited attempts to solve whether the rhizo-degradation of PAHs occurs in straw-amended soils. Besides, what is needed now is further work to explore the influence of LMWOAs on the microbial communities in the rhizosphere of straw-amended soil.

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). Thus far, most researches aimed at evaluating the change of microbial communities or PAH-RHDα genes in soils contaminated by PAHs (Jurelevicius et al. 2011; Kong et al. 2018). Network analysis (NA) was widely applied to study the connection of entities and the co-occurrence patterns between genes and microbial groups, which could help us to speculate the potential host of functional genes (Barberán et al. 2012; Li et al. 2015; Zhang et al. 2020). NA was a reliable tool that offered us new insights into the antibiotic resistance genes and their potential hosts during composting process (Zhang et al. 2016; Bao et al. 2019a). However, few studies have attempted to research the co-occurrence patterns between PAH-RHDα genes and microbial taxa, which are helpful to explore the relationship between the association of bacteria and PAH-RHDα genes.

In this research, we hypothesized the increase of PAHs biodegradation in rhizosphere soil via enhancing bioavailability of PAHs by citric acid and stimulation of both microbial activity and metabolic capability

of microorganisms added with corn straw. The aim of current research were to: (1) investigate whether combined application of corn straw and root exudates (citric acid) could enhance PAHs degradation in soils; (2) study the change of the copies of PAH-RHDα genes and microbial community structures; (3) further predict the potential PAH-RHD genes host by NA.

Materials And Methods

Corn straw and soil

A PAHs-contaminated soil at a depth of 0-20 cm was obtained from the surrounding of a coal-power plant with 59 years history and the detailed description of location was reported in our previous study (Bao et al., 2020). Soil pH was determined with a pH meter (water: soil = 2.5:1, w/v, pH = 8.37). The potassium dichromate volumetric method (external heating method) was used to determine the soil organic matter (SOM, 15.16 g kg^{-1}). The texture of soil was classified as a sandy loam, which contains silt (58.76%), sand (36.27%) and clay (4.77%). The individual PAHs concentration was shown in Table S1. The source and physicochemical properties of corn straw have been described by Bao et al. (2019). Citric acid used was of analytical purity and obtained from Sinopharm Chemical Reagent Co., Ltd of China.

Pot experiments

The indoor simulation included four treatments: without added with citric acid or corn straw (CK), added with 100 mg kg^{-1} citric acid (N100), 5% corn straw (Y) or combined application of citric acid (100 mg kg^{-1}) and 5% corn straw (YN100). The level of citric acid and corn straw were based on previous studies (Bao et al. 2019b; Li et al. 2019c; Vázquez-Cuevas et al. 2020). 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. The water holding capacity of soil was adjusted to approximate 70%. After 28 days, part of each soil sample was reserved for determination of soil PAHs, and the others were stored at -80 °C for soil microbial community structure and qPCR analysis.

PAHs measurement

Soxhlet extraction was performed to extract PAHs from soil and 120 mL of acetone and dichloromethane (DCM) mixture (1:3, v/v) were used to extract each soil sample (approximately 4.0 g) according to USEPA Standard Method 3540C (USEPA 1996). The methods of sample purification and detection were same as our previous studies (Tian et al. 2017; Bao et al. 2018). High performance liquid chromatography fluorescence detector (HPLC-FLD, Shimadzu, LC-20A) equipped with an ultraviolet detector (PF-20A) and a fluorescence detector (SPD-20A) was used to determine PAHs. The HPLC system was fitted with a PAH-specific reverse column (Φ4.6 × 150-mm Intersil ODS-P column, 5 μm, Shimadzu, Kyoto, Japan). A mixture of ultrapure water and methanol (1:1, v/v) as the mobile phase and the flow rate was 0.6 mL min^{-1}. The surrogate standard, random injection of solvent blanks and standard reference material (NIST SRM 2706 New Jersey soil) and HPLC detection limits were used as quality control. D8-Nap, d10-Ace, d10-Phe, d10-Chr, and d12- perylene was used to identify PAHs according to their relative retention time. The

variation coefficients of Σ_{15}PAHs concentrations (the value of acenaphthene did not detect in our soil sample) for duplicate samples were less than 10%. The detection limits were 0.06 to 1.39 μg kg^{-1}. The recoveries of surrogate standards were 80.0% for Pyr to 125% for Nap.

DNA extraction and quantification of PAH-RHD$_\alpha$ gene

Soil DNA was extracted from 0.5 g soil by using the E.Z.N.A.®Soil DNA Kit for soil (Omega Bio-tek, Norcross, GA, USA) following the manufacturer's instructions. The DNA solutions were stored in dark at -20 °C until further analysis.

PAH-RHD genes from Gram positive bacteria (GP, 642f/933r) and Gram-negative bacteria gram-negative (610f/911r) were quantified by the Real-time PCR (qPCR) on a Bio-Rad CFX96 instrument based on SYBR Green chemistry (Cébron et al. 2008). The primer pairs were described in Table S2. The detailed steps of qPCR were described in S1.

Microbial community analysis

The sequencing of 16S rRNA gene was performed at LC-Bio Technology Co., Ltd, Hang Zhou, Zhejiang Province, China. The universal primers 338F (50-ACTCCTACGGGAGGCAGCA-30) and 806R (50-GGACTACHVGGGTWTCTAAT-30) was used to amplifie the V3-V4 region of the bacterial 16S rRNA. The 2% agarose gel electrophoresis was used to detect the PCR amplification products, and the AxyPrep PCR Cleanup Kit was used to recover the target fragment. The Qbit fluorescence quantification system was used to quantify the purified PCR product through a Quant-iTPicoGreendsDNA Assay Kit. IlluminaMiSeq-PE300 sequencing platform was used to determine the amplified products.

Data analysis

One-way ANOVA and LSD post-hoc comparison tests, linear regression analysis and correlation analysis were conducted by SPSS 23.0. Heatmap and Circos graphs were performed using R v.4.0.1. 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

PAHs biodegradation in soils

The degradation of PAHs was monitored after 28 days incubation of soil for all the treatments (Fig. 1). The total PAHs concentration decreased from 2275 to 1873 μg·kg^{-1} in the CK treatment, suggesting the major contribution of degradation to PAHs by the indigenous microbes and in agreement with previous studies (Huang et al. 2019; Li et al. 2019c). There was no significant difference in the final concentration of PAHs between the CK and N100 treatment which was in line with previous studies. Li et al. (2019) demonstrated that compared to bulk soils the biodegradation efficiency of phenanthrene (10.7%) in ryegrass rhizosphere soil was significantly increased, while not in soils added with ryegrass root

exudates. Moreover, Vázquez-Cuevas et al. (2020) confirmed 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 degradation of ^{14}C-phenanthrene in soils. Compared to CK, Y treatment significantly ($P < 0.05$) increased PAHs degradation in contaminated soils. Our previous study revealed that high addition of corn straw (4% or 6%) enhanced the PAHs degradation in soils (Bao et al. 2019). Organic substrates could improve soil aeration and nutrient levels, as well as provide shelter for soil microorganisms, thus improving the activities of microorganism and enhancing the degradation of organic pollutants (Barathi and Vasudevan 2003). Similarly, it has been reported that returning organic substrates (sawdust, mushroom cultivation substrate, pea straw and wheat stalk) to field could stimulate the biodegradation process of PAHs-contaminated soil (Huang et al. 2019; Li et al. 2012; Han et al. 2017; Koshlaf et al. 2019). Compared to other three treatments, YN100 treatment significantly decreased ($P < 0.05$) the final PAHs concentration in soil. The increase of PAHs degradation in soils could be explained from two possible perspectives. Firstly, the addition of citric acid could promote the desorption of PAHs in soil, which has been indicated by previous researches (Ling et al. 2009; Gao et al. 2015; Zhang et al. 2017). Secondly, returning corn straw to soil stimulated the growth of degraders related to the degradation of PAHs, thus may increase the biodegradation of citric acid-desorbed PAHs.

Based on the different numbers of aromatic rings, the 15 PAHs were divided into two groups, low molecular weight (LMW, 2–3 rings) PAHs and high molecular weight (HMW , 4–6 rings) PAHs. It is well known that PAHs have a strong adsorption trend to SOM and the its aqueous solubility decrease with the increase of molecular weight, which in turn decrease their bioavailability (Dachs and Eisenreich 2000). Compared with CK, N100 treatment did not increase the degradation of LMW PAHs but enhanced the HMW PAHs degradation in soils (Fig. 1). It has been reported that the effect of organic acid (oxalic acid) on the desorption of HMW PAHs in soil was greater than the LMW PAHs (Li et al. 2019c). However, when combined with corn straw, the stimulatory effect of citric acid on the dissipation of low-ring PAHs may be increased, resulting in a significant difference between YN100 and CK for LMW PAHs. Compared with CK, Y and YN100 treatment significantly ($P < 0.05$) enhanced HMW PAHs degradation in soil by 11.98% and 20.95, respectively. Similarly, Huang et al. (2019) concluded that adding sawdust to soils results in the higher degradation of 5-6 rings PAHs in soils than 2-4 rings PAHs. Cellulose enzymes and ligninolytic enzymes, such as laccase, manganese peroxidase and lignin peroxidase formed during the decomposition of straw have exceptional capacities for bioconversion of PAHs and may contribute to soil HMW PAHs dissipation through co-metabolic mechanisms (Li et al. 2012).

The change of PAH-RHDα genes

The abundance and composition of PAHs degradation related genes can reflect the ability of PAHs degradation in soil (Haleyur et al. 2019). The abundance of PAH-RHDα genes from Gram-positive and Gram-negative bacteria could indicate the potential of PAH degradation by soil microbial communities, mainly because the RHDα genes encode enzymes for the first step of PAH degradation, which were often used as the main biomarker to reflect the PAHs degradation in soils (Ding et al. 2010; Cébron et al. 2008). Thus, the influence of different treatments on the copies of GP or GN PAH-degrading genes was

quantitatively studied by qPCR. The results showed that compared to GP-RHDα gene, the abundance of GN-RHDα gene was relatively high in all treatments, indicating that the degradation of PAHs by Gram negative bacteria was more active than that by Gram positive bacteria, which were consistent with previous researches. As shown in Table 1, the degradation rate of total PAHs had a significant ($P < 0.05$) positive correlation with the copies of PAH-RHD GN or PAH-RHD GP genes. Similarly, it was previously reported that PAH degradation in soils was related to the copies of PAH-RHDα genes (Ding et al. 2010; Li et al. 2019a). In addition, the PAH-RHD GN gene had a strong significant ($P < 0.01$) and positive 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) for PAH-RHD GN and PAH-RHD GP degrader abundance in soils. As shown in Fig.2, both Y and YN100 significantly ($P < 0.05$) enhanced GP and GN genes copies, compared with CK and N100 treatments. In coincides with previous study, addition of mushroom cultivation substrate waste, cow manure and wheat stalk could significantly enhance the copies of pdo1 and nah genes, which were related to PAHs degradation (Han et al. 2017). Interestingly, the copies of PAH-RHDα GP and GN genes under YN100 treatment were higher than those under Y treatment, and especially YN100 significantly ($P < 0.05$) increased the copies of PAH-RHDα GN genes compared with Y treatment. 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. This may have occurred due to that citric acid, selected as source of carbon by a highly selective bacterial community, may be utilized after 28 day incubation. Similarly, Li et al. (2019a) found that there was a minor effect of root exudates on the change of PAH-RHDα gene in soils and Wu et al. (2018) indicated the copies of PAH-RHDα genes remained stable in ryegrass rhizosphere soil.

Changes in bacterial community structures

To further understand the PAHs degradation in soils, the variation in the soil microbial community's abundance and diversity through high throughput sequencing of the soil bacteria were investigated. Principal coordinate analysis (PcoA) was applied to investigate the change of the soil bacterial community based on OTU composition under different treatments. As shown in Fig. 3, The bacterial community of CK treatment was similar to N100 treatment, but different from corn straw treatment (Y and YN100 treatment). The Fig. 4 showed the changes of bacterial communities at the phylum level in 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. S2, the degradation rate of PAHs was significantly ($P < 0.01$) positive correlation with the abundance of *Proteobacteria*, *Bacteroidetes* and *Firmicutes*. *Proteobacteria* was showed as the most dominant phyla, accounting for 49.49%, 48.32%, 28.10% and 29.75% under Y, YN100, CK and N100 treatment, respectively. Previous studies indicated that Alphaproteobacteria, Gammaproteobacteria and Betaproteobacteria were found to be the potential

bioindicator of PAHs in soils (Martin et al. 2012; Niepceron et al. 2013; Li et al. 2019b). In addition, *Firmicutes* and *Bacteroidetes* have been reported to show great potential for PAHs degradation (Zhu et al. 2017; Guo et al. 2020). The present results indicated that Alphaproteobacteria, Gammaproteobacteria and Betaproteobacteria were the dominant classes under Y or YN100 treatments, which is similar with Koshlaf et al. (2019). 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.

Changes in PAH-related degrading genus

High-throughput sequencing mainly focused on the structure of the bacterial communities in soils. However, it is hard to find the change of the bacteria genus related to PAHs degradation. In this research, network analysis was used to analyze the relationship between microbial community and PAH-RHDα genes and determine the possible hosts of PAH-RHDα genes. As shown in Fig. 5, 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 of asphaltene in soils (Song et al. 2018). In addition, *Massilia*, *Phenylobacterium* and *Steroidobacter* were regarded as key genera for PAHs degradation in soils (Li et al. 2019c; Cebron et al. 2015; Huang et al. 2019). However, there is currently no information on the role of Ramlibacter and microvriga for PAH degradation, because it has not been reported to be associated with PAHs degradation, and their roles in PAH contaminated soil are still unclear.

The changes in the relative abundances of PAHs bacteria at genus level were shown in Fig. 6. 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 ($P < 0.05$) correlated with the degradation rate of HMW PAHs (r=0.725–0.834) and total PAHs (r=0.708–0.835), respectively (Fig. S1). 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 degradation rate of PAHs was low in soil treated with citric acid.

Proposed degradation mechanism of PAHs in YN100 treatment

The highest degradation rate of PAHs was found in soil under YN100 treatment than other three treatments. Fig. 7 illustrates the potential degradation mechanism of PAHs added with citric acid and corn straw. Citric acid facilitated the desorption of PAHs in soils, which indicated by previous researches (Zhu et al. 2009; Jia et al. 2016). Corn straw increased soil nutrients and improved soil aeration condition,

which was beneficial to enhance the PAH-degrading bacterial biomass in PAH-contaminated soil. Citric acid-desorbed PAHs was degraded by PAHs degraders via increase the expression of PAH-RHDα genes.

Conclusions

In the present study, the possibility of rhizo-degradation of PAHs occuring in corn straw-amended soil was explored. In addition, qPCR and high throughput sequencing analysis were applied to investigate the change of PAH-RHDα genes and the bacterial community composition in soil under different treatments. Combined application of corn straw and citric acid significantly increased the degradation efficiency of PAHs, but citric acid alone offers a slight contribution to accelerate PAHs degradation in soil. The increased biodegradation 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 addition increased the copies of PAH-degrading genes and abundance of PAHs degraders. On the whole, the present study provided fundamental insights into the rhizo-degradation of PAHs in corn straw-amended soil. Further study to investigate the changing of PAHs bioavailability treated with corn straw and citric acid will contribute to a better understanding of the potential mechanisms of this soil system for the degradation of PAHs.

Declarations

Authors' contributions Huanyu Bao: Methodology, Investigation, Software, Data curation, Formal analysis, Writing-Original Draft. Jinfeng Wang: Methodology, Investigation, Software, Data curation, Formal analysis, Writing-Original Draft. He Zhang: Investigation. Jiao Li: Investigation. Fuyong Wu: Conceptualization, Methodology, Resources, Supervision, Funding acquisition, Project administration, Writing-review & editing.

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Data availability The authors declare that all relevant data supporting the findings of this study are included in this article and its supplementary information files.

Compliance with ethical standards

Ethical approval and consent to participate Not applicable.

Consent for publication Not applicable.

Competing interests The authors declare that they have no competing interests.

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Table 1

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)

Figures

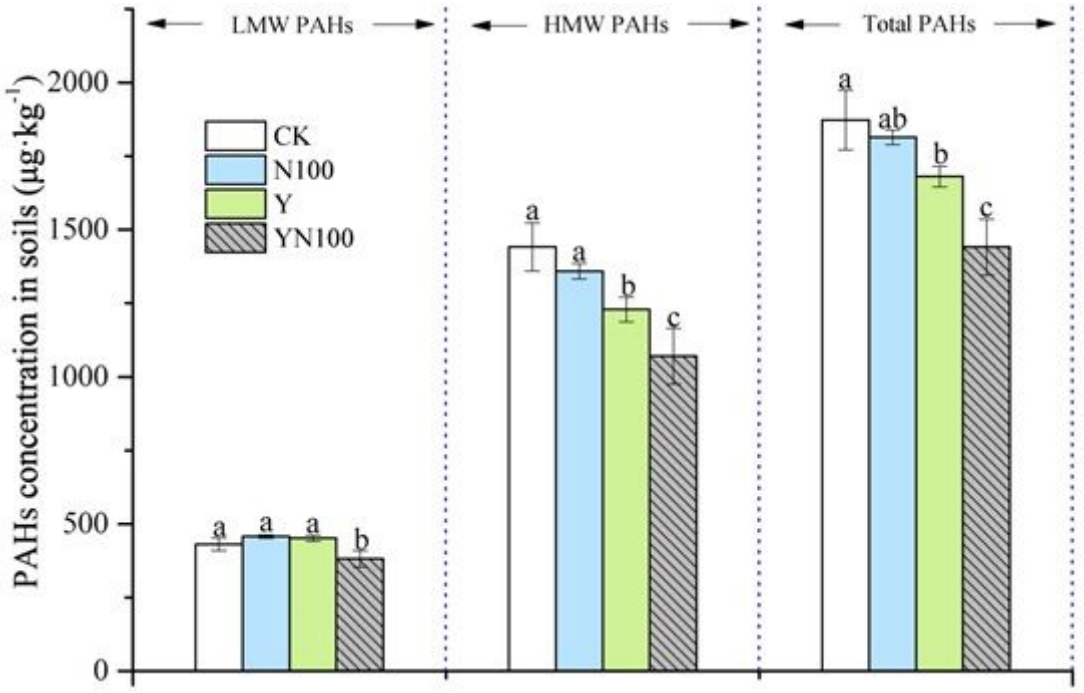

Figure 1

Concentration of LMW PAHs, HMW PAHs and Total PAHs in PAHs-contaminated soils under different treatments after 28 days of incubation. CK: no citric acid and no corn straw addition; N100: soil amended with 100 mg·kg-1 citric acid; Y: soil amended with 5 % corn straw; YN100: soil amended with 5 % corn straw and 100 mg·kg-1 citric acid. 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).

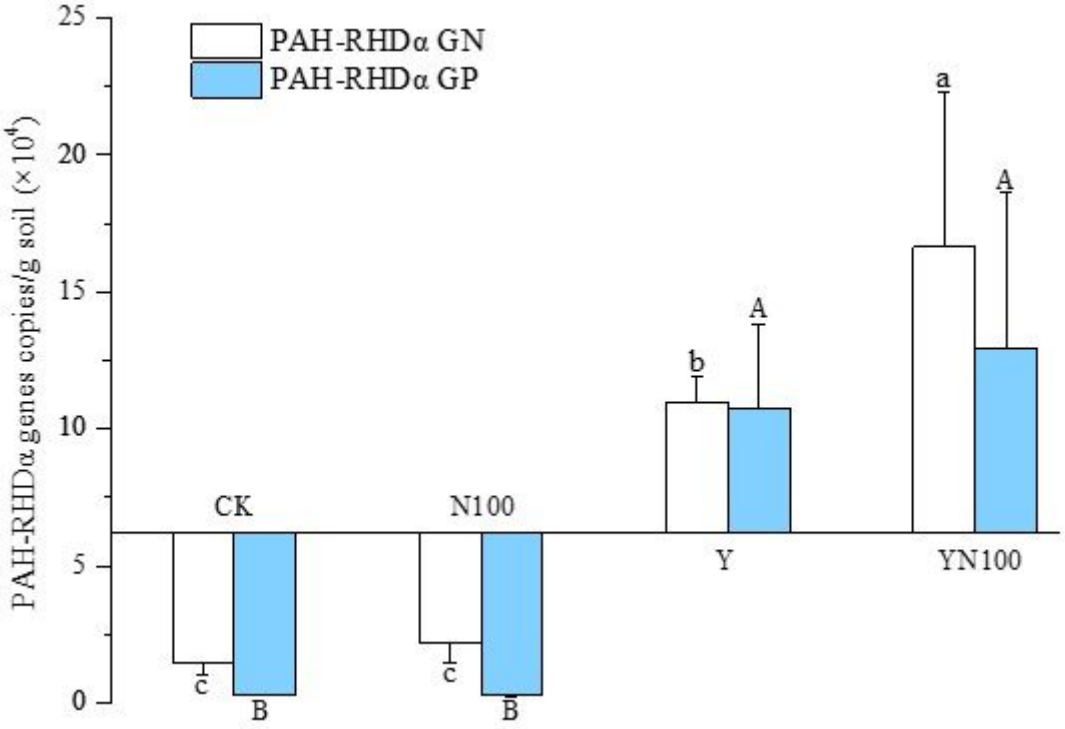

Figure 2

Variation in abundance of PAH-RHDα genes numbers detected by quantitative PCR in PAHs-contaminated soils under different treatments after 28 days of incubation. Data are expressed as the mean ± standard deviation of three replicated treatments. Different uppercase and lowercase letters indicate significant differences of PAH-RHDα GN genes copies and PAH-RHDα GP genes copies among different treatments. CK: no citric acid and no corn straw addition; N100: soil amended with 100 mg·kg-1 citric acid; Y: soil amended with 5 % corn straw; YN100: soil amended with 5 % corn straw and 100 mg·kg-1 citric acid.

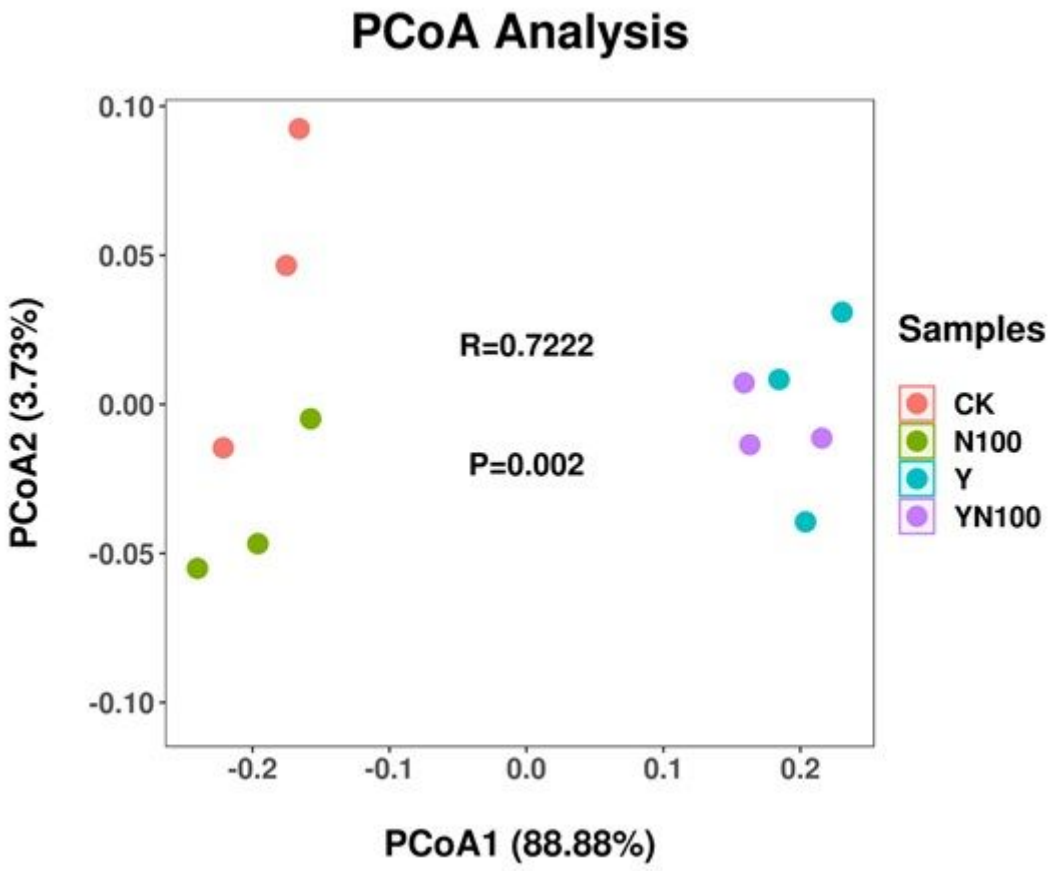

Figure 3

Principal coordinates analysis (PCoA) of bacterial communities in PAHs-contaminated soils under different treatments. CK: no citric acid and no corn straw addition; N100: soil amended with 100 mg·kg-1 citric acid; Y: soil amended with 5 % corn straw; YN100: soil amended with 5 % corn straw and 100 mg·kg-1 citric acid.

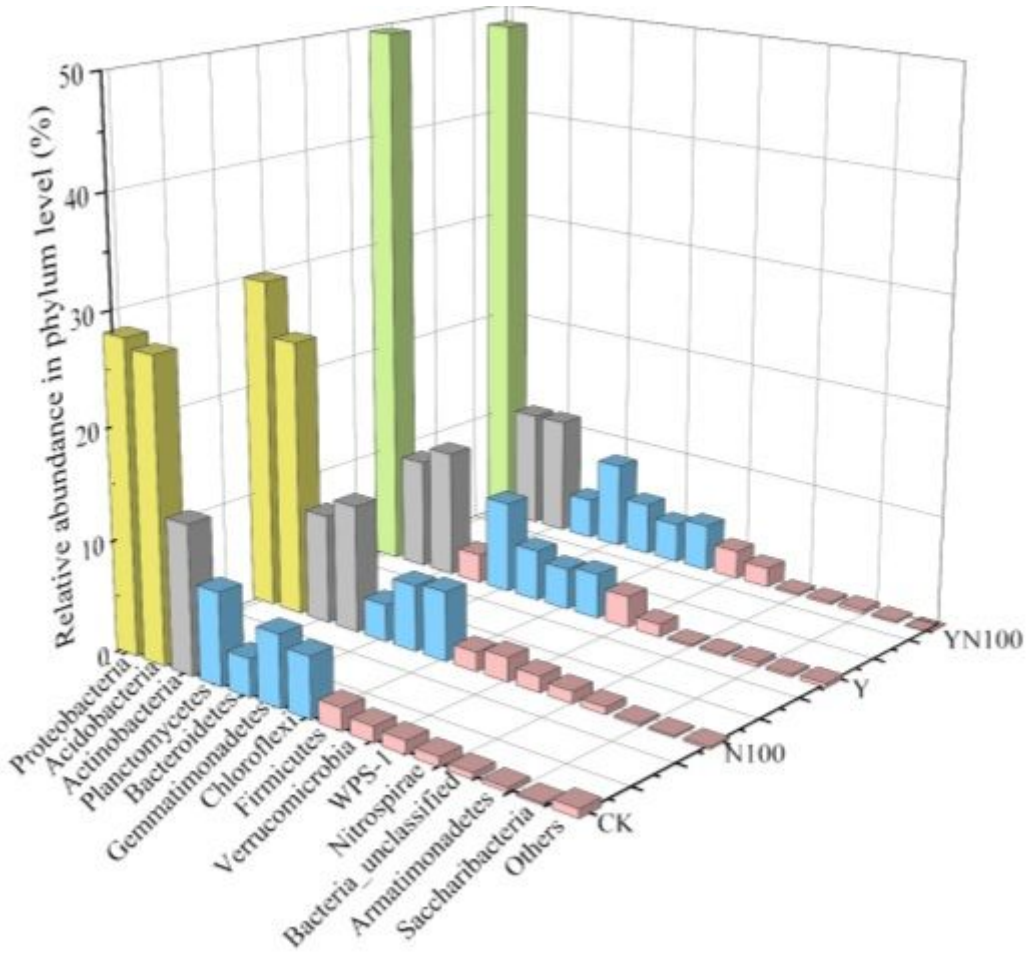

Figure 4

Relative abundances of bacterial phylum in PAHs-contaminated soils under different treatments after 28 days of incubation. CK: no citric acid and no corn straw addition; N100: soil amended with 100 mg·kg-1 citric acid; Y: soil amended with 5 % corn straw; YN100: soil amended with 5 % corn straw and 100 mg·kg-1 citric acid.

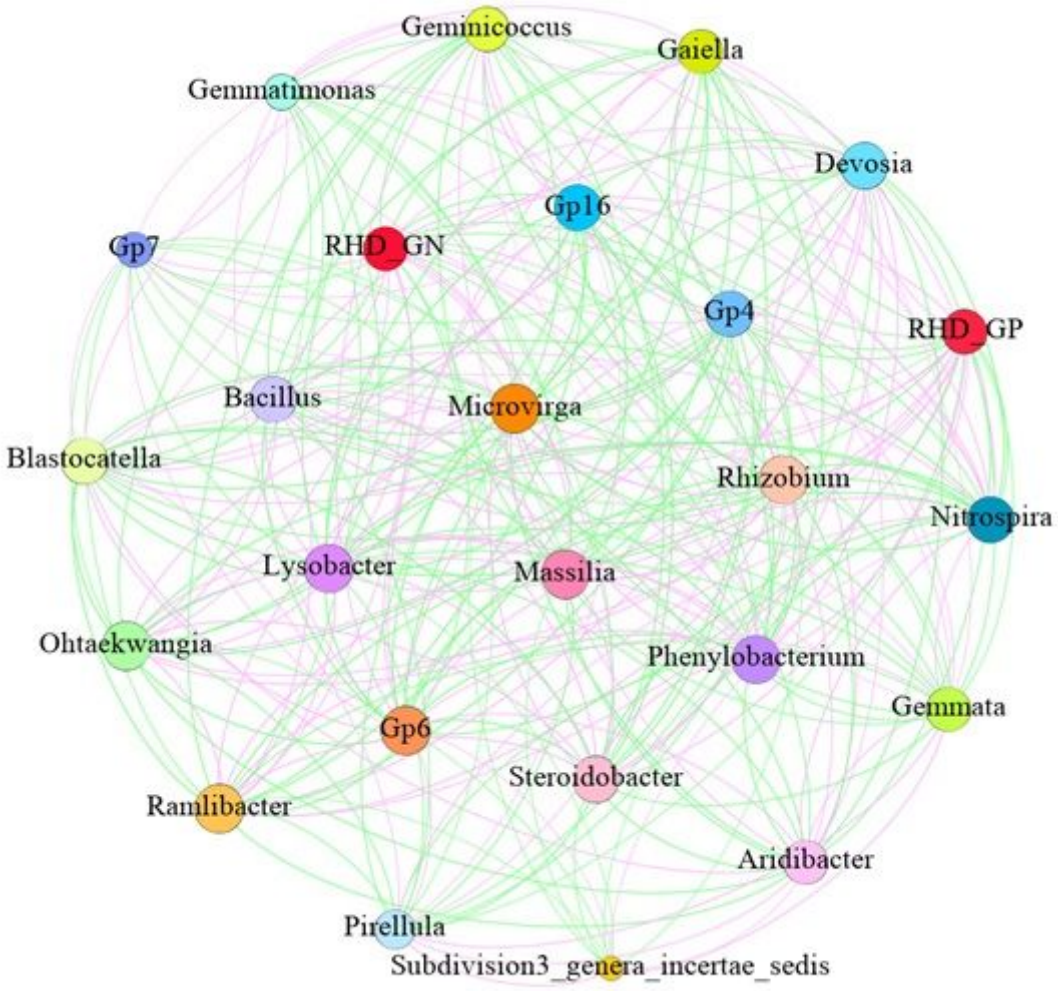

Figure 5

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.

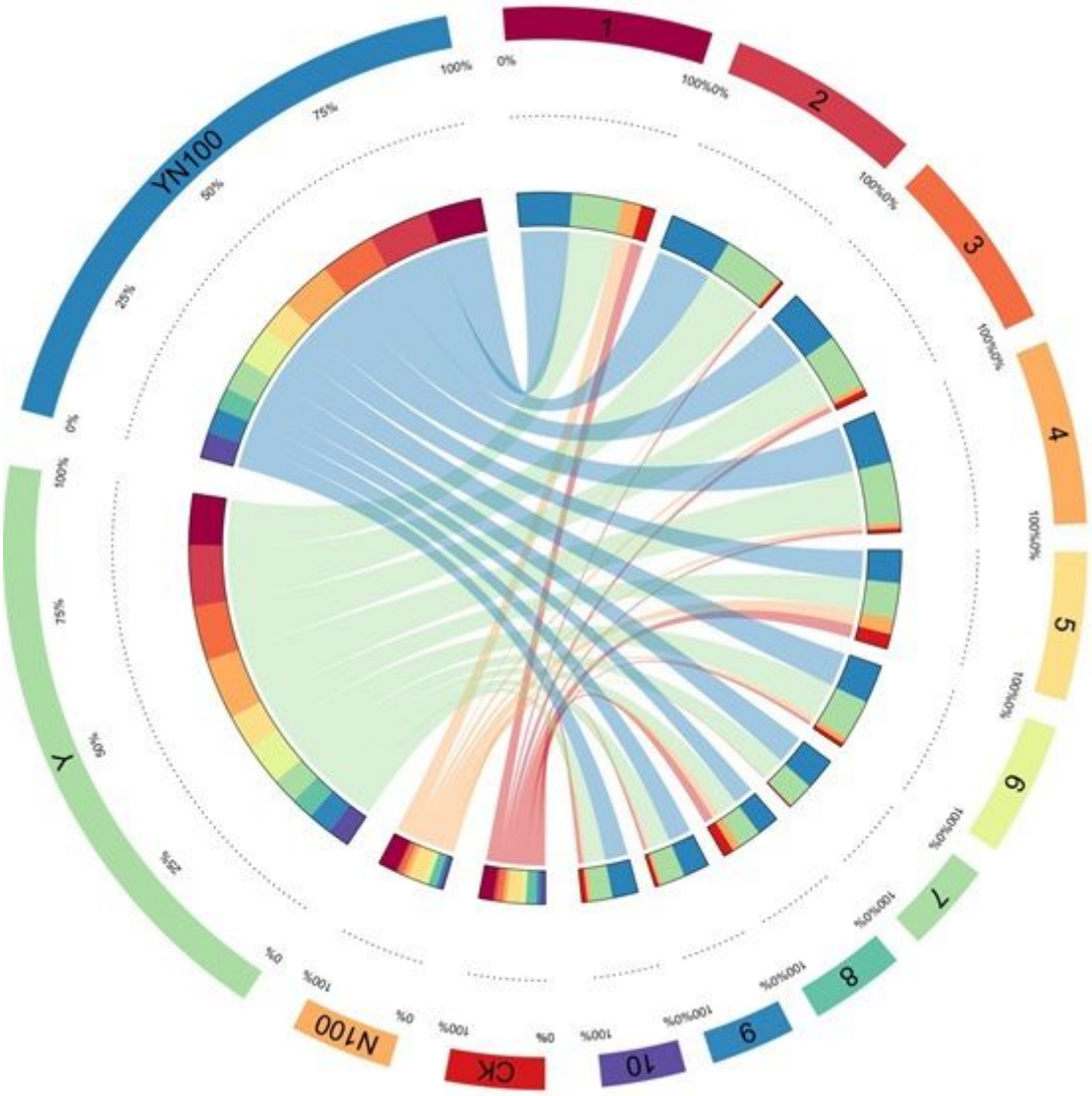

Figure 6

Changes in the relative abundances of PAHs degrader genus in PAHs-contaminated soils under different amendment treatments during 28 days of incubation. 1: Lysobacter; 2: Rhizobium; 3: Bacillus; 4: Devosia; 5: Ohtaekwangia; 6: Ramlibacter; 7: Massilia; 8: Steroidobacter; 9: Phenylobacterium; 10: Microvirga.

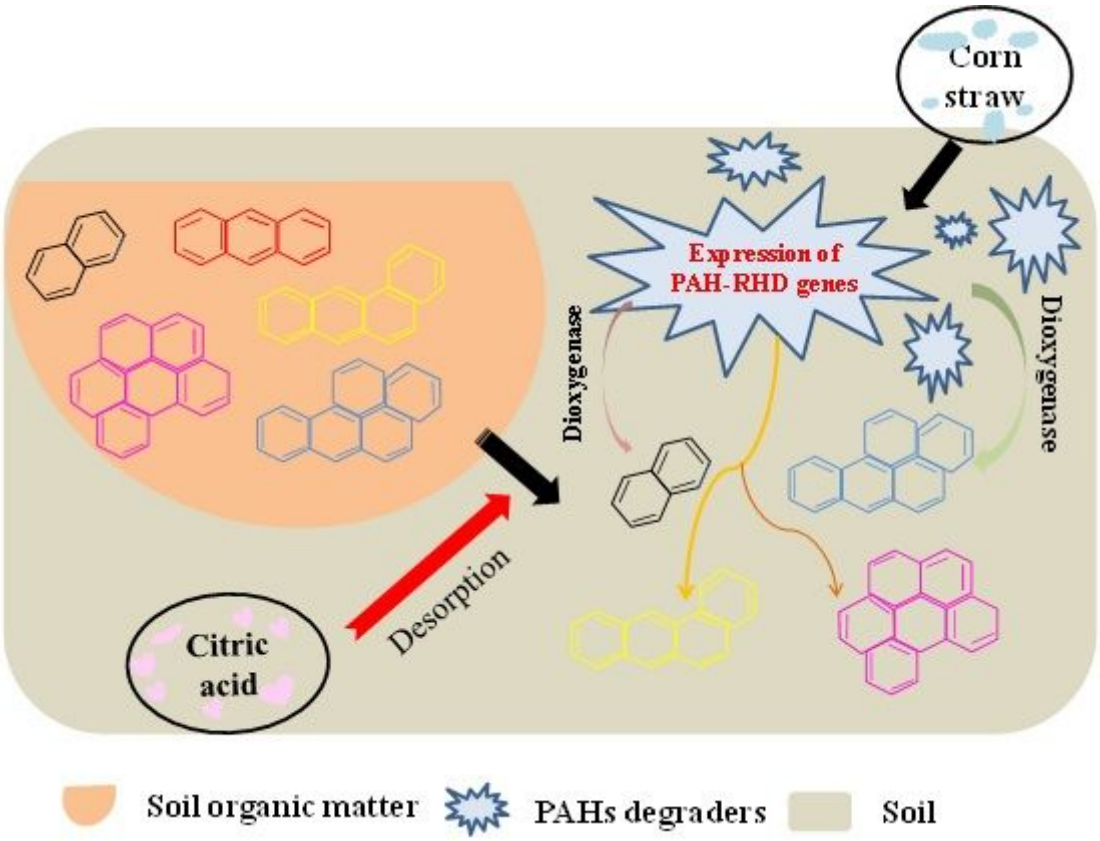

Figure 7

The possible degradation mechanism of PAHs in soil treated with corn straw and citric acid.

Supplementary Files

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- Supplementarydata.docx