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Exploring the PAHs dissipation and indigenous bacteria response in soil amended with two different microbial inoculants

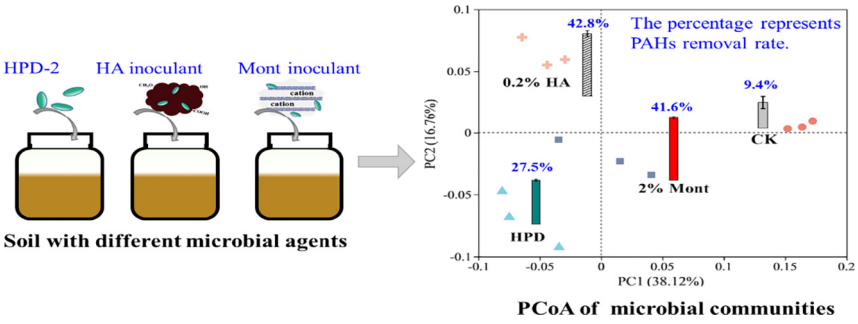
Beibei Wang [a,b], Ying Teng [a,*], Ran Li [a], Ke Meng [a,c], Yongfeng Xu [a], Shiliang Liu [b], Yongming Luo [a]

[a] Key Laboratory of Soil Environment and Pollution Remediation, Institute of Soil Science, Chinese Academy of Sciences, Nanjing 210008, China
[b] College of Resources and Environment, Henan Agricultural University, Zhengzhou 450002, China
[c] Soil Physics and Land Management Group, Wageningen University & Research, Droevendaalsesteeg 3, 6708PB Wageningen, the Netherlands

HIGHLIGHTS

- HA inoculant and Mont inoculant could dramatically accelerate PAHs removal in soil.
- HA inoculant assist PAHs removal by improving bioavailability and bioaugmentation.
- Mont inoculant enhance bioremediation by stimulating and protecting microorganisms.
- The maximum diversity of PAH degrading genera was found in 2 % Mont treatment.

GRAPHICAL ABSTRACT



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ABSTRACT

This study investigated the bioremediation of PAHs in soil by two different microbial inoculants prepared with *Paracoccus aminovorans* HPD-2 and the carrier humic acid (HA) or montmorillonite (Mont). After incubation for 42 d, the greatest removal of PAHs, 42.8 % or 41.6 %, was observed in microcosms with 0.2 % HA inoculant or 2 % Mont inoculant. The PAH removal efficiency in these treatments was significantly greater than that in soil amended only with planktonic HPD-2. Bacterial community analysis showed that the survival of *Paracoccus aminovorans* was enhanced in the treatments with Mont inoculant compared with the treatments with HA inoculant or with HPD-2 alone. Moreover, the diversity of PAH-degrading bacterial genera was greater in the treatments containing Mont inoculant than in the treatments containing HA inoculant. These results indicate that the organic material HA and inorganic material Mont promote PAH removal in different ways. Specifically, HA promotes PAHs bioavailability to accelerate the degradation of PAHs in soil, whereas Mont protects PAH-degrading microorganisms to promote pollutant removal. Overall, the findings suggest that HA and Mont are promising materials for microbial immobilization for the bioremediation of PAH-contaminated soil.

1. Introduction

Remediating soil contaminated with polycyclic aromatic hydrocarbons (PAHs) is an urgent concern due to the potential toxicity, mutagenicity and carcinogenicity of PAHs (Rathankumar et al., 2022). Biotic degradation is the main natural attenuation mechanism for PAHs, and researchers are increasingly favoring bioremediation because of its safe, energy-efficient and eco-friendly profile (Liu et al., 2021a). Previous work suggests that bioaugmentation or biostimulation of PAH-degrading bacteria and fungi holds potential for environmental PAHs remediation (Brzeszcz et al., 2020; Wu

* Corresponding author at: Key Laboratory of Soil Environment and Pollution Remediation, Institute of Soil Science, Chinese Academy of Sciences, 71 East Beijing Road, Nanjing, Jiangsu Province 210008, China.
E-mail address: yteng@issas.ac.cn (Y. Teng).

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et al., 2016). However, microbial remediation remains challenging due to the limited survival of degrading microorganisms, the low bioavailability of contaminants on the spatial and temporal scales, and inappropriate ambient temperatures and nutrient levels (Megharaj et al., 2011). For example, we previously reported that the bacterium *Paracoccus aminovorans* HPD-2 degraded 89.7 % of benzo[*a*]pyrene (BaP) in medium but only 26 % of BaP in soil (Mao et al., 2008; Teng et al., 2010). These barriers are not unique to microbial remediation, as abiotic factors such as pH, pollutant properties, and solid-phase mineral and organic matter also influence the efficiency of physical and chemical bioremediation (Megharaj et al., 2011; Punetha et al., 2022).

Depending on the conditions, soil minerals and soil organic matter either enhance or retard the removal of organic contaminants (Biswas et al., 2015). For example, the clay mineral montmorillonite significantly promoted BaP degradation in a previous study (Gan et al., 2022), whereas the clay mineral kaolinite significantly reduced BaP degradation efficiency, primarily by decreasing the bioavailability of BaP (Gan et al., 2022). In summary, clay minerals may enhance biodegradation by alleviating unfavorable factors (e.g., pH and redox potential (Eh)) and providing protection for microorganisms, or may suppress biodegradation by reducing enzyme activities or inhibiting microbial growth (Gan et al., 2022). A large fraction of hydrophobic organic compounds, including PAHs, associate with soil aggregates and persist in soil through strong associations with soil organic matter (Chen et al., 2017). Humic acid (HA) and humin (HM) are the major carbon-containing components of soil organic matter. The abundant hydrophobic carbon domains of HA and HM have high absorption capacity for organic compounds (Zhang et al., 2009), but the high adsorption capacity and affinity for humic substances does not mean that HA certainly inhibits microbial degradation of organic pollutant. As reported, HA significantly enhances the solubility of pyrene and accelerates its biodegradation (Tejeda-Agredano et al., 2014) because HA has a micellar microstructure similar to that of surfactants, which promotes the bioavailability of hydrophobic organic compounds (Smith et al., 2009).

The poor efficiency of bioremediation is often due to the short residence time of exogenous microorganisms (Luo et al., 2022). An attractive option for prolonging this residence time is to use carrier materials to deliver and protect microbial cells in natural ecosystems (Luo et al., 2022). Applying immobilized microorganisms frequently improves the efficiency of pollutant biodegradation (Zhou et al., 2021). Eco-friendly and low-cost materials are typically used as carriers to provide a favorable environment for microbial inoculants via a protective surface or pore spaces (Kauppi et al., 2011; Luo et al., 2022). As important components of inorganic and organic matter in soil, clay minerals such as montmorillonite (Mont) and humic substances such as HA might function as carriers for microbial growth and biofilm formation (Cebron et al., 2015; Han et al., 2021). The high specific surface areas and porous structures of Mont and HA might facilitate microbial colonization and contaminant retention in soil (Han et al., 2021). However, application research on the use of Mont or HA as carriers of microbial inoculants for the bioremediation of industrial PAH-contaminated soil is limited.

The present study investigated the ability of HA and Mont to function as eco-friendly carrier materials to protect microorganisms and promote PAH removal in soil. *Paracoccus aminovorans* HPD-2 was selected as the degrader to be carried on Mont or HA. Because metabolic cooperation among microorganisms is key for PAH degradation (Megharaj et al., 2011), the responses and shifts of the bacterial community and functional bacteria were also assessed to explore changes in function and metabolism. The results provide insights into the function and mechanism of microbial inoculants in bioremediation as well as convincing evidence for the application of microbial remediation.

2. Materials and methods

2.1. Soil and reagents

PAH-contaminated soil was sampled at an industrial site in Nanjing, China. The physicochemical properties of the soil were characterized in accordance with the methods reported by Lu (1999), and the following results were obtained: pH 8.32, 21.09 g kg^{-1} organic matter, 17.20 cmol kg^{-1} cation exchange capacity, 0.51 g kg^{-1} total nitrogen, 0.64 g kg^{-1} total phosphorus, 17.35 g kg^{-1} total potassium, 61.25 mg kg^{-1} alkali-hydrolyzable nitrogen, 8.42 mg kg^{-1} available phosphorus, and 118.67 mg kg^{-1} available potassium. Thirteen PAHs (≥3-ring) were determined: fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo[*a*]anthracene, chrysene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*a*]pyrene, dibenzo[*a*,*h*]anthracene, benzo[*g*,*h*,*i*]perylene and indeno[1,2,3-*cd*]pyrene. It was worth noting that total content of PAHs is up to 452.76 mg kg^{-1}, which is classified as seriously contaminated soil. The content of each PAH in the selected soil is shown in Fig. S1. The 4-ring PAHs pyrene and fluoranthene were present at the highest concentrations, 100.59 mg kg^{-1} and 69.03 mg kg^{-1}, respectively, and accounted for 22 % and 15 % of the total PAH content. Pyrene and fluoranthene were followed by the 5-ring PAH BaP, the 6-ring PAH indeno[1,2,3-cd] pyrene, and the 3-ring PAH phenanthrene, which accounted for 10 %, 10 % and 9 % of the total PAH content with concentrations of 45.13 mg kg^{-1}, 44.97 mg kg^{-1} and 42.96 mg kg^{-1}, respectively.

Mont ($Al_2O_9Si_3$) and HA were obtained from Yuanye Biotechnology Co., Ltd. (Shanghai, China). According to the manufacturer, the specific surface area of Mont was 240 m^2 g^{-1}, with a purity of >98 % and mean particle size of ~20 μm. HA had an organic material content of >75 % and a particle size of 250 μm, and ^{13}C NMR analysis indicated that the carbon fraction of HA consisted of 7.43 % aliphatic C, 11.37 % carboxylic C, and 73.65 % aromatic C (Dai et al., 2018). The surface functional groups and morphology of HA were characterized by attenuated total reflection Fourier transform infrared (ATR-FTIR) spectroscopy and scanning electron microscopy (SEM), and the results are shown in Fig. S2A and B, respectively. High-performance liquid chromatography (HPLC)-grade acetonitrile was purchased from Merck KGaA (Darmstadt, Germany). Hexane and other analytical grade reagents were provided by Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China).

2.2. Preparation of bacterial inoculants and the microcosm experiment

Paracoccus aminovorans HPD-2 is a high-efficiency high-molecular-weight PAH (HMW PAH)-degrading bacterium that was originally isolated from historically PAH-contaminated soil in Wuxi, Jiangsu Province (Mao et al., 2008). Strain HPD-2 was cultured in LB medium, and the cells were concentrated and resuspended in sterile water. Mont inoculants were separately prepared with HPD-2 (10^7–10^8 colony forming units (CFUs) g^{-1} soil) and 1 % Mont (weight of Mont: weight soil) or 2 % Mont (weight of Mont: weight soil). HA inoculants were also prepared as described in Mont inoculants with 0.2 % (weight of HA: weight soil) or 0.5 % HA (weight of HA: weight soil).

Each microcosm was prepared by placing 20 g of soil (dry weight) in a 100-mL canning jar covered with a PTFE breathable membrane. The microcosm experiments were set up with the following treatments in triplicate: (1) CK, the initial soil; (2) HPD, amended with planktonic HPD-2 (10^7–10^8 CFUs g^{-1}); (3) 0.2 % HA, amended with 0.2 wt% HA inoculant; (4) 0.5 % HA, amended with 0.5 wt% HA inoculant; (5) 1 % Mont, amended with 1 wt% Mont inoculant; (6) 2 % Mont, amended with 2 wt % Mont inoculant. The initial content of HPD-2 in treatments with HPD-2 and inoculants was kept 10^7–10^8 CFU g^{-1}. The microcosms were incubated for 42 d at 30 °C without supplementary illumination, and the moisture was adjusted to 60 % of the soil water holding capacity.

2.3. Extraction and detection of PAHs in soil

PAHs were extracted using a modification of the method of Huang et al. (2013). In brief, 2.00 g of dried and homogenized soil was extracted in a Soxhlet extraction system with 70 mL of dichloromethane for 24 h. Next, the sample was purified and filtered, and then analyzed by HPLC with fluorescence detection (HPLC–FLD) (Shimadzu, Kyoto, Japan) according to Huang et al. (2013). The PAH content was quantified by reference to a

series of PAH mixtures prepared by diluting a standard solution with acetonitrile. The R^2 values for the 13 PAHs ranged from 0.993 to 0.997, and the detection limit ranged from 0.01 to 0.10 μg L^{-1} (Table S1). The recovery rates for PAH extraction from soil were also determined and are listed in Table S1.

2.4. DNA extraction and Illumina sequencing

Total genomic DNA was extracted from 0.5 g of soil sample using the FastDNA™ SPIN Kit for soil (MP Bio., USA) according to the manufacturer's protocols. The V4-V5 region of the bacterial 16S rRNA gene was amplified using the primers 515F (5'-GTGCCAGCMGCCGCGG) and 907R (5'-CCGTCAATTCM TTTRAGTTT), and the amplicons were purified and submitted to Majorbio Bio-Pharm Technology Co., Ltd. (Shanghai, China) for sequencing on the Illumina MiSeq platform according to standard protocols. The sequencing data were then analyzed on the Majorbio cloud platform. The sequences were deposited in the Sequence Read Archive (SRA) of the National Center for Biotechnology Information (NCBI) under accession number PRJNA825660.

2.5. Statistical analysis

Figures were prepared using Rstudio, Origin and TBtools. Statistical analysis was performed in SPSS 20.0 (IBM, Chicago, IL). Duncan's multiple range test was used to compare the mean values of different groups.

3. Results

3.1. PAHs dissipation in soil

After incubation for 42 d, the removal of PAHs in the different treatments was analyzed (Fig. 1). The total PAHs removal after 42 d ranged from 9.4 % to 42.8 %. The PAHs content in CK decreased from 451.1 mg kg^{-1} to 408.7 mg kg^{-1}, corresponding to a removal efficiency of 9.4 %. Thus, the optimal incubation environment stimulated the natural reduction capacity of the long-term contaminated soil. In the other treatments, >25 % of the PAHs were removed. Specifically, in the HPD treatment, 27.5 % of the PAHs were eliminated, a significant increase compared with CK. Interestingly, compared with the CK and HPD treatments, the addition of 2 % Mont inoculant or 0.2 % HA inoculant increased PAHs removal significantly, to 41.6 % and 42.8 %, respectively. By contrast, there were no significant differences in PAHs removal efficiency between the 0.5 % HA inoculant, 1 % Mont inoculant and HPD treatments, suggesting that the ratio of carrier material is an important factor influencing PAHs removal by microbial agents in soil.

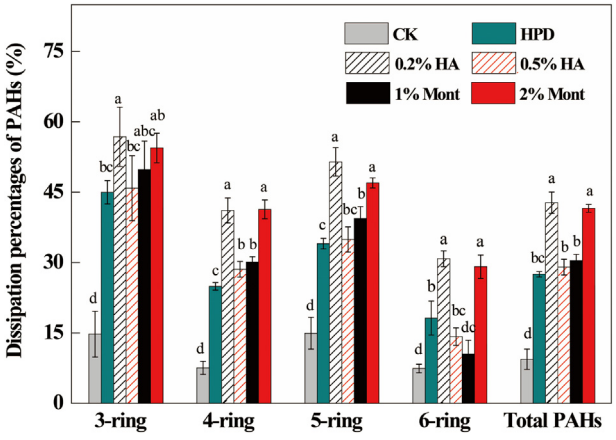

Fig. 1. Removal efficiency of 3-ring, 4-ring, 5-ring, 6-ring and total PAHs in soil amended with different bacterial inoculants after 42 d of incubation. CK, initial contaminated soil; HPD, soil amended with planktonic HPD-2; 0.2 % HA, soil amended with 0.2 wt% HA inoculant; 0.5 % HA, soil amended with 0.5 wt% HA inoculant; 1 % Mont, soil amended with 1 wt% Mont inoculant; 2 % Mont, soil amended with 2 wt% Mont inoculant. In all treatments except CK, the initial content of HPD-2 was 10^7–10^8 CFUs g^{-1}. Different letters indicate significant differences at $p < 0.05$ according to Duncan's multiple range test.

The trends of the removal of PAHs with different ring numbers were similar to that of total PAHs. The content of 3-ring PAHs was markedly lower in the treatments inoculated with HPD-2 and inoculants than in CK, and the removal efficiencies ranged from 41.6 % to 56.8 %. The best removal efficiencies for 3-ring PAHs were observed in the 2 % Mont and 0.2 % HA treatments. The maximum removal of 4-ring PAHs also occurred in the 2 % Mont and 0.2 % HA treatments, but the removal efficiency did not differ significantly between these two treatments. The removal efficiency of 5-ring PAHs was 34.0 %–51.5 %, superior to those of 4-ring and 6-ring PAHs. In the 0.2 % HA and 2 % Mont treatments, the removal efficiencies of 5-ring PAHs reached 51.5 % and 47.0 %, respectively. These results indicated that combining planktonic HPD-2 with an appropriate ratio of HA or Mont can effectively enhance the rate of PAHs removal in soil.

3.2. Shifts in bacterial diversity

The shifts in bacterial diversity in the different treatments were compared by evaluating the α and β diversities of the bacterial community based on the Ace, Shannon, and Shannon even indices and principal coordinate analysis (PCoA) (Figs. 2A, B and S3). In the initial state (0 d), the Ace, Shannon even and Shannon indices were significantly higher in CK than in the other treatments. However, at 42 days, Ace was similar among the treatments, including CK. Thus, the effects of inoculation with HPD-2 with or without carrier on Ace were temporary due to the resistance and resilience of the indigenous soil bacterial community. Over the course of the experiment, the Shannon even and Shannon indices gradually increased in the treatments inoculated with HPD-2 with or without carrier but decreased in CK; nonetheless, these two indices remained significantly higher in CK than in the other treatments after 42 d. These results indicate that the diversity of bacterial community in CK gradually decreased due to the enrichment of PAH-degrading bacteria, while the impact of the exogenous bacteria on the indigenous soil bacterial community was temporary in the other treatments.

PCoA of Bray–Curtis dissimilarity matrices of OTUs at the 97 % cutoff was performed to reveal community-level differences among the different treatments. Samples from different treatment times clustered separately, whereas samples collected at the same time clustered together (Fig. S4). As shown in Fig. 2C, the bacterial community composition of CK at 0 d was separated from those of the other treatments, indicating that inoculation with HPD-2 with or without carrier significantly changed the initial bacterial community structure. In addition, the other treatments clustered together, suggesting that the consistent inoculum of HPD-2 in these treatments resulted in similar changes in bacterial community composition and diversity. During the 42-d incubation, the bacterial communities of the different treatments clustered separately (Figs. 2D and S4B). The bacterial community of the HPD treatment clustered separately from the bacterial communities of the Mont and HA treatments. Moreover, the bacterial communities of the treatments with the same carrier, such as the 0.2 % HA and 0.5 % HA treatments, clustered together. These results suggest that both the degrader HPD-2 and the carrier materials shaped bacterial community structure.

3.3. Analysis of bacterial community composition

Valid sequences of bacterial 16S rRNA were classified using a Ribosomal Database Project naïve Bayesian rRNA classifier with a confidence of 80 %, and the community composition at the phylum level is shown in Fig. S5. At the beginning of the experiment, Proteobacteria dominated in CK, with a relative abundance of 31.4 %, followed by Actinobacteria, Chloroflexi and Acidobacteria. As expected, inoculation with HPD-2 with or without carrier augmented the abundance of its parent phylum, Proteobacteria (Fig. S5), which ranged from 80.7 % to 90.5 %. At the end

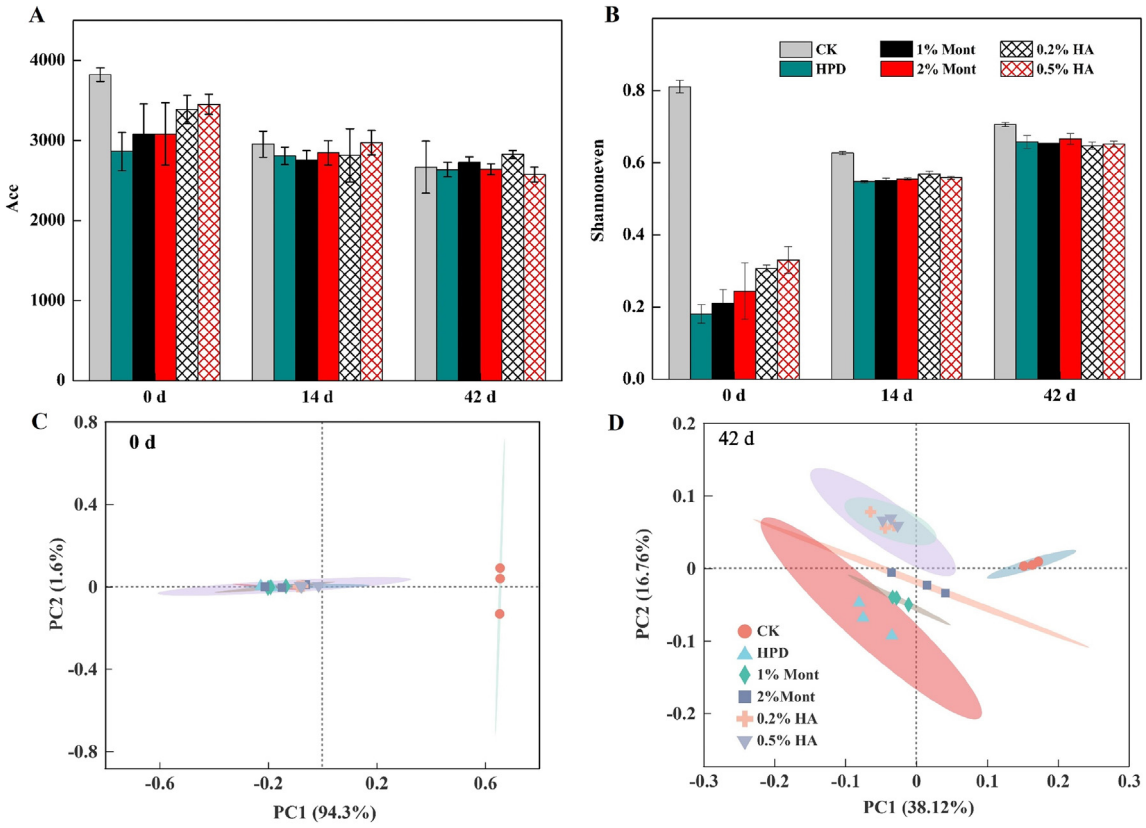

Fig. 2. The α and β diversities of the bacterial communities in the different treatments. The Ace and Shannon even indices of α diversity are shown in A and B; principal coordinate analyses (PCoAs) of the communities at 0 d and 42 d are shown in C and D. The colored regions are the confidence ellipses.

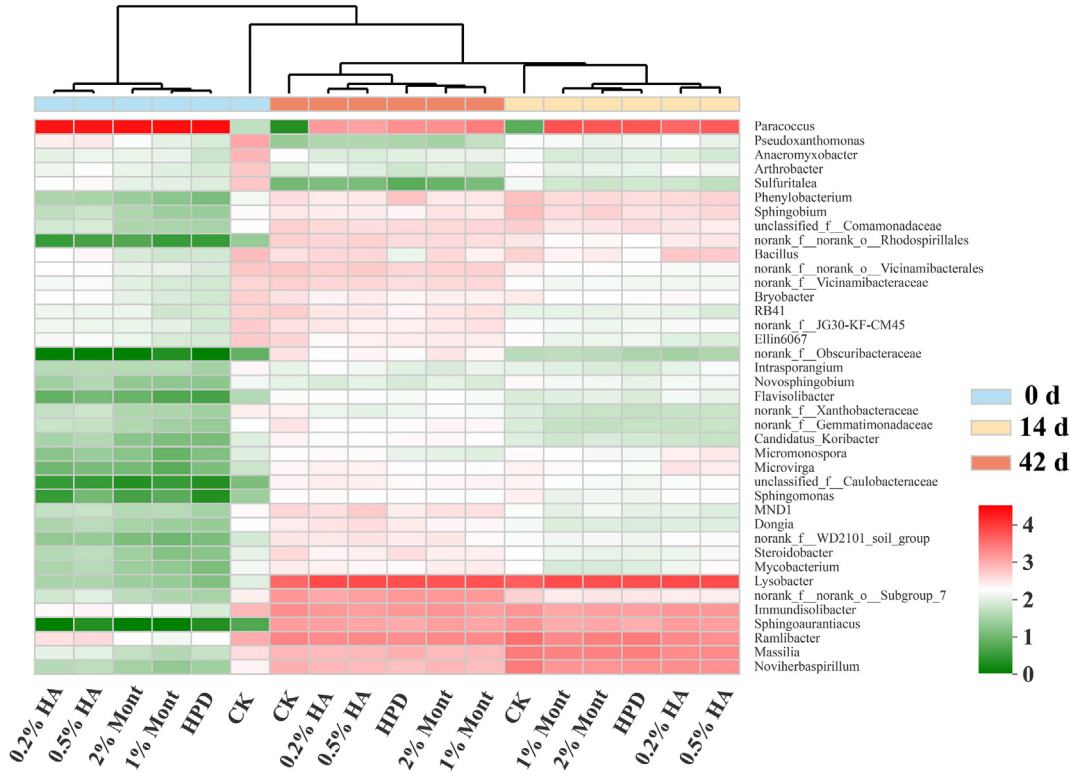

Fig. 3. Community heatmap of the top 50 bacterial genera in the different treatments at different times. The clustering trees of the treatments at each time point are shown at top.

of the experiment, the relative abundances of Proteobacteria and Acidobacteria in CK increased to 61.6 % and 14.9 %, respectively. In the other treatments, the relative abundance of Proteobacteria decreased during the experiment but remained higher than that in CK (Fig. S5).

The variation in bacterial community composition in each treatment was further analyzed and compared at the genus level. As shown in Fig. 3, the clustering of the different treatments was consistent with that in the PCoA. At 0 d, the abundance of *Paracoccus* was obviously higher in the HPD, 1 % Mont, 2 % Mont, 0.2 % HA and 0.5 % HA treatments than in CK because of the addition of HPD-2. The bacterial community at the genus level changed obviously during the course of the experiment. In particular, the relative abundance of *Paracoccus* gradually declined, indicating that this genus could not maintain its dominance in the long-term and was only temporarily enhanced inoculation with HPD-2 with or without carrier. Genera such as *Sphingomonas*, *Micromonospora*, *Microvirga*, *Flavisolibacter*, *Steroidobacter*, *Mycobacterium*, and *Lysobacter*, which have been reported as PAH-degrading bacteria in previous studies (Li et al., 2019), significantly increased in abundance. These results indicated that the addition of HPD-2 with or without carrier induced changes in the bacterial community, with gradual enrichment of bacteria associated with PAHs degradation.

3.4. Degrader survival and the functional bacterial community response

The normalized relative abundance of *Paracoccus aminovorans* was compared among the treatments to investigate degrader survival. As shown in Fig. 4A, the relative abundance of *Paracoccus aminovorans* remained stable in CK throughout the incubation period. By contrast, the relative abundance of *Paracoccus aminovorans* decreased dramatically with incubation time in the treatments inoculated with HPD-2 with and without carrier. At 42 d, the relative abundance of *Paracoccus aminovorans* in the different treatments followed the order 1 % Mont (6.6 %) > 2 % Mont (4.8 %) > HPD (4.4 %) > 0.2 % HA (4.1 %) > 0.5 % HA (3.7 %), suggesting that Mont but not HA promoted degrader survival.

Fourteen genera associated with PAHs degradation were detected (Fig. 4B). Compared with CK, *Lysobacter*, *Flavihumibacter*, and *Pseudoxanthomonas* were obviously enriched in the other treatments. Specifically, *Lysobacter* abundance increased by 88.9 % and 81.5 % in the 0.2 % HA and 0.5 % HA treatments, respectively. *Flavihumibacter* abundance increased by 64.0 % and 105.1 % in the 0.2 % HA and 0.5 % HA treatments; these increases were much greater than those observed in the 1 % Mont (51.1 %), 2 % Mont (34.7 %) and HPD (10.5 %) treatments. By contrast, the abundances of *Ramlibacter*, *Acidibacter*, *Pseudomonas*, *Novosphingobium* and *Nocardioides* decreased to varying extents in all treatments. The enriched genera were the same in treatments with the same carrier but differed between treatments with different carriers. The diversity of PAH-degrading genera was highest in the 2 % Mont treatment, which included *Lysobacter*, *Mycobacterium*, *Flavihumibacter*, *Pseudoxanthomonas*, *Sphingobium*, *Bacillus*, *Rhodococcus* and *Burkholderia*. The PAH-degrading genera that were enriched in the HA treatments, which included *Lysobacter*, *Flavihumibacter*, *Bacillus* and *Pseudoxanthomonas*, were similar to those that were enriched in the HPD treatment. Thus, both HPD-2 and the carriers induced shifts in the abundances of different functional bacteria associated with PAHs degradation in soil.

3.5. Functional prediction

Functional prediction was performed using PICRUST2. KEGG pathways related to 10 types of PAH-degrading enzymes were analyzed: acetyl-CoA *C*-acetyltransferase, protocatechuate 3,4-dioxygenase (alpha subunit), protocatechuate 3,4-dioxygenase (beta subunit), protocatechuate 4,5-dioxygenase (alpha chain), protocatechuate 4,5-dioxygenase (beta chain), 3-hydroxyanthranilate 3,4-dioxygenase, 4-hydroxy-acetophenone monooxygenase, 4,5-dihydroxyphthalate decarboxylase, naphthalene 1,2-dioxygenase ferredoxin component and naphthalene 1,2-dioxygenase ferredoxin reductase component. As shown in Fig. 5, the relative abundances of functional KEGG orthologs (KOs) increased in all treatments during the experiment, and K00626 (acetyl-CoA *C*-acetyltransferase) was dominant at all-time points. The abundances of K00626, K14578 (naphthalene 1,2-dioxygenase ferredoxin component) and K14581 (naphthalene 1,2-dioxygenase ferredoxin reductase component) increased in CK after 42 d; the corresponding genes in the KEGG database were *ACAT*, *nahAb* and *nahAa*, respectively. CK exhibited the greatest increases in the abundances of KOs but the lowest diversity and PAHs removal efficiency. In the treatments inoculated with HPD-2 with or without carrier, the abundances of all KOs except K00626 increased to varying degrees. The enriched KOs were identical in the treatments inoculated with HPD-2 with or without carrier, which had higher diversity and PAHs removal efficiencies than CK.

4. Discussion

4.1. Bacterial inoculants accelerate PAHs removal

Indigenous microorganisms play essential roles in PAHs removal (Liu et al., 2022). In this study, nonnegligible PAHs dissipation was observed

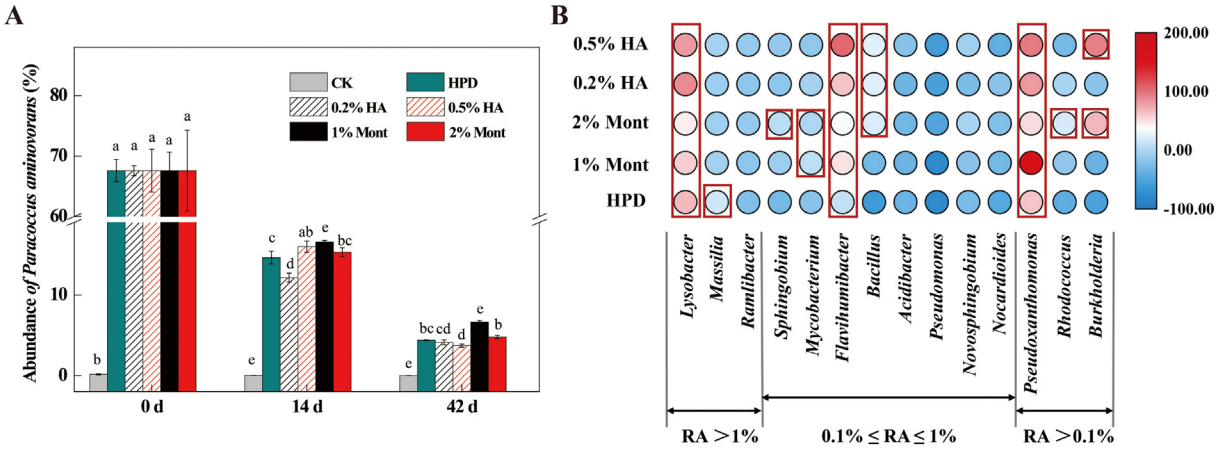

Fig. 4. The variation in the relative abundance (RA) of *Paracoccus aminovorans* (A) and PAH-degrading bacterial genera (B). Different letters indicate significant differences at $p < 0.05$ according to Duncan's multiple range test. The relative abundance of *Paracoccus aminovorans* in the different treatments at the indicated time points (0 d, 14 d and 42d) is shown in A. To represent the changes in abundance, the species abundance at 0 d was normalized to that in CK, and the normalized coefficient were applied to calculate the *Paracoccus aminovorans* abundance in the treatments inoculated with HPD-2 with or without carrier at 14 d and 42 d. The RA values shown in B were calculated using the equation $(RA_{T\text{-}42} - RA_{CK\text{-}42}) / (RA_{CK\text{-}42}) \times 100$, where $RA_{T\text{-}42}$ is the RA of the genera in a given treatment at 42 d and $RA_{CK\text{-}42}$ is the RA of the genera in CK at 42 d.

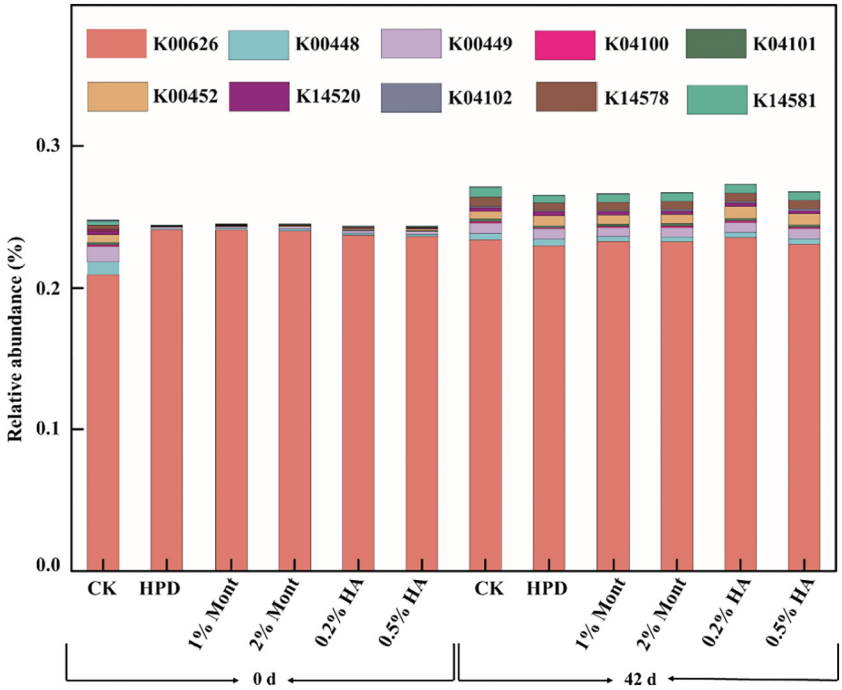

Fig. 5. Relative abundances of functional genes associated with PAHs degradation in soil. K00626, acetyl-CoA *C*-acetyltransferase [EC: 2.3.1.9]; K00448, protocatechuate 3,4-dioxygenase, alpha subunit [EC: 1.13.11.3]; K00449, protocatechuate 3,4-dioxygenase, beta subunit [EC: 1.13.11.3]; K04100, protocatechuate 4,5-dioxygenase, alpha chain [EC: 1.13.11.8]; K04101, protocatechuate 4,5-dioxygenase, beta chain [EC: 1.13.11.8]; K00452, 3-hydroxyanthranilate 3,4-dioxygenase [EC: 1.13.11.6]; K14520, 4-hydroxy-acetophenone monooxygenase [EC: 1.14.13.84]; K04102, 4,5-dihydroxyphthalate decarboxylase [EC: 4.1.1.55]; K14578, naphthalene 1,2-dioxygenase ferredoxin component; K14581, naphthalene 1,2-dioxygenase ferredoxin reductase component [EC: 1.18.1.7].

in the control treatment (CK) (Fig. 1), with a removal efficiency of 9.4 %. This removal efficiency was higher for low-molecular-weight (LMW) PAHs and reached 14.8 % for 3-ring PAHs, suggesting that an optimal incubation environment can stimulate the natural PAH-degradation capacity of contaminated soil. We found that inoculation with HPD-2 without carrier accelerated PAHs removal and increased the removal efficiency significantly (27.5 %) compared with CK, consistent with a previous report by Teng et al. (2010). These result support the use of bioaugmentation to enhance the effective biodegradation of PAHs by PAH-degrading bacteria in contaminated soils.

The HPD-2 strain of *Paracoccus aminovorans* used in this study was isolated from historically PAH-contaminated soil and can utilize fluoranthene, pyrene or BaP as the sole source of carbon and energy for growth (Teng et al., 2010). For 5-ring PAHs, the removal efficiency reached 34 % in the HPD treatment, much higher than that in CK (Fig. 1). *Paracoccus aminovorans* HPD-2 may have unique advantages for the bioremediation of HMW PAHs because of its remarkable capacity to degrade pyrene and BaP (Mao et al., 2008). The literature provides abundant evidence that members of the genus *Paracoccus* can be applied to the bioremediation of LMW and HMW PAHs (Liu et al., 2019; Teng et al., 2010). *Paracoccus* have been isolated from PAH-contaminated soil and denitrifying activated sludge systems, including *Paracoccus* sp. LXC, which can degrade acenaphthene (3-ring PAH) (Liu et al., 2019), and *Paracoccus huijuniae*-related strains that can degrade anthracene (3-ring PAH) (Ntougias et al., 2015).

Supplementation of bacterial inoculants with 0.2 % HA or 2 % Mont effectively promoted PAHs reduction, but no obvious promotion of PAHs removal was observed in the 0.5 % HA and 1 % Mont treatments. These results suggest that the addition of an appropriate amount of carrier agent to the soil can enhance PAHs removal. In general, HA acts as a surfactant to enhance PAHs bioavailability, but it can also tightly associate with PAHs to retain them in the soil (Brown, 2007; Chen et al., 2017). We speculate that the function of HA depends on the concentration; when HA was added to the inoculant at a dose of 0.5 %, PAHs were more tightly associated with the soil. Mont has been reported to alleviate stress from adverse factors by buffering toxic intermediates and pH and by providing a surface for microbial attachment and colonization (Gan et al., 2022); these effects may explain why 2 % Mont was more beneficial for microbial survival and PAH degradation than 1 % Mont. Similarly, Su et al. (2008) reported that the degradation rate of BaP was higher when an immobilized fungus (68 %) was used than when free mobile fungus (52 %) was used. These observations indicate that carrier agents can enhance both the degradation efficiency and survival rate of microorganisms in contaminated soil (Figs. 1 and 4A). Therefore, the success of a biodegradation strategy may depend not only on the type of microorganisms used but also on the carrier materials used for immobilization (Punetha et al., 2022). Both HA and Mont have been shown to impact PAHs solubility and bioavailability and to disturb the indigenous microflora in the soil (Han et al., 2021; Liu et al., 2021b).

4.2. Bacterial inoculation and carrier agents alter the indigenous bacterial response

As microbial degradation is the main route of PAHs dissipation in soil, bacterial community structure and diversity were also investigated in this study. As shown in Fig. 3, the bacterial community in the PAH-contaminated soil changed significantly during the experiment. The enriched bacterial genera included *Lysobacter*, *Ramlibacter*, *Massilia* and *Steroidobacter*, which were identified as PAH-degrading bacteria in a previous study (Li et al., 2019). In contaminated soil without amendment (CK), PAHs dissipation occurred, and PAH-degrading bacteria were enriched. Thus, under appropriate environmental conditions, the microbial community can develop in a direction that favors the survival of PAH-degrading members. While bioaugmentation with planktonic HPD-2 induced obvious changes in the indigenous soil bacterial community, these changes were gradually lost over the course of the experiment (Figs. 2 and 3). Moreover, in the treatments inoculated with HPD-2 with or without carrier, the relative abundance of *Paracoccus aminovorans* dramatically decreased during the incubation period. These observations highlight the resistance and resilience of the bacterial community, that is the ability withstand a perturbation or stress and its ability to recover to pre-perturbation levels (Picariello et al., 2021).

Factors such as pH, nutrient status and the intrinsic soil microflora directly and significantly impact the growth and development of exogenous degraders (Punetha et al., 2022). Interactions between microorganisms are hypothesized to play a key role in determining ecosystem biodiversity and function (Mougi and Kondoh, 2012). Bioaugmentation of contaminated environments with PAH-degraders can significantly improve the dissipation rate of PAHs but may also modify soil bacterial diversity and the degrading community (Li et al., 2018). Compared with CK, a distinct PAH-degrading community that included *Lysobacter*, *Massilia*, *Flavihumibacter* and *Pseudoxanthomonas* developed in the HPD treatment, whereas other genera decreased in abundance, such as *Ramlibacter*, *Rhodococcus* and *Burkholderia*. Thus, adding HPD-2 to PAH-contaminated soil affected autochthonous species and altered the composition and abundance of the functional bacterial community. These changes can be ascribed to antagonistic and cooperative interactions between microbial species in soil (Palmer and Foster, 2022).

4.3. The mechanisms of microbial inoculants in bioremediation differ

Biodegradation of pollutants is a complicated process that is controlled by both microbiological and physicochemical factors (Teng et al., 2010). PAHs that enter the soil may tightly associate with inorganic and organic components of soil, such as clay minerals and humic substances (Chen et al., 2017). The clay mineral Mont has a high cation exchange capacity, specific surface area and surface charge density, which could significantly impact the fate of pollutants (Ugochukwu and Fialips, 2017). We previously found that Mont colloidal particles assist the biodegradation of BaP by *Paracoccus aminovorans* HPD-2 by alleviating stress from adverse factors (e.g., harmful intermediates, pH, and Eh) and providing a surface for HPD-2 attachment and colonization (Gan et al., 2022). In the present study, inoculation with HPD-2 using Mont as a carrier altered the bacterial community in the contaminated soil (Figs. 3 and 4). Compared with CK, PAH-degrading bacterial genera were enriched in the treatments with Mont. In addition to *Lysobacter*, *Mycobacterium*, *Flavihumibacter* and *Pseudoxanthomona*, which were enriched in the 1 % Mont treatment; *Sphingobium*, *Bacillus*, *Rhodococcus* and *Burkholderia* were enriched in the 2 % Mont treatment (Fig. 4). These results indicate that Mont can serve as a carrier for degrading bacteria in the bioremediation of organic pollutants. Consistent with our findings, a previous study reported that Mont enhanced the biodegradation of crude oil PAHs by a hydrocarbon-degrading microbial community predominantly composed of *Alcanivorax* spp. (Ugochukwu and Fialips, 2017). In addition, Mont enhances *Pseudomonas putida* activity and stimulates the bioavailability of carbaryl (Chen et al., 2009). These studies confirm that the addition of Mont to microorganisms may have synergistic effects on bioremediation. The interlayer cations of Mont likely stimulate microbial growth by improving the accessibility of nutrients to cells on the clay surface (Ugochukwu and Fialips, 2017). Moreover, Mont provide the niche for bacteria and promote the adhesion and colonization of HPD-2 and other degraders on the surface (Gan et al., 2022), potentially increasing the exposure of microorganisms to pollutants and stimulating PAHs degradation (Fig. 6).

Although the rates of PAHs dissipation were similar in the 0.2 % HA and 2 % Mont treatments, the different carrier materials resulted in divergent responses of the bacterial communities. In a previous study, biofilm formation on HA and Mont significantly enhanced BaP biodegradation (Han et al., 2021), although distinct biodegradation capabilities were observed due to the different morphologies of the biofilms between the two carriers (Han et al., 2021). Furthermore, HA has been shown to aid the growth of PAH-degrading bacteria and increase bacterial diversity (Liu et al., 2019). The bacterial communities in the 0.2 % HA and 0.5 % HA treatments clustered together but separated from the communities in the 1 % and 2 % Mont treatments (Fig. 2). Moreover, the PAH-degrading bacteria that were enriched in the HA treatments were the same as those enriched in the HPD treatment, i.e., *Lysobacter*, *Flavihumibacter*, *Bacillus* and *Pseudoxanthomonas*, but differed from those enriched in the Mont treatments. Similarly, the bacterial diversity in the HA treatments was lower than that in the Mont treatments but the same as that in the HPD treatment.

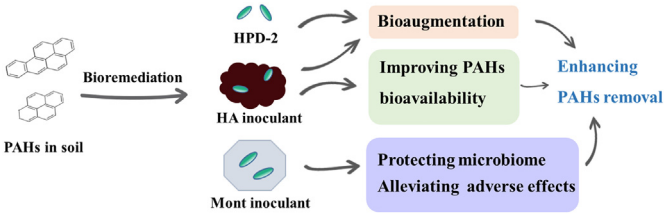

Fig. 6. Proposed mechanisms of the different bacterial inoculants in PAHs bioremediation.

Thus, we speculate that the shifts in the bacterial community in the HA treatments were attributable to inoculation with HPD-2 and that the main mechanism of bioremediation differed between the HA and Mont treatments (Fig. 6). Whereas Mont provided a habitat for functional microbes, HA likely enhanced the solubility of hydrophobic organic compounds such as PAHs, thereby improving their bioavailability (Smith et al., 2009). HA has been shown to dramatically accelerate pyrene biodegradation by improving its bioavailability; HA competes with PAHs for adsorption to soil minerals, resulting in the release of PAHs residues bound to soil particles (Brown, 2007). PAHs bound in HA micelles could also be directly bioavailable to degrading bacteria in soil; considerable degradation of PAHs was observed in soil amended with *Paracoccus* sp. LXC and HA (Liu et al., 2019). Therefore, the increase in PAH remediation in the HA treatments was likely due not only to bioaugmentation with the degrader HPD-2 but also to improved PAHs bioavailability mediated by HA (Fig. 6).

5. Conclusion

This study demonstrates that applying *Paracoccus aminovorans* HPD-2 in combination with either HA or Mont is a feasible strategy for the bioremediation of PAH-contaminated soil. The amendment of contaminated soil with these eco-friendly microbial carriers and HPD-2 resulted in superior PAHs removal compared with amendment with planktonic HPD-2 alone, especially for HMW PAHs bioremediation. The removal efficiency of PAHs reached 42.8 % and 41.6 % in the 0.2 % HA and 2 % Mont treatments, respectively. In addition, enrichment of key PAH-degrading bacterial genera such as *Sphingobium*, *Bacillus*, *Rhodococcus* and *Burkholderia* was observed. HA and Mont are model organic and inorganic soil components that not only directly interact with PAHs in soil but also stimulate microbial activity. Mont likely contributes to the enrichment of indigenous bacterial degraders by providing a protective environment for HPD-2 and indigenous microorganisms. These findings provide a foundation for future studies of the mechanisms underlying the bioremediation of PAHs and other hazardous organic contaminants in the presence of eco-friendly microbial carriers.

CRediT authorship contribution statement

Beibei Wang and Ying Teng conceived the research and designed the experiments. Beibei Wang, Ran Li, Ke Meng and Yongfeng Xu performed the experiments. Beibei Wang contributed to the analyses of data and the writing of original draft. Ying Teng contributed to the project administration, funding acquisition, supervision of experiments, review and editing of this paper. Shiliang Liu and Yongming Luo contributed to revise the language and monitored for scientific accuracy.

Data availability

Data will be made available on request.

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

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2022.160186.

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