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Biochar

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Sustained and efficient remediation of biochar immobilized with *Sphingobium abikonense* on phenanthrene-copper co-contaminated soil and microbial preferences of the bacteria colonized in biochar

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Abstract

Immobilized microbial technology has been widely used in wastewater treatment, but it has been used less frequently for soil remediation, particularly in sites that are co-contaminated with organic compounds and heavy metals. In addition, there is limited knowledge on the efficiency of remediation and microbial preferences to colonize the immobilized carriers. In this study, biochar immobilized with *Sphingobium abikonense* was introduced to remediate soils that were co-contaminated with phenanthrene (PHE) and copper (Cu), and the mechanisms of microbial assemblage were investigated. The immobilized microbial biochar maintained a degradation rate of more than 96% in both the first (0–6 d) and second (6–12 d) contamination periods. The addition of biochar increased the proportion of Cu bound to organic matter, and Fe–Mn oxide bound Cu in the soil. In addition, both Cu and PHE could be adsorbed into biochar pellets in the presence or absence of immobilized *S. abikonense*. The presence of biochar significantly increased the abundance of bacteria, such as *Luteibacter*, *Bordetella* and *Dyella*, that could degrade organic matter and tolerate heavy metals. Notably, the biochar could specifically select host microbes from the soil for colonization, while the presence of *S. abikonense* affected this preference. The autonomous selection facilitates the degradation of PHE and/or the immobilization of Cu in the soil. These results provide a green approach to efficiently and sustainably remediate soil co-contaminated with PHE and Cu and highlight the importance of microbial preference colonized in immobilized carriers.

Highlights

- Biochar immobilized with *S. abikonense* could degrade PHE efficiently and sustainably.
- Pellets of biochar immobilized with S. abikonense adsorbed more Cu on its surface.
- Biochar had a selective preference for its colonized microbial communities.

Keywords Co-contamination, Immobilized bacteria, Microbial selection, Biochar, Microbial communities

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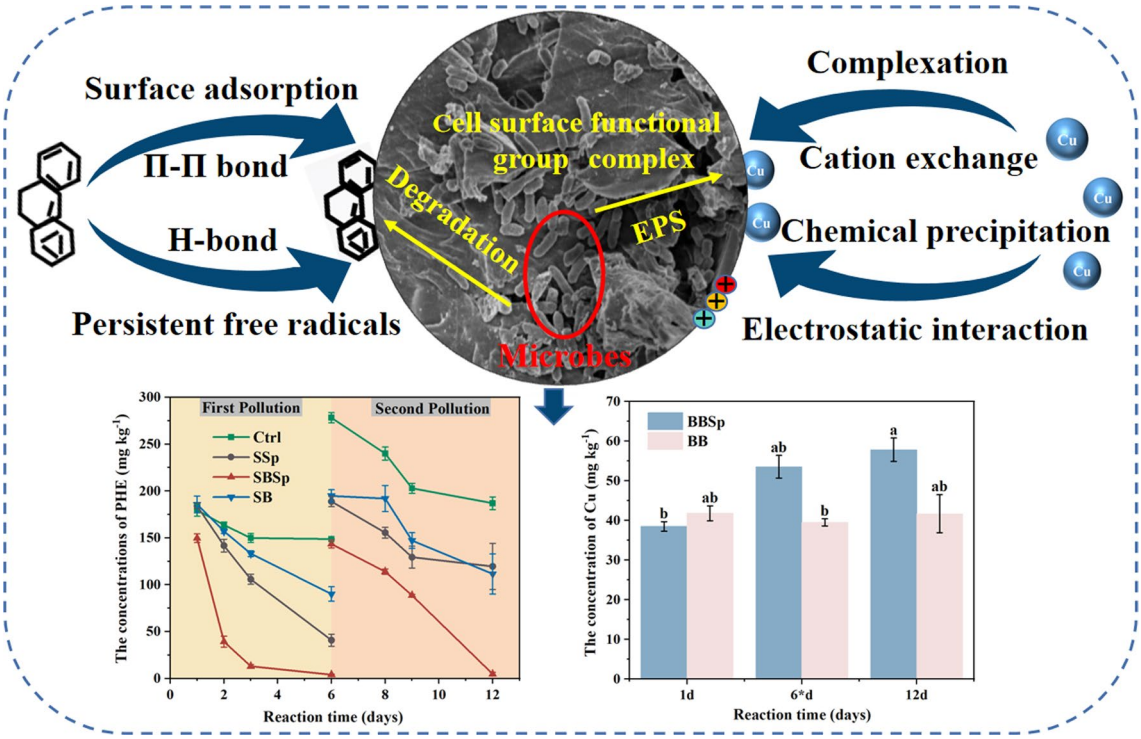
Graphical Abstract



1 Introduction

Soil that is co-contaminated with both organic and inorganic pollutants is increasingly common across the globe, and some sites are severely contaminated owing to continuous discharge (Ma et al. 2010; Hua et al. 2022; Zhang et al. 2022). Heavy metals are non-biodegradable, cause organ, neurological and genetic problems and pose serious health hazards to humans (Samuel et al. 2015, 2018a, 2021a; b). PAHs are widespread organic pollutants in soil, which affect normal human metabolism and may also damage human endocrine system and nervous system due to their stable chemical properties and triptogenicity (carcinogenic, teratogenic and mutagenic), causing serious hazards to the ecosystem and human health. Phenanthrene is widely distributed in the environment as a model substrate for PAHs, and the continuous release of pollutants makes the long-term and persistent presence of phenanthrene in the environment, making remediation more difficult (Ma et al. 2010). As reported, soils contaminated with polycyclic aromatic hydrocarbons (PAHs) always co-exist with heavy metals (HMs) (Lu et al. 2014; Chen et al. 2016a), and their interaction often causes a synergistic effect on the soil and human health (Lu et al. 2014; Samuel et al. 2018b, 2020, 2021c). Wu et al. (2016) collected a total of 20 shallow groundwater samples from a metal smelting area in southeastern China, and found that the total concentrations of the 16 PAHs (∑ PAHs) in the shallow groundwater ranged from 9.62 to 1663.93 ng L^{-1}, which were higher than the limit set by US EPA, posing a potentially serious health risk. Thus, the effective and sustainable remediation of soils co-contaminated with PAHs and HMs can be challenging owing to many environmental and technical issues.

Removal techniques for heavy metals have been reported to include chemical precipitation, ion exchange, reduction, adsorption, solvent extraction, etc. (Samuel et al. 2018b). The removal of organic pollutants includes chemical drenching, thermal treatment, phytoremediation, etc. (Sun et al. 2020; Xu et al. 2021; Su et al. 2022). However, incomplete removal and susceptibility to secondary contamination are the limiting factors of these technologies. Microbe-based bioremediation is one of the most effective and environmentally friendly methods to remediate contaminated soils (Perez et al. 2010; Song et al. 2021). However, bioaugmentation using specialized microbial inocula is susceptible to unfavorable soil environments, which results in failure or a poor effect of remediation on the contaminated soils (Canet et al.

2001). Recently, the use of porous carrier materials to immobilize microbes has been reported to provide protection for microbes from the adverse effects induced by contaminants, unsuitable environmental conditions, and competition with indigenous microbial communities in contaminated sites (Tao et al. 2019; Song et al. 2021). Zhuang et al. (2015) found that immobilized cells could tolerate higher concentrations of quinoline and much more effectively protect the bacteria against changes in temperature (20–45 °C) and pH (5–10). In addition, immobilized microbes can also improve the passivation of HMs in soil and reduce their biological effectiveness (Park et al. 2011; Tu et al. 2020). However, immobilized microbial technology has primarily been focused on wastewater treatments (Lou et al. 2019; Zhao et al. 2020; Li et al. 2022a). In addition, there have been few cases of its successful application in soil remediation, which was primarily owing to the complex soil conditions, as well as the applicability and efficiency of immobilized carrier materials and functional microbes (Li et al. 2022a).

Activated carbon, graphene, carbon nanotubes, polymers, and biochar are more adsorbent for contaminants (Samuel et al. 2018c, 2021d, 2022). Due to the complex soil environment, the adsorbent materials should be selected as non-toxic and non-secondary contamination materials. Biochar is an ideal carrier material for tailored microbial immobilization owing to its cost-effectiveness, wide availability and good physicochemical performance (Hale et al. 2015). The potential interaction mechanisms between biochar and immobilized microbes include the following: (1) the large surface area and porous structure of biochar can provide nutrients and shelter for microorganisms, thus protecting them from competition with indigenous microbes (Bandara et al. 2019); (2) biochar can improve the survival of microbes and affect the microbial community structures by changing soil properties, such as pH and nutrient conditions (Tu et al. 2020); (3) biochar can act as an electron shuttle between microorganisms and pollutants to enhance microbial degradation (Zhu et al. 2017); and (4) biochar has a strong ability to adsorb and stabilize the contaminants in its surface, which alleviates their toxic effect on the microbes (Bandara et al. 2019).

Typically, the efficiency of remediation is determined by both the ability to have a high microbial load and strong microbial functional activity when using the immobilized microbial technology (Tu et al. 2020). However, as reported, not all cultured microbes can be successfully immobilized to the biochar skeleton, which is primarily related to the preference of biochar for its colonized microbes (Dai et al. 2017). Microbial communities that can survive in the interior and surface of biochar vary depending on the properties and types of biochar, microbial species, and environmental conditions (Bandara et al. 2019). Dai et al. (2017) found that Actinobacteria and Proteobacteria preferred to become enriched on pyrogenic organic matter pyrolyzed at 300 °C, while Chloroflexi and Acidobacteria preferred to colonize pyrogenic organic matter pyrolyzed at 700 °C. Thus, the specific properties of biochar result in its different selection of colonized microbial species. However, most studies have primarily focused on the effect of biochar on soil microbial communities and the effects of remediation with biochar-immobilized cells on contaminated soils (Hale et al. 2015; Tao et al. 2019). There is still a limited understanding of the detailed microbial community composition in the interior and surface of biochar. In particular, the selection preference of the colonized microbes for different types of biochar, as well as their internal associations, remains unclear. Moreover, the stability and sustainability of the immobilized microbial technology on the soil remediation remain to be elucidated.

We hypothesize that biochar embedded with Sphingobium abikonense might accelerate the removal of phenanthrene in contaminated soils and have a longer validity for phenanthrene degradation and accelerate the passivation of copper in the soil.The objectives of this study were as follows: (1) to elucidate the effect of biochar-immobilized bacteria on remediating soil co-contaminated with PHE-Cu; (2) to evaluate the stability and durability of bacteria-immobilized biochar pellets on the degradation of PHE when the soil is contaminated again; (3) to investigate the influence of biochar-immobilized bacteria on the soil microbial communities; and (4) to reveal the preference of biochar in the selection of its colonized microbiome and the interactions between these microbes.

2 Materials and methods

2.1 Bacteria cultivation and characteristic analysis

Lysogeny broth (LB) medium: 10 g NaCl, 10 g tryptone and 5 g yeast powder were added into 1000 mL distilled water and the pH of the solution was adjusted to 7.0 ~ 7.2 with 1 mol/L $NaOH_2$ solution and 0.1 mol/L HCl solution.

Mineral salt medium (MSM): 0.5 g $NaNO_3$, 1.0 g $KH_2PO_4{\cdot}3H_2O$, 0.02 g $CaCl_2$, 0.2 g $MgSO_4{\cdot}7H_2O$, 0.5 g $(NH_4)_2SO_4{\cdot}7\ H_2O$ and 1.0 g $NaH_2PO_4{\cdot}12H_2O$ were added into 1000 mL distilled water. The pH was adjusted to 7.0 ~ 7.2 with 1 mol L^{-1} NaOH solution and 0.1 mol L^{-1} HCl solution.

In this study, *Sphingobium abikonense* (*S. abikonense*) was isolated from an incineration plant located in Xinyang, Henan Province, China (32.241°N, 114.499°E) and the strain storage number was CGMCC23559 (China General Microbiological Culture Collection Center). The

bacteria were cultivated for 16 h in a shaker at 30 °C in LB medium, and then centrifuged at 4000 rpm. The supernatant was discarded and cells were suspended in sterile water. This process was repeated two or three times and the optical density (OD) of the suspended cells was adjusted to 0.8 (cell density of about 1.6×10^9 CFU mL^{-1}) by UV-spectrophotometer (L4, Youke, China) at 600 nm.

The tolerance of *S. abikonense* to copper was tested with 18 mL LB medium and 2 mL bacterial suspension was added with copper (Cu) concentrations of 0, 50, 100, 150 and 200 mg L^{-1}. Two mL sterile water was used instead of bacterial suspension as the control group (Ctrl). Then, the microcultures were incubated in a thermostatic oscillation incubator (SHZ-82, Danrui, China) at 170 rpm and 30 °C for 48 h. After incubation, OD_{600} was detected to evaluate the growth status of the bacteria.

The ability of *S. abikonense* to degrade phenanthrene (PHE) was tested with 9 mL MSM medium and 1 mL bacterial suspension (rate of 10%) was added into a 50 mL flask following the adding PHE concentrations of 50, 100, 200, 500, 800, 1000, 1200 and 1500 mg L^{-1}. Meanwhile, MSM medium without bacteria addition was considered as the control. All cultures were incubated in a thermostatic oscillation incubator (SHZ-82, Danrui, China) at 30 °C and 170 rpm for 7 d in the dark. After incubation, the cultures were sampled and stored at 4 °C to analyze the residual PHE.

2.2 Biochar preparation and characterization

The fungal sticks were cleaned with deionized water and oven-dried overnight at 70 °C, then ground to obtain particles <2.0 mm by passing through a sieve. The powdered feedstock was collected in a stainless-steel reactor and placed in a muffle furnace. Then the temperature was increased to 400 °C at a rate of 20 °C min^{-1}, with the holding temperature of 400 °C for 2 h. To ensure an anaerobic environment, N_2 was continuously supplied to the muffle furnace at a flow rate of 50 mL min^{-1} during the whole pyrolysis process. The pyrolyzed biochar was collected after it had cooled to room temperature. The biochar was pulverized and passed through a 100-mesh sieve before it was used (Meng et al. 2014). The characteristics of biochar were analyzed as follows: the components of the biochar were determined using an elemental analyzer (Vario EL cube, Elementar, Germany), and the specific surface areas were determined by a multipoint Brunauer–Emmett–Teller (ASAP2460, Micromeritics, America) analysis of the adsorption data points with relative pressures (p/p_0) between 0.05 and 0.3. The total pore volume was estimated from a single N_2 adsorption point at a N_2 relative pressure of 0.95. The detected results are listed in Additional file 1: Table S1. The microstructure of biochar was observed by field emission scanning electron microscopy (Sigma 300, ZEISS, Germany) and the functional groups were investigated by Fourier transform infrared (FTIR) spectroscopy (Nicolet iS5, Thermo Scientific, America) with KBr press method. Biochar charge was determined using a nanoparticle size and zeta potential analyzer (Malvern Zetasizer Nano ZS90).

2.3 Preparation of immobilized-bacteria biochar pellets

Biochar and bacterial suspension (w:v = 1:20) were added into a 250 mL flask, and then incubated in a thermostatic oscillation incubator (SHZ-82, Danrui, China) at 30 °C and 170 rpm for 6 h. Then the solution was mixed with 3% (w/v) sodium alginate solution in equal volume and shaken for 30 min. The bacteria-involved biochar pellets (BBSp) were formed by adding the mixtures to the 2% (w/v) $CaCl_2$ solution dropwise with a sterile syringe and cross-linked for 12 h. In addition, biochar pellets without bacteria (BB) were also prepared (Chen et al. 2012). All operations were performed under strict sterile conditions. The microstructures of the biochar pellets with or without *S. abikonense* were observed by field emission scanning electron microscopy (Sigma 300, ZEISS, Germany). The specific surface areas of pellets (value was 6.91 m^2 g^{-1}) were determined by a multipoint Brunauer–Emmett–Teller (ASAP2460, Micromeritics, America) analysis of the adsorption data points with relative pressures (p/p_0) between 0.05 and 0.3. The pellet diameter measured with vernier calipers was about 2.5 mm.

2.4 The remediation of immobilized-bacteria biochar on soils co-contaminated with PHE and Cu

Soils were collected from the 0–20 cm surface layers of uncontaminated farmland in Jimo, Qingdao, Shandong Province (36° 5′ 8″ N, 120° 5′ 12″ E). The soil was air-dried before use and passed through a 100-mesh sieve. The basic properties of the soil are listed in Additional file 1: Table S1. The contaminated soil was prepared as follows: 150 mL of PHE (5 g L^{-1} dissolved in acetone) and 50 mL of $CuCl_2$ (7.5 g L^{-1} dissolved in sterilized water) were spiked uniformly into 2.5 kg of soil. The soils were thoroughly mixed and to prevent the volatilization of phenanthrene from interfering with the experimental effect, we incubated the samples under dark conditions at room temperature. Soil moisture content was 25%. The final concentrations of PHE and Cu in the soils were 248.6 mg $kg^{-1}$and 153.84 mg kg^{-1}, respectively.

A batch incubation experiment of four treatments was conducted in weighing bottles that contained 20 g of contaminated soils as follows: (1) Ctrl: only soil; (2) SSp: soil and a 10% suspension of free *S. abikonense* (w/v); (3) SBSp: soil and 10% bacteria-immobilized biochar pellets (w/v); and (4) SB: soil and 10% biochar (w/w). The

soil moisture was kept at 40% of the field capacity during incubation. All the treatments were performed in triplicate and conducted at 30 °C in the dark. Sterile water was supplemented every 2 days to maintain the samples at the fixed weight. After 6 d of incubation, secondary contamination was conducted with a concentration of 150 mg kg^{-1} PHE in the soil. Incubation of the mixture at 30 °C was continued in the dark. Samples were collected at 1, 2, 3, 6, 8, 9 and 12 d. At each sampling point, biochar pellets were picked out with sterile forceps and stored at −20 °C after the surface had been rinsed with sterile water.

2.5 Analysis of PHE and Cu

For the detection of residual PHE in the MSM liquid medium: PHE was extracted with dichloromethane and then filtered through a 0.22 μm microporous membrane before being analyzed by GC–MS. A gas chromatograph (GC) (Agilent 7890 A, Agilent, Santa Clara, CA, United States) and a DB-5 MS capillary column (30 m by 0.32 mm diameter by 0.25 mm) (J&W Scientific, Inc., Folsom, CA, United States) were used to analyze PHE concentration. The method proceeded as follows: the initial temperature was set to 45 °C for 2.25 min, and increased at 40 °C min^{-1} to 300 °C (holding for 3 min) (Su et al. 2022).

For the detection of residual PHE in soils and biochar pellets: Soils and biochar pellets were freeze-dried and then extracted in a 1:1 mixture of dichloromethane and acetone by ultrasonication for 30 min (Xu et al. 2021). After centrifugation, the supernatants were transferred to a new serum bottle. Extraction of the sample was performed three times. The combined supernatants were concentrated with a pressure-blowing concentrator and redissolved in 2 mL hexane, and then analyzed by GC–MS (Li et al. 2022b).

A logistical model was used to interpret the extractable PHE residue data through nonlinear curve fitting as follows (Liu et al. 2013):

$$C_t = C_0 - C_0\left[a/\left(1 + be^{(-kt)}\right)\right] \quad (1)$$

where C_0 is the initial concentration of PHE (mg kg^{-1}); C_t is the PHE concentration at time t (mg kg^{-1}); t is the incubation time (d); a is the maximum PHE degradation capacity (100%); b is a regression coefficient, and k is the reaction rate constant of PHE degradation (d^{-1}). Based on the equation, the half-life of PHE (d), $T_{1/2}$, was ln(b/(2a−1))/k. The maximum reaction rate of the PHE degradation (mg (kg d)$^{-1}$), Vmax, was obtained from 0.25 ak.

The total copper in soils and biochar pellets was analyzed by digestion with a mixture of HNO_3 and H_2O_2 (1:4, v/v) using microwave digestion (Xu et al. 2021), and the conditions of microwave digestion are shown in Table 1. The concentration of bioavailable Cu in soils was extracted with diethylene triamine peracetic acid (DTPA) following protocols described previously (Tu et al. 2020). Changes in the speciation and redistribution of Cu in soils were analyzed using the Tessier sequential extraction method (Tu et al. 2020). The concentrations of Cu were measured by atomic absorption spectrophotometer (TAS-990 AAS, Beijing Persee, China), and the measurement methods were as follows: the standard solutions of 0.2, 0.5, 1, 1.5 and 2 mg L^{-1} of Cu^{2+} were prepared, the corresponding absorbance (wavelength 328.4 nm) was measured by atomic absorption spectrometer. The standard curve was plotted with the concentration as the horizontal coordinate and the corresponding absorbance as the vertical coordinate. The concentration of Cu^{2+} in the sample can be calculated by bringing the measured absorbance of the sample into the regression equation of the standard curve.

2.6 DNA extraction and sequencing

DNA from soils and biochar pellets were extracted using the OMEGA Soil DNA Kit (Omega Bio-Tek, Norcross, GA, USA). The quantity and quality of extracted DNAs were measured using a NanoDrop NC2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) and agarose gel electrophoresis, respectively. PCR amplification of the bacterial 16S rRNA genes of V3-V4 region was performed using the forward primer 799F and the reverse primer 1193R. Upstream primer sequence was AACMGGATTAGATACCCKG, and downstream primer sequence was: ACGTCATCCCCACCTTCC . Sample-specific 7-bp barcodes were incorporated into the primers for multiplex sequencing. PCR amplicons were purified with Vazyme VAHTSTM DNA Clean Beads (Vazyme, Nanjing, China) and quantified using the Quant-iT PicoGreen dsDNA Assay Kit (Invitrogen, Carlsbad, CA, USA). After the individual quantification step, amplicons were pooled in equal amounts, and pair-end 2×250 bp sequencing was performed using the Illumina NovaSeq platform with NovaSeq 6000 SP Reagent Kit (500 cycles) at Shanghai Personal Biotechnology Co., Ltd (Shanghai, China).

Table 1 The conditions of microwave digestion

Process	Temperature/°C	Heating up time °C/min	Hold time/min
1	120	5	5
2	160	5	10
3	200	5	20

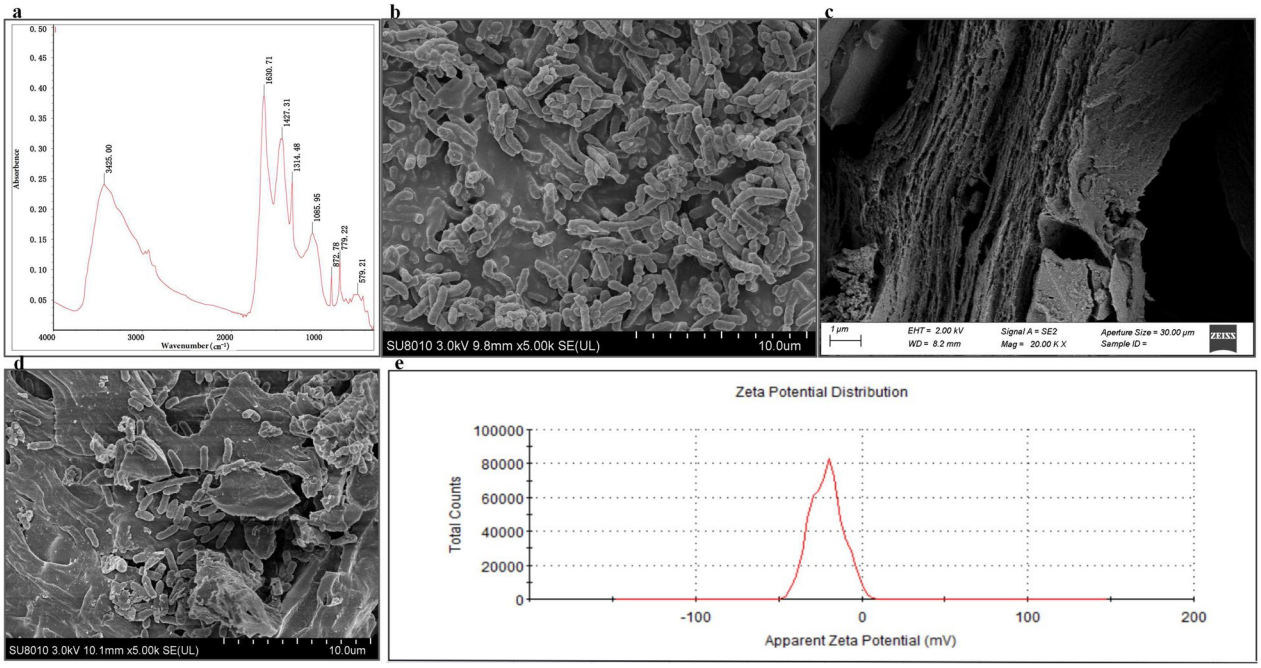


Fig. 1 FTIR spectra of biochar (**a**) and scanning electron micrographs of *S. abikonense* strain (**b**), biochar (**c**), and immobilized bacterial biochar (**d**), Zeta potential of biochar (**e**)

Microbiome bioinformatics were performed with QIIME2 2019.4 with slight modifications according to the official tutorials (https://docs.qiime2.org/2019.4/tutorials/). Briefly, raw sequence data was demultiplexed using the demux plugin following by primers cutting with the cutadapt plugin. Sequences were then quality filtered, denoised, merged and chimera removed using the DADA2 plugin. Non-singleton amplicon sequence variants (ASVs) were aligned with mafft and used to construct a phylogeny with fasttree2. Taxonomy was assigned to ASVs using the classify-sklearn naïve Bayes taxonomy classifier in feature-classifier plugin against the SILVA Release 132 Database. All DNA sequences were submitted to the NCBI Sequence Read Archive (SRA) database under accession number SRP379822.

2.7 Statistical analysis

All the statistical analyses and graphs were developed in R×64 4.1.3 and Origin 2022 (OriginLab, Northampton, MA, USA). The α-diversity value of the Shannon index was determined using the *vegan* package in R. Principal coordinate analyses (PCoAs) of weighted UniFrac distance metrics were performed to evaluate the effect of time and treatments on microbial communities using the *ape* package. Significance was tested with a permutational multivariate analysis of variance (PERMANOVA) using the *vegan* package. Additionally, to investigate the amplicon sequence variants (ASVs) that were significantly enriched in the treatments of SB, BB and BBSp, the *EdgeR* package was used to differentially calculate the ASVs between SB and BB, BB and BBSp. $p < 0.05$ was considered to be the level of statistical significance. The co-occurrence network was conducted to evaluate the microbial community complexity of biochar pellets using the molecular ecological network analysis (MENA) (http://ieg4.rccc.ou.edu/mena).

3 Results

3.1 The properties of prepared biochar, *S. abikonense* and bacteria-immobilized biochar pellets

The Fourier transform infrared spectroscopy (FTIR) spectra of biochar showed that the peak at 3425.0 cm^{-1} was –NH stretching of the primary amide, and the peak at approximately 1630 cm^{-1} was attributed to the C=O stretching vibration of aromatic rings (Fig. 1a). In addition, other functional groups, such as C=C, –OH and the P-O bond of phosphate, were also detected on the surface of biochar. The morphological structure of free *S. abikonense* cells, biochar, and bacteria-immobilized biochar pellets were imaged using scanning electron microscopy (SEM) (Fig. 1b–d). S. abikonense was short rods with smooth edges (Fig. 1b). The prepared biochar exhibited a large and porous lamellar structure, which suggested that it would serve as a good matrix for cell adhesion (Fig. 1c). As expected, many *S. abikonense* cells were attached to the interior pores of biochar pellets (Fig. 1d). In addition,

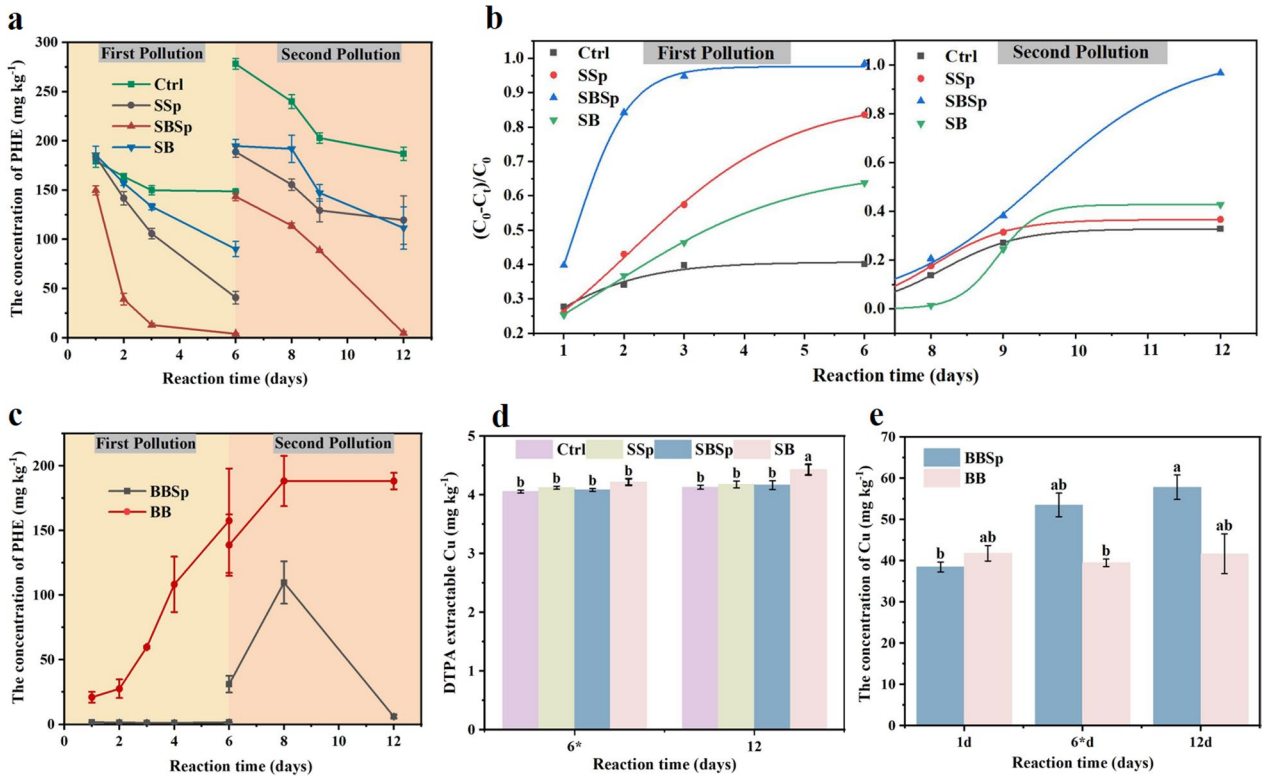


Fig. 2 The concentration variations of phenanthrene (**a**, **c**) and DTPA-Cu (**d**, **e**) in soils (**a**, **d**) and biochar pellets (**c**, **e**); And the logistic model analysis of phenanthrene degradation at first and second pollution stages (**b**). The abbreviation meanings: SSp, soils treated with free *S. abikonense*; SB, soils treated with biochar; SBSp, soils treated with immobilized bacterial biochar pellets; Ctrl, untreated soils; BB, biochar-only; BBSp, biochar pellets immobilized with *S. abikonense*. 6*d, samples were taken immediately after the second contamination on the sixth day. Lowercase letters above the error bars indicate significant differences among different treatments analyzed by LSD test ($p < 0.05$)

the bacteria grew well after 24 h of incubation in the mineral medium, which verified that the immobilized protocols used in this study are feasible to ensure that the microbes remain active. A negative value of −21.3 mV was recorded in a zeta potential analyser (Fig. 1e), which means that biochar was negatively charged and could adsorb heavy metal ions.

3.2 Long-lasting effects of immobilized-bacteria biochar pellets on PHE degradation

Phenanthrene was spiked into the soil twice to evaluate the long-lasting effects of bacteria-immobilized biochar pellets on its degradation (Fig. 2). As shown, the concentration of PHE in the soil decreased continuously during incubation (Fig. 2a). The residual PHE concentrations in SBSp were the lowest during both the first (0–6 d) and second pollution stages (6–12 d), with residual concentrations of 4.02 mg kg^{-1} and 4.67 mg kg^{-1}, respectively. In contrast, the rate of PHE degradation in SSp gradually decreased to 36.70%, which was significantly lower than that in the first pollution stage (88.0%). The addition of biochar also increased the dissipation of PHE when compared with the control treatment, with a ratio that reached 67.8% and 42.7% at the first and second pollution stages, respectively. The logistical model showed that SBSp had the highest rate of degradation of PHE in both the first and second pollution stages, and its reaction rates were maximal at (V_{max}) of 0.54 (mg [kg d]$^{-1}$) during the first stage (Fig. 2b, Additional file 1: Table S2). These results indicated that the bacteria-immobilized biochar pellets substantially enhanced the efficiency of remediation and could be utilized as an appropriate and practical strategy for soil remediation.

Notably, we also detected the content of adsorbed PHE in the biochar pellets with and without immobilized bacteria (Fig. 2c). The concentration of adsorbed PHE in the biochar pellets gradually increased and stabilized after 8 d of incubation. The concentration of adsorbed PHE decreased dramatically when the *S. abikonense* cells were immobilized into the biochar pellets (Fig. 2c). This suggested that the immobilized bacteria substantially contributed to the degradation of PHE adsorbed by biochar pellets.

3.3 The effects of immobilized-bacteria biochar pellets on Cu forms

The contents of total and diethylenetriaminepentaacetic acid (DTPA)-extractable Cu in the soil were measured at 6 and 12 d, respectively (Fig. 2d, Additional file 1: Fig. S1a). The total content of Cu did not vary significantly among the four treatments in both sampling times (Additional file 1: Fig. S1a). In contrast, the content of DTPA-extractable Cu was higher in SB than in the other three treatment groups on day 12. The variation of four fractions of Cu speciation changed slightly during incubation in all four treatments. The concentration of exchangeable Cu and that bound to carbonate decreased in both the SBSp and SB treatments when compared with the Ctrl in contrast to the organic content of Cu (Additional file 1: Fig. S1b). Similarly, the content of Cu adsorbed by biochar immobilized with or without *S. abikonense* was also measurable (Fig. 2e), and the content of Cu absorbed by BB was stable, with an average value of approximately 40 mg kg^{-1}. However, when *S. abikonense* was immobilized by biochar pellets, the absorbed content of Cu gradually increased during incubation, with an average value ranging from 38.5 mg kg^{-1} to 57.85 mg kg^{-1} after 12 days. This indicated that *S. abikonense* promoted the adsorption of Cu by biochar pellets.

3.4 Microbial community changes in the soil and biochar pellets

The Shannon index was used to measure microbial α-diversity in the soils and biochar pellets (Fig. 3a and b). The Shannon indices in the SBSp (4.97) and SSp (5.30) treatments were significantly lower than those in the treatments of SB (5.88) and Ctrl (6.21) on day 12 (Fig. 3a). Compared with the soil, the Shannon index values were relatively lower in the biochar pellets (Fig. 3b).

The microbial composition and relative abundance in both the soil and biochar pellets were studied at the phylum and genus levels (Fig. 4). Different treatments and temporal variations significantly influenced the microbial community composition in both the soil and biochar pellets. In the soil environment, Proteobacteria, Actinobacteria, Firmicutes, Chloroflexi, Acidobacteria and Gemmatimonadetes were predominant (Fig. 4a). However, the proportion of Actinobacteria was much lower in the treatments of biochar pellets immobilized with and without *S. abikonense* than in the control and free *S. abikonense* treatments. The abundance of Proteobacteria kept increasing and comprised more than 50% in the SBSp and SB treatments (Fig. 4a). The proportion of *Sphingobium* affiliated with Proteobacteria was observed to gradually increase in SSp or SBSp, indicating that the extraneous addition of bacteria successfully colonized and grew in the contaminated soils (Fig. 4c).

Compared with the soil environment, the microbial community structure was comparatively simple in the interior biochar pellets, with prominent groups of Proteobacteria, Actinobacteria and Firmicutes that successfully colonized the biochar pellets (Fig. 4b and d). The relative abundance of *Sphingobium* comprised more than 80% in the BBSp and remained stable during the whole incubation period, which suggested that the biochar pellets could successfully protect *S. abikonense* from the adverse environmental conditions. Interestingly, the microbial species that colonized the biochar were found to have some preference. For example, the microbes *Bordetella*, *Lysobacter* and *Methylobacterium*, members of Proteobacteria, and *Micromonospora, Promicromonospora* and *Kribbella*, members of Actinobacteria tended to survive in the interior of biochar pellets.

A PCoA analysis of the soil environment that was calculated on the weighted uniform distance metrics showed that the bacterial communities were clearly separated along the x-axis at different incubation times and distinguished along the y-axis at different treatments (Fig. 3c). A PERMANOVA analysis provided additional confirmation that time was the most influential factor that contributed to the differences in microbial communities in the soil (50.4% of the variance) ($p < 0.001$), followed by the different treatments (22.7% of the variance) ($p < 0.001$) (Additional file 1: Table S3). Microbial communities in the biochar pellets showed clear differences between the treatments immobilized with and without *S. abikonense*, as indicated by both the PCoA analysis and PERMANOVA results (Fig. 3d, Additional file 1: Table S3).

3.5 Significantly differential microbes colonized in biochar immobilized with and without *S. abikonense*

To clearly identify which soil microbes tended to colonize in the biochar pellets, ASVs that were specifically enriched in the biochar pellets were selected to compare to the corresponding soil microbial communities (Fig. 5a). A notable differentiation was observed in the microbial communities between those found in the biochar pellets or corresponding soils. This strongly suggested that biochar had a significant selective effect on its colonized microbiome. Microbes that were significantly enriched in the soil (28 ASVs) were primarily composed of Proteobacteria (23 ASVs) and Chloroflexi (three ASVs), whereas the microbes that were significantly enriched in the biochar pellets (32 ASVs) were primarily composed of Proteobacteria (19 ASVs), Actinobacteria (nine ASVs) and Firmicutes (four ASVs). Among them, HM-tolerant and PAH-degrading bacteria, such as

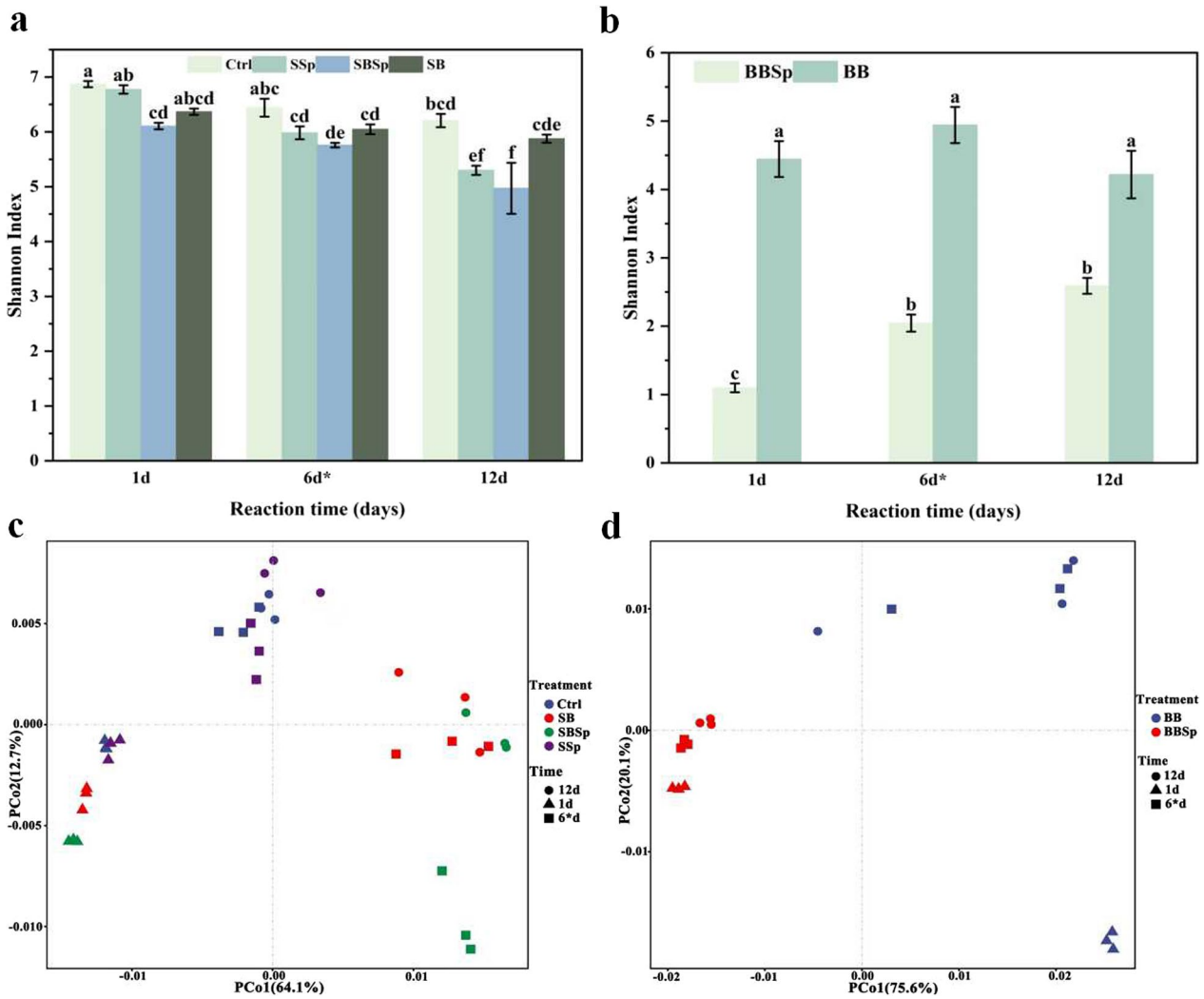


Fig. 3 Microbial variations of Shannon index and PCoA analysis of the microbial communities in soils (**a**, **c**) and biochar pellets (**b**, **d**). The abbreviation meanings are as same as in Fig. 2. Lowercase letters above the error bars indicate significant differences among different treatments analyzed by LSD test ($p < 0.05$)

Micromonospora, *Lysobacter*, and *Bacillus*, were shown to be enriched in the biochar pellets (Fig. 5a, Additional file 1: Table S4).

Additionally, the variation in microbial composition in the types of biochar with and without immobilized *S. abikonense* was observed to further evaluate the influence of immobilized bacteria on the microbial selection of biochar (Fig. 5b, Additional file 1: Table S5), and the microbial composition and abundance of the colonizing bacteria differed significantly in BBSp and BB. The microbes significantly enriched in BB (25 ASVs) were primarily composed of Actinobacteria (6 ASVs), Alphaproteobacteria (6 ASVs) and Gammaproteobacteria (10 ASVs), which comprised 35.8% of the total microbial content. The microbes significantly enriched in the BBSp (8 ASVs) were primarily Actinobacteria (2 ASVs), Bacilli (2 ASVs), and Gammaproteobacteria (4 ASVs), which only comprised less than 5.6% of the total microbial content except for the added *S. abikonense* that comprised approximately 80%. At the genus level, microbes affiliated to *Micromonospora*, *Microvirga*, *Lysobacter* and *unclassified_Burkholderiaceae* tended to grow in the biochar pellets, while *Bacillus*, *Steroidobacter* and *Nonomuraea* could grow in the biochar pellets immobilized with *S. abikonense*.

To further reveal the potential ecological interactions among the microbial members in the biochar pellets immobilized with and without *S. abikonense* cells, bacterial co-occurrence networks were constructed (Fig. 5c

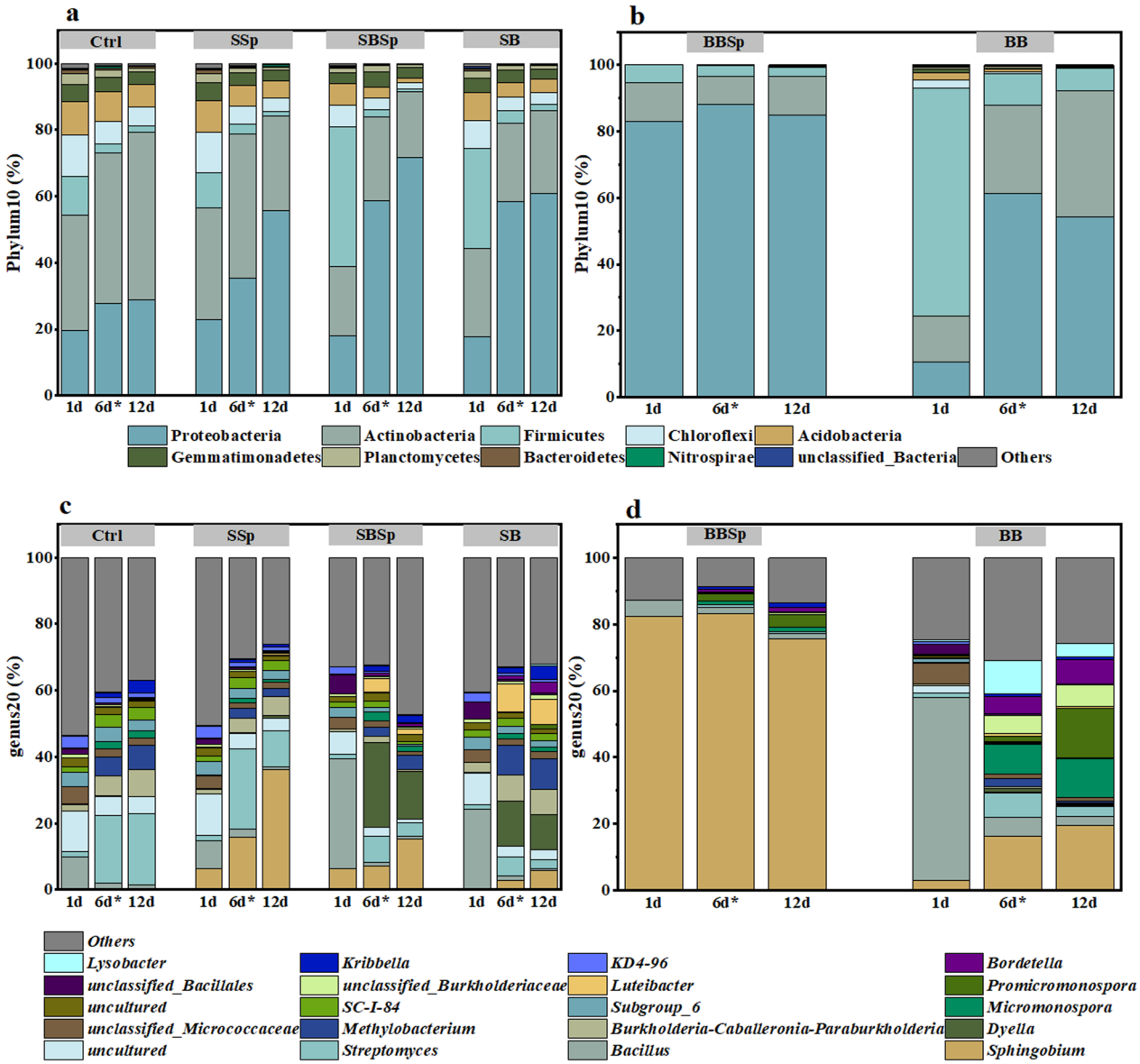


Fig. 4 Microbial community composition in soils (**a**, **c**) and biochar pellets (**b**, **d**). The abbreviation meanings were as same as in Fig. 2

and d). It is apparent that the topological properties differed between these two conditions (Additional file 1: Table S6). The co-occurrence network in BB had more nodes, edges, and average degree than that in BBSp, suggesting that immobilized *S. abikonense* decreased the tightness and complexity of the microbial communities in biochar pellets. The nodes primarily consisted of the species belonging to the phyla Actinobacteria, Proteobacteria and Firmicute, which suggested that these phyla preferred to host in the biochar.

4 Discussion

4.1 Biochar is a good carrier for microbial immobilization

S. abikonense cells immobilized by biochar pellets exhibited excellent remediation efficiency and persistence in the PHE-Cu co-contaminated soils in this study (Fig. 2). The key to successfully using the immobilized microbial technique in practical application primarily depends on the efficiency of dissipation of immobilized microbes and the microbial loading capacity of the carrier. *S. abikonense* was used in this study owing to its powerful degradative ability toward persistent organic

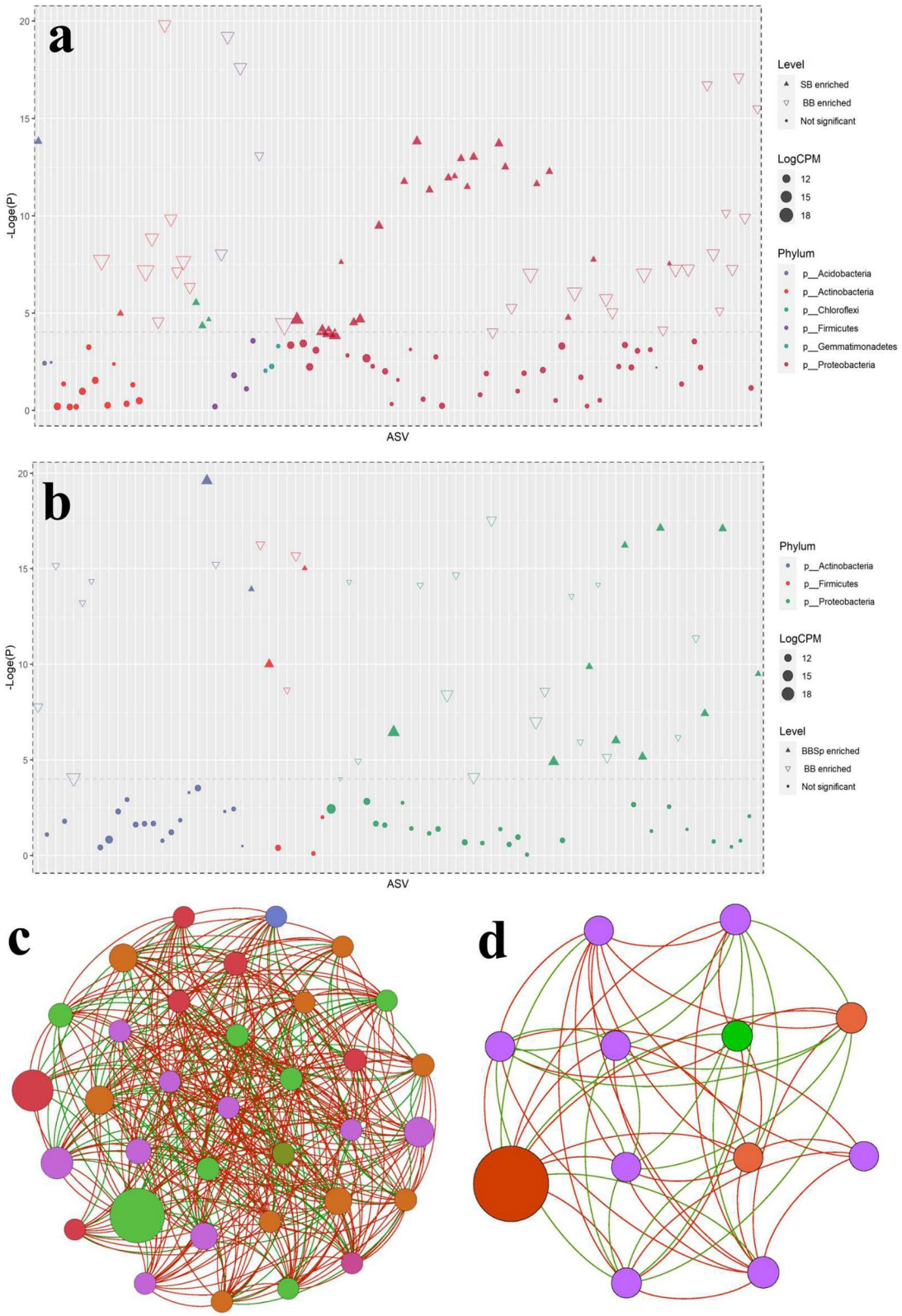


Fig. 5 Significantly changed ASVs between biochar pellets and soils treated with biochar pellets (**a**), biochar pellets and biochar pellets immobilized with *S. abikonense* (**b**), as well as the microbial networks in the biochar pellets (**c**) and biochar pellets immobilized with *S. abikonense* (**d**). The abbreviation meanings are as same as in Fig. 2

pollutants (POPs), such as hexachlorocyclohexane, phenanthrene, and pentachlorophenol (Zilouei et al. 2008; Bashir et al. 2013; Chen et al. 2016a). Indeed, *S. abikonense* showed an excellent potential for degradation, with more than 90% of the phenanthrene dissipated in one week of incubation (Additional file 1: Fig. S2b). In addition, it had strong tolerance to degrade a high concentration of phenanthrene (< 1,500 mg L^{-1}) and could survive in conditions with a content of Cu as high as 200 mg L^{-1} (Additional file 1: Fig. S2a). Thus, *S. abikonense* was the preferred choice to serve as the immobilized microbe to remediate soil contaminated with phenanthrene.

Unique attributes of biochar like high surface area, internal porosity and capability to adsorb organic compounds and bacteria have increased its feasibility for use as biocarrier (Bandara et al. 2019). In this study, biochar provided a suitable habitat for microbial colonization (Fig. 1, Additional file 1: Table S1) since it had abundant pore and tube structures, which could provide sufficient space for the growth of microbes, and subsequently enhance the removal of pollutants in contaminated environments (Song et al. 2021). Clearly, *S. abikonense* was successfully loaded in the pores of biochar as shown by the SEM (Fig. 1d). In addition, the test of incubation in liquid media showed that the *S. abikonense* immobilized by biochar pellets grew well after 24 h of incubation (unpublished results). This suggested that the bacteria-immobilized biochar pellets could keep the microbes intact and enable them to be active to the greatest extent, which is highly important for their utilization in soil remediation.

4.2 High efficiency of immobilized microbial biochar pellets on the soil remediation

In this study, the highest rate of PHE degradation, which reached approximately 98%, was detected within a relatively short time of incubation (6 days) in treatments with immobilized bacterial biochar pellets (Fig. 2). This was primarily related to the combined remediation effect of both the biochar and functional bacteria. Numerous studies have emphasized the positive effect of biochar on soil remediation (Meng et al. 2014; Nie et al. 2018; Tu et al. 2020; Li et al. 2022a), which is probably owing to several reasons. First, biochar improves the soil quality and provides nutrients to soil indigenous microbes, which facilitates the growth of functional bacteria to both directly and indirectly degrade pollutants (Zhu et al. 2017). The results of this study showed that the relative abundance of the microbes *Sphingobium*, *Promicromonospora* and *Bordetella* that degrade PHE gradually increased in soils to which biochar had been added, which was probably owing to the biostimulation effect of biochar on the soil microbial communities (Fig. 4c). In addition, biochar can protect the immobilized bacteria from competition with indigenous soil microorganisms (Song et al. 2021). Secondly, biochar can adsorb pollutants to its surface and inside through various mechanisms, thus reducing the biological effects of contaminants on soil microbial communities (Nie et al. 2018). There are abundant functional groups in biochar. Therefore, its process of adsorbing chemicals could also include chemical reactions between the pollutants and biochar surfaces, such as Π-Π electron donor–acceptor interactions (Tu et al. 2020), the H-bonding effect (Bandara et al. 2019), and redox reactions between organic contaminants and persistent free radicals on the surfaces of biochar (Odinga et al. 2020). In addition, the persistent free radicals in biochar can stimulate the activity of microorganisms (Zhao et al. 2020) and facilitate the transport of electrons between microorganisms and pollutants (Odinga et al. 2020). The biochar used in this study contains abundant functional groups, such as –NH, C–O, C=C, –OH, and P–O (Fig. 1a), which could have a positive effect on the remediation of Cu-PHE contaminated soils. The ratio of PHE degradation induced by biochar-stimulated microbial activity and functional groups was calculated with a rate of 17.56%, which further confirmed the enhanced effect of biochar itself on the soil remediation.

In contrast to the free *S. abikonense* cells, the immobilized bacteria exhibited stronger environmental adaptability and activities, as evidenced by a higher rate of PHE degradation in the first and second pollution stages. *S. abikonense* had a stable relative abundance in the biochar pellets (Fig. 4d), indicating that biochar acted as an effective shelter for the exogenous microbes. The retention of pollutants onto the surface of biochar via the weak binding forces, including Van der Waals and electrostatic interactions (Wang et al. 2020), could be utilized further by the immobilized functional microbes. As measured, the content of PHE adsorbed by just biochar increased continuously during both the first and second stages of pollution, while the content of PHE adsorbed in the biochar pellets that were immobilized with *S. abikonense* remained low (Fig. 2c). This could be explained by the fact that the PHE in the soil was adsorbed to the surface of biochar first and then rapidly degraded by immobilized *S. abikonense*. However, a previous study obtained different results and concluded that the immobilized bacteria can limit the availability of pollutants (Cai et al. 2020). This is probably owing to the difference in immobilization methods, biochar species, pollutant types, and soil properties. In summary, the results of this study showed that immobilized bacterial biochar pellets efficiently enhanced the degradation of PHE in both contaminated soils and biochar pellet surfaces, thus further enhancing the efficiency of remediation through their combination

and also avoiding the potential risk of secondary contamination of the soil induced by contaminants that desorbed from the biochar.

4.3 Long-lasting effect of immobilized-bacteria biochar pellets on PHE degradation

As is commonly known, long-lasting efficiency and stability are crucial factors for the applicability of immobilized technology to practical sites (Lou et al. 2019). In this study, the soil was contaminated twice with PHE, and the amount of PHE degraded in the treatment with free *S. abikonens*e cells decreased to 36.7% in the second pollution stage compared with the first stage of pollution of 88.0% (Fig. 2a and b). In contrast, in the treatment with immobilized bacterial biochar pellets, the PHE was highly efficiently degraded even at the second pollution stage and reached 96.74% (Fig. 2a and b). The relative abundance of *S. abikonens*e immobilized in the biochar pellets remained stable on day 12 compared with the day 6 with a high percentage of approximately 80% in the biochar pellets (Fig. 4d). These results indicated that the immobilized *S. abikonens*e cells remained functionally efficient for a long time when sheltered by biochar, which could efficiently and persistently remediate soil that was contaminated with a high content of PHE.

The activities of free *S. abikonens*e gradually weakened as the time of incubation extended (Fig. 2a and b), which was primarily owing to various environmental factors. These factors included competition with indigenous microorganisms, the stress of continual Cu and PHE toxicity, and unfavorable environmental conditions. Similar results were found by Lou et al. (2019) in a study on the degradation of nonylphenol in water by biochar immobilized cells, after eight rounds of application, the ability of free bacteria to degrade these pollutants decreased significantly. Thus, using biochar as a carrier and shelter is highly important for the growth of exogenous free bacteria. The biochar can provide nutrients for the immobilized bacteria, as well as alleviate the toxicity of intermediate metabolites or Cu, thus maintaining the bacterial activity, as previous studies have indicated (Wang et al. 2020; Zhao et al. 2020). In addition, the biochar pellets remained intact after incubation in the soil for 12 d, which was crucial to maintaining their mechanical stability to protect the immobilized bacterial activities (Additional file 1: Fig. S3). Chan et al. (2011) investigated the strength of beads with different compositions and found that the type and concentration of sodium alginate and gelling cation affected their stability. In addition, the cell load and culture conditions can also influence mechanical stability (Bhujbal et al. 2014). Most studies primarily focus on the efficiency of soil remediation effects by immobilized technologies (Tu et al. 2020; Song et al. 2021), while in these results, the stability and long-lasting potential were also evaluated to comprehensively evaluate the practicability of immobilized microbial technology. In total, the strong mechanical stability and effect of the biochar on protecting the microbes were the key to perpetually efficiently remediating contaminated soil.

4.4 Cu stabilization by immobilized-bacteria biochar pellets

Compared with the PHE degradation, variation in the concentration of Cu in the soil was not readily apparent among the different treatments (Fig. 2d, Additional file 1: Fig. S1). More than 35 mg kg^{-1} of Cu has been detected in biochar pellets, which was primarily attributed to the ability of biochar to adsorb this HM (Fig. 2e). Various studies have indicated that biochar could directly sorb HM by complexation, cation exchange (Tu et al. 2020), electrostatic interactions, and chemical precipitation processes (Bandara et al. 2019; Zhu et al. 2017) owing to its adsorptive properties, which include a large surface area, highly porous structure, high surface alkalinity and the presence of various surface functional groups (Nie et al. 2018). Moreover, the negative charge value on the surface of biochar (Fig. 1e) indicated that it may be favorable to the attraction between active sites and positive charges of Cu^{2+} (Meng et al. 2014). In addition, the biochar that was added gradually aged as the incubation time increased, which could lead to the formation of oxygen-containing functional groups and hydrophilic surfaces, thus facilitating the adsorption of HM (Tu et al. 2020). Interestingly, we found that the amounts of Cu adsorbed by the immobilized microbial biochar pellets were significantly higher than those in the biochar pellets that did not contain microbes (Fig. 2e), which was primarily related to the effects of immobilized bacteria. Microorganisms can remediate HM pollution by their own adsorption (Xu et al. 2020). For example, Ren et al. (2009) reported that the organic acids produced by the fungus *Aspergillus niger* could bind Cu and Cd. Yue et al. (2015) also showed that the extracellular polymeric substances (EPS) secreted by sulfate-reducing bacteria can reduce HM toxicity by adsorbing heavy metal ions, such as Cd^{2+} and Zn^{2+}, through their surface –COOH, –OH, $–NH_2$, and PO_4^{3-} groups. In addition, functional groups on the cell surface can complex HM ions and immobilize them on this surface (Xu et al. 2020). Examples include the presence of functional groups, such as $–CH_2$, C=O, C–N, N–H, –COOH and $–SO_3$, on the cell surface of *Pseudomonas* sp. that can adsorb Pb^{2+} or Cd^{2+} (Xu et al. 2017, 2020). Thus, all these microbial adsorption mechanisms of the complexation of metals inside a cell, the adsorption of metal ions onto the cell wall, as well as

precipitation in vitro, contributed to the high ability to adsorb HM in this study.

The addition of biochar reduced the proportion of exchangeable Cu and carbonate bound to Cu but increased the organic matter bound to Cu and the Fe–Mn oxide that was bound to Cu (Additional file 1: Fig. S1b), which was similar to the findings of a study by Tu et al.(2020). They found that both biochar and biochar loaded with *Pseudomonas frederiksbergensis* induced the transformation of Cd and Cu from labile fractions to recalcitrant fractions after a 75-d pot experiment. This was particularly true for the formation of Fe–Mn oxide bound and residential fractions. Cu has a strong affinity for soil organic matter, and the proportion of organically bound Cu increased significantly after the application of biochar (Park et al. 2011). In combination with the results of PHE degradation, these results considered that the immobilized microbial biochar technology exhibited a strong potential for remediation in soils co-contaminated with Cu and PHE.

4.5 Various factors influenced the microbial community structures in soils

PCoA and PERMANOVA analyses showed that time was the largest factor that contributed to differences in the soil microbial community composition, while different treatments were the second major factor (Fig. 3c). These findings were similar to those of various studies (Lou et al. 2019; Lefevre et al. 2018). The variation of soil microbial communities in the treatment of free *S. abikonense* cells was similar to that of the control, while the treatments of biochar that were immobilized with and without *S. abikonense* cells had a similar microbial succession (Fig. 4a and c). This suggested that the presence of biochar exhibited a stronger influence on soil microbial communities than the *S. abikonense* cells. This may be due to the fact that free bacteria added to the soil may be less active, and less alteration of the soil microenvironment. And biochar can improve soil physio-chemical properties like soil pH, electrical conductivity, organic carbon, total nitrogen, available phosphorous, cation exchange capacity etc., and may induce heterogeneous reactions in microbial communities and alter microbial community structure (Wang et al. 2019). In addition the "biochar effect" also results in increased diversity and changes in the metabolic potential of the microbial community (Kolton et al. 2017).

Biochar changed the soil pH and physicochemical properties, causing changes in some native microbial communities (Nie et al. 2018). The relative abundance of Proteobacteria in biochar-treated soils increased significantly (Fig. 4a). Proteobacteria has been considered to be the dominant bacteria in the remediation of organic pollutants and HMs (Xu et al. 2021). Therefore, the increase in abundance of Proteobacteria under biochar stimulation probably contributed to the degradation of PHE and the immobilization of Cu in the contaminated soil. However, the abundance of Acidobacteria continued to decrease over time (Fig. 4a), which could be owing to the increase in soil pH after the addition of biochar. Additionally, the abundances of *Luteibacter*, *Bordetella* and *Dyella* increased in soils with added biochar (Fig. 4c), and these bacteria had previously been shown to be immobilized in the degradation of organic pollutants (Bianchi et al. 2005; Li et al. 2009; Bresciani et al. 2014). Notably, the addition of biochar promoted the growth of indigenous populations of *Sphingomonas* in the soil that could degrade PAH. This provided additional confirmation that the addition of biochar is an effective strategy for remediation and a suitable carrier for immobilizing *S. abikonense* to apply in the remediation of PHE-Cu contaminated soils.

4.6 Selective preference of biochar for its colonized microbes

The microbial community composition in the biochar pellets was diverse (Fig. 4b and d). *Sphingobium*, *Methylobacterium*, *SC-I-84* and *Bordetella*, which were affiliated to Proteobacteria; *Streptomyces*, *Micromonospora*, *Promicromonospora* and *Kribbella*, which were affiliated to Actinobacteria; and *Bacillus*, which was affiliated to Firmicutes, were predominately enriched in the biochar pellets. As is well known, the microbes that colonize the biochar pellets primarily originate from their surrounding soil environment. However, the dominant soil microbial groups that were stimulated by the biochar were not synchronously enriched in the biochar pellets, which implied that biochar itself had a selective preference for its colonized microbial communities. For example, the addition of biochar promoted the growth of *Dyella* and *Luteibacter* in the soil, with a high abundance of 10.55% and 7.63% on day 12, respectively. However, they were not enriched in the interior of biochar pellets, with a relatively low abundance of less than 0.8% (Fig. 4c and d). In contrast, the predominant microbes of *Lysobacter* in the biochar pellets were not enriched in their adjacent soils.

We calculated the amounts of significantly different microbes that existed between biochar pellets and their surrounding soils ($p < 0.05$) and found that more than 52.2% of the microbial communities varied between these two conditions, which further confirmed that biochar itself could autonomously select their microbial assemblage. The specific physicochemical properties of biochar, such as pH and functional groups, were probably the primary cause for microbial selection. The easily mineralized charcoal contained in biochar can be used

as an energy source by most bacteria and can enhance microbial activity, suitable for the colonization of some microorganisms that prefer to grow in nutrient-rich environments, such as Proteobacteria (Taketani et al. 2013). In addition, biochar has more pores, and microorganisms colonizing the pores can mitigate the hazards of high concentrations of contaminants in the soil. Biochar is alkaline, which can attract some alkalophilic bacteria to colonize. Moreover, biochar contains phosphate functional groups, which can attract phosphorus-solubilizing bacteria to colonize. The types of biochar, properties (e.g., aromaticity, pH, surface area), pyrolysis temperature (Sun et al. 2014), soil characteristics, etc., vary in the types of microorganisms to be hosted (Noyce et al. 2016). The significantly enriched microbes of *Bacillus* have been reported to preferably survive in conditions of high pH (Banala et al. 2021). In addition, the phosphate functional groups on the biochar surface could be suitable for the growth of phosphate solubilizing bacteria, such as *Burkholderia* (Peng et al. 2021). Additionally, HM-tolerant and PAH-degrading bacteria were shown to be enriched in biochar pellets and included *Sphingobium*, *Micromonospora*, *Lysobacter*, and *Bacillus* (Figs. 4d and 5a) (Gojgic-Cvijovic et al. 2012; Wang et al. 2021; Tan et al. 2020; Li et al. 2020). Functional microorganisms can immobilize HMs by secreting a variety of compounds, such as polysaccharides, and simultaneously degrade the PHE adsorbed by biochar. These results showed that biochar can specifically choose its host microbes to colonize in their surface and pores, and this autonomous selection facilitates the degradation of PHE and/or the immobilization of Cu in the contaminated soils.

It is notable that the selection effect on its colonized microbiome was dramatically minimized when the biochar pellets were immobilized with *S. abikonense* (Fig. 4b and d). The immobilized *S. abikonense* cells in the biochar pellets remained relatively high in abundance and comprised approximately 80% during the first and second addition of PHE, which facilitated the sustainable remediation of soil. Notably, we also observed that a low abundance of diverse microbes that comprised less than 10% of the total co-existed with *S. abikonense* in the biochar pellets (Fig. 4d). Most of these rare microbes were positively connected with *S. abikonense*, such as ASVs affiliated to *Microbacterium* and *Bacillus* (Fig. 5d), which are both known to degrade PAHs and tolerate HMs (Wongbunmak et al. 2017; Li et al. 2020). In addition, *Bacillus* has been shown to degrade cellulose and lignin (Chen et al. 2016b; Hero et al. 2017), which suggested that *S. abikonense* could act synergistically with *Bacillus* to promote the decay of biochar and facilitate the mobilization of phosphorus in the soil environment (Zhang et al. 2021), which thus provides nutrients to the microbial communities that grow in the soil and biochar pellets. However, there were more negative connections among these rare microbes in the immobilized bacterial biochar pellets, including such genera as *Proticromonospora*, *Steroidobacter*, *Nonomuraea*, *Micromonospora*, *Kribbella*, and *Bordetella*. These bacteria are often present in contaminated environments and are effective in competing for survival by using space and nutrients (Wang et al. 2007; Domínguez-Mendoza et al. 2014; Zhou et al. 2019; Aguilar-Romero et al. 2022; Han et al. 2022). In summary, this study demonstrated the predominant microbes that could survive in the biochar pores and surface, which could provide a theoretical basis for future studies on the selection of specific biochar-embedded bacteria in the remediation of co-contaminated soils.

5 Conclusion

Biochar immobilized *S. abikonense* could efficiently and consistently degrade phenanthrene in soil compared to biochar-only and free *S. abikonense* treatments. Besides, the addition of biochar promoted the fixation of Cu in the soil, and the immobilized microbial biochar pellets adsorbed more Cu in soil than biochar-only pellets. The presence of biochar significantly increased the abundance of bacteria with organic matter degrading and heavy metal tolerant functions such as *Luteibacter*, *Bordetella* and *Dyella*, and promoted the growth of *S. abikonense* in the soil. We noticed that biochar has a selective preference for its colonized microbes, which provided the basis for the selection of suitable immobilized strains that could survive in its surface and pores. And also, the immobilized *S. abikonense* was predominantly enriched in the biochar pellets during the continuous pollution, indicating that biochar was a perfect immobilized carrier to protect microbes from the competition of indigenous soil microbes and unfavorable environmental conditions. In this study, we determined the concentrations of phenanthrene and copper in soil and biochar pellets by using biochar immobilized *S. abikonense* to remediate PHE-Cu co-contaminated soils, and gained a more comprehensive understanding of the remediation effect of biochar immobilization microbial technology, which provided an efficient and durable green remediation technology for PHE-Cu co-contaminated soils. Moreover, the structural composition and changes of microbial communities in soil and biochar particles were analyzed by high-throughput sequencing technology, and the selection preference of biochar for immobilized microorganisms was investigated, which provided the theoretical basis for the selection of strains of biochar-embedded microorganisms in the future. Since the study was conducted under controlled laboratory conditions, further research is still needed to determine whether the application effect

of actual contaminated soil is optimistic. Also, checking the leaching of immobilized biochar and investigating the desorption-regeneration effect of biochar on contaminants are necessary. In addition, the question of whether the selectivity of biochar for microorganisms is random or oriented deserves further attention. The compound pollution of heavy metals and organic pollutants in groundwater is also more serious, and immobilized microbial technology should be applied to groundwater/real wastewater pollution remediation in the future.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1007/s42773-023-00241-x.

Additional file 1: Figure S1. Changes in total Cu (a) and Cu speciation (b) in the soil. **Figure S2.** The OD_{600} values of *S. abikonense* grew under different Cu concentrations (a) and the degradation potential of *S. abikonense* to phenanthrene (b). **Figure S3.** The morphology of biochar pellets before and after incubation. **Table S1.** Basic properties of prepared biochar and soil. **Table S2.** Logistic model parameters of PHE degradation in soil treatments. **Table S3.** Permutational MANOVA results using weighted UniFrac as distance metrics for the microbial communities in soils and biochar pellets. **Table S4.** Detailed classification of enriched ASVs between biochar pellets (BB) and soils treated with biochar pellets (SB). **Table S5.** Detailed classification of enriched ASVs between biochar pellets (BB) and biochar pellets immobilized with *S. abikonense* (BBSp). **Table S6.** Topological parameters of the microbial co-occurrence networks in biochar pellets (BB) and biochar pellets immobilized with *S. abikonense* (BBSp).

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Author contributions
YZ: Methodology, Formal analysis, Investigation, Writing–original draft, Visualization. SL and LN: Formal analysis, Investigation, Validation, Visualization. AS and ML: Investigation, Methodology. YW: Investigation, Validation. YX: Conceptualization, Supervision, Writing–review & editing, Funding acquisition.

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Availability of data and materials
The datasets used or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Competing interests
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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