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Short Communication

Effects of short and long-term thermal exposure on microbial compositions in soils contaminated with mixed benzene and benzo[*a*]pyrene: A short communication

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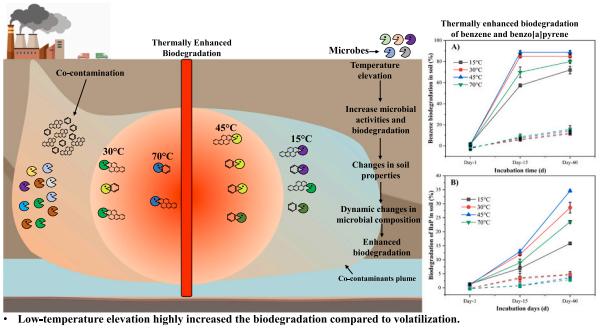
HIGHLIGHTS

- The specific microbial abundances at elevated temperatures accounted for enhanced biodegradation.
- The bacterial phylum of Firmicute plays an essential role under elevated temperature conditions.
- Fungal abundance and compositions changed more significantly than bacteria at elevated temperatures.
- *Brevibacillus* was first reported to prosper at a temperature of 45 °C.
- Temperature affects microbial compositions directly via microbial specificity and indirectly by the soil properties.

GRAPHICAL ABSTRACT



- Low-temperature elevation highly increased the biodegradation compared to volatilization.
- Temperature affects microbial composition directly via microbial specificity and indirectly by the soil properties.

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ABSTRACT

Polycyclic aromatic hydrocarbons (PAHs) and benzene, toluene, ethylbenzene, and xylene (BTEX) are the most persistent and toxic organic contaminants often found co-contaminated in anthropogenic and petrochemical industrial sites. Therefore, an experiment was performed for the safe biodegradation of benzene and benzo[*a*] pyrene (BaP) through thermally-enhanced biodegradation, and to explore the influence of elevated thermal treatments on microbial diversity and composition. The results revealed that elevated thermal treatments (15 to 45 °C) significantly enhanced the diversity of both bacteria and fungi. The composition analysis revealed that short-term and long-term elevated temperature conditions can directly enhance the specificity of microorganisms that play a crucial role in the biodegradation of benzene and BaP co-contaminated soil. Moreover, the indirect role of elevated temperature conditions on microbial compositions was through the fluctuations of soil properties, especially soil pH, moisture, TOC, potassium, phosphorous, total Fe, Fe(II), and Fe(III). In addition, the correlation analyses revealed that thermal exposure enhances the synergistic association (fungal-fungal, fungal-bacterial, bacterial-bacterial) of microbes to degrade the toxic contaminants and to cope with harsh

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environmental conditions. These results concluded that the biodegradation of benzene and BaP co-contamination was efficiently enhanced under the thermally-enhanced biodegradation approach and the elevation of temperature can affect the microbial compositions directly via microbial specificity or indirectly by influencing the soil properties.

1. Introduction

Co-contamination of polycyclic aromatic hydrocarbons (PAHs) and benzene, toluene, ethylbenzene, and xylene group (BTEX) are very common due to anthropogenic activities, such as petroleum oil spills. Benzo[*a*]pyrene (BaP) and benzene, representative compounds for PAHs and BTEX groups respectively, are ranked 6th and 8th on the most toxic priority contaminants list (ATSDR, 2019). Therefore, immediate safe remedial actions are required for soils co-contaminated with these toxic chemicals. Among the different remediation technologies, bioremediation, a promising strategy to degrade toxic contaminants, has been studied extensively due to its cost-effective and eco-benign remediation (Ali et al., 2022a). According to the United States Environmental Protection Agency (USEPA), bioremediation accounted for ~24 % of soil and groundwater remediation technologies. However, due to the lack of contaminants bioavailability and low microbial sustenance, bioremediation efficiency under harsh conditions is often very low. The success of bioremediation is highly dependent on the contaminant's bioavailability, the presence of potential microorganisms, and environmental conditions. It has been reported that increasing temperature enhances the bioavailability of contaminants and microbial metabolisms (Wang et al., 2022). Therefore, thermally enhanced biodegradation (TEB) can be an effective strategy to improve the remediation efficiency of hazardous contaminants.

Temperature plays a key role in controlling the bioavailability of contaminants, the nature and extent of microbial growth, and microbial metabolism. Previous research has shown that an increase in temperature can promote the biodegradation of aromatic hydrocarbons (Wang et al., 2022; Kaur et al., 2021). This is because higher temperatures can speed up the rate of microbial metabolism, resulting in faster degradation of the contaminants. Additionally, conditions with elevated temperatures can also increase the solubility of hydrophobic contaminants, making them more accessible to microbial attack. For example, (Yadav et al., 2012) reported that the biodegradation of toluene increased two-fold with a 10 °C rise in the soil temperature. Similarly, (Raju et al., 2017) reported that the highest microbial degradation rate of diesel oil was observed by increasing temperature to 30 and 40 °C. The temperature elevation is often accompanied by an increase in mass transfer and a decrease in the viscosity of organic contaminants, resulting in the increased bioavailability of contaminants, and enhanced biodegradation efficacy (Kaur et al., 2021; Wang et al., 2022; Yadav et al., 2012). Therefore, thermally-enhanced biodegradation (TEB), an emerging concept proposed in recent years to combine the two conventional remediation strategies of bioremediation and thermal treatments in an attempt to improve the biodegradation of BaP and benzene co-contamination, could be an effective way to remediate these hazardous pollutants from the environment. In addition, TEB can also overcome the disadvantages of bioremediation (slow degradation and low microbial sustenance) and thermal treatments (high temperature can damage soil properties). Detailed reviews conducted by Da et al. (2019) and Wang et al. (2022) reported that temperature has a high influence on fluctuation in soil properties. In our previous work, we also reported that low-temperature treatments can influence certain soil properties such as soil pH, TOC, moisture, total iron, oxidized iron, and reduced iron (Ali et al., 2023). These changes in soil properties might exert influences on the compositions of bacteria and fungi during TEB under contamination conditions.

Microorganisms have specific temperature ranges at which they can function optimally under different contaminated sites. For instance, some microorganisms can thrive in temperatures as low as 0 °C, while others require temperatures as high as 100 °C (Smith et al., 2019) and the optimal temperature range for most microorganisms is between 20 °C and 45 °C (Gou et al., 2023; Gou et al., 2020). These temperature ranges differ based on the type of microorganism and the environment where they are found. For example, (Taha et al., 2014) reported the efficient involvement of a thermophilic fungus *Thermomucor indicae-sudatica* in a high contaminant concentration under 55 °C temperature treatments. Several measures have been studied to enhance the biodegradation of different organic contaminants (Wang et al., 2022; Kaur et al., 2021). However, there is a lack of knowledge regarding the effects of enhanced temperature on soil bacterial and fungal communities, enzymes, and potential genes involved in the biodegradation of benzene and BaP co-contamination, and the soil properties influence on microbial composition under different temperature conditions. Therefore, in this study, we reported the short and long-term TEB treatment's impact on the dynamic changes in soil bacterial and fungal communities, soil properties and the subsequent changed soil properties influence on microbial composition.

2. Material and methods

2.1. Collection of soil and preparation

The soil collected from the BD-1458 site of a steel-making factory located in Hangzhou, Zhejiang province underwent a process of air-drying. The collected soil was then analyzed to determine its physicochemical properties. These properties were documented and are presented in Table S1. In addition, total (Σ15) PAHs were also analyzed, and the results revealed that the concentration of benzene and BaP in the collected soil samples was lower than the average level of contamination in the factory. Therefore, the soil was contaminated artificially with benzene and BaP.

2.2. Preparation of soil

Artificial contamination was accomplished for both BaP and benzene, and around 4 kg of soil was first spiked with 50 mg BaP kg^{-1} (Picariello et al., 2020). A 5000 mg L^{-1} standard solution was prepared by dissolving 0.2 g of BaP in 40 mL of acetone. The solution was then spiked with 320 g of soil and mixed thoroughly. The mixture was left overnight to evaporate the acetone in a fume-hood. In order to obtain a final concentration of 50 mg BaP kg^{-1} soil, the contaminated soil was mixed with the remaining 3.6 kg of clean soil. Following the mixing process, six random samples were collected and analyzed for BaP concentration. The results indicated that the BaP concentration in the soil samples was 48 ± 0.02 mg kg^{-1}. The aging of soil prior to experimentation is an essential process that ensures the interaction of the contaminant with soil particles. In the case of BaP-contaminated soil, it is necessary to keep the soil in the dark for at least three months to allow the aging process to occur. However, due to the volatilization of benzene, it was not possible to keep the soil for aging, and the experiment was started directly after spiking the BaP-contaminated soil with benzene.

2.3. Experiment setup

Two experimental setups, including microbial-kill (biocide) and microbial treatment, were carried out in this study, with the biocide

setup being selected to evaluate the abiotic (adsorption + volatilization) remediation of contaminants. In each experimental setup, four different temperature treatments, 15 °C, 30 °C, 45 °C, and 70 °C, were chosen for the purpose of analyzing the microbial capacity for the degradation of mixed contamination of benzene and BaP. Two conditions, including short-term (ST) and long-term (LT), comprised of 15 and 60 days TEB incubation time, respectively, were selected to analyze the biodegradation of benzene and BaP co-contamination, enzymatic activities, bacterial-fungal diversity, and compositions, total bacterial and fungal gene copy number, and PAH-RHD gene in gram-positive and gram-negative bacteria. The duration rates for short-term (15 days) and long-term (60 days) were chosen based on the degradation rates observed in this study.

2.4. Analytical condition

The extraction and analytical conditions of benzene and BaP has been discussed by (Ali et al., 2022b,2023).

2.5. 16S rRNA and ITS analysis

Soil total DNA extraction was accomplished in all samples to classify the microbial diversity and composition in each sample under different treatments. The Fast DNA SPIN Kit from MP Biomedicals, USA was utilized for extracting DNA from each sample following the manufacturer's instructions. This kit is a reliable and efficient method for extracting DNA from a variety of environmental samples, including soil. The extracted DNA was quantitatively verified using a NanoDrop ND-2000 spectrophotometer (NanoDrop Technologies, USA) based on spectral absorbance at 260 nm. The spectrophotometer measures the amount of DNA in a sample by calculating the absorbance of light at a specific wavelength. To determine the quality of the extracted DNA, agarose gel electrophoresis was performed. This technique is used to separate DNA fragments based on their size. After verification and quality determination, the extracted DNA was dissolved in 80 μL of TE buffer and stored at −20 °C until PCR amplification. TE buffer is commonly used to dissolve DNA samples as it helps to stabilize the DNA and maintain its integrity. The storage temperature of −20 °C is ideal for long-term storage of DNA as it prevents degradation and maintains its quality. The microbial communities in the sample were analyzed by high-throughput sequencing using Illumina MiSeq technology (Illumina Inc.). To amplify the V4 and V5 regions of the bacterial 16S rRNA gene, the primers 338F and 806R were used, following the protocol described by Lu et al. (2019). More information about sequencing is presented in the supplementary file.

Soil fungal communities (ITS) were analyzed during short- and long-term thermal exposure through high-throughput sequencing on the Illumina MiSeq platform. For this, the ITS1 region of the rRNA gene was first amplified with ITS1F_ITS2R primers with specific illumine adapters as described by (Crowther et al., 2014). The PCR was performed through an EasyCycler PCR system via a program of 95 for 5 min, followed by 35 cycles at 95 for 10 min. Three replicated PCR reactions were conducted for each DNA soil sample. The products of each triplicate reaction were collected and purified. A 2nd eight-cycle PCR was performed to Illumina sequencing adapters and added dual index using a Nextera XT Index Kit. The PCR products were then purified and quantified to a normalized pooled. Finally, the pooled DNA library was paired-end sequenced on an Illumina MiSeq platform (Illumina Inc., San Diego, USA).

To quantify the 16S rRNA gene copy number, real-time quantitative PCR (qPCR) was employed to determine the bacterial abundance using the primers 5′-TTCCCGAGTACGAGGGATAC and 5′-TCACGTTGATGAACGACAAA (Wang et al., 2018). Clones containing the 16S rRNA gene copy numbers were prepared using Peasy-T1 Simple Cloning Kit (TransGen Biotechnology Co., Ltd., China), according to the manufacturer's instructions. The cloned strains were cultured to extract the plasmid DNA. A standard solution was prepared using serial dilution (10-fold) of the extracted plasmid DNA. The reaction mixture comprised 20 μL of ChamQTM SYBR Color qPCR Master Mix, 2 μL of template DNA (1–10 ng), and 0.5 μmol of each primer. The conditions for the qPCR were as follows: initial denaturation at 95 °C for 30 s and elongation for 39 cycles at 95 °C for 15 s, 56 °C for 30 s, and 72 °C for 30 s prior to the final plate reading. The amplification efficiency (R^2 values) for 16S rRNA genes copy number ranged from 0.998 to 0.999.2.6 Statistical analysis.

The mean differences were measured using $\leq$0.05 probability with the least significant differences. For the calculation of alpha diversity indices, Mothur software was used to observe the number of OTUs. Principal coordinate analysis (PCoA) and dimension analysis were carried out to correlate the differences among the different treatments and to measure the dynamic changes in soil properties. R statistical language was used to calculate the sample microbiota composition diagram under the short- and long-term thermal exposures.

3. Result and discussion

3.1. Microbial diversity abundance and richness

The degradation of benzene and BaP, enzymatic activities, and potential gene quantitation have been published previously by (Ali et al., 2023). Briefly, with the mass loss to the gas phase considered, the highest biodegradation efficiency of benzene and BaP occurred at 45 °C (Fig. 1). The results revealed that the contribution of benzene biodegradation was much higher than its volatilization during the low-temperature thermal treatments (Fig. 1a). The biodegradation rates of benzene increased from 57.4 % to 88.7 % and 84.9 %, and the biodegradation efficiency of BaP was enhanced from 15.8 % to 34.6 % and 28.6 %, when the temperature was raised from the ambient temperature of 15 °C to 45 °C and 30 °C, respectively. The elevated temperature from 15 °C to 45 °C highly increased the microbial degradation of benzene and BaP, which could be due to the increased metabolic potential and microbial activities, as evidenced by the abundance of genes and microbial activities (production of CO_2 and CH_4) in the supplementary file (Figs. S1).

This study focused on the exploration of the effects on microbial compositions due to short-term and long-term thermal exposures. Both bacterial and fungal diversity richness and evenness were analyzed through Ace, Chao1, Shannon, Simpson, and Pielou diversity indices under short- and long-term (ST and LT) thermal treatments of benzene and BaP co-contaminated soil (Fig. S2). The results revealed that the highest bacterial diversity and richness was recorded in ST-30 °C, whereas LT-45 °C resulted in the most responses in fungal diversity and richness. The comparison results demonstrated that long-term elevated thermal exposure, especially 30 and 70 °C, significantly reduced the diversity of bacteria and fungi, whereas in 45 °C treatments, the bacterial diversity was non-significantly influenced by the exposure duration. This might be due to the presence of easily degradable benzene during the ST treatment that was degraded by the number of bacteria, whereas at LT only the difficultly degradable BaP highly reduced the microbial diversity. On the other hand, the fungal richness, and evenness increased at elevated thermal treatments under LT treatments. These results indicated that the bacterial diversity richness and evenness fluctuate under the short- and long-term elevated thermal treatments, whereas fungal diversity and evenness further increased under the long-term elevated thermal treatments compared to the short-term temperature elevation (Fig. S2F). One of the key factors that may contribute to this phenomenon is the way in which fungi respond to changes in temperature. For example, as temperatures rise, fungi may become more resistant to heat and may be better able to survive in low elevated temperature environments.

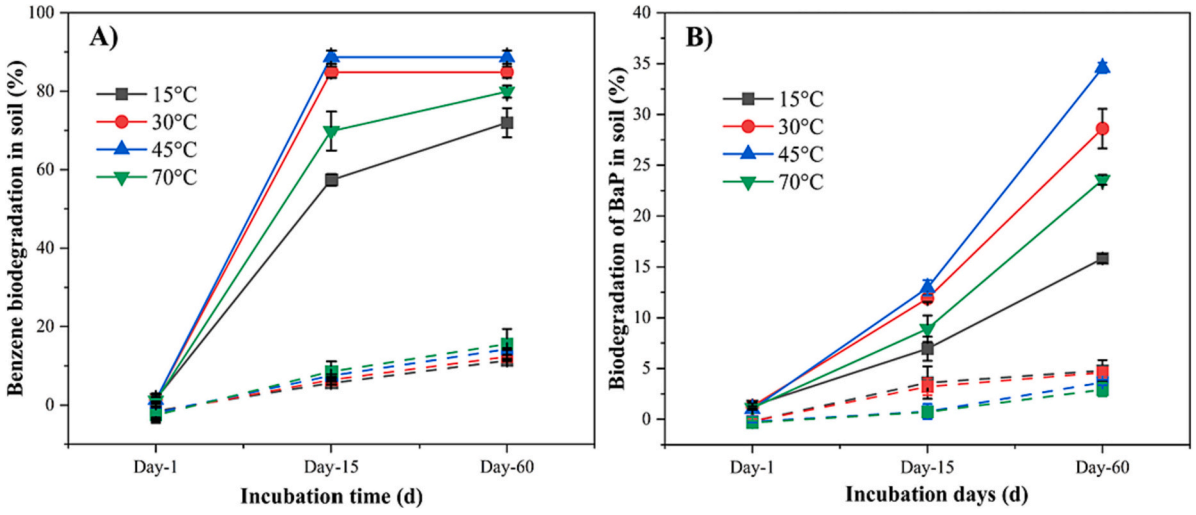

Fig. 1. Soil benzene and BaP biodegradation (%) during the TEB of benzene and BaP co-contaminated soil. The dashed lines represent the biocide control and the solid lines represents the treatments with microorganisms.

3.2. Microbial compositions and their correlations under short and long-term temperature treatments

3.2.1. Microbial compositions under short and long-term temperature treatments

Bacterial and fungal composition on phylum and genus levels were analyzed to further explore the microbial composition affected by short- and long-term thermal exposure under benzene and BaP co-contaminated condition (Fig. 2). The results revealed that Firmicutes, Proteobacteria, Actinobacteriota, Chloroflexi, and Acidobacteria were the dominant recognized phyla in ST and LT TEB of benzene and BaP (Fig. 2A). Interestingly, the elevation of temperature from 15 to 70 °C increased the abundance of Firmicutes, while the abundance of Proteobacteria reduced with increased temperature from 30 to 70 °C. Comparing the ST and LT treatments, the abundance of Actinobacteriota and Firmicutes decreased under LT compared to ST in 15 °C treatment, whereas, the abundance of Firmicutes increased and Proteobacteria decreased upon thermal time prolongation in 30 and 45 °C treatments. This indicates that Firmicutes have evolved with better mechanisms to cope with environmental changes and fluctuations. For example,

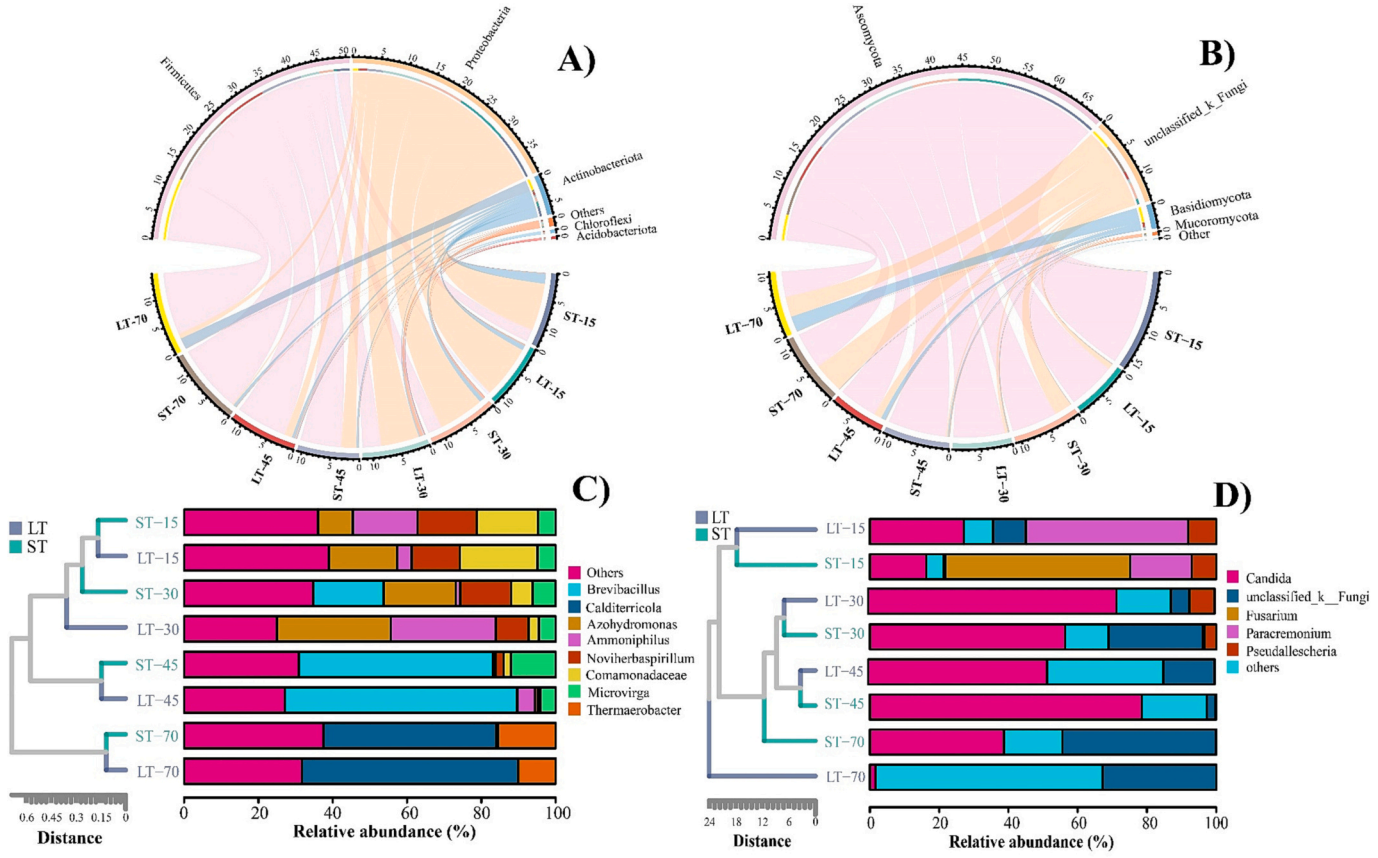

Fig. 2. Compositions of soil bacteria at A) phylum, C) genus level, and soil fungi at B) phylum, and D) genus level during the short- and long-term thermal exposure.

(Filippidou et al., 2016) conducted an experiment to explore the impacts of extreme environmental conditions on Firmicute abundance. The results revealed that Firmicutes can tolerate the extreme thermal condition of 55 °C, and Firmicutes can produce certain kinds of endospores, making them able to tolerate extreme stress conditions (Benammar et al., 2020; Filippidou et al., 2016). On the other hand, Ascomycota, unclassified_k_Fungi, Basidiomycota, and Mucoromycete were the dominant recognized fungal phyla under ST and LT treatments exposure (Fig. 2B). Ascomycota was the dominant phyla in all the treatments and a high abundance of Ascomycota was recorded at 15–45 °C elevated temperature treatments, while reduction of Ascomycota abundance was noted at 70 °C. Unclassified_k_Fungi and Basidiomycota were dominant at 70 °C temperature conditions, indicating their tolerance to high thermal treatments. (Osinska-Jaroszuk et al., 2015) reported that fungi belonging to Ascomycota and Basidiomycota phyla have the capability to secrete exopolysaccharides, making them able to tolerate elevated temperature conditions (Alves and Murray, 2022). Comparing ST and LT treatments, the abundance of Ascomycota decreased in LT treatments of 15, 45, and 70 °C thermal conditions, whereas, Ascomycota abundance increased in LT treatment of 30 °C thermal conditions compared to ST, indicating that elevation of temperature and prolongation of time reduced the abundance of Ascomycota. Alternatively, the abundance of unclassified_k_Fungi and Basidiomycota increased in LT treatments of 45 and 70 °C, indicating their tolerance/adoption to the elevated thermal conditions.

Fig. 2C and D showed the tree-bar plot for bacterial and fungal abundance on the genus level and distinguished different groups on the basis of bray-Curtis analysis. Bacterial compositions on the genus level revealed that *Ammoniphilus*, *Azohydromonas*, *Comamonadaceae*, and *Noviherbaspirillum* were the dominant genera at 15 and 30 °C thermal treatments. *Comamonadaceae* was the dominant bacteria at 15 °C, and increased temperature to 30 °C changed the dominant bacteria to *Azohydromonas*. The enrichment of *Brevibacillus* was noted when the temperature was raised from 15 to 45 °C temperature conditions, and further increasing temperature to 70 °C enriched *Caldeterricola*. The findings, as shown in Fig. 2C, indicated that thermal elevation causes fluctuations in the bacterial community structures, suggesting that changes in temperature have a considerable impact on the compositions of bacterial communities. Several studies reported the abundance of *Comamonadaceae* in contaminated subsurface and groundwater, indicating that the genus favors the subsurface temperature (15 °C) to degrade the hazardous contaminants (Feld et al., 2016; Höckenreiner et al., 2013). Moreover, (Li et al., 2018) conducted an experiment to reveal the mechanisms of bioaugmentation in PAHs-contaminated wastewater via DNA-stable-isotope-probing (DNA-SIP), and *Ammoniphilus* was the first time reported to degrade PAHs. In our experiment, *Ammoniphilus* was also observed and the abundance of *Ammoniphilus* was increased at LT-30 °C. Similarly, (Zhao et al., 2021) reported that *Azohydromonas* genera encoding methyl parathion hydrolase genes have the capability to grow at 50 °C; however, in our study reduced abundance of *Azohydromonas* was noted at >30 °C, which might be due to the toxic influence of BaP and benzene co-contamination.

The elevation of temperature from 30 to 45 °C highly stimulated the abundance of *Brevibacillus* and *Microvirga*, and further elevation of temperature to 70 °C specifically enhanced the abundance of *Caldeterricola and Thermaerobacter*. For example, (Zhao et al., 2023) reported a high abundance of *Caldeterricola*, *Thermaerobacter*, *Microvirga*, and *Brevibacillus* in a retired coal gas plant post-thermal remediation site, indicating the high potential of these genera to degrade hazardous contaminants under elevated thermal conditions. Moreover, (Reddy et al., 2010) reported that *Brevibacillus* has the biosurfactant-producing capability that can enhance the bioavailability of hydrophobic contaminants resulting in high biodegradation. Similarly, Hadad et al. (2005) reported that *Brevibacillus* is a thermophilic bacterium, capable of producing biofilms that further increase the biodegradation of contaminants through hydrophobic-hydrophobic interactions. The high abundance of *Brevibacillus* can clearly explain the high biodegradation rate of benzene and BaP at 45 °C.

On the other hand, *Candida* was the most dominant fungal genus in all the treatments, and elevation of temperature from 15 to 40 °C highly enhanced the abundance of *Candida*, whereas, the elevation of temperature to 70 °C increased the abundance of *unclassified_k_Fungi*. Previous studies reported that *Candida* has the potential to produce biosurfactants under normal pH and temperature conditions (Alao and Adebayo, 2022; Fan et al., 2014; Zhang et al., 2014); however, the *Candida* biodegradation potential under different thermal conditions has not been explored yet. The abundance of *Candida* increased with the prolongation of time (ST → LT) in 30 °C treatment, whereas, a reduction in *Candida* abundance was noted in 45 °C treatment. The decrease in 45 °C treatments might be due to the toxic influence of hazardous benzene and BaP contamination, or the instability of *Candida* with the prolongation of time at elevated temperature conditions. In addition, the genus *unclassified_k_Fungi* needs to be further explored for its high biodegradation potential at 70 °C. The results revealed that prolongation of time under the elevated thermal treatments increased the abundance of specific microbial genera, which have the capability to biodegrade the co-contamination of benzene and benzo[*a*]pyrene.

3.2.2. Correlation analysis under short- and long-term temperature treatments

Spearman pairwise correlation was conducted to further explore the correlations among the biodegradation of benzene and BaP, bacteria, fungi, and PAH-RHD gene in GP and GN bacteria due to short- and long-term thermal exposure (Fig. 3A and B). The results revealed that *Candida*, a fungal genus was significantly positively correlated with benzene and BaP biodegradation under short-term thermal treatments, whereas in the long-term treatment, the significant positive correlation of *Candida* was noted with *Fusarium* and *Ammoniphilus*, indicating that during long-term exposure *Candida* might play a synergistic correlation with other fungi and bacteria. For example, Shirtliff et al. (2009) reported that bacteria and fungi are found together in a myriad of environments, particularly in a biofilm, where adherent species interact through diverse signaling mechanisms. In addition, bacterial genera *Brevibacillus* and *Microvirga* were positively correlated with benzene and BaP biodegradation under short-term thermal exposure, while long-term thermal exposure highly reduced the abundance of *Microvirga* and had a negative correlation with benzene and BaP biodegradation. These results indicate that *Brevibacillus* and *Microvirga* together are highly involved in the early stage of benzene biodegradation, and due to the time prolongation and toxic influence of BaP, the abundance of *Microvirga* reduced.

Biodegradation of benzene and BaP was high under the low-temperature elevated condition might be attributed to the abundance of potential microbes capable of degrading PAHs and BTEX. For example, the bacterial genus *Brevibacillus,* belongs to the phylum Firmicute capable of secreting biosurfactants under PAHs contaminated soils (Reddy et al., 2010) while *Microvirga* is an aerobic genus belonging to phylum Proteobacteria capable of degrading aromatic hydrocarbons (Lv et al., 2022). The results concluded that *Brevibacillus* was significantly positively correlated with the genus of *Microvirga*, indicating their (bacterial-bacterial) synergistic role in contaminants biodegradation under different temperature conditions. In addition, the PAH-RHD gene in GN bacteria was positively correlated with the abundance of *Azohydromonas*. Previous studies reported that *Azohydromonas* is a gram-negative bacterium capable of degrading aromatic hydrocarbons (Llado et al., 2009; Zhu et al., 2020) as reported in this study. This observation implies that the complex synergistic biochemical association among these genera might contribute to the high biodegradation potential under different thermal treatments. In addition, it was observed that long-term thermal exposure further strengthens the fungal-bacterial and bacterial-bacterial synergistic correlation, as evidenced by (Shirtliff et al., 2009; Gu et al., 2022). It is generally believed

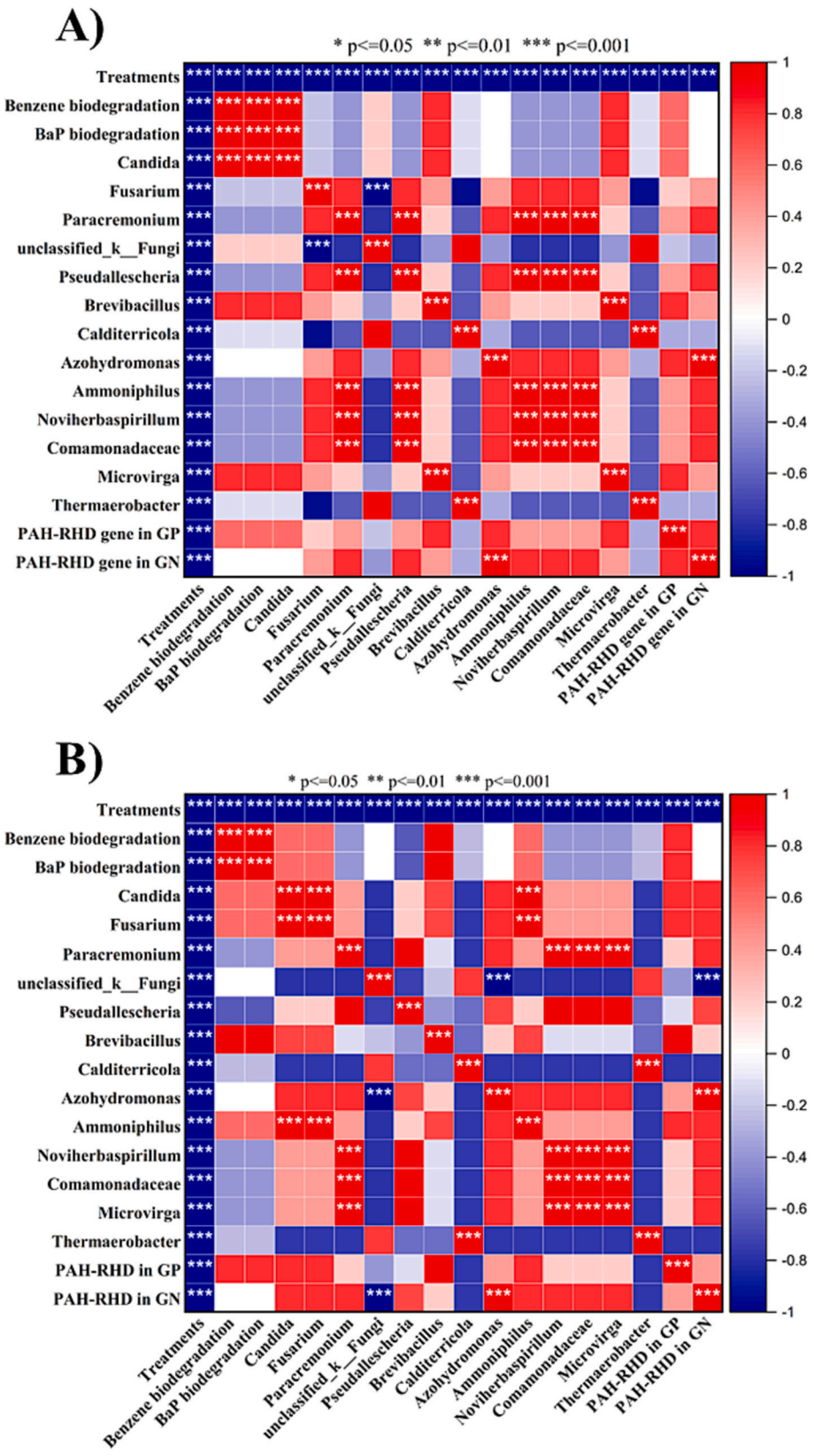

Fig. 3. Spearman pairwise correlation of benzene and BaP biodegradation, abundance of dominant bacteria and fungi genera, and PAH-RHD gene in gram-positive and gram-negative bacteria under A) short-term, and B) long-term thermal treatments.

that the conversion of PAHs by fungal enzymes forms more hydrophilic and polar intermediates, which have higher bioavailability than the parent compounds and may degrade through different bacterial pathways (Gu et al., 2022). This can conclude the possible synergism between bacteria and fungi during the long-term low-temperature elevation condition.

Furthermore, fungal-fungal, and bacterial-bacterial synergistic correlations among bacterial and fungal genera were also observed under the short-term thermal exposure condition. For example, the fungal genera of *Paracremonium* and *Pseudallescheria* were significantly positively correlated with the bacterial genera of *Ammoniphilus*, *Noviherbaspirillum*, and *Comamonadaceae*, indicating that these fungi and bacteria acted synergistically to deal with contaminants degradation under different temperature conditions. Certain bacteria have the capability to secrete extracellular polymeric substances and biosurfactants that can increase the bioavailability of contaminants, allowing the other potential degraders to degrade PAHs under stressed conditions (Ali et al., 2022b).

3.3. Effect of long-term thermal treatments on soil properties

According to our previous studies (Da et al., 2019; Ali et al., 2022b; Wang et al., 2022), thermal elevation of temperature can cause fluctuations in soil properties. For example, Hart et al. (2022) reported that the elevation of temperature (at ~40 °C) caused the reduction in soil moisture, dissolution of total organic carbon (TOC), and fluctuation in soil pH and other elements. Therefore, this study explored the dynamic changes in soil properties under low-temperature thermal treatments (15–70 °C), and their subsequent influences on bacterial and fungal composition. The results revealed that the elevation of temperature has a significant influence on soil properties such as soil pH, moisture, total organic carbon (TOC), total Fe, Fe(II), and Fe(III) concentration (Ali et al., 2023). The principal component analysis (PCA) biplot was performed to measure the dynamic changes in soil properties under different thermal treatments after 60 days (long-term) of the incubation time (Fig. 4). The results showed that thermal treatments caused significant changes in soil properties, and 45 °C share the same distances with 30 and 70 °C. Elevation of temperature from 30 to 70 °C caused changes in soil total phosphorus (TP), total potassium (TK), TOC, pH, total iron (Fe), Fe(II), and Fe(III). On the other hand, soil electrical conductivity (EC) and moisture concentration were inversely proportional to the elevated temperature condition. Increasing temperature enhances soil basification, which causes the reduction of H^+ ions during microbial processes (Fu et al., 2018). Similarly, TOC, total Fe, TP, and TK were also in direct correlation with the elevation of temperature, possibly owing to the dissolution of organic carbon, phosphorous, and potassium from mineral clay, and the conversion of strictly bound Fe to loosely bound Fe (Hart et al., 2022; Martinez et al., 2003).

3.4. Soil properties effects on microbial compositions under TEB

The redundancy analysis results revealed that RDA1 and RDA2 contributed about 91.1 % and 87.67 % for bacterial and fungal phyla, respectively (Fig. 5A and C). Thermal influence on bacterial phyla (Fig. 5A) was distinctly distributed compared to fungal phyla (Fig. 5C), and the phyla Proteobacteria, Chloroflexi, and Acidobacteria were abundant at 30 °C, and positively correlated with the soil moisture content. Similarly, Firmicutes were more abundant at 45 °C and were

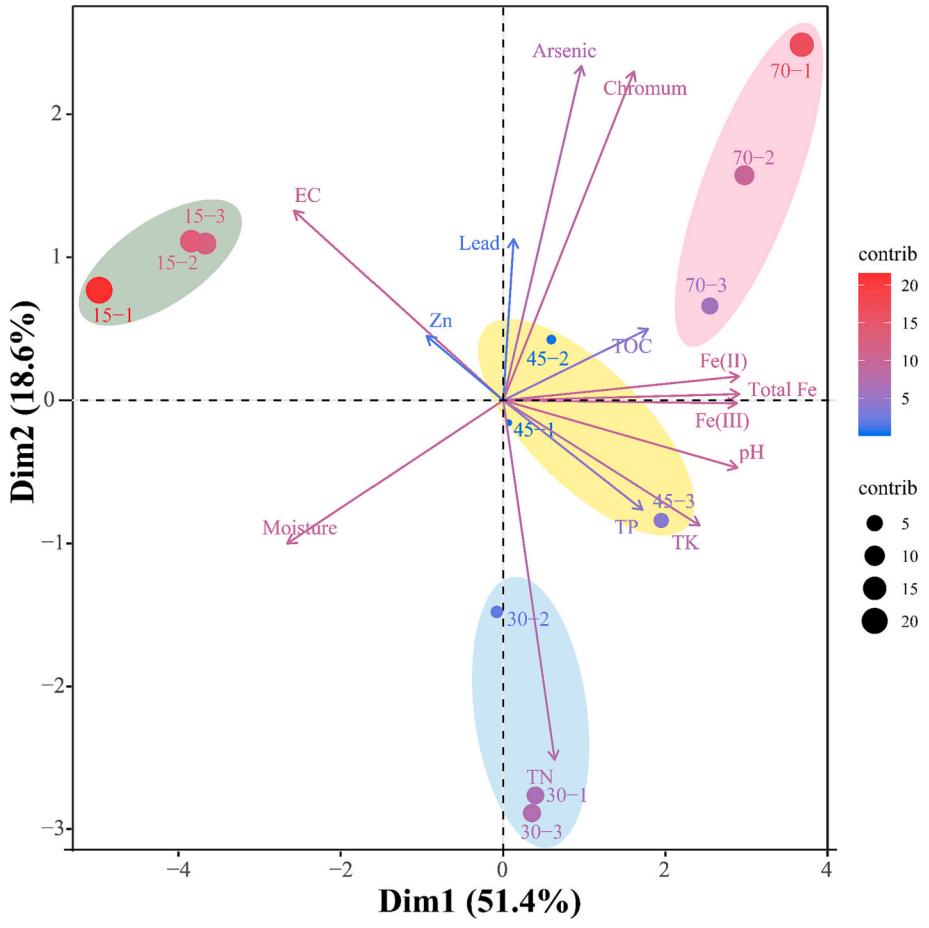

Fig. 4. Principal component analysis (PCA)-biplot for the fluctuation in soil properties under the thermally enhanced biodegradation of benzene and BaP co-contamination.

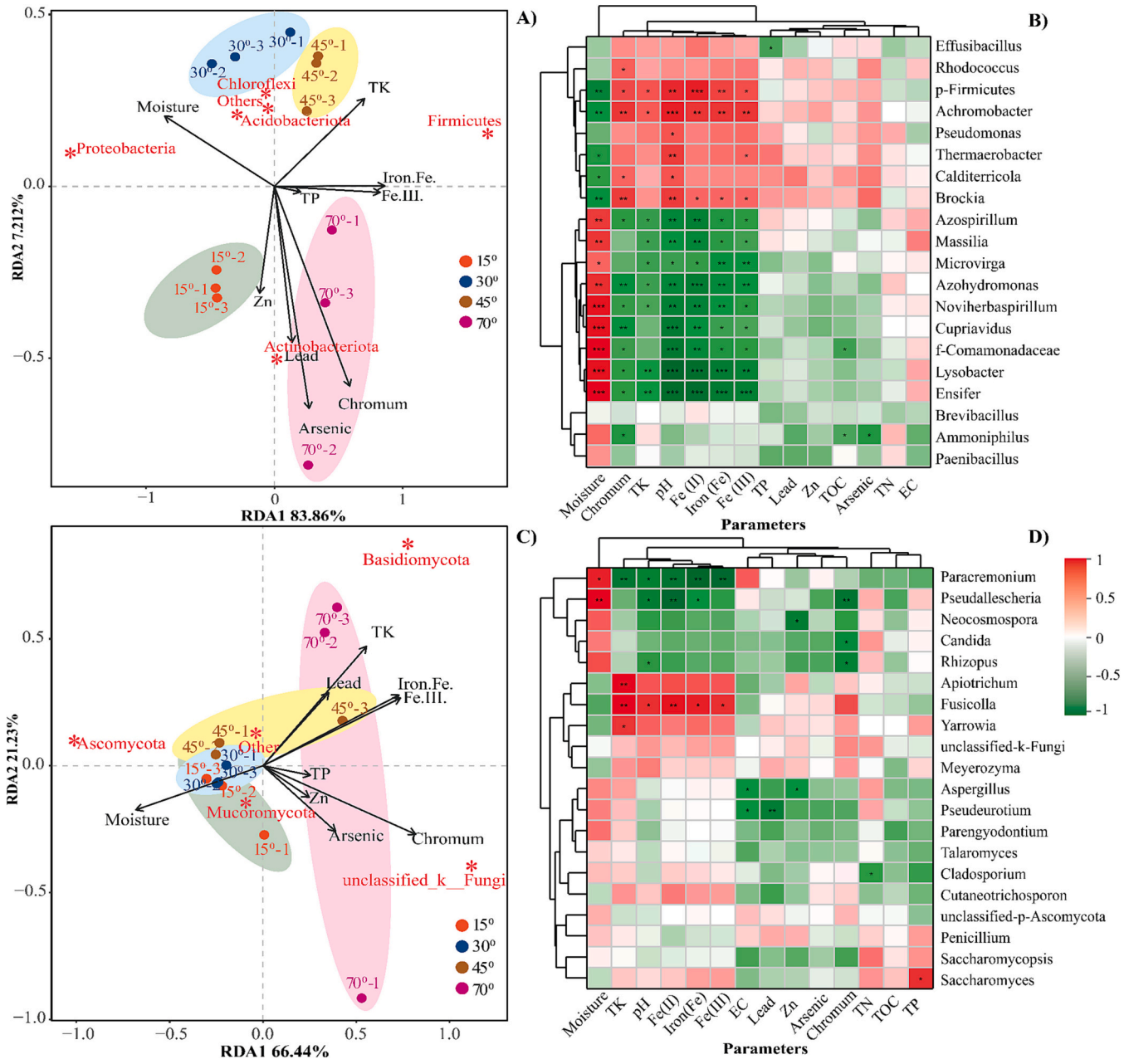

Fig. 5. Redundancy analysis (RDA) for the fluctuation of the dominant A) bacterial and c) fungal phyla and soil properties under the thermally enhanced biodegradation of benzene and BaP co-contamination.

positively correlated with soil pH and potassium concentration, whereas Actinobacteriota was abundant at 70 °C, and was positively correlated with soil phosphorus, lead (Pb), chromium (Cr), and arsenic (As) concentration in soil. The main factor involved in the fluctuation of Proteobacteria and Firmicutes is the dynamic changes in soil properties under different treatments (Zhang et al., 2023; Gupta et al., 2018). Moreover, it has been reported that increasing pH and total Fe concentration were the main factors associated with the high abundance of Firmicutes. In our study, these two factors were also correlated with the abundance of Firmicutes, indicating that temperature plays a crucial role cause dynamic changes in these parameters. On the other hand, fungal phyla were clustered together, especially at 15–45 °C temperature and 70 °C caused a slight distinctness. Ascomycota was abundant in 45 °C treatment and was negatively correlated with soil As and Cr concentration, indicating that metal concentration can influence the activity of Ascomycota. These results are in line with (Passarini et al., 2022) who reported that the presence of Cr caused a reduction of 12.5 % in the Ascomycota phylum. Mucoromycete was abundant in 15 °C treatment and was positively associated with soil moisture content. Unclassified_k_Fungi was dominated at 70 °C treatment and was positively associated with soil As, Cr, Pb, and Fe concentration, indicating that this phylum might contain the potential genes to tolerate high-temperature conditions and metals concentration; however, the phyla need further exploration to be recognized on genus and specie level.

The correlations between bacterial and fungal compositions at the genus level and the changed soil properties under TEB of benzene and BaP co-contaminated soil are presented in Fig. 5B, D. The analysis showed that bacterial genera abundance (Fig. 5B) was more significantly affected by the changed soil properties compared to fungal genera (Fig. 5D). Soil parameters, especially soil pH, TK, moisture, total Cr, total Fe, Fe(II), and Fe(III), were significantly positively or negatively correlated with the composition of bacterial and fungi genera. The

elevation of temperature (15–70 °C) increased soil pH, total Fe, Fe(II), Fe (III), and TK, which were significantly positively associated with the *Rhodococcus*, *Effusibacillus*, *p-Firmicutes*, *Achromobacter*, *Pseudomonas*, *Thermoaerobacter*, *Calditricola* and *Brockia*, indicating that elevation of temperature influences the soil properties that resulted in the dynamic changes in compositions and abundance of these genera. On the other hand, *Lysobacter*, *Ensifer*, *f-Comamonadaceae*, *Cupriavidus*, *Noviherbaspirillum*, *Azohydromonas*, *Microvirga*, *Massilia*, and *Azospirillum* were significantly negatively affected by soil pH, TK, Cr, total Fe, Fe(II) and Fe(III) under TEB of benzene and BaP contaminated soil. Similar phenomena were observed for the fungal analysis. The fungal genera, especially *Yarrowia*, *Fusicolla*, *Apiotrichum*, *Saccharomyces*, *Paracremonium*, and *Pseudallescheria* were significantly positively affected by soil TK, pH, total Fe, Fe(II), Fe(III), moisture and TP. On the other hand, *Cladosporium*, *Pseudeurotium*, *Aspergillus*, *Rhizopus*, *Candida*, *Neocosmospora*, *Pseudallescheria*, and *Paracremonium* were significantly negatively influenced by the soil EC, Pb, Zn, Cr, TK, pH, total Fe, Fe(II) and Fe(III), changed during the TEB biodegradation of benzene and BaP co-contaminated soil. These results revealed that the biodegradation of benzene and BaP co-contamination were efficiently enhanced under the thermally-enhanced biodegradation approach and the elevation of temperature can affect the microbial compositions directly via microbial specificity or indirectly by the influences on the soil properties.

4. Conclusions

An experiment was conducted to explore the impacts of short- and long-term thermal exposure on benzene and BaP biodegradation in mixed contaminated soil, and their subsequent influence on microbial (bacteria and fungi) compositions. The results revealed that low-temperature elevation (15 °C → 45 °C) highly increased the biodegradation of benzene and BaP. Subsequently, microbial composition analysis revealed that increasing temperature enhances the abundance of Firmicutes, whereas the Proteobacteria was abundant at low temperature, and elevation of temperature highly reduced the abundance of Proteobacteria. Moreover, it was found that the elevation of temperature increases the bacterial diversity, and further prolongation of time from short- to long-term increased the bacterial genera specificity. On the other hand, fungal specificity was reduced with the prolongation of time during elevated temperature conditions. The correlation analysis revealed that elevated temperature condition stimulates the abundance of potential microbes capable of degrading PAHs and BTEX, and long-term thermal exposure can increase the synergistic associations between bacteria-fungi, fungi-fungi, and bacteria-bacteria that can further promote the biodegradation efficiency. Moreover, it was found that the elevation of temperature influences the soil properties, which were positively and negatively associated with the abundance of bacteria, fungi, and PAH-RHD genes. These results provide an insight that the elevation of temperature can affect the microbial composition directly via microbial specificity or indirectly by influencing the soil properties under mixed contaminated conditions.

CRediT authorship contribution statement

Mukhtiar Ali: Conceptualization, Methodology, Writing – original draft. **Xin Song:** Conceptualization, Writing – review & editing, Supervision, Project administration. **Qing Wang:** Methodology, Investigation, Data curation. **Zhuanxia Zhang:** Conceptualization, Investigation, Visualization. **Meng Zhang:** Investigation, Visualization. **Min Ma:** Investigation, Data curation. **Jilu Che:** Methodology. **Rui Li:** Investigation, Methodology. **Xing Chen:** Investigation, Data curation. **Zhiwen Tang:** Investigation, Methodology. **Biao Tang:** Methodology. **Xiangfeng Huang:** Investigation.

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.

Data availability

Data will be made available on request.

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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.2023.168862.

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