

Co-occurrence of PFASs, TPHs, and BTEX in subsurface soils: Impacts on native microbial communities and implications for bioremediation

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Abstract

This study investigates the co-occurrence of per- and polyfluoroalkyl substances (PFASs), petroleum hydrocarbons (TPHs) and benzene, toluene, ethylbenzene, and xylene (BTEX) and their effects on the indigenous microbial communities in soils at a contaminated site with a history of petroleum refinery operations. PFASs concentrations were in the range of 5.65 to 6.73 ng/g, and fluorooctane sulfonate (PFOS) and perfluorobutane sulfonic acid (PFBS) were the dominating compounds. No significant difference was observed in the PFASs concentration profiles between the site and background locations, indicating that PFASs detected in the soil samples were mainly contributed from non-point sources, due to the long-distance transport of PFASs in the region. The concentrations of TPHs and BTEX ranged from 227 to 72,360 mg/kg and 0.06 to 2109.77 mg/kg, respectively, mainly contributed by the historical refinery activities. The presence of PFASs, TPHs, and BTEX significantly impacted soil microbial community diversity and abundance, altering microbial compositions and enriching bacteria with higher resistance or metabolic capabilities against contamination. Strong correlations were observed between TPHs and its degraders such as *Pseudomonas*, *Azoarcus*, and *Polaromonas*. Significant positive relationship between PFASs and *Trichlorobacter* implied the potential defluorination capabilities of *Trichlorobacter*, warranting further investigation. Moreover, the higher energy metabolism including carbon, nitrogen and sulfur metabolisms and higher abundance of metabolic enzymes for alkane, cyclohexane and toluene in the refinery site revealed the potential occurrence of natural biodegradation of contaminants with indigenous microbial community. These findings highlight the complexity of sites contaminated with a mixture of traditional and emerging contaminants, providing valuable insights into the potential for biodegradation of mixed contaminants and underscoring the need for integrated approaches in environmental remediation strategies. This study contributes to understanding the ecological impacts of co-occurring contaminants and emphasizes the importance of considering multiple contaminant types in environmental risk assessments and remediation efforts.

Keywords: PFASs; Co-contamination; Microbial community; Ecological responses; Metabolism function

1. Introduction

Per- and polyfluoroalkyl substances (PFASs) are widely used in industrial and commercial products, owing to their surface activity, water and oil repellent properties, chemical and thermal stability. PFASs have been detected in various environmental media, such as surface and subsurface water, industrial soils, farmland and landfill soil and plants (Wei et al., 2018) (Chen et al., 2016; Ding et al., 2022; Wei et al., 2018; Xu et al., 2021b). Due to the broad detections of PFASs in multiple environmental media, the concern on co-occurrence of PFASs with other contaminants of concern has been rising on the horizon in recent years. Total petroleum hydrocarbons (TPHs), including benzene, toluene, ethylbenzene, and xylene (BTEX), are often accidentally released into the environment due to spills and leaks in industrial activities such as oil refining, drilling and transportation, as well as improper disposal practices. TPHs and BTEX in soil and groundwater posed severe threats to human health and ecological system (Eze et al., 2021). PFASs have been reported at alarming levels with traditional contaminants including TPHs, polycyclic aromatic hydrocarbons, chlorinated hydrocarbons and BTEX in soil and groundwater (Tang et al., 2022; Zhang et al., 2023).

PFASs can be used as surfactants for oil drilling and fire extinguishing agents, which may cause the presence of PFASs at refinery facilities (Li et al., 2019; Yao et al., 2022). Furthermore, it was reported that elevated PFASs were detected in an oilfield, in which the major traditional contaminants were TPHs and BTEX, suggesting the possibility of co-contamination with PFASs, TPHs, and BTEX (Li et al., 2019; Meng et al., 2021). There have been many studies of the effects on native microbial consortium resulted from long-term exposure to various contaminants (Bao et al., 2018; Sun et al., 2016; Tang et al., 2022; Truskewycz et al., 2019). Under natural conditions, the indigenous microbial community has the ability for degradation of contaminants and tolerance under long-term contamination. The degrading genera for hydrocarbons widely exists in soil and groundwater contaminated with TPHs and BTEX, including *Bacillus*, *Pseudomonas*, *OPB41* and *Desulfoprunum* (Khomyakova et al., 2022; Taylor et al., 2021; Wu and Wang, 2022).

Moreover, it has been reported that the microbial consortia could survive in PFASs pollution, such as *Acinetobacter*, *Pseudomonas* and *norank_p__GAL15* (Tang et al., 2022; Zhang et al., 2023). However, the microbial community composition profile and the responses to the co-occurrence of PFASs, TPHs and BTEX are not clear. In addition, the influence of contaminants on shaping the community composition and the microbial metabolic activity for contaminants under natural condition also need to be revealed, which is looking forward to providing helpful insight for bioremediation with indigenous microorganisms. Furthermore, due to the broad presence of PFASs in all environmental media, the ecosystem risk of PFASs on the regional level has been a major concern. It has been reported that the regional exposure of PFASs can influence the microbial energy metabolism, as reported by Tang et al. (2022), and the carbon metabolism and xenobiotic metabolism via cytochrome P450 were induced under contamination of PFASs.

In this study, the co-occurrence profiles of PFASs, TPHs and BTEX at a refinery site were investigated and the effects of mixed contaminants on the abundance and composition of indigenous microbial communities were explored. Moreover, the energy metabolism and contaminants degradation analysis were conducted to study the influence of co-contaminants on microbial community and shed lights on the implications for bioremediation of PFASs, TPHs and BTEX.

2. Materials and methods

2.1 Study area description and soil sampling

The study area was located in the petroleum production zone at a petroleum refinery site, located in Gansu province, China. The refinery, having a history of operation for over 30 years, is no longer used for production. Based on our previous investigation of the horizontal and vertical distributions of TPH and BTEX at the refinery site, two representative sampling locations in the area were chosen: one in the area with low levels of TPHs and BTEX concentrations (S1), and another in the source area with relatively higher concentrations of

TPHs and BTEX (S2) (**Figure 1a**). In addition, the control location (designated as BK), where no contamination of TPH and BTEX was expected, was chosen to represent the background conditions.

The subsurface stratigraphic profile was shown in **Figure 1b**. The geologic formations consist of refill, silt, silty sand and coarse sand, with a groundwater level laying at approximately 7.0 m below ground surface (bgs). Groundwater flows in the direction of southeast, suggesting that S1 is located downgradient of S2. Soil samples were obtained by a drilling rig (SM200A, Shangming, China) at three sampling locations at 5 m bgs (labeled with S1-5, S2-5 and BK-5) and 10 m bgs (S1-10, S5-10 and BK-10), representing soils from unsaturated and saturated aquifers, respectively. Since the surface samples do not represent the natural soil conditions with hard concrete and refill laid below the surface in the refinery site, no samples were collected. All the soil samples analyzed for PFASs, TPHs and BTEX were stored in polypropylene bags and samples for physicochemical properties analysis were collected in 250 mL brown bottles. Soil samples for microbial community analysis were collected in sterile bags. All the samples were kept in dry ice for transportation to the laboratory for further analysis.

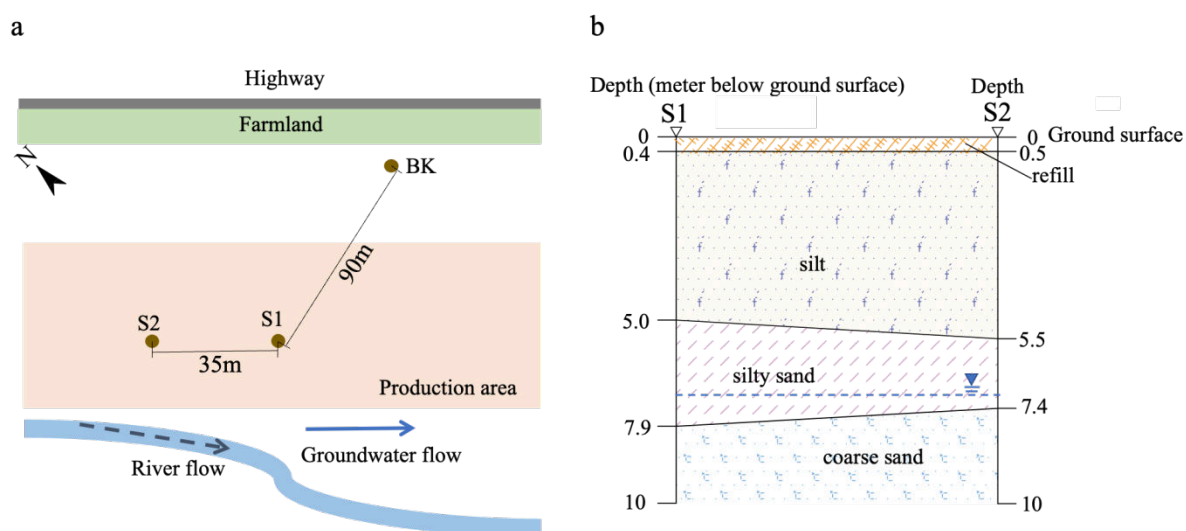


Figure 1. Site layout (a) and stratigraphic profile (b) of the study area.

2.2 Determination of soil physicochemical properties

Moisture content (MC) was determined by gravimetric method before and after drying soil

samples in an oven at 105 °C for 24 h. The pH of the soil was measured by creating a mixture of soil to distilled water (1: 5), followed by analysis with a pH meter, as outlined by Gao et al., (2022). Soil total nitrogen (TN) was analyzed with the Kjeldahl method (Standard LY/T 1228-2015) and total organic carbon (TOC) was measured with the combustion oxidation nondispersive infrared absorption method using a TOC-V CPH 200V instrument (Shimadzu, Japan). Total phosphorus (TP), ammonium ($\text{NH}_4^+\text{-N}$), nitrite ($\text{NO}_2^-\text{-N}$), nitrate ($\text{NO}_3^-\text{-N}$) and cation exchange capacity (CEC) were determined with an ultraviolet-visible spectrophotometer (UV-2600, Shimadzu, Japan) as described in previous study (Liu et al., 2023). Soil organic matter (SOM) was determined according to the Standard procedure NY/T 1121.6-2006.

2.3 Analysis of PFASs, TPHs and BTEX in soil samples

In this study, 13 target PFASs, including 9 perfluorocarboxylic acids (PFCAs) (perfluorobutanoic acid (PFBA), perfluoropentanoic acid (PFPeA), perfluorohexanoic acid (PFHxA), perfluoroheptanoic acid (PFHpA), perfluorooctanoic acid (PFOA), perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), perfluoroundecanoic acid (PFUdA) and perfluorododecanoic acid (PFDoA)), 3 perfluorosulfonic acids (PFSAs) (perfluorobutane sulfonate (PFBS), perfluorohexane sulfonate (PFHxS), and perfluorooctane sulfonate (PFOS) and 1 novel alternatives of PFOS (6:2 chlorinated polyfluorinated ether sulfonic acid (F-53B) were analyzed in soil samples. Soil samples for PFASs analysis were air-dried, grounded, and sieved through a 2 mm mesh, as previously described by Xu et al. (2021a). Briefly, 2 g of dry soil sample was spiked with 5 ng of internal standard, followed by ultrasonic extraction with 100 mM NaOH acetonitrile-water solution serving as the extraction solvent. The extract was cleaned using ENVI-Carb cartridges (Supelco: 250 mg, 3 mL, USA) packed with graphite and diluted with Milli-Q water to reduce organic solvent. PFASs were subsequently extracted by loading them through Oasis WAX cartridges (Waters: 6cc/150 mg, 30 μm , USA). The detailed information of standards and reagents for PFASs analysis was provided in **Text S1**.

The analysis of the 13 targeted PFASs was performed with an ultra-high-performance liquid

chromatography-tandem mass spectrometer (HPLC-MS AB Sciex 5500 TripleQuad, USA), and the PFASs separation was achieved using a C18 column (Waters: 3.0 × 100 mm, 1.7 μm, USA). The instrumental analysis parameters for PFASs were described in **Text S2 and Table S1**. TPHs were analyzed according to different carbon chain length of the compounds with C6-C10 and C10-C40. The analysis of C6-C9 fraction of TPHs and BTEX were conducted with a gas chromatography equipped with a flame ionization detector (GC-FID) with headspace injection (7697A-7890B GC, Agilent, USA). The C10-C40 fraction of TPHs was analyzed using a gas chromatograph with a flame ionization detector (GC-FID) (7890B GC, Agilent, USA) according to the methods described by Patowary et al. (2023).

2.4 Quality assurance and quality control

To ensure the accuracy and reliability of soil hydrocarbon analysis for the C6-C9 and C10-C40 fractions, including BTEX, our focused QA/QC strategy encompasses several key aspects. Sample collection protocols are meticulously designed to prevent contamination and preserve sample integrity. The GC-FID analytical methods are rigorously validated for precision and accuracy, with calibration procedures employing certified standards. Quality control measures include the utilization of laboratory blanks to monitor for any potential contamination, matrix spikes to verify recovery rates, and duplicate samples to confirm reproducibility. Regular instrument maintenance and comprehensive documentation of all processes, from calibration to sample analysis, underpin the integrity of our data. This streamlined approach ensures that our soil hydrocarbon analysis yields trustworthy and consistent results.

For the analysis of 13 target PFASs in soil samples, quality control was stringently applied throughout the sampling and analytical processes. This involved the inclusion of blanks for field, transport, procedure, and solvent with every batch of samples to ensure the reliability of the results. Quantification was achieved by spiking samples with 5 ng of isotope-labeled internal standards (¹³C₄-PFBA, ¹³C₄-PFHxA, ¹³C₄-PFOA, ¹³C₄-PFNA, ¹³C₄-PFDA, ¹³C₄-PFUdA, ¹³C₂-PFDoA, ¹⁸O₂-PFHxS and ¹³C₄-PFOS), and calibration curves were constructed over a range of concentrations, demonstrating excellent linearity ($R^2 > 0.99$). The level of

detection (LOD) and level of quantification (LOQ) were carefully determined based on the signal-to-noise ratios of 3 and 10, respectively. To validate the analytical results, each batch included procedural blank samples, as well as quality control samples, with matrix spike recoveries indicating recoveries for the PFASs ranging from 72.9% to 100.1%. This meticulous QA/QC regimen, detailed further in **Table S2**, assures the reliability and validity of our analytical outcomes.

2.5 DNA extraction and Illumina sequencing

The DNA from each soil sample was meticulously extracted employing FastDNA Spin Kits (MP Bio, USA), following the guidelines provided by the manufacturer. To assess the quantity of the extracted DNA, a NanoDrop ND-2000 spectrophotometer (NanoDrop Technologies, USA) was utilized, while the integrity of the DNA was confirmed through agarose gel electrophoresis. The evaluation of DNA yield was based on the optical density measured at 260 nm wavelength, which is a reliable indicator of nucleic acid concentration. The bacterial 16S rRNA gene was targeted for amplification using the well-established bacterial primer set, which included primers 338F and 806R (Munyaka et al., 2015). The obtained data were deposited in the NCBI Sequence Read Archive with the project number (PRJNA1137589). Sequencing was performed on the Illumina MiSeq PE300 platform (Majorbio Biotechnology Co., Ltd., China). In the post-sequencing phase, a focus was placed on the delineation of amplicon sequence variants (ASVs). The sequences obtained were first subjected to stringent quality filtering to discard reads not meeting quality thresholds. Subsequent to quality control, ASVs were determined using a pipeline that included denoising algorithms such as DADA2 or Deblur, which are designed to recognize and correct for sequencing errors, thus enhancing the accuracy of variant identification. The resulting ASVs were then used for comprehensive bioinformatics analyses, including the calculation of diversity metrics and the elucidation of community composition.

2.6 Statistical analyses

The statistical analyses in this study were conducted using R statistical software (version 4.2.1). Differences in soil physicochemical properties were analyzed using the agricolae package (version 1.3-5). Fisher's Least Significant Difference (LSD) method was applied to determine statistically significant differences among samples. Alpha diversity indices, including community richness (Chao index) and diversity (Shannon index), were calculated with Mothur (version v.1.30.1) based on ASVs level and differences in microbial community diversity indices were analyzed using the Wilcoxon rank test. The beta diversity of microbial community composition in soil samples was assessed using Principal Coordinate Analysis (PCoA) based on the weighted UniFrac distances matrix. The Mantel tests were conducted using the R software (version 3.3.1). The analyses of energy metabolism and xenobiotics biodegradation and metabolism as well as the abundance of functional enzymes was analyzed in PICRUST2 based on the Kyoto Encyclopedia of Genes and Genomes (KEGG) information.

3. Results and Discussion

3.1 Concentration of PFASs, TPHs and BTEX in soil samples

Figure 2 showed the concentration and abundance profiles of PFASs, TPHs and BTEX in soil samples. The total concentrations of PFASs (Σ_{13} PFASs) were similar, ranging from 5.65 to 6.73 ng/g across all six samples collected from three sampling locations (**Table S3**). No significant differences were observed among samples at BK location (5.79-6.24 ng/g), S1 (5.65-5.96 ng/g) and S2 locations (6.29-6.73 ng/g), indicating the ubiquitous occurrence of PFASs as regional contamination, which could be caused by dry and wet depositions (Zhang et al., 2023). It is worth noting that the PFASs concentrations observed in this study area were lower compared to those observed in landfills sediment (7.91-164 ng/g), landfills soil (5.31-108.00 ng/g), aquifer solids in a fluorochemical plant (7.65-20.20 ng/g) and soils in a farmland (maximum 641 ng/g) in previous studies (Ding et al., 2022; Liu et al., 2017; Xu et al., 2021a; Xu et al., 2021b). Additionally, the similar PFASs composition profiles observed in both unsaturated (5

m bgs) and saturated aquifers (10 m bgs) soils suggested that PFASs can migrate from the shallow to deep soils in the subsurface, which is consistent with our previous investigations and indicates that PFASs pose a great concern in the subsurface, in particular to the groundwater quality (Tang et al., 2022; Zhang et al., 2023).

There are limited reports on the vertical migration of PFASs in the subsurface. Jia et al. (2023) reported that the highest PFAS concentrations were found in the topsoil at two plants, and PFAS generally showed decreasing trends from shallow layers to the bottom of the soil core and demonstrated some downward migrations at different soil depths with time. Tang et al. (2022) demonstrated that PFASs can migrate downward showing similar concentration profiles at different depths. However, the highest concentrations were observed at 60 meters below ground surface, primarily due to the influence of dense non-aqueous liquid (DNAPL) co-existing at the sampling location. Therefore, it can be stated that the spatial distributions (both horizontal and vertical) of PFASs, like many other persistent organic compounds, are generally to be distinctly site-specific and influenced by historic production activities of facilities, accidental discharge and/or leakage, site hydrogeology, variations in the physicochemical properties of compounds. We speculated that the similar concentrations detected at different depths can be attributed to the regional contribution, which is more uniform compared to the accidental leakage in most facilities, yet more investigations on the PFASs vertical migration trends are needed in the near future.

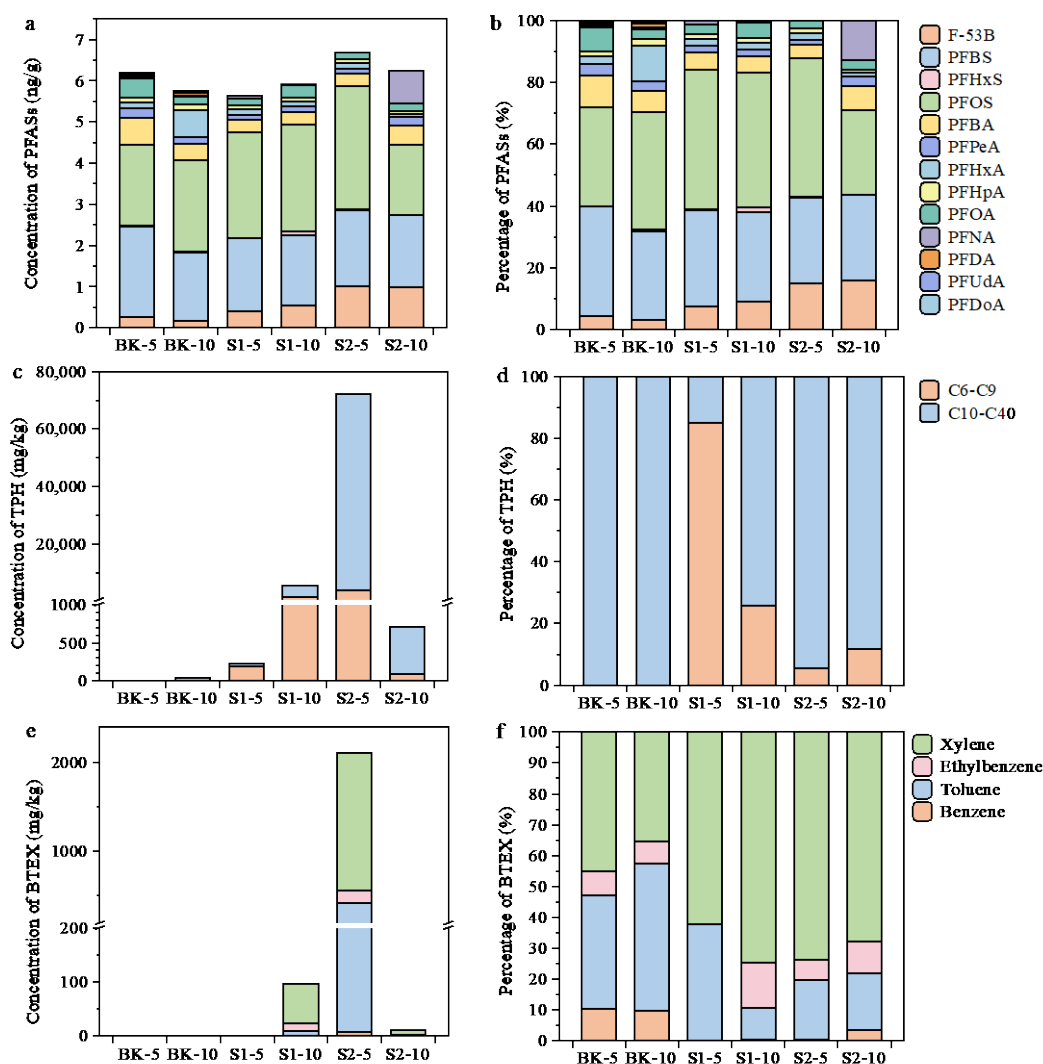


Figure 2. Concentration and abundance profiles of PFASs (a, b), TPHs (c, d) and BTEX (e, f) in soil samples.

Notably, PFOS and PFBS were the two significant dominant compounds observed in this study, accounting for 27.18-45.31% and 27.63-35.41% of the total PFASs detected, respectively, followed by F-53B (an alternative of PFOS) contributing 3.10-15.74%. This was consistent with our previous studies that the dominance of PFOS, PFBS and F-53B was observed in a steel-manufacturing plant (Zhang et al., 2023). PFBS, an alternative of PFOS, is anticipated to progressively rise in the coming years with the increasing usage, which may cause PFBS dominating in the environment (Charbonnet et al., 2022). The relative abundances of F-53B and PFBS indicated the wide usage of substitutes, due to the banned use of PFOS (Ding et al.,

2020; Xin et al., 2018). Furthermore, it was noted that PFSAAs (70.47-87.52%, primarily PFOS and PFBS) contributed more significantly than PFCAs (12.48-29.53%, primarily PFOA and PFBA) in the study area, which in contrast to our previous investigations where PFCAs were the dominant substances in multiple environmental media with point sources such as industrial parks and landfill in China (Ding et al., 2022; Tang et al., 2022; Xu et al., 2021b).

TPHs were a group of common contaminants of concern identified in refinery sites, as reported by Vazquez et al. (2017). TPHs concentrations ranged from 227 mg/kg (in S1-5) to 72360 mg/kg (in S2-5) with long-chain TPHs (C10-C40) dominating, except for in S1-5 which had higher abundance of short-chain TPHs (C6-C9). TPHs concentrations at BK location ranged from 2.39 mg/kg to 33.8 mg/kg, much less than those at S1 and S2 locations, indicating that the presence of TPHs in the study area was mainly contributed with the historical industrial production activities at the site. The presence of TPHs at BK, albeit lower than the cleanup goal of 826 mg/kg, indicated that the historical operational activities exerted an impact on the environment nearby outside the site. BTEX, a group of aromatic compounds within the range of C6-C9, were also widely present in the soil samples except at BK location. Consistent with the profiles of TPHs, the highest concentration of BTEX was 2109.77 mg/kg in S2-5, followed by 97.47 mg/kg in S1-10, whereas the concentration of BTEX at BK was below 1 mg/kg. Among BTEX, xylene was the main dominating compound in all soil samples, followed by toluene and benzene was the least abundant. The concentrations of xylene, toluene and benzene were 0.04-1553 mg/kg, 0.02-404 mg/kg and 0-8.77 mg/kg, respectively. The abundance profile of BTEX could be also due to the historical production as the refinery products. S2 was located in a source area and S1 was a downgradient location, the much higher concentrations of TPH and BTEX at S2, along with the significant differences were expected, even though they were 35 m apart. In addition, the vertical distributions of TPH and BTEX at S1 showed the concentrations at S1-10 can be influenced by the contaminant migration from upgradient, as well as the leaching contribution from unsaturated aquifer, while those at S1-5 tend to be affected by leaching from shallower soils. Previous studies have demonstrated similar small-scale spatial variability in hydrocarbon-contaminated soils (Yao et al., 2022; Zhang et al., 2023).

These results reflect the complexity of contaminant behaviors in soil environments, which can be affected by subsurface heterogeneities, contaminant properties, historical site activities, etc., which pose significant challenges in delineate and remediate the plume in the subsurface.

It is noteworthy that TPHs and BTEX, typical contaminants from refineries, were primarily found within the production area, indicating that their distribution was mainly due to production activities. However, PFASs were observed at similar concentrations and with similar occurrence profiles at both the BK location and the production area soils, suggesting ubiquitous distribution. This indicated that PFASs at the site was a regional issue, not related to the refinery activities. PFASs are capable of migrating vertically in soil and groundwater, depending on the site geology. Our previous study reported that the PFASs detections at 60 m bgs in a tight formation consisting of silt, silty sand (Ding et al., 2022). In addition, it was reported that PFASs vertical migration can be affected by chlorinated aliphatic hydrocarbons, such as trichloromethane, which formed dense non-aqueous phase liquids and carried PFAS migrating into deep aquifer. Therefore, it was concluded that the distribution patterns of PFASs conform the site geology and their properties. Additionally, the PFASs concentrations, ranging from 5.65 to 6.73 ng/g, are significantly higher than samples in soil from a global survey conducted by Rankin et al. (2016), indicating that regional PFASs contamination cannot be ignored. Evich et al. (2022) reported that PFASs in the environment can migrate via atmospheric movements and subsequently enter distant environments through dry and wet deposition, and this mechanism accounts for the widespread of PFASs. Atmospheric long-distance transport and wet/dry deposition can lead to the wide distribution of PFASs, even in areas far from fluorochemical production industries. To the best of our knowledge, the co-contamination of PFASs with other contaminants, including polycyclic aromatic hydrocarbons (Nakata et al., 2006) and TPH (Meng et al., 2021), heavy metals (Zhang et al., 2023), and chlorinated solvents (Tang et al., 2022), is widely reported. Thus, the effect of co-contamination on the native microbial community needs further investigation, which is expected to provide important information for *in-situ* bioremediation.

3.2 Effect on microbial community diversity

The Chao and Shannon indices, indicative of the alpha-diversity within the microbial community across the six samples were shown in **Figure 3a and 3b**. It was found that the Ace and Shannon indices were lower in S1 and S2 locations (except for S2-10) compared to BK location, demonstrating that the long-term presence of TPHs and BTEX reduced the microbial richness and diversity in soil (Wu et al., 2019). The lower Chao and Shannon indices was observed in S1-10 and S2-5 with the high concentrations of TPHs and BTEX implied that high concentrations of co-contaminants had an adverse effect on the microbial community, leading to less species richness and diversity, consistent with observations reported in previous study (Zhang et al., 2023). Moreover, the reduction on bacterial community diversity and richness were observed with the presence of PFASs, indicating their negative effect on microbial community (Bao et al., 2018; Sun et al., 2016). Additionally, the effects of regionally occurring PFASs, as co-contaminants of BTEX and TPHs, on the soil health and ecological functions, were highlighted in the study by Xu et al. (2022b).

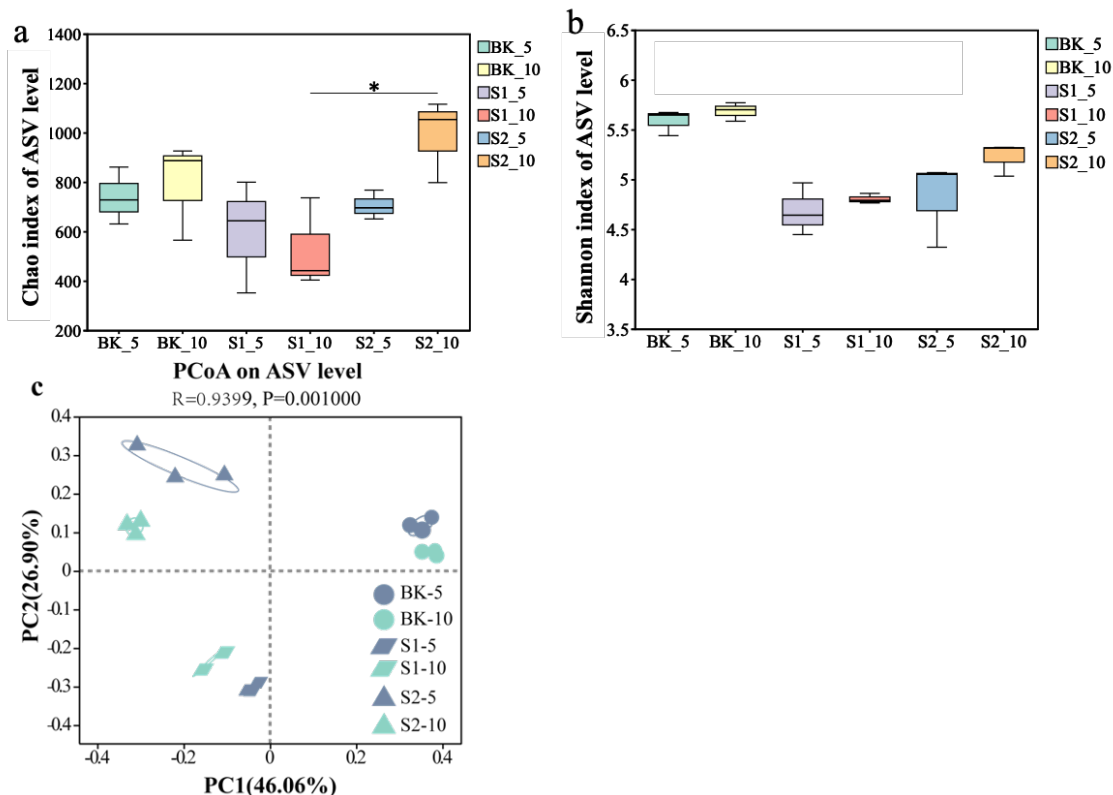


Figure 3. Alpha diversity with Chao index (a) and Shannon index (b) ($***p \leq 0.001$, $*0.001 < p \leq 0.01$, $*p \leq 0.05$), and beta diversity of PCoA based on weighted UniFrac scores of ASVs level (c) in microbial community.

The PCoA results were shown in **Figure 3c**, to assess the overall variations in the microbial community. The first and second principal coordinates accounted for 46.06% and 26.90%, respectively. Microbial community profiles were clustered into BK, S1 and S2 three groups, suggesting a significant difference in the compositions of microbial communities at the three sampling locations, which fit the findings reported by Wu et al. (2023) that the microbial community structure altered according to sampling locations in a PFASs contaminated area. No difference was observed in clusters at different depth for each sampling locations, consistent with the observation of Zhang et al. (2023). Similar conclusion was drawn based on insignificant differences of the Chao and Shannon indices of soil microbial communities observed at both depths in the BK and S1 locations (**Figure 3b**). The observed significant difference on microbial diversity among different sampling locations other than sampling depth could be attributed by the co-contamination and soil physicochemical parameters as reported previously (Li et al., 2017b; Zhang et al., 2023).

3.3 Microbial community compositions under co-contamination of PFASs, TPHs and BTEX

The composition of microbial community at the genus level was shown in **Figure 4 and Table S4**, offering deeper insights into the indigenous microorganisms under co-occurrence of PFASs, TPHs and BTEX. Dominant genera varied across the three sampling locations, with *norank_p__GAL15* and *MB-A2-108* dominating at location BK, accounting for 10.83-12.14% and 7.27-10.12%, respectively. The enrichment of *norank_p__GAL15* and *MB-A2-108* with the presence of PFASs at location BK, suggests that these genera may possess survival strategies under PFASs stress. Similarly, Zhang et al. (2023) reported that *norank_p__GAL15* was dominating genus with high concentrations of PFASs and heavy metals being found, suggesting the important potential of *norank_p__GAL15* for resistant survival strategies to

tolerate complex contamination condition. In addition, *MB-A2-108* has been reported to be a keystone genus in a microbial community under the contamination of polycyclic aromatic compounds and showed negative correlation with the content of PAHs (Sazykina et al., 2022; Xu et al., 2022a), which is consistent with our study with lower TPHs and highest abundance of *MB-A2-108* observed at location BK. For S1 location, genera *OPB41* and *Desulfoprimum* were dominant, accounting for 13.53-15.76% and 8.62-9.50%, respectively. It has been reported that some strains of *OPB41* can utilize aromatic compounds, indicating the metabolic ability for TPHs and BTEX at location S1 (Khomyakova et al., 2022). Wu et al. (2022) have reported the degradation of BTEX by *Desulfoprimum* with sulfate reduction and methane production under anaerobic condition. Since the limited supply of oxygen in subsurface soil, the anaerobic degradation is an important intrinsic mechanism for biodegradation of BTEX with native degrading microorganisms. In addition, it has been reported that *Desulfoprimum* was highly enriched during the biodegradation of hydrocarbons (Taylor et al., 2021).

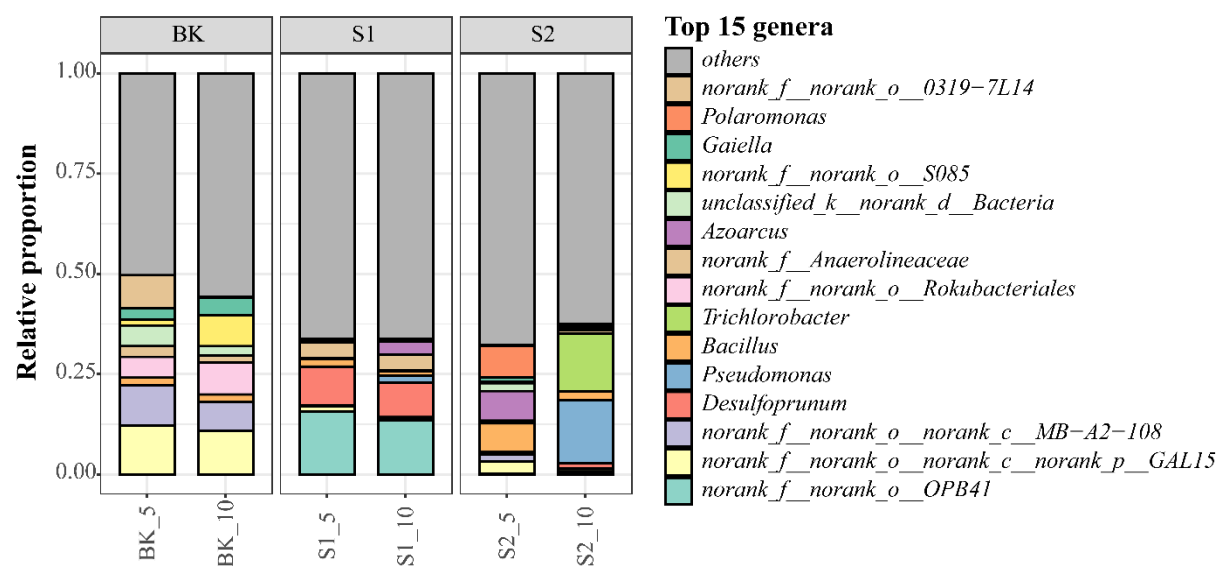


Figure 4. Microbial Community compositions at the genus levels.

Unlike the microbial compositions at location BK and S1, it was worth noting that dominant genera were distinct at different depth at location S2. It has been reported that the microbial community compositions and abundance changed with soil depth in previous studies with observation of co-contamination (Li et al., 2017a; Zhang et al., 2023). The difference can be

explained with extremely high concentration of TPHs in S2-5, serving as organic carbon, which played an important role for shaping microbial community composition. *Bacillus*, *Azoarcus*, and *Polaromonas* were the dominant genera in S2-5 with relative abundances of 2.25%, 7.39% and 7.92%, respectively. In contrast, *Pseudomonas* and *Trichlorobacter* dominated in S2-10, accounting for 15.80% and 14.34%, respectively. *Bacillus* has been reported with metabolic ability for BTEX, implying the potential of natural biodegradation of BTEX at S2 (Wu et al., 2022). *Azoarcus* and *Polaromonas* have been identified in various petroleum-contaminated environmental media, including soil, groundwater, and sediment, indicating their potential for hydrocarbon utilization and TPHs and BTEX removal (Kundu et al., 2023; Raittz et al., 2021; Sperfeld et al., 2018; Wang et al., 2023). Moreover, *Pseudomonas* is commonly found in contaminated soils and exhibits metabolic activity towards contaminants, such as TPH, chlorinated hydrocarbons, with reported potential for PFASs removal (Mansur et al., 2016; Martinez-Toledo and Rodriguez-Vazquez, 2013; Muthukumar et al., 2023). Additionally, *Trichlorobacter* is a dominant genus in S2-10, which is capable to survive under high levels of chlorinated volatile organic compounds, indicating its resistance to adverse conditions (Hussain et al., 2023). In addition, there was significantly high concentrations of NO_3^- -N (2.74 mg/kg) and NH_4^+ -N (83.1 mg/kg) detected in S2-5 as shown in **Table S5**, indicating the active nitrate reduction ability of microorganisms in this community. The denitrification process in unsaturated soil was reported previously by Luo et al. (1999) and Ma et al. (2020). Notably, the moisture in soil was an important factor influencing denitrification, which could influence the redox conditions the available nitrate. Ma et al. (2020) reported the denitrification of nitrate occurred with the moisture of 17.70%, which was comparable to that of S2-5 with 11.40%, indicating the suitable condition for denitrification of S2-5 in current study. Furthermore, the reduction of nitrate in unsaturated anoxic soil coupled with biodegradation of TPHs, toluene and ethylbenzene was also observed by Yang et al. (2016), implying the presence of coupled bioprocesses. The highest TPHs in S2-5 indicating active carbon metabolism, which has been reported that the carbon metabolism in soil contributed to the nitrogen cycling (Nelson et al., 2016), may lead to the high concentration of NH_4^+ observed in this study. The dominating

genera *Bacillus*, *Azoarcus*, *Polaromonas* in S2-5 are all reported to be involved in nitrogen metabolism (Jang et al., 2019; Kim et al., 2005; Sperfeld et al., 2018).

Studies have also found that indigenous microorganisms have the ability to transform precursors, resulting in the observed perfluoroalkyl acids (PFAAs) (Lewis et al., 2016b; Yi et al., 2022). In addition, the biodegradation of PFAAs by native microbial consortium has been reported. Tang et al. (2022) reported the microbial community under long-term exposure of PFASs and chlorinated solvents in a contaminated site, and the biodegradation of trifluoroacetate, PFOA and perfluoro-2-methyl-3-oxahexanoic acid (HFPO-DA) was observed recently in a microcosm study with aquifer microbial community from the studied site (Tang et al., 2023), suggesting the degradation potential by native microorganism. *Pseudomonas* was found to be dominant with higher PFASs in the native community and then enriched during the biotransformation of PFOA in laboratory studies (Tang et al., 2022; Tang et al., 2023). These findings suggest the crucial role of *Pseudomonas* for potential of PFASs transformation, which is promising for bioremediation of PFASs with indigenous microorganisms. Furthermore, the genera *Bacillus*, *Pseudomonas*, *Polaromonas* and *Trichlorobacter* were reported to have enzymes which may be involved in PFASs transformation, including reductive dehalogenase, cytochromes P450, fluoroacetate dehalogenase and alkane monooxygenases (Hu and Scott, 2024), underlying their importance in the potential biodegradation of PFASs.

In summary, the structure and composition of microbial communities are the result of many influencing factors, including soil physical and chemical properties, co-contaminants composition and contamination level. And in turn, the specific microbial composition can react to soil metabolism, especially the biotransformation of contaminants that we are concerned about.

3.4 Identification of key factors influencing microbial community under co-contamination

The correlations of co-contamination and environmental factors on microbial compositions

were shown in **Figure 5 and Table S6**. It was observed that PFASs exhibited a significantly positive relationship with *Trichlorobacter*. A strain of *Trichlorobacter* was reported to be able to grow via reductive dechlorination of trichloroacetate (De Wever et al., 2000). Moreover, the potential role of reductive dehalogenase for defluorination was suggested by Hu and Scott (2024), indicating that *Trichlorobacter* may have the potential for defluorination, which warrant further investigation. Genera significantly associated with TPHs were *Bacillus*, *Azoarcus* and *Polaromonas*. Previous studies have reported that several *Bacillus* strains can degrade hydrocarbons and crude oil through oxidation pathway to generate alcohols and acids in the environment (Roy et al., 2018; Van Beilen and Funhoff, 2007). Moreover, *Bacillus* can enhance the removal of petroleum hydrocarbons by producing biosurfactants to increase the bioavailability of petroleum hydrocarbons (Zhang et al., 2023). *Azoarcus* can use aromatic hydrocarbons as metabolic substrates, and use nitrate as electron acceptor to degrade organic hydrocarbons along with nitrogen cycling (Raittz et al., 2021; Sperfeld et al., 2018). In addition, *Azoarcus*, which has been identified as a dominant genus at sites contaminated with aromatic compounds, has been found to play a significant role in the degradation of BTEX (Sperfeld et al., 2018; Tancsics et al., 2013). Similarly, *Polaromonas* can metabolize straight-chain alkanes and aromatic hydrocarbons with genes encoding the alkane-monooxygenase AlkB and CYP450 enzyme families (Jurelevicius et al., 2022). Furthermore, it has been reported that *Bacillus* and *Azoarcus* can achieve BTEX removal by secreting surfactants or by catalyzing complete or non-complete mineralization of monocyclic substances (Djokic et al., 2011; Salmazo et al., 2023; Surendra et al., 2017; Wongbunmak et al., 2020), indicating their important role in BTEX degradation.

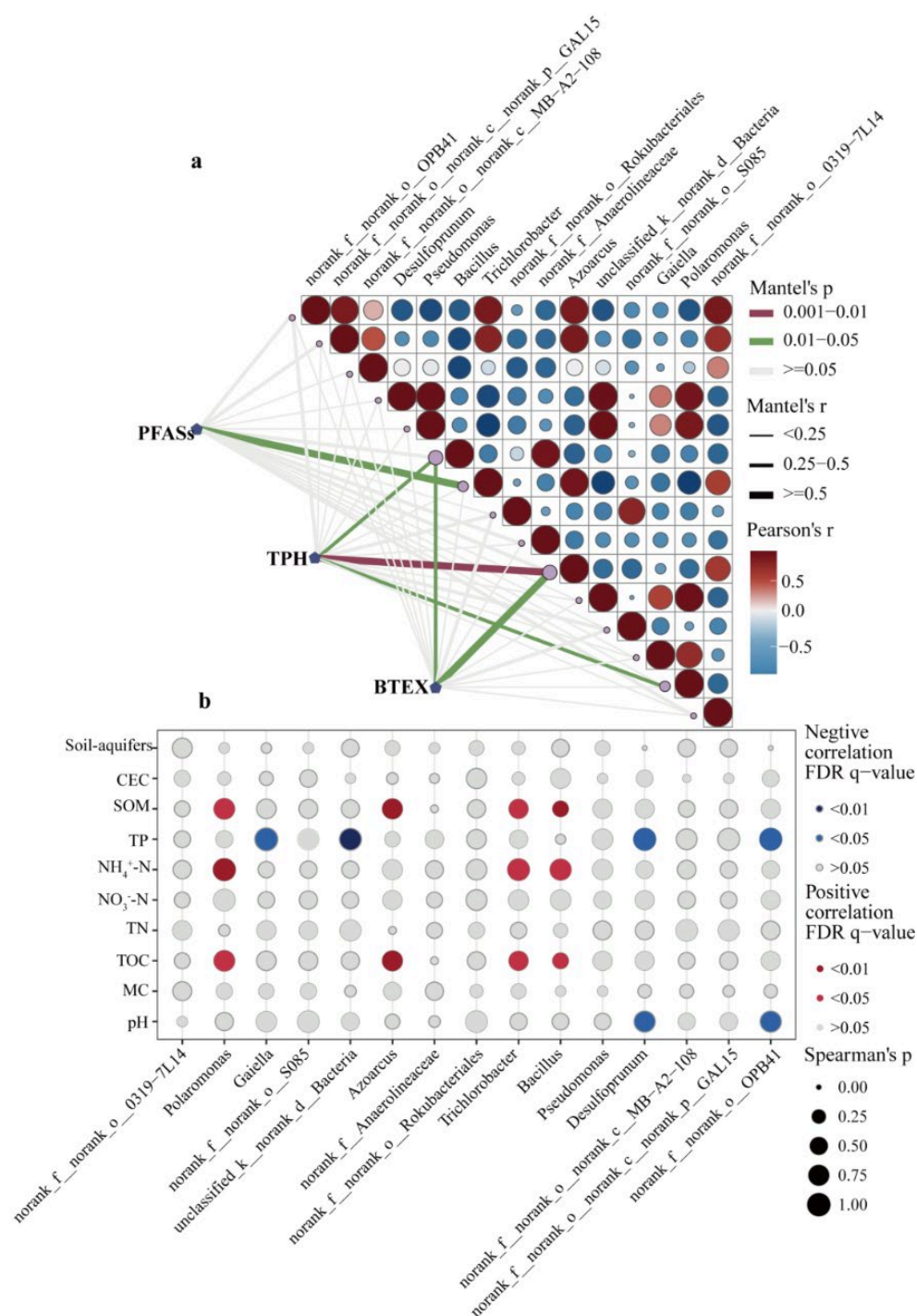


Figure 5. Correlations among Top 15 microbial genera with PFASs, TPHs and BTEX (a) and with soil physicochemical properties (b).

In addition to the effect of co-contamination, the correlations observed between microbial genera and soil physicochemical properties such as SOM, NH_4^+ , and NO_3^- indicated the influence of these factors on microbial community compositions (**Figure 5b**). Specifically, genera such as *Bacillus*, *Azoarcus*, and *Polaromonas*, known for their ability to degrade TPHs

and BTEX, showed positive correlations with SOM. Notably, the significant correlations between these genera with TPHs was also observed, which has been discussed above, suggests that the presence of TPHs and SOM contributed together to the presence of these genera. Further to this, the positive correlation between NH_4^+ and *Trichlorobacter*, *Bacillus*, and *Polaromonas*, revealed a potential role of these genera in utilizing nitrate as an electron acceptor during the biodegradation of TPHs. Moreover, the nitrogen metabolism ability of *Bacillus*, *Polaromonas* has been reported (Jang et al., 2019; Kim et al., 2005). In addition, Sorokin et al. (2023) have reported that strains of *Trichlorobacter* can grow with nitrate serving as electron acceptors to from ammonia. *Gaiella*, *Desulfoprunum* and *OPB41* were negatively associated with TP, and pH also was a significant influencing factor for presence of *Desulfoprunum* and *OPB41*. It has been reported that pH and nutrient (phosphorous and nitrate) had significant effects on the bacterial flora of the dominant bacterial species (Zhang et al., 2018), suggesting the importance of nitrogen metabolism in the biodegradation of co-contaminants under natural condition.

3.5 Implication for bioremediation of PFASs and hydrocarbons co-contamination

Contamination of TPHs and BTEX are widespread, and the microbial degradation metabolic pathways for these pollutants have been extensively confirmed, providing a typical example of environmental remediation mechanisms evolved by microorganisms under environmental stress and other biological processes, including nitrogen and sulfur cycling (Cunningham et al., 2001; Zhang et al., 2018). To further explore the potential biodegradation of mixed contaminants, the energy metabolisms and xenobiotics biodegradation and metabolism were selected from pathway level 3 (PICRUSt2) (**Figure 6a and Table S7**). The metabolism of carbon, nitrogen and sulfur were selected as the relevant energy metabolisms. It is worth noting that the abundances of energy metabolisms were highest in S2-5, followed by those in S2-10 and S1-5, suggesting the microbial energy metabolisms were enhanced with higher TPHs and BTEX in these samples. It has been reported that there exist active nitrogen and sulfur cycle during the biodegradation of BTEX and TPHs with enhanced carbon metabolism with

indigenous microorganisms (Chakraborty et al., 2005; Eziuzor et al., 2022).

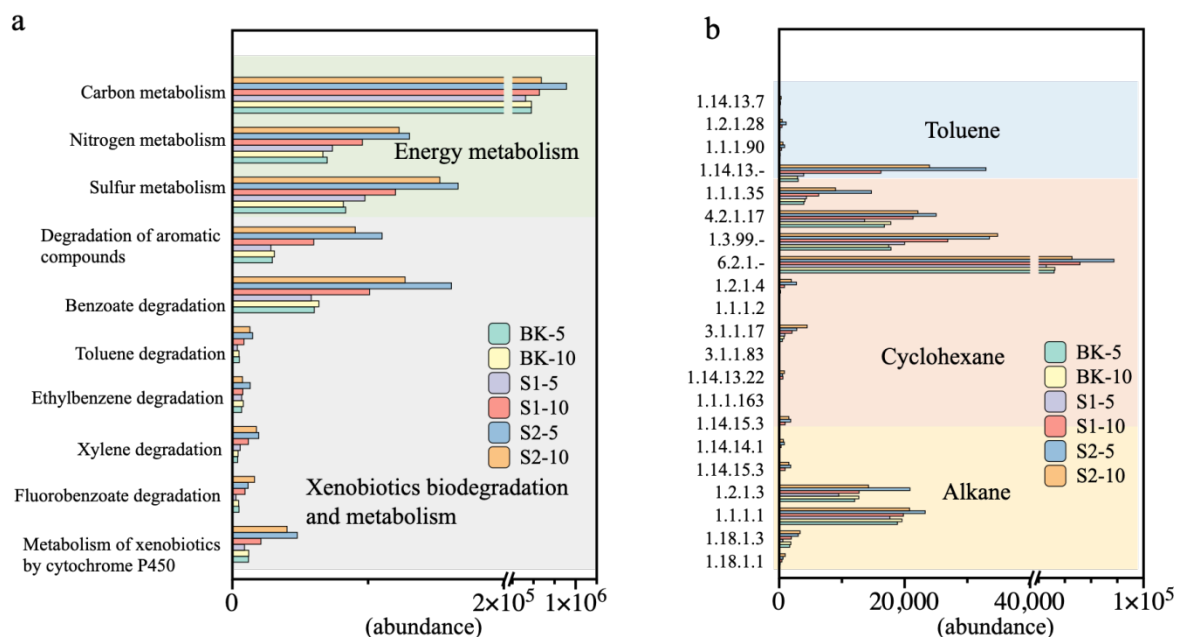


Figure 6. Abundance of energy metabolism and xenobiotics biodegradation and metabolism (a) and abundance of functional enzymes involved in three substrates metabolism based on PICRUST analysis (b).

In addition, the abundance of xenobiotics biodegradation and metabolism was similar to the energy metabolism, indicated that there exists active biodegradation of contaminants in the samples with high TPHs and BTEX concentrations under natural conditions. Among xenobiotics biodegradation and metabolism, the abundances of aromatic compounds and benzoate degradation were much higher than others, suggesting that the active degradation of total BTEX. Benzoate is one of the metabolic intermediates of cycloalkanes and BTEX and the highest abundance of degradation of benzoate was also observed in previous study (Li et al., 2022). Notably, Cytochrome P450, a group of enzymes facilitating important ability for organic contaminants biodegradation including chlorinated solvents, aromatic hydrocarbons (Lin et al., 2022) as well as the potential for PFASs transformation (Hu and Scott, 2024) was also higher in S2-10, S2-5 and S1-5. Commichau et al. (2006) reported that the presence of contaminants could domesticate microorganisms with specific functions to enhance their resistance to environmental stress, resulting in high tolerance and metabolic capacity. The same conclusion

was also drawn in another study conducted by Tang et al. (2022), in which the abundances of energy metabolism and contaminants degradation pathways (Cytochrome P450 and chloroalkane and chloroalkene degradation) in co-contaminated groundwater samples were significantly higher than those in the background wells.

The biodegradation pathways and involved functional enzymes of toluene, alkane and cyclohexane were well addressed by Li et al. (2022). Toluene was chosen as the model chemicals for BTEX and alkane with cyclohexane for TPHs to further investigate the biodegradation activities of BTEX and TPHs under natural condition in this study based on PICRUSt analysis (**Figure 6b and Table S8**). Among the functional enzymes, monooxygenase [EC: 1.14.13-] was the crucial enzyme for the degradation of toluene as well as other BTEX, with the significantly higher abundance in S1-10, S2-5 and S2-10 and higher concentration of BTEX were observed in these samples. Previous studies have confirmed the presence of monooxygenase in *Bacillus*, *Azoarcus* and *Polaromonas* (Deng et al., 2020; Hou, 2006; Nishino et al., 2013), which were also dominating in this study area. Furthermore, the potential role of monooxygenases involving in PFASs transformation was recently reviewed by Hu and Scott (2024). It has been reported that two strains possessing butane and alkane monooxygenases are able for defluorination of FTOHs and 6:2 polyfluoroalkyl phosphates through multiple-CF₂-groups elimination to form shorter-chain PFCAs (Lewis et al., 2016a; Lewis et al., 2016b). Moreover, the gene copy number of *alkB*, encoding alkane monooxygenase was positively correlated with high levels of fluoride released from biodegradation of FTOHs and 6:2 polyfluoroalkyl phosphates. These findings indicated the potential occurrence of biodegradation for TPHs, BTEX and the potential PFASs biodegradation capability evolving in indigenous microbial communities possessing functional enzymes.

Among the functional enzymes for degradation of cyclohexane, Adipate-CoA ligase [EC: 6.2.1.-] was the highest one, and it was reported that the long-chain-fatty-acid-CoA ligase in Adipate-CoA ligase was found in *Bacillus* (Zheng et al., 2018), which was the dominating

genus with highest concentration of long-chain TPHs (C10-C40) in S2-5. This result was consistent with the finding that *Bacillus* showed significant relationship with TPHs in section 3.4, indicating the role of *Bacillus* for the biodegradation of TPHs in this study. In addition, the relative abundance of functional enzymes involved in cyclohexane metabolism at S1 and S2 locations were significantly higher than those at BK location, revealing the potential of active degradation under natural conditions. The enzymes EC: 1.1.1.1 and EC: 1.2.1.3, which are responsible for transformation of alkane to aldehyde and fatty acid, respectively, were the most and second abundant enzymes in alkane biodegradation and this finding was consistent with previous study (Li et al., 2022).

Although it has been reported that PFASs are difficult to degrade by microorganisms due to the high strength of C-F bond, there have also been studies that have reported defluorination behaviors of PFASs. Jaffe et al. have carried out a series of studies on the biodegradation of PFOA by a pure culture *Acidimicrobium* sp. strain A6 under ammonium oxidation and iron reduction processes (Jaffe et al., 2024; Ruiz-Uriguen et al., 2022; Sima et al., 2023). Moreover, the stimulated biotransformation of 6:2 fluorotelomer thioether amido sulfonate under nitrate-reducing condition has been reported by Yi et al. (2022), implying the complex interactions between biological processes and PFASs metabolism. Furthermore, the defluorination of PFASs with indigenous microbial community from long-term PFASs-contaminated site was observed with noticeable fluoride releasing by Tang et al. (2023), suggesting the degradation potential by native microorganism. All these findings provided valuable insights for the possibility of PFASs bioremediation. Moreover, the genera *Polaromonas*, *Bacillus*, *Azoarcus* and *Pseudomonas* observed with high abundance in this study are also reported to be involved in PFASs metabolism as discussed (Section 3.3). Finally, some potential metabolic enzymes involved in PFASs biotransformation were also revealed, including haloacid dehalogenases, reductive dehalogenases, cytochromes P450, alkane and butane monooxygenases (Hu and Scott, 2024). Notably, cytochromes P450 and monooxygenase are also important enzymes for biotransformation of hydrocarbons, and their higher abundances were observed in this study with higher concentration of hydrocarbons, suggesting the enhanced expression of these

enzymes under contamination of hydrocarbons, which is looking forwards to being beneficial for PFASs transformation.

4. Conclusions

This study sheds light on the impacts of co-occurrence of PFASs, TPHs, and BTEX on native microbial consortia at a refinery site with varying depths. The results revealed that PFASs were primarily sourced from non-point pollution, while TPHs and BTEX concentrations exhibited significant variations due to historical refinery activities. The presence of these co-contaminants significantly altered the microbial community structure, favoring bacteria with enhanced resistance or metabolic capabilities. Moreover, the analysis of energy metabolism and functional enzymes for TPHs and BTEX biodegradation provides insights into the microbial mechanisms involved in contaminant degradation, with cytochrome P450 and alkane monooxygenases playing important role not only in hydrocarbons but also in PFASs transformations. Overall, this study contributes to advancing our understanding of the ecological impacts of co-occurring contaminants and provides crucial insights for soil health and its ecological functions. Furthermore, our findings demonstrated the need for integrated approaches to address the challenges posed by complex contaminant mixtures in soil environments, paving the way for more effective and sustainable remediation solutions in contaminated sites.

Author contributions

Zhuanxia Zhang: Conceptualization, Data curation, Writing original draft. **Zeliang Liu:** Conceptualization, Methodology, Writing original draft. **Frederic Coulon:** Writing review & editing, Conceptualization. **Gubai Luo:** Resources. **Qing Wang:** Writing review & editing, Conceptualization. **Xinyu Gao:** Investigation. **Zhongyuan Li:** Methodology. **Xin Song:** Writing review & editing, Supervision, Project administration, Funding acquisition, Conceptualization.

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References

- Bao, Y., Li, B., Xie, S. and Huang, J. 2018. Vertical profiles of microbial communities in perfluoroalkyl substance-contaminated soils. *Annals of Microbiology* 68(6), 399-408.
- Chakraborty, R., O'Connor, S.M., Chan, E. and Coates, J.D. 2005. Anaerobic degradation of benzene, toluene, ethylbenzene, and xylene compounds by *Dechloromonas* strain RCB. *Applied and Environmental Microbiology* 71(12), 8649-8655.
- Charbonnet, J.A., McDonough, C.A., Xiao, F., Schwichtenberg, T., Cao, D., Kaserzon, S., Thomas, K.V., Dewapriya, P., Place, B.J., Schymanski, E.L., Field, J.A., Helbling, D.E. and Higgins, C.P. 2022. Communicating confidence of per- and polyfluoroalkyl substance identification via high-resolution mass spectrometry. *Environmental Science & Technology Letters* 9(6), 473-481.
- Chen, S., Jiao, X.-C., Gai, N., Li, X.-J., Wang, X.-C., Lu, G.-H., Piao, H.-T., Rao, Z. and Yang, Y.-L. 2016. Perfluorinated compounds in soil, surface water, and groundwater from rural areas in eastern China. *Environmental Pollution* 211, 124-131.
- Commichau, F.M., Forchhammer, K. and Stülke, J. 2006. Regulatory links between carbon and nitrogen metabolism. *Current Opinion in Microbiology* 9(2), 167-172.
- Cunningham, J.A., Rahme, H., Hopkins, G.D., Lebron, C. and Reinhard, M. 2001. Enhanced in situ bioremediation of BTEX contaminated groundwater by combined injection of nitrate and sulfate. *Environmental Science & Technology* 35(8), 1663-1670.
- De Wever, H., Cole, J.R., Fettig, M.R., Hogan, D.A. and Tiedje, J.M. 2000. Reductive dehalogenation of trichloroacetic acid by *Trichlorobacter thiogenes* gen. nov., sp nov. *Applied and Environmental Microbiology* 66(6), 2297-2301.
- Deng, D., Dung Ngoc, P., Li, F. and Li, M. 2020. Discovery of an inducible toluene monooxygenase that cooxidizes 1,4-dioxane and 1,1-dichloroethylene in propanotrophic *Azoarcus* sp. strain DD4. *Applied and Environmental Microbiology* 86(17).
- Ding, D., Song, X., Wei, C., Hu, Z. and Liu, Z. 2020. Efficient sorptive removal of F-53B from water by layered double hydroxides: Performance and mechanisms. *Chemosphere* 252.

- Ding, X., Song, X., Xu, M., Yao, J., Xu, C., Tang, Z. and Zhang, Z. 2022. Co-occurrence and correlations of PFASs and chlorinated volatile organic compounds (cVOCs) in subsurface in a fluorochemical industrial park: Laboratory and field investigations. *Science of the Total Environment* 814.
- Djokic, L., Narancic, T., Nikodinovic-Runic, J., Savic, M. and Vasiljevic, B. 2011. Isolation and characterization of four novel gram-positive bacteria associated with the rhizosphere of two endemorelict plants capable of degrading a broad range of aromatic substrates. *Applied Microbiology and Biotechnology* 91(4), 1227-1238.
- Evich, M.G., Davis, M.J.B., McCord, J.P., Acrey, B., Awkerman, J.A., Knappe, D.R.U., Lindstrom, A.B., Speth, T.F., Tebes-Stevens, C., Strynar, M.J., Wang, Z., Weber, E.J., Henderson, W.M. and Washington, J.W. 2022. Per- and polyfluoroalkyl substances in the environment. *Science* 375(6580), 512-+.
- Eze, S.U., Ogagarue, D.O., Nnorom, S.L., Osung, W.E. and Ibitoye, T.A. 2021. Integrated geophysical and geochemical methods for environmental assessment of subsurface hydrocarbon contamination. *Environmental Monitoring and Assessment* 193(7).
- Eziuzor, S.C., Correa, F.B., Peng, S.C., Schultz, J., Kleinsteinuber, S., da Rocha, U.N., Adrian, L. and Vogt, C. 2022. Structure and functional capacity of a benzene-mineralizing, nitrate-reducing microbial community. *Journal of Applied Microbiology* 132(4), 2795-2811.
- Gao, Y., Yuan, L., Du, J., Wang, H., Yang, X., Duan, L., Zheng, L., Bahar, M.M., Zhao, Q., Zhang, W., Liu, Y., Fu, Z., Wang, W. and Naidu, R. 2022. Bacterial community profile of the crude oil-contaminated saline soil in the Yellow River Delta Natural Reserve, China. *Chemosphere* 289.
- Hou, C.T. 2006. Monooxygenase system of *Bacillus megaterium* ALA2: Studies on linoleic acid epoxidation products. *Journal of the American Oil Chemists Society* 83(8), 677-681.
- Hu, M. and Scott, C. 2024. Toward the development of a molecular toolkit for the microbial remediation of per-and polyfluoroalkyl substances. *Applied and Environmental Microbiology* 90(4).
- Hussain, B., Chen, J., Huang, S., Tsai, I.S., Rathod, J. and Hsu, B. 2023. Underpinning the ecological response of mixed chlorinated volatile organic compounds (CVOCs) associated with contaminated and bioremediated groundwaters: A potential nexus of microbial community structure and function for strategizing efficient bioremediation. *Environmental Pollution* 334.
- Jaffe, P.R., Huang, S., Park, J., Ruiz-Uriguen, M., Shuai, W. and Sima, M. 2024. Defluorination of PFAS by *Acidimicrobium* sp. strain A6 and potential applications for remediation. *Methods in enzymology* 696, 287-320.
- Jang, J., Anderson, E.L., Venterea, R.T., Sadowsky, M.J., Rosen, C.J., Feyereisen, G.W. and Ishii, S. 2019. Denitrifying bacteria active in woodchip bioreactors at low-temperature conditions. *Frontiers in Microbiology* 10.
- Jia, X., Li, X.T., Zhou, L.F., Hui, Y.M., Li, W.C., Cai, Y.Q. and Shi, Y.L. 2023. Variations of the level, profile, and distribution of PFAS around POSF manufacturing facilities in

- China: An overlooked source of PFCA. *Environmental Science & Technology* 57(13), 5264-5274.
- Jurelevicius, D., Pereira, R.d.S., da Mota, F.F., Cury, J.C., de Oliveira, I.C., Rosado, A.S., Mason, O.U., Jansson, J.K. and Seldin, L. 2022. Metagenomic analysis of microbial communities across a transect from low to highly hydrocarbon-contaminated soils in King George Island, Maritime Antarctica. *Geobiology* 20(1), 98-111.
- Khomyakova, M.A.A., Zavarzina, D.G.G., Merkel, A.Y.Y., Klyukina, A.A.A., Pikhtereva, V.A.A., Gavrilov, S.N.N. and Slobodkin, A.I.I. 2022. The first cultivated representatives of the actinobacterial lineage OPB41 isolated from subsurface environments constitute a novel order *Anaerosomatales*. *Frontiers in Microbiology* 13.
- Kim, J.K., Park, K.J., Cho, K.S., Nam, S.W., Park, T.J. and Bajpai, R. 2005. Aerobic nitrification-denitrification by heterotrophic *Bacillus* strains. *Bioresource Technology* 96(17), 1897-1906.
- Kundu, A., Harrison, O. and Ghoshal, S. 2023. Impacts of Arctic diesel contamination on microbial community composition and degradative gene abundance during hydrocarbon biodegradation with and without nutrients: A case study of seven sub-Arctic soils. *Science of the Total Environment* 871.
- Lewis, M., Kim, M., Wang, N. and Chu, K. 2016a. Engineering artificial communities for enhanced FTOH degradation. *Science of the Total Environment* 572, 935-942.
- Lewis, M., Kim, M.H., Liu, E.J., Wang, N. and Chu, K.-H. 2016b. Biotransformation of 6:2 polyfluoroalkyl phosphates (6:2 PAPs): Effects of degradative bacteria and co-substrates. *Journal of Hazardous Materials* 320, 479-486.
- Li, B., Bao, Y., Xu, Y., Xie, S. and Huang, J. 2017a. Vertical distribution of microbial communities in soils contaminated by chromium and perfluoroalkyl substances. *Science of the Total Environment* 599, 156-164.
- Li, J.H., de Toledo, R.A. and Shim, H.J. 2017b. Multivariate optimization for the simultaneous bioremoval of BTEX and chlorinated aliphatic hydrocarbons by *Pseudomonas plecoglossicida*. *Journal of Hazardous Materials* 321, 238-246.
- Li, R., Munoz, G., Liu, Y.N., Sauve, S., Ghoshal, S. and Liu, J.X. 2019. Transformation of novel polyfluoroalkyl substances (PFASs) as co-contaminants during biopile remediation of petroleum hydrocarbons. *Journal of Hazardous Materials* 362, 140-147.
- Li, Y.T., Zheng, B.P., Yang, Y.H., Chen, K.J., Chen, X.L., Huang, X. and Wang, X. 2022. Soil microbial ecological effect of shale gas oil-based drilling cuttings pyrolysis residue used as soil covering material. *Journal of Hazardous Materials* 436.
- Lin, S., Wei, J., Yang, B., Zhang, M. and Zhuo, R. 2022. Bioremediation of organic pollutants by white rot fungal cytochrome P450: The role and mechanism of CYP450 in biodegradation. *Chemosphere* 301.
- Liu, N., Wang, L., Cao, D., Li, D., Zhu, Y., Huang, S. and Shi, J. 2023. Remediation of petroleum contaminated soil by persulfate oxidation coupled with microbial degradation. *Journal of Environmental Chemical Engineering* 11(3).
- Liu, Z., Lu, Y., Shi, Y., Wang, P., Jones, K., Sweetman, A.J., Johnson, A.C., Zhang, M., Zhou, Y., Lu, X., Su, C., Sarvajayakesavaluc, S. and Khan, K. 2017. Crop bioaccumulation

- and human exposure of perfluoroalkyl acids through multi-media transport from a mega fluorochemical industrial park, China. *Environment International* 106, 37-47.
- Luo, J., Tillman, R.W. and Ball, P.R. 1999. Factors regulating denitrification in a soil under pasture. *Soil Biology & Biochemistry* 31(6), 913-927.
- Ma, L., Xiong, Z.Q., Yao, L.G., Liu, G.H., Zhang, Q.F. and Liu, W.Z. 2020. Soil properties alter plant and microbial communities to modulate denitrification rates in subtropical riparian wetlands. *Land Degradation & Development* 31(14), 1792-1802.
- Mansur, A.A., Taha, M., Shahsavari, E., Holeyur, N., Adetutu, E.M. and Ball, A.S. 2016. An effective soil slurry bioremediation protocol for the treatment of Libyan soil contaminated with crude oil tank bottom sludge. *International Biodeterioration & Biodegradation* 115, 179-185.
- Martinez-Toledo, A. and Rodriguez-Vazquez, R. 2013. *In situ* biosurfactant production and hydrocarbon removal by *Pseudomonas putida* CB-100 in bioaugmented and biostimulated oil-contaminated soil. *Brazilian Journal of Microbiology* 44(2), 595-605.
- Meng, Y., Yao, Y.M., Chen, H., Li, Q. and Sun, H.W. 2021. Legacy and emerging per- and polyfluoroalkyl substances (PFASs) in Dagang Oilfield: Multimedia distribution and contributions of unknown precursors. *Journal of Hazardous Materials* 412.
- Munyaka, P.M., Eissa, N., Bernstein, C.N., Khafipour, E. and Ghia, J.E. 2015. Antepartum antibiotic treatment increases offspring susceptibility to experimental colitis: a role of the gut microbiota. *Plos One* 10(11).
- Muthukumar, B., Surya, S., Sivakumar, K., AlSalhi, M.S., Rao, T.N., Devanesan, S., Arunkumar, P. and Rajasekar, A. 2023. Influence of bioaugmentation in crude oil contaminated soil by *Pseudomonas* species on the removal of total petroleum hydrocarbon. *Chemosphere* 310.
- Nakata, H., Kannan, K., Nasu, T., Cho, H., Sinclair, E. and Takemura, A. 2006. Perfluorinated contaminants in sediments and aquatic organisms collected from shallow water and tidal flat areas of the Ariake Sea, Japan: Environmental fate of perfluorooctane sulfonate in aquatic ecosystems. *Environmental Science & Technology* 40(16), 4916-4921.
- Nelson, M.B., Martiny, A.C. and Martiny, J.B.H. 2016. Global biogeography of microbial nitrogen-cycling traits in soil. *Proceedings of the National Academy of Sciences of the United States of America* 113(29), 8033-8040.
- Nishino, S.F., Shin, K.A., Gossett, J.M. and Spain, J.C. 2013. Cytochrome P450 initiates degradation of *cis*-dichloroethene by *Polaromonas* sp strain JS666. *Applied and Environmental Microbiology* 79(7), 2263-2272.
- Patowary, K., Bhuyan, T., Patowary, R., Mohanta, Y.K., Panda, B.P., Deka, S., Islam, N.F., Joshi, S.J. and Sarma, H. 2023. Soil treatment using a biosurfactant producing bacterial consortium in rice fields contaminated with oily sludge- a sustainable approach. *Environmental Research* 220.
- Raittz, R.T., Reginatto De Pierri, C., Maluk, M., Bueno Batista, M., Carmona, M., Junghare, M., Faoro, H., Cruz, L.M., Battistoni, F., Souza, E.d., Pedrosa, F.d.O., Chen, W.-M., Poole, P.S., Dixon, R.A. and James, E.K. 2021. Comparative genomics provides

- insights into the taxonomy of *Azoarcus* and reveals separate origins of *Nif* genes in the proposed *Azoarcus* and *Aromatoleum* genera. *Genes* 12(1).
- Rankin, K., Mabury, S.A., Jenkins, T.M. and Washington, J.W. 2016. A North American and global survey of perfluoroalkyl substances in surface soils: Distribution patterns and mode of occurrence. *Chemosphere* 161, 333-341.
- Roy, A., Dutta, A., Pal, S., Gupta, A., Sarkar, J., Chatterjee, A., Saha, A., Sarkar, P., Sar, P. and Kazy, S.K. 2018. Biostimulation and bioaugmentation of native microbial community accelerated bioremediation of oil refinery sludge. *Bioresour Technol* 253, 22-32.
- Ruiz-Uriquen, M., Shuai, W., Huang, S. and Jaffe, P.R. 2022. Biodegradation of PFOA in microbial electrolysis cells by *Acidimicrobiaceae* sp. strain A6. *Chemosphere* 292.
- Salmazo, P., De Marco, N., Soeiro, V.S., Castanho, N.R.C.M., Leite, F.G., Chaud, M.V., Grotto, D. and Jozala, A.F.F. 2023. Evaluation of *Bacillus subtilis* as a tool for biodegrading diesel oil and gasoline in experimentally contaminated water and soil. *Current Microbiology* 80(3).
- Sazykina, M.A., Minkina, T.M., Konstantinova, E.Y., Khmelevtsova, L.E., Azhogina, T.N., Antonenko, E.M., Karchava, S.K., Klimova, M.V., Sushkova, S.N., Polienko, E.A., Birukova, O.A., Mandzhieva, S.S., Kudeevskaya, E.M., Khammami, M.I., Rakin, A.V. and Sazykin, I.S. 2022. Pollution impact on microbial communities composition in natural and anthropogenically modified soils of Southern Russia. *Microbiological Research* 254.
- Sima, M.W., Huang, S. and Jaffe, P.R. 2023. Modeling the kinetics of perfluorooctanoic and perfluorooctane sulfonic acid biodegradation by *Acidimicrobium* sp. Strain A6 during the feammox process. *Journal of Hazardous Materials* 448.
- Sorokin, D.Y.Y., Tikhonova, T.V.V., Koch, H., van den Berg, E.M.M., Hinderks, R.S.S., Pabst, M., Dergousova, N.I.I., Soloveva, A.Y.Y., Kuenen, G.J.J., Popov, V.O.O., van Loosdrecht, M.C.M. and Lucker, S. 2023. *Trichlorobacter ammonificans*, a dedicated acetate-dependent ammonifier with a novel module for dissimilatory nitrate reduction to ammonia. *Isme Journal* 17(10), 1639-1648.
- Sperfeld, M., Rauschenbach, C., Diekert, G. and Studenik, S. 2018. Microbial community of a gasworks aquifer and identification of nitrate-reducing *Azoarcus* and *Georgfuchsia* as key players in BTEX degradation. *Water Research* 132, 146-157.
- Sun, Y., Wang, T., Peng, X., Wang, P. and Lu, Y. 2016. Bacterial community compositions in sediment polluted by perfluoroalkyl acids (PFAAs) using Illumina high-throughput sequencing. *Environmental Science and Pollution Research* 23(11), 10556-10565.
- Surendra, S.V., Mahalingam, B.L. and Velan, M. 2017. Degradation of monoaromatics by *Bacillus pumilus* MVS3. *Brazilian Archives of Biology and Technology* 60.
- Tancsics, A., Farkas, M., Szoboszlai, S., Szabo, I., Kukolya, J., Vajna, B., Kovacs, B., Benedek, T. and Kriszt, B. 2013. One-year monitoring of *meta*-cleavage dioxygenase gene expression and microbial community dynamics reveals the relevance of subfamily I.2.C extradiol dioxygenases in hypoxic, BTEX-contaminated groundwater. *Systematic and Applied Microbiology* 36(5), 339-350.
- Tang, Z., Song, X., Xu, M., Yao, J., Ali, M., Wang, Q., Zeng, J., Ding, X., Wang, C., Zhang, Z.

- and Liu, X. 2022. Effects of co-occurrence of PFASs and chlorinated aliphatic hydrocarbons on microbial communities in groundwater: A field study. *Journal of Hazardous Materials* 435.
- Tang, Z., Vogel, T.M., Wang, Q., Wei, C., Ali, M. and Song, X. 2023. Microbial defluorination of TFA, PFOA, and HFPO-DA by a native microbial consortium under anoxic conditions. *Journal of hazardous materials* 465, 133217.
- Taylor, N.M., Toth, C.R.A., Collins, V., Mussone, P. and Gieg, L.M. 2021. The effect of an adsorbent matrix on recovery of microorganisms from hydrocarbon-contaminated groundwater. *Microorganisms* 9(1).
- Truskewycz, A., Gundry, T.D., Khudur, L.S., Kolobaric, A., Taha, M., Aburto-Medina, A., Ball, A.S. and Shahsavari, E. 2019. Petroleum hydrocarbon contamination in terrestrial ecosystems-fate and microbial responses. *Molecules* 24(18).
- Van Beilen, J.B. and Funhoff, E.G. 2007. Alkane hydroxylases involved in microbial alkane degradation. *Applied Microbiology and Biotechnology* 74(1), 13-21.
- Vazquez, S., Monien, P., Pepino Minetti, R., Juergens, J., Curtosi, A., Villalba Primitz, J., Frickenhaus, S., Abele, D., Mac Cormack, W. and Helmke, E. 2017. Bacterial communities and chemical parameters in soils and coastal sediments in response to diesel spills at Carlini Station, Antarctica. *Science of the Total Environment* 605, 26-37.
- Wang, J.L., Zhang, Y.L., Ding, Y., Song, H.W., Liu, T., Zhang, Y., Xu, W.Q. and Shi, Y.J. 2023. Comparing the indigenous microorganism system in typical petroleum-contaminated groundwater. *Chemosphere* 311.
- Wei, C., Wang, Q., Song, X., Chen, X., Fan, R., Ding, D. and Liu, Y. 2018. Distribution, source identification and health risk assessment of PFASs and two PFOS alternatives in groundwater from non-industrial areas. *Ecotoxicology and Environmental Safety* 152, 141-150.
- Wongbunmak, A., Khiawjan, S., Suphantharika, M. and Pongtharangkul, T. 2020. BTEX biodegradation by *Bacillus amyloliquefaciens* subsp. *plantarum* W1 and its proposed BTEX biodegradation pathways. *Scientific Reports* 10(1).
- Wu, E., Wang, K., Liu, Z., Wang, J., Yan, H., Zhu, X., Zhu, X. and Chen, B. 2023. Metabolic and microbial profiling of soil microbial community under per- and polyfluoroalkyl substance (PFAS) stress. *Environmental Science & Technology* 57(51), 21855-21865.
- Wu, M., Wu, J., Zhang, X. and Ye, X. 2019. Effect of bioaugmentation and biostimulation on hydrocarbon degradation and microbial community composition in petroleum-contaminated loessal soil. *Chemosphere* 237, 124456.
- Wu, R. and Wang, S. 2022. Integration of microbial reductive dehalogenation with persulfate activation and oxidation (Bio-RD-PAO) for complete attenuation of organohalides. *Frontiers of Environmental Science & Engineering* 16(2).
- Wu, Z., Liu, G., Ji, Y., Li, P., Yu, X., Qiao, W., Wang, B., Shi, K., Liu, W., Liang, B., Wang, D., Yanuka-Golub, K., Freilich, S. and Jiang, J. 2022. Electron acceptors determine the BTEX degradation capacity of anaerobic microbiota via regulating the microbial community. *Environmental Research* 215.

- Xin, Y., Ren, X., Ruan, T., Li, C., Guo, L. and Jiang, G. 2018. Chlorinated polyfluoroalkylether sulfonates exhibit similar binding potency and activity to thyroid hormone transport proteins and nuclear receptors as perfluorooctanesulfonate. *Environmental science & technology* 52(16), 9412-9418.
- Xu, C., Liu, Z., Song, X., Ding, X. and Ding, D. 2021a. Legacy and emerging per- and polyfluoroalkyl substances (PFASs) in multi-media around a landfill in China: Implications for the usage of PFASs alternatives. *Science of the Total Environment* 751.
- Xu, C., Song, X., Liu, Z., Ding, X., Chen, H. and Ding, D. 2021b. Occurrence, source apportionment, plant bioaccumulation and human exposure of legacy and emerging per- and polyfluoroalkyl substances in soil and plant leaves near a landfill in China. *Science of the Total Environment* 776.
- Xu, G., Geng, S., Cao, W., Zuo, R., Teng, Y., Ding, A., Fan, F. and Dou, J. 2022a. Vertical distribution characteristics and interactions of polycyclic aromatic compounds and bacterial communities in contaminated soil in oil storage tank areas. *Chemosphere* 301.
- Xu, R., Tao, W., Lin, H., Huang, D., Su, P., Gao, P., Sun, X., Yang, Z. and Sun, W. 2022b. Effects of perfluorooctanoic acid (PFOA) and perfluorooctane sulfonic acid (PFOS) on soil microbial community. *Microbial Ecology* 83(4), 929-941.
- Yang, S., Song, Y., Wang, D., Wei, W., Yang, Y., Men, B. and Li, J. 2016. Application of nitrate to enhance biodegradation of gasoline components in soil by indigenous microorganisms under anoxic condition. *Environmental Technology* 37(9), 1045-1053.
- Yao, Y., Meng, Y., Chen, H., Zhu, L. and Sun, H. 2022. Non-target discovery of emerging PFAS homologues in Dagang Oilfield: Multimedia distribution and profiles in crude oil. *Journal of Hazardous Materials* 437.
- Yi, S., Harding-Marjanovic, K.C., Houtz, E.F., Antell, E., Olivares, C., Nichiporuk, R.V., Iavarone, A.T., Zhuang, W.Q., Field, J.A., Sedlak, D.L. and Alvarez-Cohen, L. 2022. Biotransformation of 6:2 fluorotelomer thioether amido sulfonate in aqueous film-forming foams under nitrate-reducing conditions. *Environmental Science & Technology* 56(15), 10646-10655.
- Zhang, M., Lin, M., Cao, X., Zhao, S., Jiang, D., Wang, B., Wang, S., Fan, Y., Guo, M. and Lin, H. 2018. Difference in pH value and nutrient and bacterial diversity in the *Carya cathayensis* forest soil under different management models. *Biodiversity Science* 26(6), 611-619.
- Zhang, M., Qing, W., Xin, S., Mukhtiar, A., Zhiwen, T., Xin, L., Zhang, Z., Shurong, M., Jinhui, B. and Zhongyuan, L. 2023. Co-occurrence of PFASs, heavy metals and PAHs and their composite impacts on microbial consortium in soil: A field study. *Pedosphere*.
- Zheng, D., Zeng, Z., Xue, B., Deng, Y., Sun, M., Tang, Y. and Ruan, L. 2018. *Bacillus thuringiensis* produces the lipopeptide thumolycin to antagonize microbes and nematodes. *Microbiological Research* 215, 22-28.



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