Structural and functional responses of soil microbial communities to petroleum pollution in eastern Gansu Province on the Loess Plateau, China: Postprint

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Abstract

Crude oil pollution is a significant global environmental challenge. The eastern Gansu Province on the Loess Plateau, an important agricultural region containing the Changqing Oilfield, is facing increasing crude oil contamination. Understanding how microbial communities respond to varying pollution levels is critical for developing effective bioremediation strategies. This study examined how different concentrations of crude oil affect soil properties and microbial communities in Qingyang City, eastern Gansu Province, China by comparing lightly polluted (1895.84–2696.54 mg/kg total petroleum hydrocarbons (TPH)), heavily polluted (4964.25–7153.61 mg/kg TPH), and uncontaminated (CK) soils. Results revealed that petroleum contamination significantly increased total organic carbon (TOC), pH, C:N:P ratio, and the activities of dehydrogenase (DHA) and polyphenol oxidase (PPO), while reducing total nitrogen (TN), available nitrogen (AN), total phosphorus (TP), available phosphorus (AP), available potassium (AK), soil organic matter (SOM), soil water content (SWC), the activities of urease (URE) and alkaline phosphatase (APA), and microbial alpha diversity ($P<0.050$). Light pollution (LP) soils demonstrated an increase in culturable microorganisms, whereas heavy pollution (HP) soils exhibited increased hydrocarbon-degrading microbes and higher expression of key functional genes, such as alkane monooxygenase (AlkB), cytochrome P450 alkane hydroxylases (P450), catechol 2,3-dioxygenase (C23O), and naphthalene dioxygenase (Nah) ($P<0.050$). Non-metric multidimensional scaling (NMDS) and redundancy analysis (RDA) indicated evident variations in microbial community structure across different oil contamination levels. LP soils were dominated by bacterial genera Pseudoxanthomonas and Solimonadaceae, whereas Pseudomonas, Nocardioides,

and hydrocarbon-degrading genera (Marinobacter, Idiomarina, and Halomonas) were predominant in HP soils. The fungal genus Pseudallescheria exhibited the most pronounced abundance shift between LP and HP soils ($P<0.050$). Environmental factor analysis identified AN, SWC, TN, SOM, and alpha diversity indices (Shannon index and Chao1 index) as the key differentiators of CK soils, whereas the pollutant levels and metal content were characterized in HP soils. Hydrocarbon-degrading microbial abundance was a defining trait of HP soils. Metabolic pathway analysis revealed enhanced aromatic hydrocarbon degradation in HP soils, indicating microbial adaptation to severe contamination. These findings demonstrated that crude oil pollution suppressed soil nutrients while reshaping the structure and function of microbial communities. Pollution intensity directly affected microbial composition and degradation potential. This study offers valuable insights into microbial responses across contamination gradients and supports the development of targeted bioremediation strategies for oil-contaminated loess soils.

Full Text

Preamble

Structural and Functional Responses of Soil Microbial Communities to Petroleum Pollution in Eastern Gansu Province on the Loess Plateau, China

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Abstract

Crude oil pollution represents a significant global environmental challenge. The eastern Gansu Province on the Loess Plateau—an important agricultural region containing the Changqing Oilfield—is experiencing increasing crude oil contamination. Understanding how microbial communities respond to varying pollution levels is critical for developing effective bioremediation strategies. This study examined the effects of different crude oil concentrations on soil properties and microbial communities in Qingyang City, eastern Gansu Province, China, by

comparing lightly polluted (1895.84–2696.54 mg/kg total petroleum hydrocarbons (TPH)), heavily polluted (4964.25–7153.61 mg/kg TPH), and uncontaminated (CK) soils.

Results revealed that petroleum contamination significantly increased total organic carbon (TOC), pH, C:N:P ratio, and the activities of dehydrogenase (DHA) and polyphenol oxidase (PPO), while reducing total nitrogen (TN), available nitrogen (AN), total phosphorus (TP), available phosphorus (AP), available potassium (AK), soil organic matter (SOM), soil water content (SWC), the activities of urease (URE) and alkaline phosphatase (APA), and microbial alpha diversity ($P<0.050$). Light pollution (LP) soils demonstrated increased culturable microorganisms, whereas heavy pollution (HP) soils exhibited increased hydrocarbon-degrading microbes and higher expression of key functional genes, including alkane monooxygenase (AlkB), cytochrome P450 alkane hydroxylases (P450), catechol 2,3-dioxygenase (C23O), and naphthalene dioxygenase (Nah) ($P<0.050$).

Non-metric multidimensional scaling (NMDS) and redundancy analysis (RDA) indicated evident variations in microbial community structure across different oil contamination levels. LP soils were dominated by bacterial genera *Pseudoxanthomonas* and *Solimonadaceae*, whereas *Pseudomonas*, *Nocardioides*, and hydrocarbon-degrading genera (*Marinobacter*, *Idiomarina*, and *Halomonas*) predominated in HP soils. The fungal genus *Pseudallescheria* exhibited the most pronounced abundance shift between LP and HP soils ($P<0.050$).

Environmental factor analysis identified AN, SWC, TN, SOM, and alpha diversity indices (Shannon index and Chao1 index) as the key differentiators of CK soils, whereas pollutant levels and metal content characterized HP soils. Hydrocarbon-degrading microbial abundance was a defining trait of HP soils. Metabolic pathway analysis revealed enhanced aromatic hydrocarbon degradation in HP soils, indicating microbial adaptation to severe contamination. These findings demonstrate that crude oil pollution suppressed soil nutrients while reshaping the structure and function of microbial communities. Pollution intensity directly affected microbial composition and degradation potential. This study offers valuable insights into microbial responses across contamination gradients and supports the development of targeted bioremediation strategies for oil-contaminated loess soils.

Keywords: crude oil pollution; microbial community; bacterial community function; soil physical-chemical properties; Loess Plateau

Introduction

Crude oil-derived hydrocarbons are among the world' s most valuable natural resources. However, inadequate safety measures and accidental spills during extraction, transportation, storage, and use frequently lead to soil contamination

(D' Ugo et al., 2021). Crude oil pollutants are complex mixtures of non-aqueous, hydrophobic substances including n-alkanes, aromatics, resins, and asphaltenes (Summers et al., 2024). These hydrocarbons readily form persistent, recalcitrant compounds in soil—often referred to as oil sludge—that can remain in the environment for extended periods. Oil sludge contains a wide range of total petroleum hydrocarbons (TPH) and polycyclic aromatic hydrocarbons (PAHs) (Khudur et al., 2019). Notably, 16 PAHs have been identified as priority pollutants by the United States Environmental Protection Agency (US EPA) due to their toxicity, mutagenicity, and carcinogenicity (Guneshwari et al., 2023).

When petroleum pollutants enter soil, they alter both biological and physical-chemical properties (Wang et al., 2021). Crude oil can adhere to soil aggregates and clog pore spaces, reducing aeration and creating anaerobic microenvironments that profoundly affect microbial communities (Gao et al., 2022). Extensive research has demonstrated that petroleum contamination affects microbial diversity, ecological clustering, functional gene expression, and metabolic pathways (Shi et al., 2022; Lu et al., 2024). For example, petroleum contaminants affect the microbiome and functional genes (Sun et al., 2019; Huang et al., 2021) while reducing microbial diversity and altering soil microbial community structure and metabolic pathways (Wang et al., 2012b; Shuaib et al., 2021; Cabral et al., 2022). They also affect soil physical-chemical properties and enzyme activities (Dindar et al., 2015; Xu et al., 2021).

Despite extensive efforts to clarify the relationship between petroleum hydrocarbons and soil microbial communities (Wang et al., 2021; Gao et al., 2022) and the identification of many petroleum-degrading strains for bioremediation (Abbasian et al., 2015; Summers et al., 2024), bioremediation effectiveness is influenced by multiple factors. These include natural conditions (Miri et al., 2019), sources of petroleum hydrocarbons (Erlacher et al., 2013), their composition and concentration (Liu et al., 2019; Hazaimeh and Ahmed, 2021), as well as the duration of pollution and the efficiency with which microbes utilize pollutants (Umeh et al., 2018). Furthermore, petroleum pollution impacts vary significantly across regions due to differences in soil physical-chemical properties (Shuaib et al., 2021; Guneshwari et al., 2023).

Studies have documented microbial impacts of petroleum pollutants in various geographic settings. For example, Liang et al. (2012), Yang et al. (2019), and Wang et al. (2023) investigated microbial communities in petroleum-contaminated soils in Northeast China. Jiao et al. (2016) and Liu et al. (2020) studied soils in the Yanchang oil field in Shaanxi Province, China. Long et al. (2017) examined petroleum impacts on the Qinghai-Xizang Plateau. However, limited research has focused on biological and physical-chemical responses to petroleum pollution in eastern Gansu Province on the Loess Plateau.

The Loess Plateau is an important agricultural base in China (Liang et al., 2024). The Changqing Oilfield Company, established in 1978 in eastern Gansu Province of the Loess Plateau, has become China' s largest oilfield since 2015,

with annual crude oil production exceeding 3.0$×10^{7}$ t (Wang et al., 2021). Eastern Gansu Province serves as the company' s main production area, but this region has experienced serious soil contamination due to crude oil extraction (Shi et al., 2022). The Loess Plateau is characterized by arid conditions and poor soil fertility (Qiu et al., 2021). Prolonged retention of petroleum hydrocarbons in loess soils increases their capacity to adsorb heavy metals and PAHs, particularly high-molecular-weight (HMW) compounds with four or more aromatic rings (Li and Li, 2021). Moreover, physical and chemical remediation methods commonly used in the region often lead to secondary pollution and soil structural degradation (Wang et al., 2021). As a sustainable and environmentally friendly approach, bioremediation remains in its early stages of application in this region (Gao et al., 2019), largely due to limited understanding of how local microbial communities respond to petroleum pollution gradients. Previous studies have indicated that analyzing microbial community structures in oil-contaminated soils can offer important insights for ecosystem restoration and environmental management (Gao et al., 2022). Therefore, investigating soil microbial communities in petroleum-polluted areas is essential to identify potential indigenous hydrocarbon-degrading microorganisms for bioremediation. However, the effects of petroleum contamination on microbial communities in eastern Gansu Province on the Loess Plateau have only been studied to a limited extent, especially regarding microbial responses to different pollution concentrations.

Against this background, the present study investigated the structural and functional responses of soil microbial communities to petroleum pollution in eastern Gansu Province on the Loess Plateau, China. This research examined differences in key environmental parameters—including contaminant levels (petroleum hydrocarbons and selected heavy metals), soil physical-chemical properties, soil enzyme activities, and soil microbial communities—across different contamination level soil groups. The main objectives were: (1) to investigate the structural and functional responses of soil microbial communities to petroleum pollution in eastern Gansu Province on the Loess Plateau; (2) to understand the relationship between microbial communities and environmental variables, including soil physical-chemical properties and oil pollution constituents; and (3) to identify potential bacterial and fungal taxa that could be utilized for remediation of petroleum-contaminated soils in this region.

2.1 Study Area and Soil Sample Collection

The study area features a gully landscape typical of the Loess Plateau, with loess as the soil type and a light loam texture. Climatically, it belongs to the warm-temperate semi-arid monsoon zone, with annual precipitation of 400–600 mm (mainly concentrated in July–September), multi-year average evaporation exceeding 1000 mm, and surface evaporation of 350–560 mm (Wang et al., 2012a). In September 2024, 30 soil samples were collected from Nanzhuang Village, Xifeng District, Qingyang City. As shown in Figure 1 [Figure 1: see

original paper], samples with varying oil contamination levels were obtained from three distinct sites, categorized into three groups: Light pollution (LP)—crude oil-contaminated soil from a decommissioned Changqing Oilfield storage station (operated during 2006–2020; 35°58 51 N, 107°56 54 E), with TPH concentrations of 1895.84–2696.54 mg/kg; Heavy pollution (HP)—soil from an active storage station (established in 2018; 35°58 52 N, 107°56 36 E), with TPH levels of 4964.25–7153.61 mg/kg; and Control (CK)—uncontaminated fallow land (35°59 13 N, 107°55 55 E), previously used for wheat and maize cultivation during 2013–2020, with minimal TPH levels of 1.81–1.92 mg/kg likely due to crude oil diffusion. Soils with TPH < 500.00 mg/kg were classified as uncontaminated (Li et al., 2022). Ten replicate samples were randomly collected from each group to ensure spatial representation. Samples were extracted from a depth of 5–25 cm using a five-point method, with composite samples prepared for each site (Wang et al., 2021). Samples for physical-chemical and TPH analyses were air-dried for one week, while those for microbial analysis were stored at -80°C for subsequent deoxyribonucleic acid (DNA) extraction (Gao et al., 2019; Liu et al., 2020).

2.2 Soil Physical-Chemical Property Measurement and Petroleum Hydrocarbon Analysis

Based on standard soil testing procedures recorded in Bao (2000), we measured soil physical-chemical properties including pH, soil water content (SWC), total nitrogen (TN), available nitrogen (AN), total phosphorus (TP), available phosphorus (AP), available potassium (AK), soil organic matter (SOM), total organic carbon (TOC), concentrations of chromium (Cr), lead (Pb), cadmium (Cd), arsenic (As), manganese (Mn), and nickel (Ni), and activities of dehydrogenase (DHA), polyphenol oxidase (PPO), urease (URE), and alkaline phosphatase (APA). After obtaining TOC, TN, and TP contents, we calculated C:N:P as the ratio of TOC:TN:TP. TPH was quantified via ultrasonic-Soxhlet gravimetry (Wang et al., 2021). Crude oil components (total saturated hydrocarbons (TSH) and total aromatic hydrocarbons (TAH)) were separated using silica gel and alumina column chromatography and analyzed using an Agilent 6890 gas chromatograph with an Agilent 5973 mass spectrometer (Agilent, Santa Clara, USA), as described previously (Gao et al., 2019). All extractions and quantifications were conducted in six replicates due to the strong sorption of PAHs in soil samples (Wang et al., 2012c). Standard compounds included five concentrations of mixtures containing 33 target n-alkanes (C8–C40) and 16 United States Environmental Protection Agency polycyclic aromatic hydrocarbons (16 US EPA PAHs) for determination of petroleum hydrocarbon components (Liu et al., 2019).

2.3 Soil Microbial Community Analysis

Total DNA was extracted from soil samples using a PowerSoil™ DNA Isolation Kit (MO BIO Laboratories, Inc., Carlsbad, USA), following the manufacturer' s instructions. Partial 16S ribosomal RNA (16S rRNA) and internal transcribed spacer (ITS) amplicons were generated for each DNA sample using barcoded primers. For bacterial 16S rRNA, primers 338F (5 -ACTCCTACGGGAGGCAGCA-3) and 806R (5 -GGACTACHVGGGTWTCTAAT-3) targeting the V3-V4 hypervariable regions were used. For the fungal ITS region, primers ITS1F (5 -CTTGGTCATTTAGAGGAAGTAA-3) and ITS2R (5 -GCTGCGTTCTTCATCGATGC-3) were used. The polymerase chain reaction (PCR) protocol was as follows: 95°C for 2 min; 25 cycles of 95°C for 30 s, 55°C for 30 s, and 72°C for 45 s; followed by 72°C for 10 min. PCR products were purified using a QIAGEN PCR Purification Kit (QIAGEN, Hilden, Germany) and pooled at equimolar concentrations (Wang et al., 2021). High-throughput sequencing was conducted by Beijing Biomarker Technologies Co., Ltd. on the Illumina HiSeq platform (Illumina, San Diego, USA). Library preparation and sequencing were performed by Beijing Biomarker Technologies Co., Ltd. Raw reads were filtered using quantitative insights into microbial ecology (QIIME) v.1.9.1 (University of Colorado, Boulder, USA) to obtain high-quality sequences. Operational taxonomic units (OTUs) were clustered at 97.00% similarity. Chao1 richness index, Shannon diversity index, and downstream bioinformatics analyses were conducted using the Biomarker Biocloud platform (https://www.biocloud.org).

2.4 Bioinformatics Pipeline

Raw sequences were quality-filtered using QIIME v.1.9.1 by removing reads with ambiguous bases, low-quality scores (Phred score < 20), or incorrect primer matches. Chimeric sequences were identified and removed using USEARCH v.10.0, developed by Dr. Robert Edgar (https://drive5.com/), against the SILVA (used for 16S sequence alignment and identification; https://www.arb-silva.de/) and UNITE (used for ITS sequence alignment and identification; https://unite.ut.ee/) reference databases. High-quality sequences were clustered into OTUs at 97.00% similarity using UPARSE. Taxonomic classification was performed with the RDP Classifier v.2.2 (the Ribosomal Database Project at Michigan State University, East Lansing, USA) using the Greengenes alignment sequence (used for identification; https://greengenes.secondgenome.com/) and UNITE (used for ITS) databases at a confidence threshold of 0.8. Alpha diversity indices (Chao1 and Shannon) and beta diversity metrics (weighted UniFrac) were calculated using QIIME v.1.9.1. Functional predictions for bacterial communities were performed using PICRUSt2 v.2.3.0 (the Huttenhower Lab at Harvard T.H. Chan School of Public Health, Boston, USA) against the KEGG (https://www.kegg.jp/kegg/) database, whereas fungal functional annotation was conducted using FUNGuild

v.1.0 (Noah Fierer' s Lab at University of Colorado, Boulder, USA).

2.5 Enumeration of Culturable Microorganisms and MBC Content

Culturable microorganisms, including number of bacteria (NB), number of fungi (NF), and number of actinomycetes (NA), were quantified by colony counting on plates using beef extract peptone, Red Bengal, and Gao No. 1 media, respectively (10 replicates) (Yao et al., 2006). The numbers of alkane-degrading bacteria (ADB) and PAH-degrading bacteria (PDB) were determined using the method described by Koohkan et al. (2023), whereas alkane-degrading fungi (ADF) and PAH-degrading fungi (PDF) were determined using the method described by Dawoodi et al. (2015). To better match regional characteristics of petroleum contaminants in eastern Gansu Province on the Loess Plateau, we supplemented the mineral salt medium with region-specific substrates: C12-C20 alkanes (N-dodecane (C12), N-tetradecane (C14), N-heptadecane (C17), and N-eicosane (C20)) for alkane degraders and a PAH mixture (10 g/L naphthalene (C13), 10 g/L phenanthrene (C14), 1 g/L pyrene (C16), and 1 g/L benzo(a)pyrene (C20), all dissolved in pentane) for PAH degraders. Microbial biomass carbon (MBC) was measured by fumigation extraction (ten replicates) (Huang et al., 2021).

2.6 Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR) Detection for TPH Degradation Function Genes

Four functional genes related to TPH degradation in the bacterial community were analyzed: alkane monooxygenase (AlkB), cytochrome P450 alkane hydroxylase (P450), catechol 2,3-dioxygenase (C23O), and naphthalene dioxygenase (Nah). Genomic DNA extracted using the PowerSoil™ DNA Isolation Kit served as the PCR template, and Ex Taq™ RR001A (TaKaRa, Kyoto, Japan) was used for amplification according to the manufacturer' s instructions. Based on the methodology of Yang et al. (2019), we processed soil samples and amplified target genes. The integrity of total DNA was normalized to 16S rRNA as an internal housekeeping gene. Relative gene expression was calculated using the $\Delta\Delta$Ct method. All experiments were performed with five replicates. Primer sequences (Table S1) were designed based on conserved regions of well-characterized hydrocarbon-degrading bacterial strains, as reported in previous studies (Park and Crowley, 2006; van Beilen et al., 2006; Xie et al., 2014; Long et al., 2017).

2.7 Statistical Analysis

Statistical analyses and mapping were performed using SPSS v.23.0 (IBM, Chicago, USA) and R v.4.2.0 (https://www.R-project.org/). Intergroup comparisons were performed using one-way analysis of variance (ANOVA) with Duncan' s multiple range test ($P<0.050$). We conducted principal component analysis (PCA) and redundancy analysis (RDA) based on Bray-Curtis dissimilarity and relative abundances of microbial communities at the phylum and genus levels using Wekemo Bioincloud (https://www.bioincloud.tech). Functional prediction of microbial metabolism was performed using the FAPROTAX database (for 16S rRNA dataset) and FUNGuild database (for ITS dataset) on the basis of KEGG data, by matching OTUs to known functional profiles within these databases. Linear Discriminant Analysis Effect Size (LEfSe) was used to identify differentially abundant taxa, with a linear discriminant analysis (LDA) score threshold over 4.0. We constructed co-occurrence networks (top 100 genera) using Spearman' s correlation ($| |>0.7$, $P<0.010$) in Gephi v.0.9.3 (The Gephi Consortium, Paris, France; https://gephi.org/) (Jiao et al., 2016), with network modules subdivided according to Jia et al. (2023). Keystone taxa were identified based on three topological properties: high degree, low betweenness centrality, and high closeness centrality (Jia et al., 2023). The niche breadth (B') and niche overlap (Oij) were calculated using the Shannon-Wiener index-based formulas (Levins, 1968; Colwell and Futuyma, 1971):

$$B' = 1 / \Sigma(Pi^2)$$
$$Oij = \Sigma[\min(Pik, Pjk)]$$

where Pi is the relative abundance of species i under specific pollution concentration; n is the total number of measurable species; Pik and Pjk are the relative abundances of species i and j under the kth pollution level, respectively; and m is the total number of pollution levels being evaluated.

Based on Bray-Curtis dissimilarity, we used non-metric multidimensional scaling (NMDS) to visualize microbial community structures. Mantel tests ($P<0.050$) were conducted to identify key environmental drivers, with variable fitting performed using the envfit function from the vegan package in R v.4.2.0. NMDS axes were rotated to maximize variance explained by the first axis (Wang et al., 2021).

3.1 Concentration of Soil Pollutants

The HP group exhibited the highest TPH content, followed by the LP group, whereas only trace amounts were detected in the CK group, likely due to diffusion ($P<0.050$) (Table 1). Compared with the CK group, the LP and HP groups demonstrated significantly higher TSH by 1304.37- and 3986.49-fold and higher TAH levels by 8830.83- and 27,481.00-fold, respectively. Similarly, concentrations of Cr, Pb, Cd, As, Mn, and Ni followed the same trend: highest in HP,

followed by LP, and lowest in CK (P<0.050), with Cr, Cd, and As undetected in CK. Among the 16 US EPA PAHs, only those present in contaminated soils (LP and HP) were analyzed. The HP group showed significantly higher concentrations of bicyclic, tricyclic, tetracyclic, pentacyclic, and hexacyclic aromatic hydrocarbons than the LP group, with increases of 51.09%, 61.83%, 299.01%, 187.87%, and 212.31%, respectively (P<0.050).

3.2 Soil Physical-Chemical Properties

Compared with the CK group, the average contents of SWC, TN, TP, AN, AP, AK, and SOM decreased by 71.90%, 55.71%, 52.39%, 58.66%, 33.45%, 51.56%, and 74.20% in the LP group, respectively, and by 76.90%, 77.14%, 61.05%, 72.22%, 63.63%, 64.15%, and 78.84% in the HP group, respectively (P<0.050) (Table 2). The average pH and TOC values increased by 0.29 and 287.48% in the LP group, and by 0.41 and 700.68% in the HP group, respectively, compared with CK (P<0.050). In addition, the C:N:P ratio significantly increased in crude oil-contaminated soils relative to CK and increased progressively with increasing pollution levels (P<0.050). These findings suggest that crude oil contamination elevated soil pH and TOC, disrupted the C:N:P balance, and had an increasingly inhibitory effect on soil nutrients as contamination worsened.

Furthermore, DHA and PPO activities increased by 116.84% and 138.88% in the LP group and by 125.00% and 294.44% in the HP group, respectively (P<0.050) (Fig. 2a [Figure 2: see original paper] and b). Conversely, URE and APA activities decreased by 41.68% and 53.01% in the LP group and by 63.86% and 77.91% in the HP group, respectively (P<0.050) (Fig. 2c and d). These trends indicate that crude oil pollution promoted DHA and PPO activities, which are key enzymes in alkane and aromatic hydrocarbon degradation, while suppressing URE and APA essential for nitrogen and phosphorus cycling, highlighting the ecotoxicological effects of petroleum contamination.

3.3 Enumeration of Different Culturable Microorganisms and MBC Content

Compared with the CK group, the numbers of NB and NF increased by 70.14% and 35.48% in the LP group, respectively, but decreased by 55.92% and 58.35% in the HP group, respectively (P<0.050) (Fig. 3a [Figure 3: see original paper] and b). NA increased significantly by 150.88% and 115.42% in the LP and HP groups, respectively, compared with the CK group (P<0.050) (Fig. 3c). The abundance of oil-degrading microorganisms, both ADB and PDB, was highest in the HP group and lowest in the CK group (P<0.050) (Fig. 3d and e). LP had the highest abundance of ADF and PDF, followed by HP, with CK showing the lowest levels (P<0.050) (Fig. 3f and g). Finally, the average MBC content

increased by 34.71% in the LP group but decreased by 32.69% in the HP group compared with the CK group (P<0.050) (Fig. 3h).

3.4 Alpha Diversity Indices of Soil Microbial Community

Compared with the CK group, the Shannon and Chao1 indices of the soil bacterial community decreased by 20.41% and 33.28%, respectively, in the LP group, and by 21.95% and 41.93%, respectively, in the HP group (P<0.050) (Fig. 4a [Figure 4: see original paper] and b). For the soil fungal community, the Shannon and Chao1 indices decreased by 13.36% and 72.20% in LP and by 42.58% and 80.37% in HP, respectively, compared with CK (P<0.050) (Fig. 4c and d). The CK group had the highest number of unique OTUs for bacteria (10,404) and fungi (2726). In contrast, the number of unique bacterial OTUs decreased by 53.04% (LP) and 55.09% (HP), and fungal OTUs decreased by 63.87% (LP) and 94.09% (HP), respectively, compared with CK (Fig. 4e and f). Moreover, the number of shared bacterial OTUs between CK and LP and HP was 251 and 266, respectively, which was significantly lower than the 971 shared by LP and HP. Similarly, shared fungal OTUs with CK were 72 and 7 for LP and HP, respectively, remarkably lower than 215 shared between LP and HP. These results suggest that crude oil pollution significantly altered the OTU distribution of soil microbial communities, especially in fungal communities, whereas communities between LP and HP remained more similar.

3.5 Soil Microbial Community Composition

In the CK group, the dominant bacterial phyla (relative abundance > 1.00%) were *Acidobacteriota* (15.90%), *Chloroflexi* (10.39%), *Myxococcota* (7.84%), *Gemmatimonadota* (3.23%), *Verrucomicrobiota* (1.64%), and *Methylomirabilota* (1.65%). Their abundances decreased significantly in the LP (52.53%–97.10%) and HP (81.67%–98.35%) groups (P<0.050), demonstrating phylum-level community differences between contaminated and uncontaminated soils (Fig. 5a [Figure 5: see original paper]). In contaminated soils, *Actinobacteriota* (23.18%), *Chloroflexi* (4.24%), and *Patescibacteria* (3.73%) were more abundant in the LP group than in the HP group (P<0.050). In contrast, *Proteobacteria* (53.99%), *Bacteroidota* (9.50%), *Firmicutes* (2.61%), *Desulfobacterota* (1.12%), and *Campylobacterota* (1.50%) were more prevalent in the HP group (P<0.050), indicating a concentration-dependent bacterial response to oil pollution.

Among fungi, CK and LP were dominated by *Ascomycota*, *Basidiomycota*, *Glomeromycota*, and *Mortierellomycota* (98.91% and 99.35%, respectively), whereas HP was dominated by *Ascomycota* and *Basidiomycota* (98.63%) (Fig. 5b). Compared with the CK group, *Ascomycota* increased by 66.28% and 99.31% in LP and HP groups, respectively (P<0.050), whereas *Basidiomycota*

decreased by 73.45% and 94.44% in the LP and HP groups, respectively. *Glomeromycota* decreased significantly in both LP (96.76%) and HP (99.82%) (P<0.050), indicating petroleum-induced suppression of this phylum. These findings confirm that oil pollution altered fungal community structure in a concentration-dependent manner.

At the genus level, soil bacterial communities demonstrated distinct distribution patterns (Fig. 5c). The CK group had significantly higher abundances of *unclassified_{Gemmatimonadaceae}* (8.35%), *unclassified_{Bacteria}* (7.36%), *unclassified_{Chloroflexi}* (2.83%), *Vicinamibacteraceae* (5.15%), *Solimonadaceae* (4.73%), *Promicromonospora* (4.44%), *Vicinamibacterales* (3.13%), and *MND1* (3.18%) than the LP and HP groups (P<0.050). In contaminated soils, the LP group was enriched in *Pseudoxanthomonas* (6.64%), *Immundisolibacter* (5.21%), *Proteiniphilum* (3.53%), *Bryobacter* (1.61%), *Iamia* (1.63%), and *unclassified_{Sandaracinaceae}* (2.00%) (P<0.050), whereas the HP group showed higher abundances of *Pseudomonas* (6.29%), *Nocardioides* (2.03%), and *Actinomyces* (1.95%) (P<0.050). Notably, *Marinobacter* (10.23%), *Idiomarina* (7.25%), *Halomonas* (3.50%), *Marinobacterium* (3.21%), *Thalassospira* (2.73%), and *Thalassolituus* (2.54%) were exclusively detected in HP and absent in LP and CK.

For fungi, the CK group had higher relative abundances of *unclassified_{Glomeraceae}* (4.91%), *Glomus* (3.26%), *Filobasidium* (2.90%), and *unclassified_{Pleosporales}* (2.76%) than others (P<0.050) (Fig. 5d). The LP and HP groups were enriched in *unclassified_{Sordariomycetes}* (7.98% and 2.73%, respectively), *Schizothecium* (6.39% and 3.34%, respectively), *Podospora* (4.70% and 2.15%, respectively), and additional taxa (P<0.050), with generally higher fungal abundances in LP than in HP. *Pseudallescheria* was the most distinctive genus: its relative abundance was 50.68% in HP, 0.14% in LP, and absent in CK. PCA revealed distinct genus-level community structures (Fig. 5e and f), with LP and CK separated along principal component 1 (PC1), whereas HP was separated along principal component 2 (PC2), indicating significant compositional differences between uncontaminated, lightly contaminated, and heavily contaminated soils.

3.6 Co-Occurrence Network Analysis

Network analysis revealed significant structural changes in microbial communities under oil contamination. Bacterial modularity indices were 0.406 (CK), 0.488 (LP), and 0.419 (HP); all values exceeded 0.400, indicating modularity. Fungal modularity indices were 0.620 (CK), 0.492 (LP), and 0.428 (HP). Compared with CK, bacterial network edges increased by 72.46% (LP) and 141.68% (HP), whereas fungal edges increased by 54.55% (LP) and 109.76% (HP), reflecting enhanced microbial interactions and complexity (Table 3). Contaminated soils exhibited shorter average path lengths (for bacteria: decreasing from 2.436

to 1.870, then to 1.738; and for fungi: decreasing from 3.535 to 2.771, then to 2.701) and higher cluster coefficients (for bacteria: increasing from 0.551 to 0.748; and for fungi: increasing from 0.386 to 0.661), indicating tighter microbial connectivity with increasing oil concentrations.

Positive correlations (symbiosis) in bacterial networks increased from 65.99% (CK) to 68.68% (LP) and 84.19% (HP), and those in fungal networks increased from 58.61% (CK) to 69.02% (LP) and 89.89% (HP) (Fig. S2). Bacterial modularity decreased in LP and HP (Fig. 7a–c [Figure 7: see original paper]), indicating simplified functional modules, whereas fungal modularity became more complex in HP (Fig. 7d–f). This divergence reflected distinct degradation strategies: bacteria streamlined interactions for efficiency, while fungi developed specialized modules for synergistic pollutant degradation. These structural shifts indicated that crude oil contamination reshaped microbial communities, enhanced cooperation in polluted soils, and promoted functional adaptation to hydrocarbon degradation.

The keystone taxa and their niche traits were also analyzed across CK, LP, and HP (Fig. 8 [Figure 8: see original paper]). Bacterial keystones in CK included *Nordella*, *unclassified_{env}.OPS_{17}*, and *unclassified_{Bacteria}*. LP harbored *Defluviicoccus*, *Pontibacter*, *Promicromonospora*, and others (Fig. 8a). HP had a more diverse set, including *Promicromonospora*, *Lysobacter*, and *Acinetobacter*. Fungal keystones in CK included *Penicillium*, *Curvularia*, and *Mortierella*, whereas those in LP were enriched in *Mortierella*, *Exophiala*, *Pseudogymnoascus*, *Schizothecium*, and *Wickerhamomyces* (Fig. 8d). Notably, CK keystones (e.g., *Nordella* and *Mortierella*) were associated with broad nutrient cycling niches, whereas LP and HP keystones (e.g., *Promicromonospora* and *Schizothecium*) were linked to crude oil degradation (Fig. 8b and e). The niche overlap of keystones was highest in contaminated soils (Fig. 8c and f), suggesting broader microbial participation and higher overlap due to the complex composition of crude oil (alkanes and aromatics).

3.7 Change in Soil Microbial Metabolic Pathway

Figure 9 [Figure 9: see original paper] illustrates functional pathways in the three soil types, as predicted by FAPROTAX (based on the 16S rRNA dataset) and FUNGuild (ITS dataset). Bacterial metabolic pathways varied significantly with contamination level. Compared with CK, LP showed enrichment in fermentation, nitrogen respiration, and nitrogen fixation (Fig. 9a1 and a2). HP showed greater enrichment in aromatic compound degradation, hydrocarbon degradation, methanol oxidation, methylotrophy, and oil bioremediation compared with CK (Fig. 9b1 and b2). HP also had an advantage in hydrocarbon metabolism, methanol oxidation, and methylotrophy over LP (Fig. 9c1 and c2), suggesting enhanced metabolic function in highly polluted soils. For fungi, saprotrophs dominated in LP and HP, whereas symbiotrophs and pathotrophs were more

abundant in CK (Fig. 9d1, d2, e1, and e2). In contaminated soils, saprotroph abundance peaked in HP, whereas symbiotrophs and pathotrophs were more prevalent in LP (Fig. 9f1 and f2).

3.8 Change in Relative Expression of Functional Genes Related to TPH Degradation

Compared with CK, the relative expression levels of two alkane degradation genes (AlkB and P450) increased 11.85- and 42.61-fold in LP (Fig. 10a [Figure 10: see original paper]) and 5.25- and 6.64-fold in HP (Fig. 10b), respectively ($P<0.050$). For two aromatic hydrocarbon degradation genes (C23O and Nah), expression increased 17.42- and 110.53-fold in LP (Fig. 10c) and 87.39- and 213.29-fold in HP (Fig. 10d), respectively ($P<0.050$). These results suggest that increasing crude oil pollution stimulated the growth of oil-degrading bacteria and upregulated functional genes linked to TPH degradation, supporting the potential use of indigenous microbes in bioremediation of soils in eastern Gansu Province on the Loess Plateau.

3.9 Response of Soil Microbial Communities to Environmental Factors

Figures 11a and 11b [Figure 11: see original paper] present relationships between soil microbial communities (at the genus level) and environmental factors (crude oil contamination, nutrients, culturable microbes, alpha diversity, and functional genes) based on NMDS. Bacterial communities in the CK group primarily clustered on the positive half of NMDS1, whereas LP- and HP-contaminated groups mainly clustered on the negative half (Fig. 11a). In contrast, fungal communities in CK were mainly on the negative half of NMDS2, whereas LP and HP clustered on the positive half (Fig. 11b). Mantel test results ($P<0.050$) identified the following key discriminatory factors. For bacteria, AN, SWC, TN, SOM, AP, Shannon index, and Chao1 index distinguished CK from contaminated soils. C23O, Cr, Nah, TPH, pH, PPO, Cd, and TOC separated HP from LP. NB, NA, PDB, ADB, MBC, and DHA differentiated LP from HP. For fungi, Chao1, TN, SWC, Shannon index, AN, SOM, and URE differentiated CK from LP and HP. NF, PDF, ADF, and MBC separated LP from HP. Mn, TPH, Cr, pH, TOC, PPO, and DHA further distinguished HP from LP (Figs. 11b and S3). Uncontaminated soils were characterized by higher nutrient content, enzyme activity, and diversity, whereas contaminated soils were mainly differentiated by pollutant concentrations, especially between LP and HP groups.

RDA further clarified environmental-microbial associations (Figs. 11c, d, and S4). For bacteria, Shannon index, Chao1 index, SWC, SOM, AN, and TN were positively correlated with *unclassified_{Gemmatimonadaceae}*, *Vicinamibacter-*

aceae, and *Subgroup_7*, clustered on the negative side of RDA1 (Fig. 11c). NB and MBC positively correlated with *Bryobacter*, *Cellulomonas*, and *Streptomyces* on the positive side of RDA2. NA, DHA, Cr, TPH, and 16 US EPA PAHs were positively correlated with *Thalassolituus*, *Pseudomonas*, and *Marinobacter*, located on the negative side of RDA2. For fungi, Shannon index, Chao1 index, SWC, AN, SOM, and URE positively correlated with *unclassified_{Glomeraceae}*, *Glomus*, and *Vishniacozyma*, clustered on the negative side of RDA1 (Fig. 11d). MBC, NF, and PDF were positively correlated with *Apodus*, *Podospora*, and *Cladosporium* on the positive side of RDA2. Moreover, 16 US EPA PAHs, TPH, Cr, and DHA showed strong correlations with *Alternaria* and *Fusarium* on the negative side of RDA2.

4.1 Crude Oil Pollutants Significantly Inhibited Soil Nutrients and Alpha Diversity of Microbial Community

The Loess Plateau, a vital agricultural region in China, is experiencing increasing environmental degradation due to crude oil pollution (Shi et al., 2022; Liang et al., 2024). In line with previous studies showing that co-contamination with crude oil and heavy metals can reduce soil nutrients and microbial diversity, with adverse effects intensifying with pollution severity (Dindar et al., 2015; Li et al., 2020; Shuaib et al., 2021), our findings revealed that contaminated soils had significantly lower nutrient levels (TN, TP, AN, AP, AK, SOM, and SWC) and reduced microbial alpha diversity than uncontaminated controls. This inhibitory effect was more pronounced at higher pollution levels. In contrast, TOC and pH were elevated in polluted soils (Table 2; Fig. 4). These changes could be influenced by inherent soil physical-chemical properties, hydrocarbon types, and pollution duration (Erlacher et al., 2013; Chanokporn et al., 2018; Guneshwari et al., 2023).

Three main mechanisms may be involved. First, petroleum pollutants can exert direct toxic effects. HP soils contained significantly higher concentrations of toxic and carcinogenic 16 US EPA PAHs and heavy metals (Table 1). High-molecular-weight (HMW) PAHs ($ $4aromaticrings), $whicharemorepersistentandrecalcitrantthanlow−molecular−weight(LMW)PAHs(Wangetal., 2012c), $tendtograduallyaccumulateinsoilmicroporesthroughme lowers exchangeable acidity and effective cation exchange capacity, leading to elevated soil pH (Wang et al., 2013). NMDS and RDA results identified pH and SWC as key drivers shaping microbial community structure (Fig. 11). High pH and low SWC restrict pollutant migration and promote accumulation of toxic substances in dry loess soils, thereby depleting nutrients and impairing biogeochemical cycling (Chanokporn et al., 2018; Umeh et al., 2018).

Third, microbial biodegradation processes can accelerate nutrient depletion. Crude oil pollution can trigger shifts in microbial communities (Gao et al., 2019; Liu et al., 2019). LP soils had higher abundances of culturable bacteria and fungi, whereas HP soils showed increased levels of ADB, PDB, ADF,

and PDF compared with CK soils (Fig. 3), indicating active biodegradation. Although this process promotes proliferation of oil-degrading microbes, high contamination levels can reduce microbial diversity and richness. These specialized degraders tend to prioritize hydrocarbon metabolism over native organic matter decomposition, accelerating nutrient consumption and disrupting natural cycling (Chen et al., 2023). This creates a feedback loop that favors stress-tolerant specialist taxa (Chanokporn et al., 2018; Hazaimeh and Ahmed, 2021). The abundance of actinomycetes was significantly higher in polluted soils, and MBC peaked in LP soils but declined under HP conditions (Fig. 3c and h), consistent with previous studies (Liu et al., 2020; Shuaib et al., 2021; Wang et al., 2023; Xu et al., 2023). Although low pollution levels may supply carbon substrates that stimulate microbial activity, high levels may exert toxic effects. The strong hydrocarbon-degrading capacity of *Actinobacteria* may explain their enrichment in polluted environments. These findings highlight the dual role of microorganisms: while they support bioremediation of oil-contaminated soils, they may also contribute to declining soil fertility, underscoring the importance of integrating nutrient management into bioremediation strategies.

4.2 Response of Soil Microbial Community Structure to Crude Oil Contamination

Previous studies have shown that soil microbial community structure undergoes substantial shifts under the influence of crude oil concentration, hydrocarbon composition, and pollution duration (Erlacher et al., 2013; Chanokporn et al., 2018; Wang et al., 2022; Guneshwari et al., 2023). In this study, microbial communities at the phylum level demonstrated clear differentiation between contaminated and uncontaminated soils. *Chloroflexi*, *Gemmatimonadota*, *Myxococcota*, *Verrucomicrobiota*, and *Methylomirabilota* were enriched in uncontaminated soils, whereas *Proteobacteria*, *Actinobacteriota*, *Bacteroidota*, and *Patescibacteria* dominated in contaminated soils (bacterial phyla), along with *Ascomycota* (fungal phylum) (Fig. 5a and b). These findings are consistent with previous reports identifying *Proteobacteria*, *Actinobacteriota*, and *Bacteroidota* as key bacterial phyla involved in petroleum degradation (Chanokporn et al., 2018; Li et al., 2022; Jia et al., 2023; Xu et al., 2023), while *Ascomycota* (along with *Basidiomycota* and *Mucor* fungi) are major fungal groups contributing to hydrocarbon degradation (Wang et al., 2012b; Simister et al., 2015; Maryam et al., 2023), indicating adaptive responses of phylum-level communities to pollutant stress (Wang et al., 2021; Xu et al., 2023).

At the genus level, LEfSe analysis confirmed distinct enrichment patterns (Fig. 6 [Figure 6: see original paper]). LP soils were dominated by *Pseudoxanthomonas*, *Immundisolibacter*, *Solimonadaceae*, *Promicromonospora*, and *Proteiniphilum* (Fig. 5c). In HP soils, *Pseudomonas*, *Nocardioides*, and *Actinomyces* remained enriched, whereas *Marinobacter*, *Idiomarina*, and *Halomonas* were uniquely present in HP soils. For fungi, *Schizothecium*, *Podospora*, *Al-*

ternaria, *Pyrenochaetopsis*, and *Acremonium* were more abundant in contaminated soils (Fig. 5d). *Pseudallescheria* exhibited the most striking variation: 50.68% in HP, 0.14% in LP, and undetectable in CK. These genus-level shifts aligned with TPH concentration gradients (Table 1), consistent with previously reported activity thresholds (Wang et al., 2010; Xu et al., 2023). Alkanes accounting for 50.00%–75.00% of crude oil are preferentially degraded; however, microbial consortia often co-metabolize aromatic hydrocarbons, reflecting their broad metabolic adaptability (Ahmed et al., 2023).

In LP soils, *Pseudoxanthomonas* and *Immundisolibacter* are known to degrade pyrene (Klankeo et al., 2009; Maiysha et al., 2011) and target phenanthrene (Summers et al., 2024), while *Proteiniphilum* and *Bryobacter* participate in naphthene degradation (Zhang et al., 2021). *Immundisolibacter*, together with *Promicromonospora*, participates in naphthene degradation (Zhang et al., 2021). In HP soils, *Marinobacter*, *Pseudomonas*, and *Halomonas* were enriched and degraded both alkane and HMW PAHs. Some of these genera produce biosurfactants or function anaerobically to enhance their degradation capacity (Kodama et al., 2008; Gutierrez et al., 2015; Zenati et al., 2018; Zhu et al., 2024). Fungal taxa such as *Pseudallescheria* dominant in HP soils can degrade HMW PAHs, tolerate heavy metals, and produce surfactants critical for heavy oil breakdown (Boelter et al., 2018; Ren et al., 2020; Summers et al., 2024). *Schizothecium* also degrades HMW PAHs (Gałązka et al., 2020), whereas *Podospora* contributes to alkane degradation (Maryam et al., 2023). Contaminated soils exhibited broader ecological niches and greater niche overlap among keystone taxa (Fig. 8b, c, e, and f), indicating enhanced metabolic versatility that supports degradation of complex hydrocarbon mixtures. The increased overlap also reflects higher functional redundancy and microbial cooperation, enabling more coordinated responses to petroleum degradation under polluted conditions (Huang et al., 2021; Mekonnen et al., 2024). Co-occurrence network analysis further revealed an increase in positive correlations (symbiotic relationships) with increasing pollution levels (Figs. 7 and S2), consistent with earlier findings (Huang et al., 2021; Jia et al., 2023). These patterns suggest enhanced microbial cooperation in response to pollutant stress.

In summary, crude oil contamination substantially altered the structure of soil microbial communities in eastern Gansu Province on the Loess Plateau. Pollution severity drives selection of functionally specialized taxa. Indigenous genera including *Pseudomonas*, *Nocardioides*, and *Marinobacter* (bacteria), as well as *Pseudallescheria* and *Schizothecium* (fungi), show potential for bioremediation of oil-polluted soils in this region.

4.3 Response of Soil Microbial Community Function to Crude Oil Contamination

Crude oil pollution alters soil microbial community function due to its ecotoxicity. Although not all hydrocarbons serve as direct substrates, elevated concentrations can stimulate microbial biodegradation, with bacteria acting as primary degraders using pollutants for energy (Xu et al., 2023). Petroleum biodegradation involves a series of metabolic reactions catalyzed by diverse enzymes, such as oxygenases, oxidoreductases, hydroxylases, and dehydrogenases, which function under both aerobic and anaerobic conditions (Varjani and Upasani, 2017). Among these, alkane monooxygenases play a key role in initiating alkane degradation. Microorganisms carrying *AlkB* and *P450* genes possess enhanced short-chain alkane degradation capacities (Yang et al., 2019). In this study, Figures 10a and 10b show significantly increased expression of *AlkB* and *P450* genes in contaminated soils compared with uncontaminated soils, with greater expression at higher contamination levels. This trend was consistent with changes in DHA activity (Fig. 2a), which correlates with oxidative decomposition of alkanes (Xu et al., 2021), indicating greater abundance of *AlkB*- and *P450*-harboring degraders in HP soils than in LP soils.

NMDS results identified NB, NA, PDB, ADB, MBC, and DHA as key factors distinguishing LP bacterial communities (Fig. 11a). RDA revealed positive correlations between these indicators and crude oil-degrading genera such as *Bryobacter*, *Cellulomonas*, and *Streptomyces* on the positive side of RDA2. NA, DHA, Cr, TPH, and 16 US EPA PAHs were positively correlated with *Thalassolituus*, *Pseudomonas*, and *Marinobacter*, located on the negative side of RDA2. For aromatic pollutants, C23O and Nah serve as degradation markers. C23O is involved in breaking down compounds such as benzene, toluene, ethylbenzene, xylene, and catechol (Jiang et al., 2004), whereas Nah-related genes target biphenyl, toluene, naphthalene, and phenol (Lu et al., 2024). Our findings demonstrated increased expression of C23O and Nah at higher oil concentrations (Fig. 10c and d), consistent with PPO activity patterns (Fig. 2b) associated with aromatic degradation (Zuzanna et al., 2016; Chanokporn et al., 2018). These results suggest that C23O and Nah are key functional markers of hydrocarbon degradation in HP soils.

NMDS identified the 16 US EPA PAHs, PPO, C23O, and Nah as key drivers of bacterial community differentiation in HP soils (Fig. 11a), while RDA linked these factors to aromatic-degrading genera such as *Alternaria*, *Sarocladium*, *Acremonium*, *Bipolaris*, *Fusarium*, and *Pseudallescheria* (Fig. 11c). Although NMDS and RDA revealed no significant correlation between *AlkB* expression and LP-dominant bacteria (Fig. S4), strong positive correlations were observed between C23O and Nah expression and HP-dominant communities. This could be due to higher concentrations of aromatic pollutants in HP soils (Table 1), which imposed stronger selective pressure. These patterns aligned with increased abundance of PDB and PDF in HP soils (Fig. 3e and g), underscoring the greater influence of aromatic hydrocarbons on microbial function (Xu et al.,

2023). Metabolic pathway analysis further demonstrated higher abundances of aromatic compound degradation, aerobic heterotrophy, and hydrocarbon degradation pathways in HP soils than in LP soils (Fig. 9c). These findings were consistent with trends in functional gene expression and enzyme activity, suggesting that pollutant concentration is a key driver of pathway enrichment. In summary, crude oil concentration shaped microbial community structure, which could govern functional responses to pollution stress.

Co-occurrence network analysis revealed a greater proportion of positive microbial correlations with increasing contamination levels (Figs. 7 and S2), consistent with Jiao et al. (2016), who noted that high contamination promoted niche specialization and reduced competition. This reflects resource limitation under high hydrocarbon stress (Qiu et al., 2021), where cooperation replaces competition. In less polluted soils, networks enhance interactions based on shared carbon use (Jia et al., 2023), whereas in highly polluted conditions, interactions can reflect functional complementarity (Bertness and Callaway, 1994). This promotes functional convergence as oil-degrading species dominate. Microbes have adopted buffering strategies to collectively mitigate pollution impacts (Durán et al., 2018). Overall, crude oil-contaminated soils, particularly heavily polluted soils, exhibit stronger microbial responses, including shifts in both taxonomic composition and functional roles, supporting a stress-driven microbial adaptation mechanism for pollutant degradation.

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5 Conclusions

This study investigated the ecological impacts of crude oil pollution and associated microbial adaptive mechanisms in eastern Gansu Province on the Loess Plateau. Our results showed that contamination significantly reduced key soil nutrients (TN, TP, AN, AP, AK, and SOM) and microbial alpha diversity, while simultaneously increasing TOC and pH levels. Microbial communities displayed pollution-dependent structural changes: *Pseudoxanthomonas* dominated in lightly polluted soils, whereas *Pseudomonas*, *Nocardioides*, and *Pseudallescheria* became prominent hydrocarbon degraders in heavily polluted areas. Enhanced expression of functional genes (*AlkB*, *P450*, *C23O*, and *Nah*) and a high proportion (85.00%) of positive microbial interactions indicated adaptive synergistic responses. These findings deepen our understanding of pollution ecology in arid environments, provide a scientific foundation for bioremediation using indigenous microbial consortia, and support targeted strategies for soil health recovery in the ecologically vulnerable eastern Gansu Province on the Loess Plateau.

Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix

Table S1. Sequence information of gene primer pair

Primer	Primer sequence	Reference
AlkB-F	5 -AACTACMTCGARCAYTACGG-3	Long et al. (2017)
AlkB-R	5 -TGAMGATGTGGTYRCTGTTCC-3	Long et al. (2017)
P450-F	5 -GATGAAGAAGGGCGATTGGA-3	van Beilen et al. (2006)
P450-R	5 -CCTTGATGTTGGCAGGTAGGA-3	van Beilen et al. (2006)
C23O-F	5 -ATTTAGGTGCTCGGTTTCTATCTGTTTA-3	Xie et al. (2014)
C23O-R	5 -ATTTATGGTCTTGCCGTGAGTGTTTA-3	Xie et al. (2014)

Primer	Primer sequence	Reference
Nah-F	5 -TGAMGATGTGGTYRCTGTTCC-3	Park et al. (2006)
Nah-R	5 -CAGGTCAGCATGCTGTTGTT-3	Park et al. (2006)

Note: F, forward; R, reverse.

Fig. S1. Indicator bacterial (a) and fungal (b) groups within the three groups with LDA scores higher than 4.0. Denote microbial taxon at the phylum, class, order, family, and genus level with the prefix "p___ "," c **"," o"** , "f **", and" g"** .

Fig. S2. Co-occurrence network of bacterial (a–c) and fungal (d–f) genera based on positive and negative correlation analysis across different oil contaminated soil groups. Nodes are connected by lines only when significantly correlated (Spearman' s | |>0.70, P<0.010). Node size reflects the number of connections; colors indicate modularity classes.

Fig. S3. Variance in bacterial (a) and fungal (b) communities explained by different soil environmental factors. R^2 reflects the proportion of community variation explained by each factor. *, significance at P<0.050 level; **, significance at P<0.010 level.

Fig. S4. Heatmap of Spearman' s correlation coefficient between the relative abundance of bacterial (a) and fungal (b) communities at genus level and environmental factors. Dendrograms (top) illustrate hierarchical clustering of taxa (left) and environmental factors (bottom) based on similarity in correlation patterns. *, significance at P<0.050 level;* ***, significance at P<0.010 level;*** , significance at P<0.001 level. To control the family-wise error rate, this study adopted Bonferroni correction.

Note: Figure translations are in progress. See original paper for figures.