



Effect of petroleum hydrocarbon pollution levels on the soil microecosystem and ecological function[☆]

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ABSTRACT

Petroleum hydrocarbon pollution is a global problem. However, the effects of different petroleum pollution levels on soil microbial communities and ecological functions are still not clear. In this study, we analyzed the changes in microbial community structures and carbon and nitrogen transformation functions in oil-contaminated soils at different concentrations by chemical analysis, high-throughput sequencing techniques, cooccurrence networks, and KEGG database comparison functional gene annotation. The results showed that heavy petroleum concentrations (petroleum concentrations greater than 20,000 mg kg⁻¹) significantly decreased soil microbial diversity ($p = 0.01$), soil microbiome network complexity, species coexistence patterns, and prokaryotic carbon and nitrogen fixation genes. In medium petroleum contamination (petroleum concentrations of between 4000 mg kg⁻¹ and 20,000 mg kg⁻¹), microbial diversity ($p > 0.05$) and carbon and nitrogen transformation genes showed no evident change but promoted species coexistence patterns. Heavy petroleum contamination increased the *Proteobacteria* phylum abundance by 3.91%–57.01%, while medium petroleum contamination increased the *Actinobacteria* phylum abundance by 1.69%–0.26%. The results suggested that petroleum concentrations played a significant role in shifting soil microbial community structures, ecological functions, and species diversities.

1. Introduction

Soil pollution due to increasing human activity is one of the major issues faced by developing and developed industrial societies (Brown et al., 2017; Ramadass et al., 2018; Fanaei et al., 2020). One of the major soil pollutants is petroleum, which negatively affects terrestrial and aquatic ecosystems by releasing toxic hydrocarbons during production, operation, use, and transportation (Karthick et al., 2019; Liu et al., 2020b; Benguenab and Chibani, 2021). Petroleum contamination alters the metabolic activities of microorganisms (Obafemi et al., 2018; Wood et al., 2017; Tao et al., 2019; Baoune et al., 2018). In China, northern Shaanxi Province has suffered severe petroleum pollution due to numerous and scattered oil wells in the regions. (Wu et al., 2019; Wu et al., 2020).

Many biological strategies developed for the remediation of oil-contaminated soils utilize microorganisms to metabolize these toxic

compounds as substrates (Wang et al., 2019a; Ferguson et al., 2020; Liu et al., 2020c). Previous research has focused on the isolation and screening of pure strains for degrading petroleum hydrocarbons (Ojewumi et al., 2018; Durval et al., 2020). However, bioremediation is a complex process that involves cometabolism and cross-induction among different microorganisms (Dean-Ross et al., 2002; Maletić et al., 2021). Information on the impacts of different petroleum contents on microbial communities can provide guidance for the rehabilitation of polluted environments. Thus, it is necessary to understand the responses of microbiomes toward petroleum hydrocarbons for the establishment of remediation technologies (Yousuf et al., 2019; Wang et al., 2019b).

Soil has special ecological functions, including nitrogen fixation, transformation, and carbon sequestration (Duchesneau et al., 2020; Hesnawi and Adbeib, 2013; Rudi et al., 2007). Carbon and nitrogen are important nutrients that affect plant physiology and photosynthesis. Some studies indicated that petroleum contamination did not greatly

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affect soil N and P but increased soil organic matter and pH (Zhao et al., 2020). However, the way in which oil pollution concentrations affect the soil carbon and nitrogen transformation function is still not clear.

At present, next-generation sequencing (NGS) has been highly popular in analyzing the microbial diversity of biological systems, such as amplicon sequencing used for variant identification and phylogenetic surveys and genome shotgun sequencing for single organism genomes and metagenomes. These methods provide a new perspective on studying soil microbial communities and their functional characteristics (Okoye et al., 2020; AlKaabi et al., 2020; Morgan and Huttenhower, 2014; Cordero and Datta, 2016; Acin-Albiac et al., 2020). Essential information required for the development of bioremediation technologies includes the response of microbes to petroleum hydrocarbons and their functional change with the immediate environment. Although much work has been done to explore the microbial community composition shift caused by oil pollution, few have studied the microecosystem and ecological function in different degrees of oil pollution. In this study, we collected 20 soil samples from oil exploration regions in northern Shaanxi Province, China, to study the impacts of different petroleum pollution levels on soil microbial communities, bacterial interactions, and soil carbon and nitrogen transformation functions by using high-throughput sequencing, chemical analysis and bioinformatics. Our study sheds light on the soil microecological function shift when petroleum concentration levels are different, and it provides a theoretical basis for soil bioremediation.

2. Materials and methods

2.1. Soil samples

Petroleum-contaminated soils were collected from 10 oil-producing counties, including Dingbian (DB), Jingbian (JB), Wuqi (WQ), Zhidan (ZD), Ansai (AS), Zichang (ZC), Suide (SD), Yanan (YA), Fuxian (FX), and Yanchang (YC), in northern Shaanxi Province, China. The sampling sites are shown in Fig. 1. Detailed information on these sites, including longitude, latitude, annual average temperature, mean annual precipitation, and annual sunshine duration, is listed in Table S1. In each

sampling site, we set a 20 m × 20 m quadrat. Then, five sampling points were set at the four corners and center of each quadrat. At each sampling point, the topsoil (0–20 cm) samples were gathered in triplicate using a sterilizing shovel and then mixed (Wu et al., 2019). In addition, 10 uncontaminated soils were collected from local nearby farmland soils as the control samples. Soil samples were placed in a cooler and transferred to the laboratory for further analysis. The sampling time was December 12, 2019.

The total petroleum hydrocarbon content was determined by the gravimetric method (Kuang et al., 2021). In brief, 1 g of soil was poured into a weighted glass vial, and 10 mL of solvent (mixture of 5 mL dichloromethane and 5 mL acetone) was added. The vial was placed in an oscillator (Guohua water bath thermostatic oscillator SHA-B, China), oscillated for 1 h at 120 rpm, and then centrifuged at 1300 rpm for 10 min. After three replicates of extraction, the total supernatant was combined into another weighted vial. The TPH concentration was measured by the gravimetric method after solvent evaporation using a nitrogen blowing instrument.

The 10 petroleum-polluted soil samples were designated DB-W, JB-W, WQ-W, ZD-W, AS-W, ZC-W, SD-W, YA-W, FX-W, and YC-W. The 10 uncontaminated soil samples were designated DB-C, JB-C, WQ-C, ZD-C, AS-C, ZC-C, SD-C, YA-C, FX-C, and YC-C. The petroleum concentrations in the 10 polluted soils are shown in Fig. 1. In the 10 uncontaminated soils, the petroleum contents were less than 500 mg kg⁻¹.

2.2. Soil physicochemical analysis

The physicochemical properties, including soil pH, total nitrogen (TN), total carbon (TC), nitrate nitrogen (NO₃⁻), ammonium nitrogen (NH₄⁺), and total organic carbon (TOC), were determined according to the *Handbook of soil agrochemical analysis* (Bao, 2000). In detail, pH values were determined using a pH meter (Hach, USA); TN and TC were determined using the Kjeldahl method and carbon/sulfur analyzer (LC-CS6D, China), respectively; NO₃⁻ concentrations were determined by phenoldisulfonic acid colorimetry; NH₄⁺ concentrations were determined by indophenol blue colorimetry; TOC was measured by the Walkley-Black potassium dichromate-sulfuric acid oxidation method;

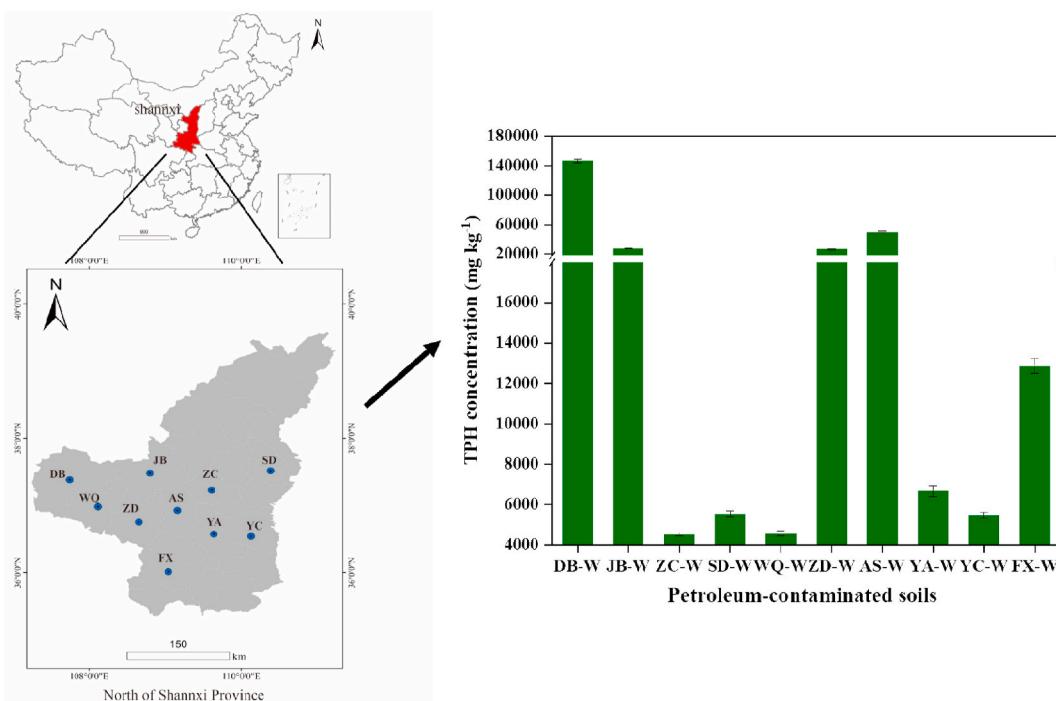


Fig. 1. The map showed the sampling locations of soils in 10 oil-producing counties of the north of Shaanxi province, China, and the total petroleum hydrocarbon concentrations in the contaminated soils.

and soil moisture and soil oxidation reduction potential (ORP) were measured using a soil moisture meter (VM-210 S) and redox potentiometer (DP-422), respectively. Total petroleum hydrocarbons (TPHs) were analyzed using gas chromatography (Wu et al., 2019).

2.3. DNA extraction, PCR, and high-throughput sequencing

The DNA of soil samples was extracted using a MolPure Soil DNA kit (Shanghai, China) following the manufacturers' protocol. Agarose gel electrophoresis was used to detect the integrity of DNA. Only when there was no band in the negative controls, we continued sequencing to ensure the accuracy of the experiment. The resulting DNA was amplified with the primer set 515 F/806 R (Walters et al., 2016; Amoo et al., 2020) to construct amplicon libraries and then subjected to high-throughput sequencing by Sangon Biotech Co, Ltd, Shanghai, China, using the Illumina MiSeq sequencing platform.

The 16 S rRNA gene sequencing reads were demultiplexed, quality filtered by Trimmomatic and merged by Flash, after which these sequences were clustered in operational taxonomic units (OTUs) with a cutoff value of 97% similarity using Usearch. Blast was used to annotate the representative sequences taxonomically (Yang et al., 2018a). The functional annotation of genes was conducted to understand the potential functions of the microbiome using KEGG databases (Devpura et al., 2017). The genomic inventory of each metagenome was predicted via PICRUSt analysis. For this the pick_closed_reference_otus.py command in QIIME was used with Greengenes version 13.5 as a reference database for OTU picking, and the resulting OTU table was uploaded in the Galaxy server. Functional predictions of metagenomes were done with Nearest Sequenced Taxon Index (NSTI) values followed by reconstruction of the metabolic pathways using KEGG database.

2.4. Bioinformatics analysis

Laboratory-derived raw sequences were processed using QIIME (Quantitative Insights Into Microbial Ecology) (<http://qiime.sourceforge.net/>). Observed OTUs (sequencing depth index), Shannon's diversity (bacterial diversity index), and evenness index (bacterial evenness index) were determined using the Mothur software package. We used weighted unifrac distance in principal coordinate analysis. We used the vegan package and vegdist, cmdscale and ordiplot functions of R. A heat map among taxa (the taxa with greater than 1% abundance) at the genus level was generated using the Pheatmap software package. The bacterial community was visualized by using Cytoscape.

2.5. Statistical analysis

Soil physicochemical properties and alpha diversity were analyzed using analysis of variance (ANOVA). Principal coordinate analysis (PCoA) was used to visualize the differences in microbiomes. We used PERMANOVA analysis in our PCoA plots using the Adonis function in the vegan package of the R platform. Spearman's correlation analysis was performed to test the effects of the microbiome at the taxonomic level, and Spearman's correlation coefficient >0.6 was considered to indicate a strong correlation. All data processing and statistical analyses were performed in the R platform (3.3.1). A statistically significant trend was detected using a significance level (p) less than 0.05. We used False-Discovery Rate (FDR) to make multiple test correction.

2.6. Nucleotide sequence accession number

This study generated the original sequencing data submitted to the NCBI Sequence Read Archive (SRA) database (<http://www.ncbi.nlm.nih.gov/sra>), registration number for SRP303774.

3. Results

3.1. Soil characterization

In the 10 petroleum-polluted soils, four polluted samples with petroleum concentrations over 20,000 mg kg⁻¹ were classified as heavy petroleum-contaminated soils, including DB-W, AS-W, JB-W, and ZD-W samples; the remaining six polluted samples, including ZC-W, SD-W, WQ-W, YA-W, YC-W, and FX-W, were classified as medium petroleum contamination with petroleum concentrations between 4000 and 20,000 mg kg⁻¹ (Fig. 1). Soils with petroleum hydrocarbon concentrations below 500 mg kg⁻¹ were classified as uncontaminated (clean) soils, which acted as a control. This classification was based on the ecotoxicity risk of petroleum (Oklahoma, 2010; Kansas, 2000) and our following study in this paper.

The physicochemical parameters of the soil samples are shown in Table S2. In the 10 uncontaminated soils, the pH values were 7.14–8.62; soil moisture contents were 0.12%–14.76%; soil oxidation reduction potentials (ORPs) were 128–180 mV; nitrate nitrogen contents were 0.28–8.04 mg kg⁻¹; and ammonia nitrogen contents were 4.11–58.11 mg kg⁻¹. The pH value of soil in northwestern China is alkaline since the soils contain a large amount of calcium carbonate (Sun et al., 2020). Because soil ORPs were less than 200 mV, soil inorganic nitrogen mainly existed in the form of ammonium nitrogen. Our results are consistent with Li et al. (2021).

For the 10 oil-polluted soils, the pH ranged from 6.82 to 8.79, and the ORP ranged from 135 to 170 mV. The ammonia nitrogen contents in the heavy and medium petroleum-contaminated soils were 17.5–38.1 mg kg⁻¹ and 18–68.1 mg kg⁻¹, respectively. The concentrations of nitrate nitrogen were 0.6–3.79 mg kg⁻¹ in the heavy contamination soils and 1.76–7.33 mg kg⁻¹ in the medium contaminated soils.

3.2. Microbial communities

The Shannon and evenness indices presented a significant decrease ($p < 0.05$) in the four heavy petroleum-contaminated soils but no significant difference in the moderately contaminated soils when compared with their uncontaminated soils ($p > 0.05$) (Fig. 2 a-f). The beta diversity of the bacterial community was analyzed using PCoA based on weighted UniFrac distances at the OTU level (Fig. 2g). Compared with the uncontaminated soils, the microbial community structures in the heavily contaminated soils presented significant differences, but there was no obvious difference in the moderately contaminated soils. Our study suggested that heavy petroleum contamination led to significant changes in soil microbial diversities and community structures.

In this study, the microbial alpha and beta diversities were significantly reduced ($p < 0.05$) for soils with petroleum contents over 20,000 mg kg⁻¹. This result suggested that only microorganisms with degradation abilities or strong tolerance toward high petroleum concentrations could survive in heavily contaminated soils.

In the four heavy petroleum-contaminated soils, DB-W, AS-W, JB-W, and ZD-W, the relative abundance of the *Proteobacteria* phylum increased by 36.59%, 30.49%, 57.01%, and 3.91%, respectively, when compared with their corresponding uncontaminated soil samples ($p < 0.01$) (DB-C, AS-C, JB-C, and ZD-C) (Fig. 3a). The relative abundance of genera affiliated with the *Proteobacteria* phylum also increased. For example, *Pseudomonas*, *Alkanindiges*, and *Methylobacterium* in DB increased significantly ($p < 0.01$). The relative abundance of *Pseudomonas*, *Acinetobacter*, and *Candidimomas* increased in AS. In JB and ZD, *Pseudomonas*, *Idiomarina*, *Chromohalobacter* ($p < 0.01$) and *Sphingomona* and *Acinetobacter* ($p < 0.01$) increased significantly (Fig. 3b). In the moderately polluted soils (including WQ-W, YC-W, FX-W, SD-W, YA-W), the relative abundance of the *Actinobacteria* phylum increased by 1.69%, 1.92%, 14.65%, 30.26%, and 1.19% ($p < 0.01$) (Fig. 3a). The relative abundance of genera affiliated with the *Actinobacteria* phylum also increased, such as *Mycobacterium* (WQ), *Pseudonocardia* (YC), *Gaiella*

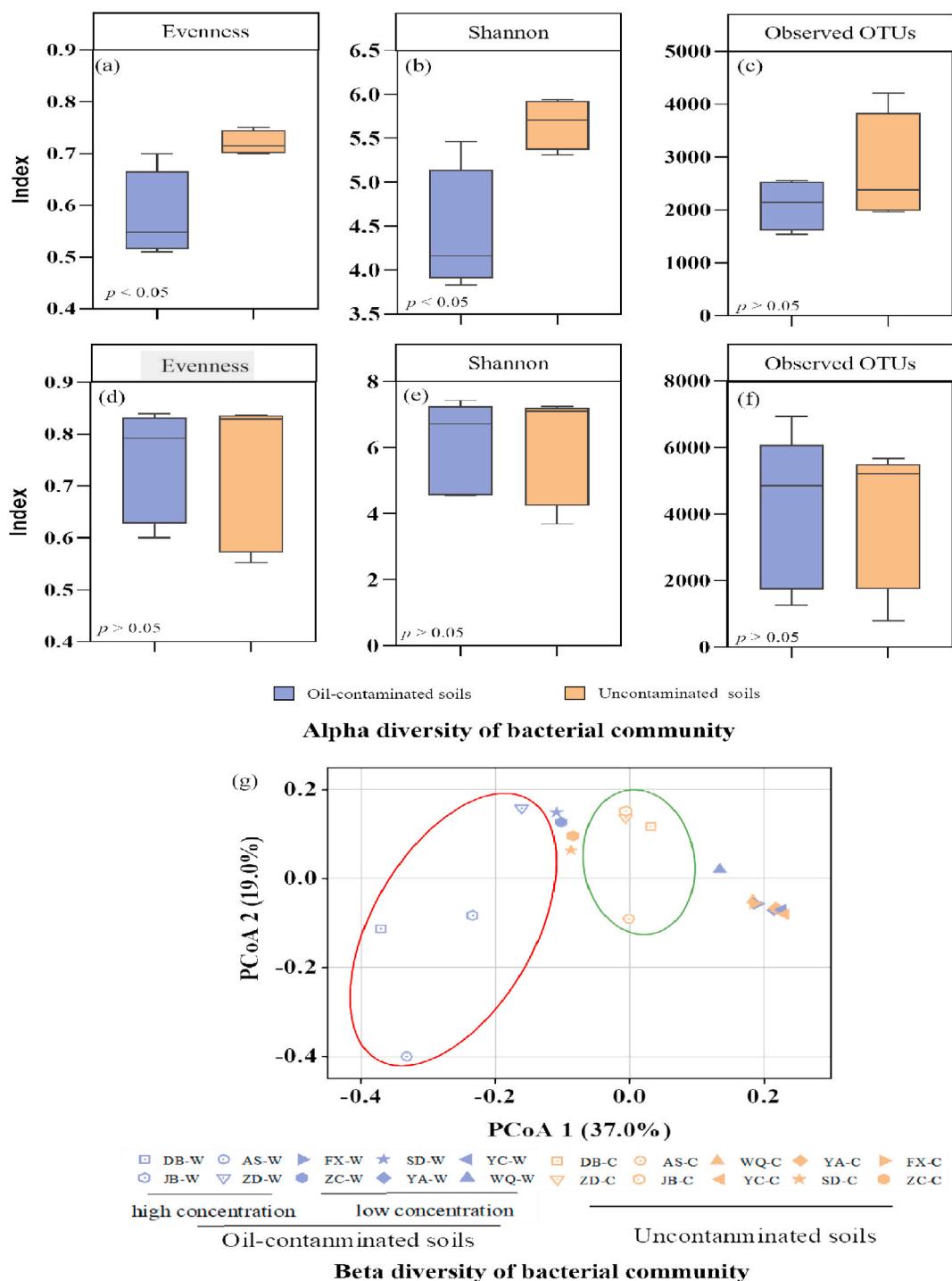


Fig. 2. Alpha and beta diversity of the soil bacterial communities. a–c: heavy petroleum-contaminated soils; d–f: medium petroleum-contaminated soils; Evenness and Shannon: Purple boxes represent contaminated soil, and yellow boxes represent uncontaminated soil. g: beta diversity. The tops and bottoms of boxes represent the 75th and 25th percentiles, respectively. The upper and lower whiskers extend to data no more than $1.5 \times$ the interquartile range from the upper edge and lower edge of the box, respectively. Beta diversity was analyzed by Principal coordinate analysis (PCoA) based on weighted unifrac distance at OTU level and displayed in scatter diagram. The samples in the two circles show significant differences between uncontaminated and heavily contaminated soils ($p < 0.05$). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

(FX), and *Nocardioides* (SD, YA) ($p < 0.01$) (Figs. 2 and 3b).

3.3. Bacterial interactions

Microbial network structures have been successfully constructed to analyze the cooccurrence patterns of microorganisms, which can allow researchers to illustrate microbial symbiotic or competitive

relationships in petroleum-contaminated environments. In this study, microbial network structures at the genus level were established to assess potential interactions of bacterial communities in oil-contaminated soils (Fig. 4). The network properties are shown in Table S3. In the four heavily contaminated soils, the network edges decreased by 13.15%, indicating that interrelationships among the microorganisms weakened and microbial community complexity

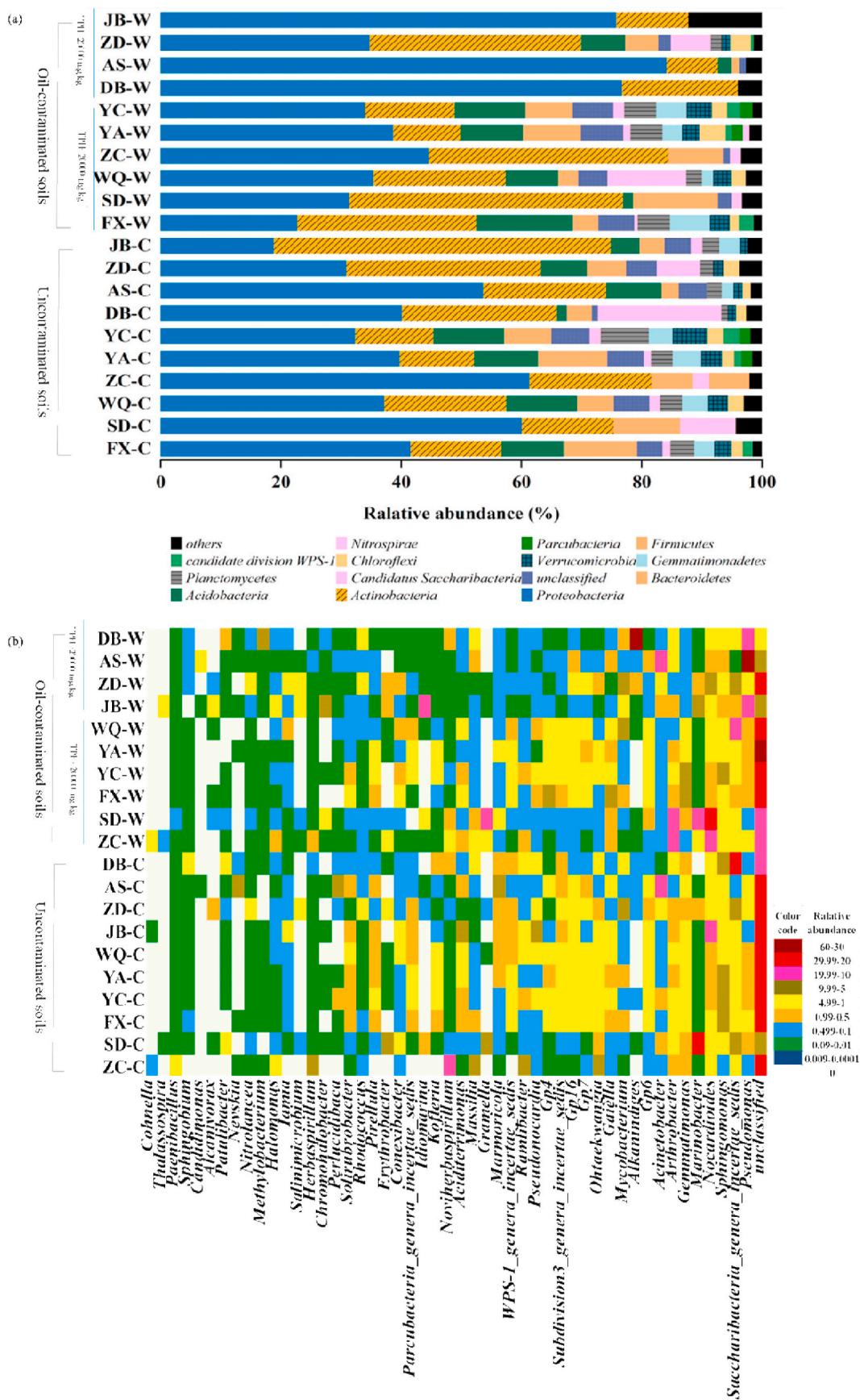


Fig. 3. Taxonomic distribution of bacterial communities in oil-contaminated and uncontaminated soils. a: Taxonomic clades detected at an average relative abundance $\geq 1\%$ at the phylum level; b: Taxonomic clades detected at an average relative abundance $\geq 1\%$ at the genus level.

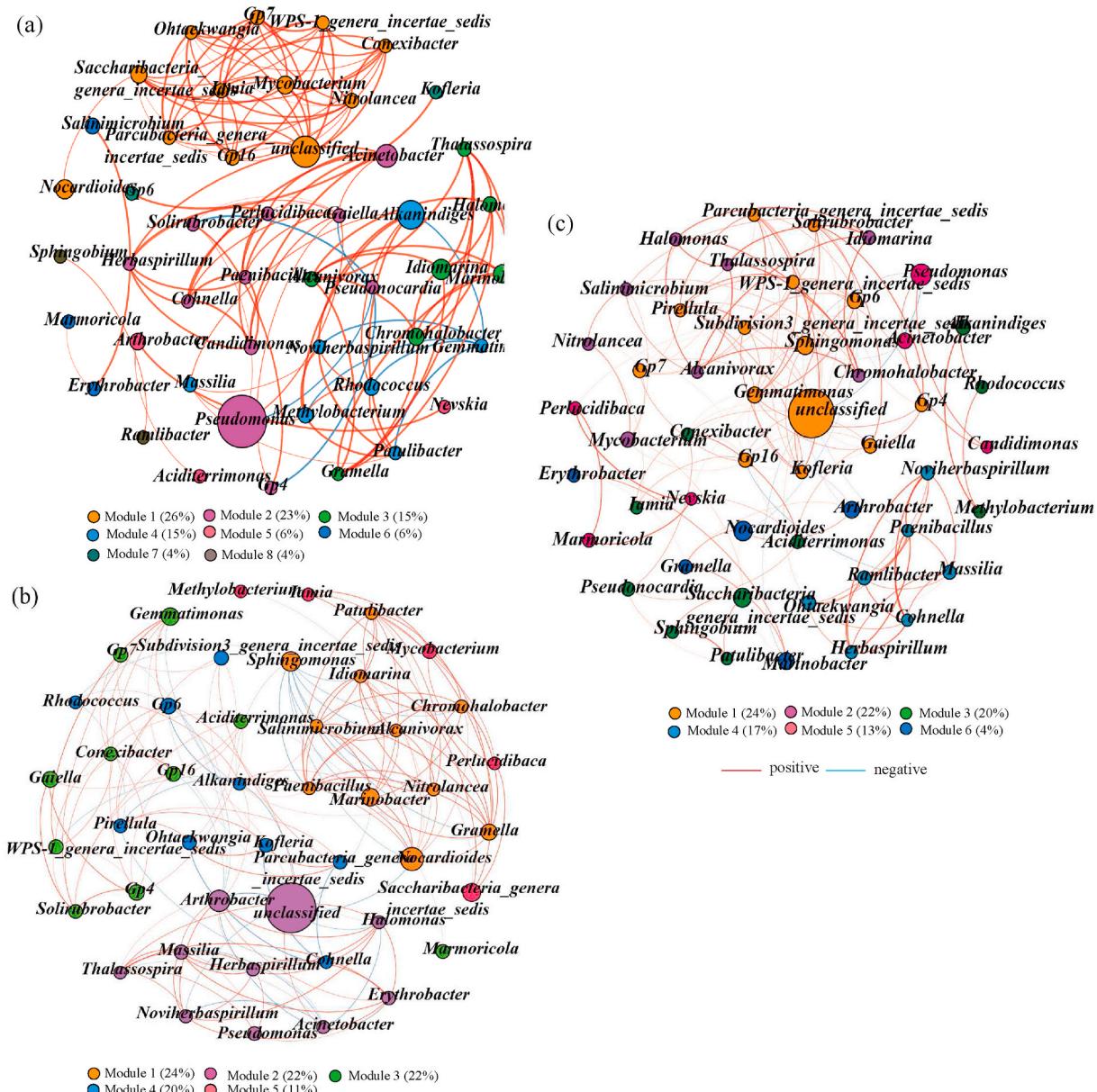


Fig. 4. Network of co-occurring denitrifying bacterial genera. The network based on Spearman's correlation significant analysis ($p < 0.05$) across soil samples. The size of node represents proportional to the relative abundance. The nodes were colored according to modularity class. The thickness of each edge (connection between two nodes) is degree to the value of correlation coefficients. a: heavy petroleum-contaminated soils; b: medium petroleum-contaminated soils; c: uncontaminated soils.

decreased. In the moderately polluted soils, the edges of bacterial networks increased by 7.89%, indicating that the soil microorganism correlations and community complexity increased. The average paths of microbial networks were 3.043, 2.648, and 1.228 in the uncontaminated, moderately contaminated, and heavily contaminated soils, respectively (corresponding to clustering coefficients of 0.702, 0.762, and 0.905 in the uncontaminated, moderately contaminated, and heavily contaminated soils). The results suggested that microorganisms in the heavily contaminated soils had a stronger response toward petroleum contamination than those in the moderately contaminated soils.

3.4. Carbon and nitrogen related pathways

The Calvin cycle was the most important photosynthetic carbon fixation pathway, followed by the reductive pentose phosphate cycle in the uncontaminated and petroleum-contaminated soils (Table S4).

There were no significant differences in gene abundances during photosynthetic carbon fixation in either the heavy or medium petroleum-contaminated soils ($p > 0.05$) when compared with the uncontaminated soils. For the prokaryotic carbon fixation pathway, the reductive citrate cycle, dicarboxylate-hydroxybutyrate cycle, and 3-hydroxypropionate bicyclic were dominant in all soil samples regardless of soil contamination levels (Table S5). The relative abundances of *porC* and *pycA* genes, relating to the incomplete reductive citrate cycle, in the heavily contaminated soils decreased by 0.09% and 0.04%, respectively ($p < 0.05$) (Fig. 5, Table S6). As a result, heavy contamination reduced the incomplete reductive citrate cycle for prokaryotic carbon fixation pathways, while medium contamination had no obvious effects on either photosynthetic carbon fixation or prokaryotic carbon fixation capacity.

When examining nitrogen transformation processes in soils with different degrees of oil pollution, the abundances of functional genes

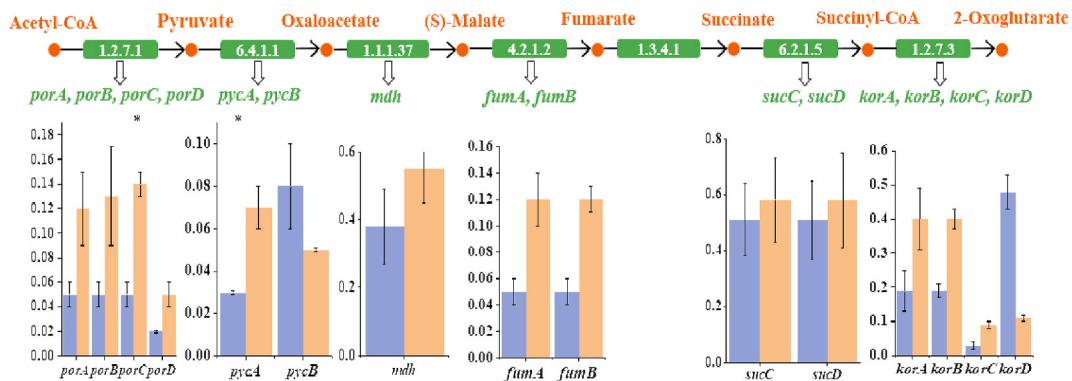


Fig. 5. The gene relative abundances of prokaryotic carbon fixation pathways: incomplete reductive citrate cycle in the heavy contaminated and uncontaminated soils. ** indicates that there are significant differences between heavy contaminated and uncontaminated soils ($p < 0.05$). The ordinate axis of bar represents the relative abundance of genes.

related to nitrate metabolic pathways changed significantly in the heavily contaminated soils. The gene abundances that control dissimilatory and assimilatory nitrate reduction were 14.84% and 3.74% in the heavily contaminated soils compared to 14.73% and 5.59% in the uncontaminated soils, respectively (Fig. 6). The abundance of nitrite reductase *nrfA*, which converts nitrate to ammonium, was 0.26% and 0.09%, respectively. The abundances of hydroxylamine oxidoreductase (converting hydroxylamine to nitrite) were 0.08% and 0.21%, respectively (Table 1). Likewise, the *nifD* and *nifK* genes (nitrogen controlling genes involved in nitrogen fixation) were less abundant in the heavily contaminated soils than in the uncontaminated soils (Table 1). The abundance of genes involved in assimilated nitrate reduction, nitrification, and nitrogen fixation decreased significantly in the heavily contaminated soil. In the soils with medium contamination, the abundances of these genes had no significant differences when compared with the uncontaminated soils (Tables S7 and S8).

4. Discussion

Oil-contaminated soils present different patterns of microbial communities and ecological functions compared with uncontaminated soils, which may be attributed to diverse soil properties and complex microbe functions (Fuentes et al., 2016; Nikolopoulou and Kalogerakis, 2018). In this study, soil microbial community structures and ecological functions under different levels of petroleum pollution conditions were analyzed to illustrate the potential ecological risk and bioremediation capability.

The pH values of soils in northwestern China are alkaline since the soils contain large amounts of calcium carbonate (Sun et al., 2020). There was a decrease in the pH value of the soil upon contamination with crude oil. This may be due to the production of acidic intermediates such as phenolic acids, organic acids, esters, and fatty acids through soil microbiological activities (John et al., 2011). Inorganic nitrogen mainly exists in the form of ammonium nitrogen since the soil ORPs are less than 200 mV. Trindade et al. (2005) and Li et al. (2021) reported that petroleum contamination provided suitable conditions for nitrogen-fixing bacteria, which led to an increase in the available N in the soils. Liu et al. (2009) determined the contents of total nitrogen and hydrolyzed nitrogen in oil-contaminated soils in approximately 46 oil wells and suggested no correlation between petroleum concentrations and nitrogen contents. Our findings are basically consistent with Trindade et al. (2005) and Li et al. (2021).

Adequate nitrogen and balanced C/N ratios are essential for the metabolic activities of microbes, especially for participation in petroleum degradation (Ouriache et al., 2020). It is beneficial for petroleum hydrocarbon biodegradation when the soil C/N ratio is 100:10 (Gainer et al., 2019). Since petroleum pollution causes a large increase in the soil carbon contents, it is necessary to supplement exogenous nitrogen to

stimulate the activities of soil microorganisms during bioremediation of polluted soil (Trindade et al., 2005).

Previous studies indicated that *Proteobacteria* and *Actinobacteria* were the two main phyla in petroleum-contaminated soils (Wang et al., 2019a; Ferguson et al., 2020). Ribeiro et al. (2013) reported that oil pollution may interfere with microbes' ecological niches, leading to a decrease in biodiversity. In this study, we further investigated the effects of different petroleum concentration levels on the two dominant phyla and soil biodiversity. Our study demonstrated that heavy petroleum contamination (petroleum contents were over 20,000 mg kg⁻¹) led to an increase in the *Proteobacteria* phylum, but that the *Actinobacteria* phylum was increased by medium petroleum levels (petroleum contents of between 4000 and 20,000 mg kg⁻¹). In addition, heavy oil contamination intensively altered the soil microbial community patterns and decreased microbial abundance, bacterial richness, and bacterial biodiversity.

According to the fundamental theory of ecology, the Intermediate Disturbance Hypothesis (IDH) suggests that species diversity is the highest when an ecosystem is moderately disturbed, but species diversity will be reduced, and only species with a strong adaptation ability can survive when the ecosystem is seriously damaged (Liu et al., 2019; Gerwing et al., 2017; Swart et al., 2019; Wang et al., 2018a). Our study demonstrated that the changes in soil microbial systems under oil stress also agreed with the IDH principle. When soil is heavily polluted by oil (petroleum concentration is over 20,000 mg kg⁻¹), the diversities and complexities of microbial community structures are significantly reduced, and the correlations among the different microbial species become weaker. In the medium petroleum-polluted soil (petroleum concentration of between 4000 and 20,000 mg kg⁻¹), the diversity of soil microorganisms remained unchanged, but the correlations of microorganisms and the complexities of community structures increased significantly.

In general, nitrogen fixation is mainly mediated by some nitrogen-fixing microorganisms, such as *Rhodococcus*, *Nocardioides*, and *Mycobacterium*, which improve soil stability and productivity (Wang et al., 2018b; Liu et al., 2020a; Lu et al., 2020; Yousuf et al., 2019; Too et al., 2021). Our study showed that the genera *Rhodococcus* and *Nocardioides* decreased in the heavy petroleum-contaminated soils, while they increased in the moderately contaminated soils (Fig. 3-b). This could explain why fewer nitrogen-related genes and nitrogen fixation pathways existed in the heavily contaminated soils.

5. Conclusions

In this study, the impacts of oil pollution on soil ecological functions and microbial community compositions were analyzed using high-throughput sequencing and molecular bioinformatics by collecting soils from different pollution levels in northern Shaanxi Province, China.

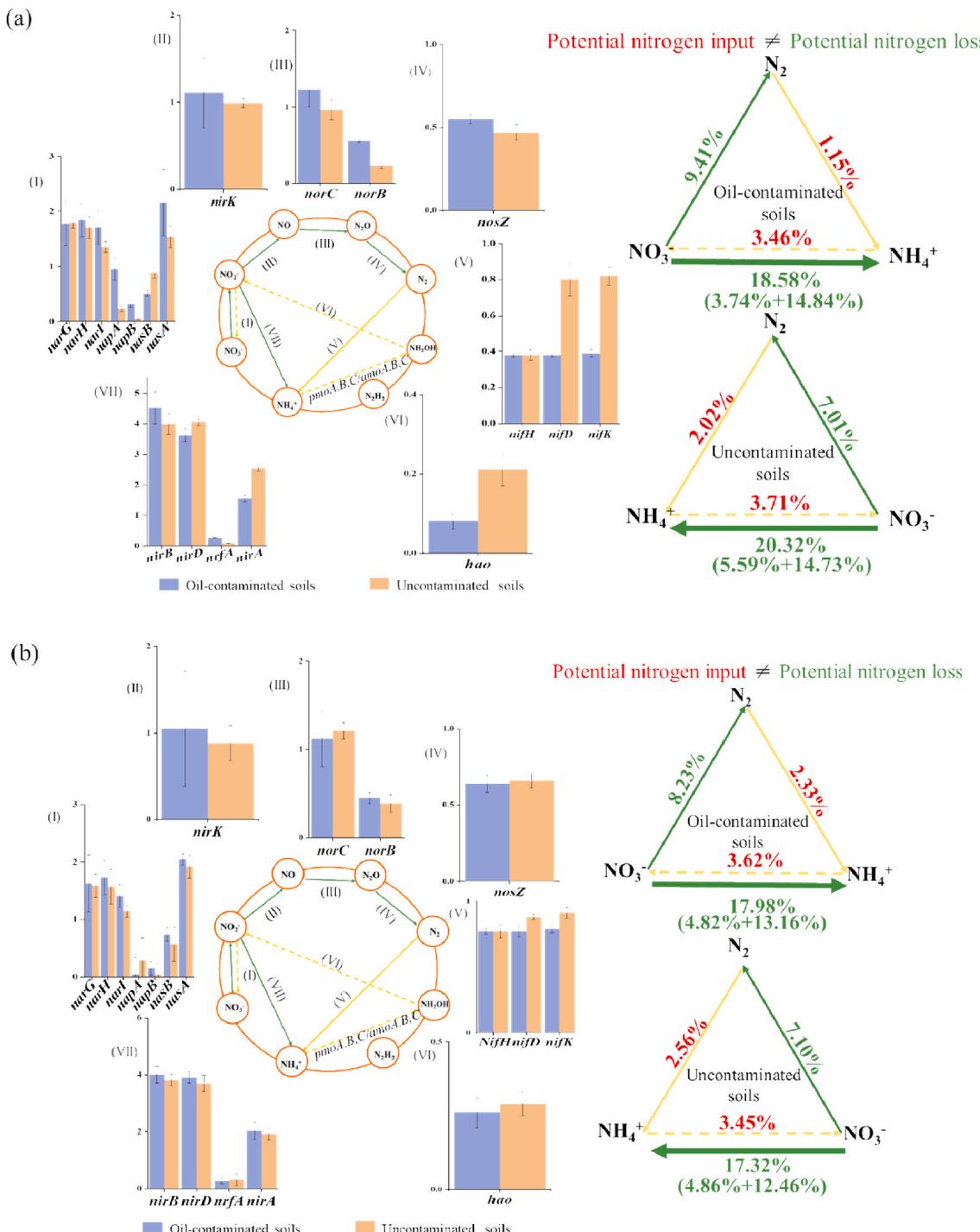


Fig. 6. The gene relative abundance of nitrogen transformation pathways in oil-contaminated and uncontaminated soils. a: heavy petroleum-contaminated soils; b: medium petroleum-contaminated soils. The yellow solid line indicates nitrogen fixation, the yellow dotted line indicates nitrification, The green thin line indicates denitrification, the thick green line indicates the assimilatory nitrate reduction and the dissimilatory nitrate reduction. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Our results suggested that petroleum concentrations played a significant role in shifting soil microbial community structures, ecological functions, and alpha diversities. When the concentrations of total petroleum hydrocarbon (TPH) were between 4000 and 20,000 mg kg⁻¹, the alpha diversities and the functional genes of carbon and nitrogen transformation remained unchanged, but the complexity of the bacterial community increased. Over 20,000 mg kg⁻¹ petroleum decreased species diversity, carbon and nitrogen functional genes, and microbial interrelationships. The changes in soil microecology caused by petroleum

contamination are in accordance with the Intermediate Disturbance Hypothesis.

Authors' contribution

Huan Gao: Methodology, Formal analysis, Writing-Original draft preparation. **Manli Wu:** Conceptualization, Writing-Reviewing and Editing. **Heng Liu:** Visualization, Investigation. **Yinrui Xu:** Data curation, Investigation. **Zeliang Liu:** Visualization, Investigation.

Table 1

Comparative analysis of the relative abundance of bacterial genes related to the nitrogen cycle in the heavy contaminated soils and uncontaminated soils.

KO	Definition	Heavy contaminated (Mean ± SD)	Uncontaminated (Mean ± SD)	p
K02586	Nitrogenase molybdenum-iron protein alpha chain (<i>nifD</i>)	0.0038 ± 0.0010	0.0080 ± 0.0009	0.002
K02591	Nitrogenase molybdenum-iron protein beta chain (<i>nifK</i>)	0.0039 ± 0.0002	0.0082 ± 0.0005	0.002
K10535	hydroxylamine oxidase (<i>hao</i>)	0.0008 ± 0.0001	0.0021 ± 0.0004	0.043
K03385	Nitrite reductase (cytochrome c-552) (<i>nrfA</i>)	0.0026 ± 0.0001	0.0009 ± 0.0002	0.028

Declaration of competing interest

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

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.envpol.2021.118511>.

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