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RESEARCH

Unraveling the Positive Effect of Soil Moisture on the Bioaugmentation of Petroleum-Contaminated Soil Using Bioinformatics

Huan Gao[1,2] · Manli Wu[1,2,3] · Heng Liu[1,2] · Yawen Ou[1,2] · Ting Zhang[1,2] · Xuhong Duan[1,2]

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Abstract

Petroleum contamination is a severe threat to the soil environment. Previous studies have demonstrated that petroleum degradation efficiency is promoted by enhancing soil moisture content (MC). However, the effects of MC on soil microbial ecological functions during bioremediation remain unclear. Here, we investigated the impacts of 5% and 15% of moisture contents on petroleum degradation, soil microbial structures and functions, and the related genes using high-throughput sequencing and gene function prediction. Results indicated that petroleum biodegradation efficiency was increased by 8.06% in the soils with 15% MC when compared to that with 5% of MC. The complexity and stability of soil microbial community structures with 15% MC were higher than those in the soils with 5% MC when hydrocarbon-degrading bacterial flora (HDBF) were inoculated into the soils. Fifteen percent of moisture content strengthened the interaction of the bacterial community network and reduced the loss of some key bacteria species including *Mycobacterium*, *Sphingomonas*, and *Gemmatimonas*. Some downregulated gene pathways relating to bioaugmentation were enhanced in the soils with 15% MC. The results suggested that the dynamic balances of microbial communities and the metabolic interactions by 15% MC treatment are the driving forces for the enhancement of bioremediation in petroleum-contaminated soil.

Keywords Petroleum contamination · Moisture content · Bioaugmentation · Microbial community · Metabolic pathway

Introduction

Soil total petroleum hydrocarbon (TPH) pollution in petroleum exploitation areas is a worldwide environmental problem [1–4]. Petroleum industrial activities and accidental spills leak into the environment, which damages both the ecosystem and human health [5, 6]. Hence, the clarification of soil and water contaminated by petroleum is an urgent task required for local environmental protection and sustainable development.

Bioremediation of oil-contaminated environments has always been a popular strategy due to its low energy consumption and reduced economic costs [7–10]. Generally, bioaugmentation with exogenous microorganisms is a common strategy for the enhancement of pollutant removal [11–13]. Previous studies have reported different bacterial classifications isolated from petroleum-contaminated sites that have the capability to degrade petroleum [14, 15]. An important issue for bioaugmentation is whether the inoculated microorganisms can survive in the new environment. The activities of the inoculum are influenced by biotic factors such as the population number, activity, relationship with the indigenous microorganisms, and diversities of soil microbial communities [16–18]. To achieve effective contaminant degradation, it is important for the inoculated microorganisms to be capable of adapting to the soil environment, as their activity directly affects the remediation efficiency [19, 20].

The activities of the microorganisms are also affected by some abiotic factors such as the soil texture, moisture content (MC), pH, and electron donors and acceptors [21, 22]. Among these parameters, the soil MC has important effects

✉ Manli Wu
wumanli1974@hotmail.com

1 Xi'an University of Architecture and Technology, Xi'an 710055, People's Republic of China

2 Key Laboratory of Northwest Water Resource, Environment and Ecology, MOE, Xi'an University of Architecture and Technology, Xi'an 710055, People's Republic of China

3 Shaanxi Key Laboratory of Environmental Engineering, Xi'an University of Architecture and Technology, Xi'an 710055, People's Republic of China

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on microbial activity for the bioremediation of organic contaminants [23]. Many studies have evaluated the effects of soil moisture on microbial activity [24–26]. Moisture functions are based on abiotic soil properties, namely that microorganisms have access to nutrients via diffusion to the cell surface [27, 28]. However, the correlative mechanism of how MC affects the microbial community during the bioremediation of petroleum-contaminated soils remains unclear. Research on the effects of soil moisture on the microbial community and respiration is based on a fixed MC level [29, 30]. It is necessary to understand the bacterial interspecies network and gene metabolism during the remediation of petroleum-contaminated soils by inoculating the hydrocarbon degraders under different MCs.

In this study, the biodegradation capacity of hydrocarbon-degrading bacterial flora toward petroleum was compared under different moisture contents. Furthermore, the responses of the indigenous microbial community, the ecological function, and the co-occurrence relationship, as well as the metabolic potential of the soil microbiomes, were investigated via molecular informatics technology.

Materials and Methods

Soil Samples

Petroleum-polluted soils were obtained from the Shanbei oilfield in China. Soil samples were collected according to the methodology previously described by Wu et al. [31]. In brief, eight topsoil samples were gathered from the area around the oil well using a sterilizing shovel. Then, these samples were mixed evenly into a single sample. The sample was bagged and kept in an ice cooler at 4 °C, which was then transported to the laboratory for further study.

Soil Physicochemical Properties

The soil pH was determined with a digital pH meter (Hach, USA) using the soil-to-water ratio of 1:2.5 (w/v). The soil MC was calculated gravimetrically and presented as the percentage of soil water to dry weight. The number of petroleum hydrocarbon-degrading bacteria was determined by the flat colony counting method.

Isolation and Identification of Petroleum Hydrocarbon-Degrading Bacterial Flora

First, 5 g of petroleum-contaminated soil was added to 50 mL of mineral salt medium (MSM), which contained 0.27 g K_2HPO_4, 0.35 g KH_2PO_4, 2.7 g NH_4Cl, 0.1 g $MgCl_2$ $6H_2O$, 0.1 g $CaCl_2$ $2H_2O$, 0.009 g $FeCl_2{\cdot}4H_2O$, and 0.004 g $MnCl_2{\cdot}4H_2O$ in 1 L sterile H_2O, and the pH was adjusted to 7.0. The cultures were autoclaved at 121 °C for 30 min prior to the addition of 1% of sterile crude oil (obtained from the Shanbei oilfield) as the sole carbon and energy source. The experiment was conducted at room temperature (28–30 °C) with a shaking incubator at 150 rpm. After 7 days of incubation, 5 mL of the culture was transferred into a new 50-mL MSM medium with 1% crude oil and incubated for 7 days under the same conditions. After 5 cycles of enrichment, petroleum hydrocarbon-degrading bacterial flora (HDBF) were obtained by centrifugation at 5000 rpm for 15 min and then suspended in PBS buffer solution (NaCl 8 g, Na_2HPO_4 1.42 g, KH_2PO_4 0.27 g, KCl 0.2 g, 1 L sterile water). HDBF was identified via high-throughput sequencing analysis by Sangon Biotech Co., Ltd., China (ftp://ftp.sangon.com:21148). The HDBF sequences obtained were deposited in the GenBank database under accession numbers PRJNA939509.

Crude Oil Biodegradation by Inoculating the HDBF in the Liquid Phase

The capacity of the HDBF to degrade petroleum was determined by liquid flask experiments at 150 rpm and 30 °C. In brief, 20 mL of sterilized MSM medium (sterilized by autoclaving at 121 °C for 30 min) was respectively placed in 18 50-mL flasks, and 200 mg of crude oil (10,000 mg L^{-1}) was added to each flask. Inoculation was then carried out by respectively adding HDBF in the amounts of 10^3, 10^4, 10^5, 10^7, and 10^8 CFU mL^{-1} to each flask. Experiments were performed in triplicate and the control was conducted without the inoculation of HDBF throughout the experiment. After 30 days of incubation, the cultures were extracted and analyzed.

Crude Oil Biodegradation by Inoculating HDBF in Soil with MCs of 5% and 15%

Experiments were conducted under natural soil moisture of 5% (AN) and soil moisture enhanced to 15% (A15). HDBF in the amounts of 10^3, 10^4, 10^5, 10^7, and 10^8 CFU g^{-1} soil was respectively inoculated into the petroleum-contaminated soil samples with a petroleum concentration of 15,233 mg kg^{-1} and incubated at 24 °C for 60 days. Experiments were performed in triplicate, and the control without HDBF inoculation was set up throughout the whole experiment (AN-CK, A15-CK). Petroleum was extracted and analyzed at 7, 15, 30, 45, and 60 days.

The residual petroleum hydrocarbons in the liquid phase were extracted by ultrasonic extraction and determined by GC. For the extraction of the crude oil culture, the broth was mixed with equal volumes of N-hexane and dichloromethane (1:1) in a centrifugal tube, after which ultrasonic extraction was carried out for 10 min followed by centrifugation (4 °C,

8000 r·min^{-1}). GC analysis was carried out on a PE CLARUS 680 device equipped with a flame ionization detector (FID) and a 30-m-long HP-5-MS capillary column (internal diameter, 0.25 mm; film thickness, 0.25 µm). Helium was used as the carrier gas. The detector temperature was 430 °C. Moreover, the initial and final temperatures of the inlet were 40 °C and 320 °C, respectively. The inlet temperature program and final hold time were 10 °C·min^{-1} and 30 °C·min^{-1}, respectively. The biodegradation efficiency was calculated using the formula as follow [32].

$$\text{Biodegradation efficiency (\%)} = 100 - \left(A_s * 100/A_{ac}\right) \quad (1)$$

where A_s is the total area of peaks in each test sample and A_{ac} is the total area of peaks in the appropriate abiotic control.

The ultrasonic extraction method was adopted for the extraction of petroleum from the soil, and the specific operation was carried out with reference to the steps of the liquid-phase ultrasonic extraction of petroleum. The filtrate obtained through the separator funnel after centrifugation was collected into a weighing bottle that had previously been weighed. The weighing bottle was then weighed after the volatilization of N-hexane and dichloromethane. The gravimetric estimation of residual oil left after biodegradation was carried out by weighing the quantity of oil. The biodegradation efficiency was calculated using the formula as follow [31].

$$\text{Biodegradation efficiency (\%)} = \left(C_o - C_i\right)/C_o * 100\% \quad (2)$$

where C_o is the petroleum hydrocarbon concentration in the soil at 0 day (mg·kg^{-1}) and C is the petroleum hydrocarbon concentration in the soil at day i (mg·kg^{-1}).

DNA Extraction and High-Throughput Sequencing

Five percent and 15% MC soil samples (inoculation and control group samples) were selected for high-throughput sequencing on days 7 and 60, respectively; for the soil MC of 5%, the inoculation amount was 10^8 CFU g^{-1}, and for the soil MC of 15%, the inoculation amount was 10^7 CFU g^{-1} (AN-7-10^8, AN-60-10^8, A15-7-10^7, A15-60-10^7). Moreover, on day 60, the soil moisture of the non-inoculated soil samples was respectively 5% and 15% (AN-60-CK, A15-60-CK). The variable V3-V4 region of 16s rRNA was amplified using primers 341F (CCCTACACGACGCTCTTCCGATCTGCCTACGGGNGGCWGCAG) and 805R (GACTGGAGTTCCTTGGCACCCGAGAATTCCAGACTACHVGGGTATCTAATCC). The PCR products were then loaded on an Illumina-Miseq device. After sequencing, the data were collected and processed by Sangon Biotech Co., Ltd. (ftp://ftp.sangon.com:21148). All sequences obtained in this study were deposited in the GenBank database under accession numbers PRJNA939509.

The genomic inventory of each metagenome was predicted via Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt) analysis. For this, the pick_closed_reference_otus.py command in Quantitative Insights Into Microbial Ecology (QIIME) was used with Greengenes as a reference database for OTU picking, and the resulting OTU BIOM table was uploaded to the Galaxy server (https://huttenhower.sph.harvard.edu/galaxy). It allocated the functional attributes by comparing the identified 16S rRNA gene sequence with that of the nearest match of the known genome sequence. Functional predictions of metagenomes were carried out with Nearest Sequenced Taxon Index (NSTI) values followed by the reconstruction of the metabolic pathways using the KEGG database.

Bioinformatics Analysis

Laboratory-derived raw sequences were processed using QIIME (http://qiime.sourceforge.net/). The observed OTUs (sequencing depth index), Shannon's diversity (bacterial diversity index), and evenness index (bacterial evenness index) were determined using the Mothur software package. The weighted UniFrac distance was used in principal coordinate (PCoA) analysis. The vegan package and vegdist, cmdscale, and ordiplot functions of the R platform were used. A heat map of taxa (those with greater than 1% abundance) at the genus level was generated using the Pheatmap software package. The bacterial community was visualized by using Cytoscape. Linear discriminant analysis (LDA) effect size (LEfSe) was performed to investigate potential biomarkers within the soil microbiomes specifically enriched in one of the treatments before and after inoculation based on $P < 0.05$ and LDA score > 2.0.

Statistical Analysis

Statistical analyses were performed on the R platform (3.3.1). One-way analysis of variance (ANOVA) was used to analyze differences in the soil physicochemical properties, and statistical differences were considered significant at $P < 0.05$. PCoA was used to visualize the differences in microbiomes. Permutational multivariate ANOVA (PERMANOVA) was adopted for the PCoA plots by 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 a value of >0.6 was considered to indicate a strong correlation. All data processing and statistical analyses were performed on the R platform (3.3.1). A statistically significant trend was detected using a significance level (P) of less than 0.05. The false-discovery rate (FDR) was used for multiple test correction.

Results

The Effect of MC on TPH Degradation During the Inoculation of HDBF to the Soil

The HDBF isolated from the Shanbei petroleum-contaminated soil was mainly composed of the Proteobacteria phylum (accounting for 99.75%), *Acinetobacter sp.* (87.32%), and *Pseudomonas sp.* (12.00%) (Fig. S1).

After 30 days of the inoculation of HDBF with amounts ranging from 10^3 to 10^8 CFU mL^{-1} in liquid-phase crude oil, the TPH degradation efficiencies ranged from 89 to 91%, and the removal rate of petroleum hydrocarbon was 40.09% in the uninoculated control. The optical density (OD_{600}) increment indicates that the HDBF grew well in the liquid phase. The result illustrates that the HDBF flora grew well and had a good capacity to degrade crude oil in the liquid phase (Fig. S2).

The physicochemical properties of the petroleum-polluted soil are listed in Table S1. The soil MC was 5.0% and the number of hydrocarbon-degrading bacteria was 3.3×10^5 CFU g^{-1}. In this study, when inoculating different numbers of degrading bacteria (10^3, 10^4, 10^5, 10^7, and 10^8 CFU g^{-1}) under 15% MC, the removal efficiencies of petroleum hydrocarbons were higher than those under 5% MC. The highest TPH degradation efficiency reached 18.67% under 15% MC, which was improved by 8.06% as compared with the inoculation of HDBF under 5% MC (Fig. 1). This study indicated that TPH degradation efficiency increased from 10.61% (5% MC soil) to 18.67% (15% MC soil). Fifteen percent of moisture content improved the bioavailability of petroleum hydrocarbons in the soils. Soil moisture is an important impact factor for petroleum bioremediation effectiveness.

The Effects of the Soil Moisture Content on Microbial Ecological Functions

Taxonomic Biomarkers

In the uninoculated soils, the relative abundances of Proteobacteria and Actinobacteria respectively increased from 28.22% and 14.88% to 31.14% and 34.76% with the enhancement of the soil MC from 5 to 15% (Fig. 2, Fig. S3), indicating that the soil MC of 15% was beneficial to Proteobacteria and Actinobacteria. LEfSe analysis revealed that a total of 19 biomarkers affiliated with the two phyla and nine taxa affiliated with the order Actinomycetales (Actinobacteria phylum) were sensitive to soil moisture ($P < 0.05$, LDA > 2.0; Fig. 3, Table S2). At the genus level, *Mycobacterium* and *Leifsoni*a affiliated with the order Actinomycetales, *Bradyrhizobium* affiliated with the order Rhizobiales (Proteobacteria phylum), and *Sphingomonas* affiliated with the order Sphingomonadales (Proteobacteria phylum) were susceptible to soil moisture. There were no significant differences ($P > 0.05$) in the total number of species or the alpha and beta diversities of the bacterial communities for the 5% and 15% MC soils (Fig. S3, Fig. 4). The results indicate that increment of the soil MC changed the taxonomic biomarkers, but did not affect the diversity of the microbial communities.

After inoculating the HDBF to the soils with 5% MC, the relative abundance of Proteobacteria increased to 66.35% and 70.24% on the 7th and 60th days, respectively. Moreover, the abundances of *Acidobacteria* and *Planctomycetes* decreased from 14.88% and 10.09% to 2.85% and 1.52%, respectively (Fig. 2, Fig. S3). LEfSe analysis revealed that 15 biomarkers affiliated with Proteobacteria and Actinobacteria were sensitive to inoculation ($P < 0.05$, LDA > 2.0;

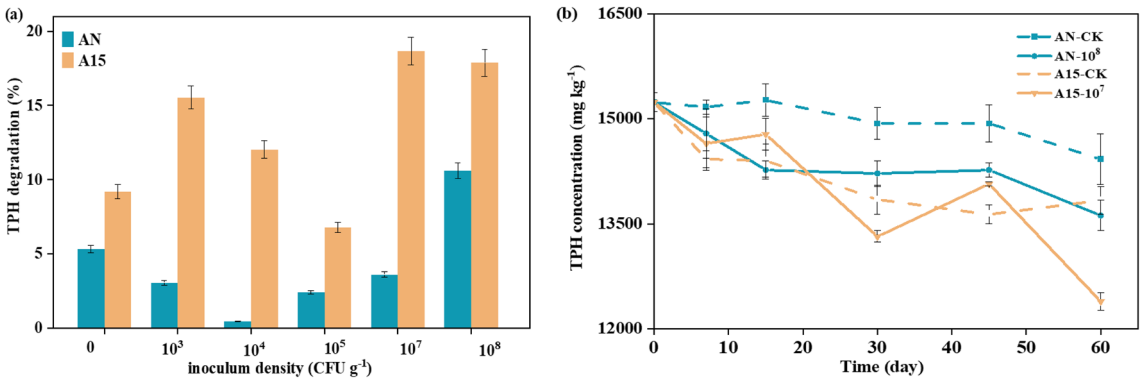

Fig. 1 The change of TPH degradation efficiency at different inoculation densities (10^3, 10^4, 10^5, 10^7, 10^8 CFU mL^{-1}) in 5% and 15% moisture oil-contaminated soil, respectively

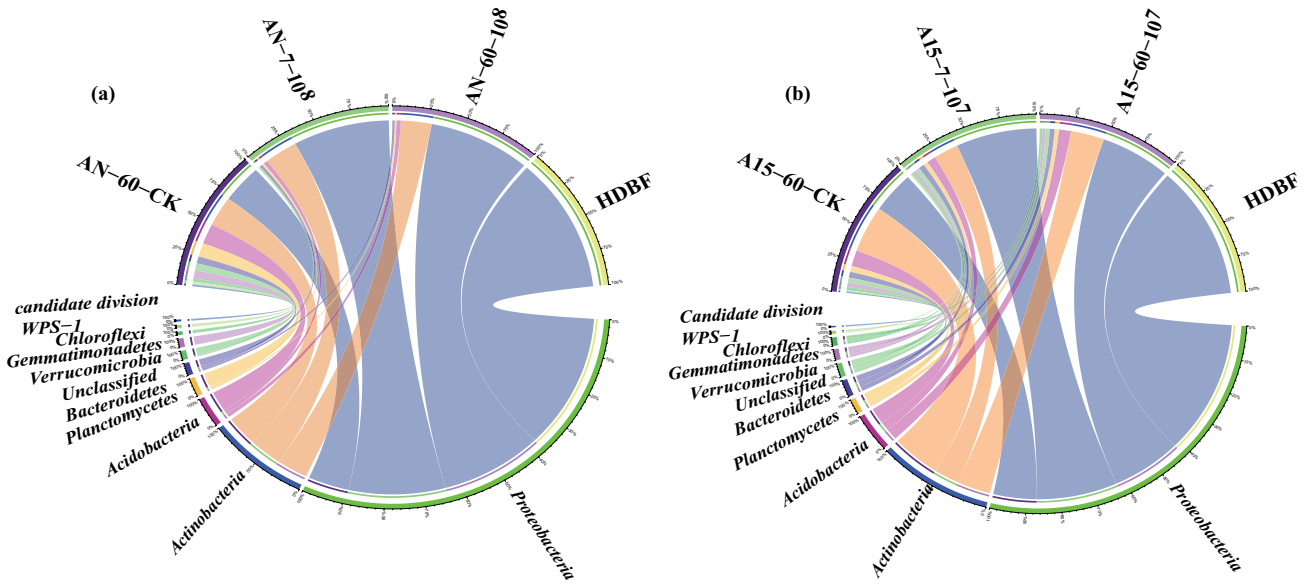

Fig. 2 Relative distribution of major bacterial (phylum) in 5% (**a**) and 15% (**b**) moisture oil-contaminated soil, respectively. Microbial community composition at phylum and class level with >1% cumulative abundance was considered. Cumulative abundance <1% was added up and represented as "Others"

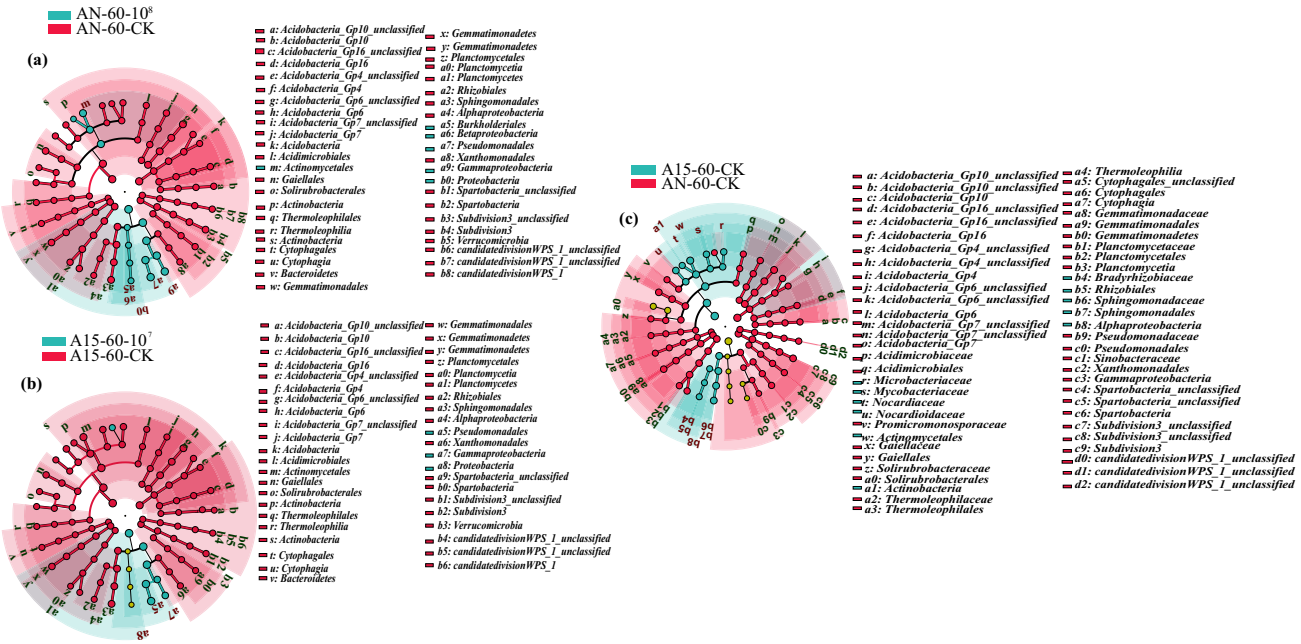

Fig. 3 LEfSe results revealed bacterial biomarkers (from phylum to genus level) sensitive to 5% (**a**) and 15% (**b**) moisture soils (without inoculation and inoculation) and control soil samples (**c**, 5% and 15% moisture). Namely, the soil moisture was 5%, the inoculation amount was 10^8 CFU g^{-1}, and the soil moisture was 15%, the inoculation amount was 10^7 CFU g^{-1}, on day 60, respectively (AN-60-10^8, A15-60-10^7), the soil moisture was 5% and 15% of the non-inoculated soil on day 60 (AN-60-CK, A15-60-CK). In each of the cladogram, the circular ring from inside to outside represents phylum, class, order, family, and genus, respectively. The node on the circular ring represents the taxonomic group affiliating within the taxonomic level. Taxa that had significantly higher relative abundance in a certain treatment within each soil type were color-coded within the cladogram

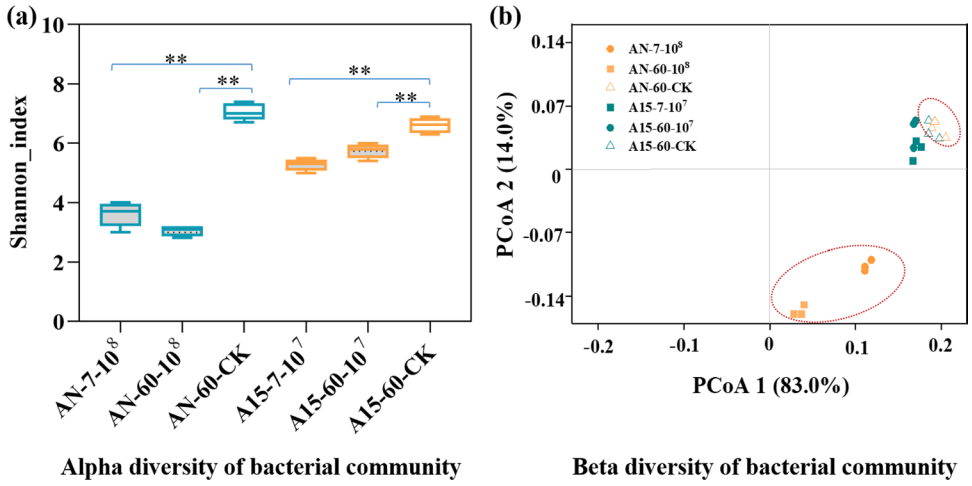

Fig. 4 Alpha (**a**) and beta (**b**) diversity of the soil bacterial community in 5% and 15% moisture oil-contaminated soil, respectively. Shannon index was calculated based on phylogenetic distance at OTU level and displayed in boxplot. Beta diversity was analyzed by principal coordinate analysis (PCoA) based on weighted unifrac distance at OTU level and displayed in scatter diagram

Fig. 3, Table S3). The genera of *Acinetobacter* and *Pseudomonas* affiliated with the order Pseudomonadales (Proteobacteria phylum) were significantly increased in the 5% MC inoculated soil. The genera of *Rhodococcus* and *Nocardia* affiliated with the order Actinomycetales (Actinobacteria phylum), as well as *Achromobacter* affiliated with the order Burkholderiales (Proteobacteria phylum), were sensitive to the inoculation of HDBF. The soil alpha diversity decreased significantly ($P = 0.001$) as compared with that of the uninoculated soils ($P = 0.02$) (Fig. 4). The results indicate that inoculation destroyed the uniformity of the indigenous microbial community under 5% MC.

When inoculating the HDBF to the soils under the 15% MC condition, the relative abundance of the Proteobacteria phylum increased from 31.14 to 57.98% and 50.22% at the 7th day and 60th day, respectively (Fig. 2). The alpha diversity of bacterial species was higher than that in the 5% MC inoculated soil (Fig. S3), but the microbial community similarity was not significantly different ($P = 0.64$) (Fig. 4), indicating that 15% soil MC was conducive to maintaining the homogeneity of the soil microbial community after inoculation with HDBF. LEfSe analysis revealed that eight biomarkers affiliated with the Proteobacteria and Actinobacteria phyla were sensitive to 15% MC ($P < 0.05$, LDA > 2.0; Fig. 3, Table S3). The genera of *Pseudomonas* and *Acinetobacter* affiliated with the Pseudomonadales order of the Proteobacteria phylum, as well as *Microbacterium* affiliated with the Actinomycetales order of the Actinobacteria phylum, were sensitive to inoculation.

Co-Occurrence of Soil Microbiomes

Figure 5 and Table 1 present the networks of the microbiome communities at the genus level based on Spearman's correlation significance analysis ($P < 0.05$). The percentage of edges, network density, and the positive correlation of the microbiomes were respectively increased by 48%, 0.26, and 4% in the uninoculated soil with 15% MC when compared with those of the 5% MC soil, indicating that 15% MC strengthened the microbiome association.

In the inoculated soil with 5% MC, the percentage of nodes, the network density, and the clustering coefficient were respectively reduced by 35.41%, 0.11, and 0.16, but the path length was increased by 1.06, as compared with those of the uninoculated soil. These results indicate that the soil microbiome associations were weaker after inoculation in the soil with 5% MC.

In the 15% MC inoculated soil, the edges and density of the microbiome network were respectively 156 and 0.31, which were higher than those in the 5% MC soil, namely 93 and 0.18, respectively. The soil microbiome formed a stronger relatedness after inoculation in the 15% MC soil as compared with the 5% MC soil.

Metabolic Characterization

Metagenomes were predicted by PICRUSt analysis to understand the metabolic potential and to identify different abundant functional features. A total of 266 KEGG pathways and 20 non-human-gene pathways changed (fold change > 2) when inoculating the HDBF in the 5% MC soil ($P < 0.05$). Among them, the abundances of nine KEGG pathways changed more than four-fold (fold change > 4) (Fig. 6). These included pathways related to cellular processes (adherens junction, tight junction, p53 signaling pathway, and flagellar assembly), organismal systems (leukocyte transendothelial migration, phototransduction, circadian rhythm, and carbohydrate digestion and absorption), and metabolism (isoflavonoid biosynthesis, fatty acid elongation in mitochondria, D-arginine and D-ornithine metabolism, biosynthesis of vancomycin group antibiotics, flavonoid biosynthesis, butirosin and neomycin biosynthesis, polyketide sugar unit biosynthesis, nitrotoluene degradation, starch and sucrose metabolism, and galactose metabolism). Among them, the metabolic pathways decreased in the 5% MC soil after inoculation. However, there

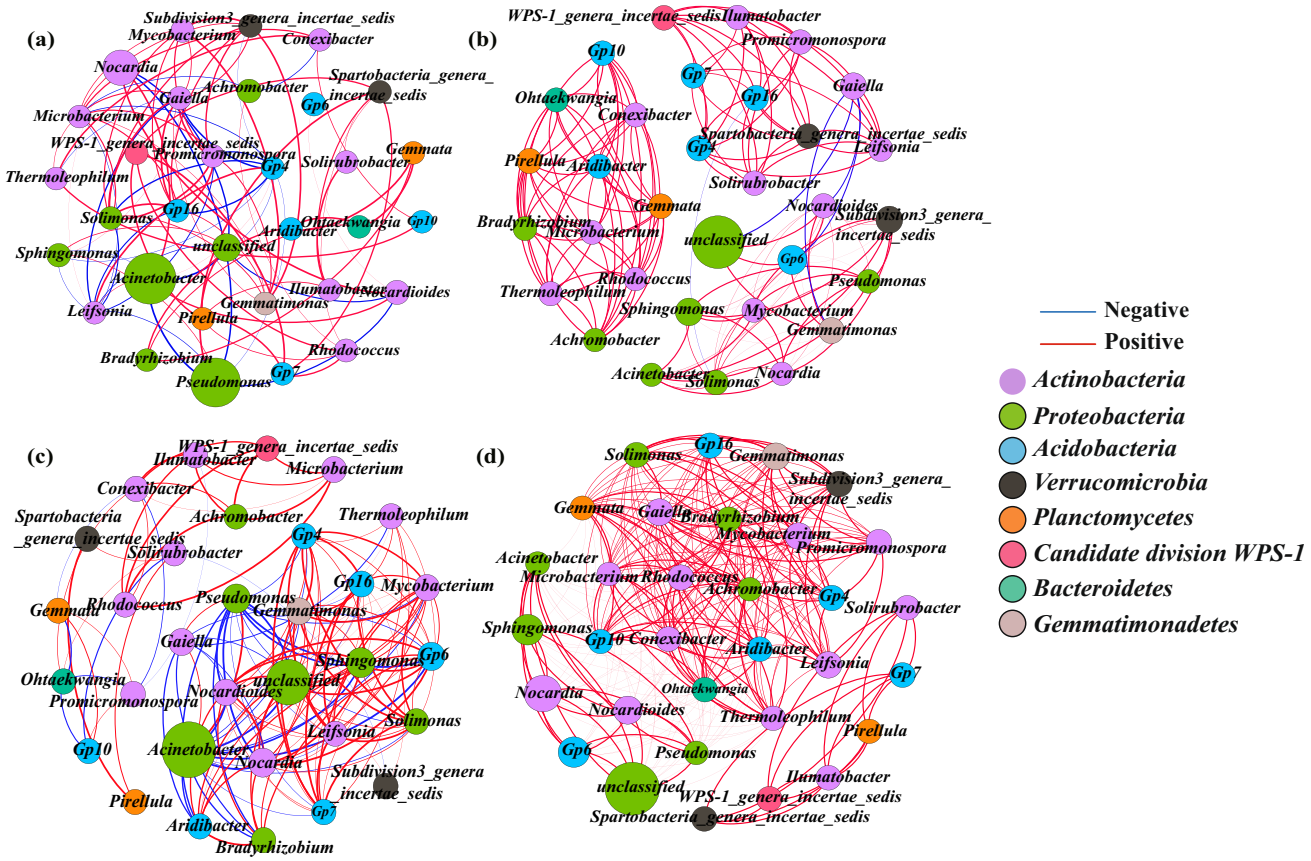

Fig. 5 Network of microbiome community at the genus level based on Spearman's correlation significant analysis ($P < 0.05$). **a**, **b**, **c**, and **d** represents AN-60-10^8, AN-60-CK, A15-60-10^7, and A15-60-CK, respectively. The node size is proportional to the abundance of taxa. The network consists of nodes representing genera, and the red edges represent positive correlations and the blue edges represent negative correlations between species

Table 1 Network properties of 5% and 15% moisture soils at genus level

Parameters	AN	AN-CK	A15	A15-CK
Average degree	5.81	9	9.75	17.31
Avg. weighted degree	5.38	8.68	8.94	15.94
Network diameter	8	4	7	2
Graph density	0.18	0.29	0.31	0.55
Modularity	0.54	0.65	0.31	0.18
Connected components	2	2	1	2
Avg. clustering coefficient	0.79	0.96	0.80	0.93
Avg. path length	2.92	1.85	3.19	1.18
Nodes	32	32	32	32
Edges	93	144	156	277

was no significant loss of metabolic pathways (fold change > 2) when inoculating the HDBF in the 15% MC soil.

There was a strong correlation between the bacterial taxonomic clades and metabolic pathways in the HDBF-inoculated soil with 5% MC (Fig. 7). Metabolic pathways that were found to be differentially abundant exhibited strong negative correlations with the biomarkers *Acinetobacter*, *Pseudomonas*, *Nocardia*, and *Rhodococcus* (Spearman's correlation > 0.7, $P < 0.001$; Fig. 7, Fig. S4). Some reduced or disappeared genera including *Gemmata*, *Gaiella*, *Pirellula*, *Sphingomonas*, and *Gemmatimonas* had strong positive correlations with metabolic pathways (Spearman's correlation > 0.7, $P < 0.001$; Fig. 7, Fig. S4). The disappearance of these bacterial species was related to petroleum biodegradation stagnation.

Discussion

According to the literature, the suitable bioremediation conditions for petroleum-contaminated soil should be 15–20% humidity and 108 CFU g^{-1} of hydrocarbon degraders [25, 31, 33, 34]. Previous studies have focused on the effects of soil moisture on the soil microenvironments during bioremediation. Although high soil humidity limits the soil oxygen flux, the enhancement of soil microbial activity is conducive

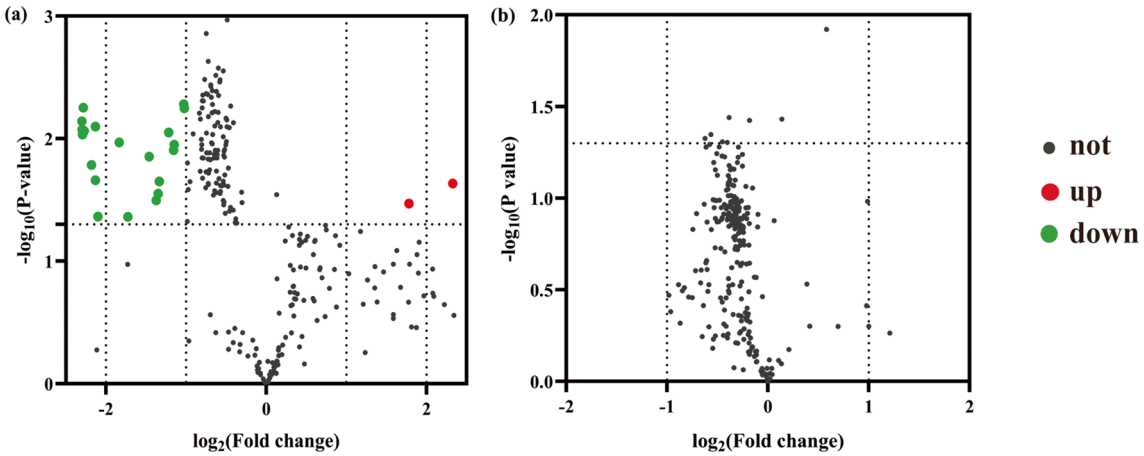

Fig. 6 Differentially abundant KEGG pathways between inoculation group and control group soils with 5% moisture (**a**), and between inoculation group and control group soils with 15% moisture (**b**). Green dots represent significantly downregulated pathways, red dots represents significantly upregulated pathways, and black represents no significant difference in pathways

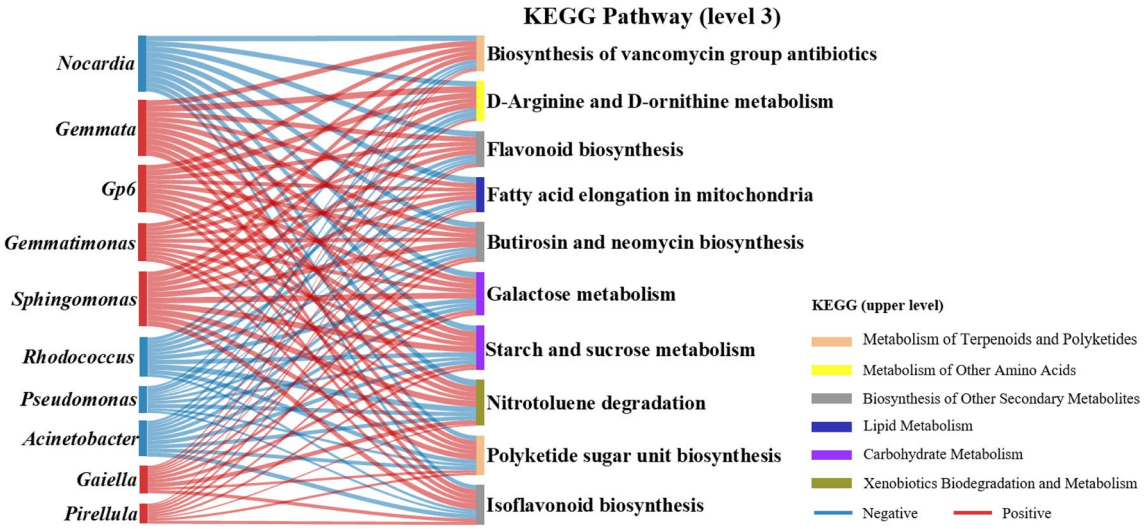

Fig. 7 Bacterial taxa associated inoculation in the soil with 5% moisture are related to several gene for metabolism pathways. Only KEGG pathways relating to metabolism and selected genera of interest are included in the heatmap

to soil microbial degradation [32]. Thus, a homogeneous environment of oxygen, bacterial mass, macro- or micronutrients, and target hydrocarbons is provided by soil moisture [35]. In addition, the uptake and transport of metabolic by-products into or out of bacterial cells are influenced by the moisture contents in the reaction medium [36, 37]. However, the changes of diversities and structures of soil microbial communities by different moisture levels have not yet been illustrated, especially for the functional microbes referring to petroleum degradation. In this study, we investigated the effects of soil moisture contents on petroleum biodegradation, the structures and compositions of hydrocarbon degrading bacteria, and their degradation-related genes.

Biodegradation does not always progress as rapidly, or to the same extent, as that observed in liquid-phase experiments due to the presence of a large number of microorganisms in the soil and the complex relationships between them. There are synergistic/competitive interactions between exogenous and indigenous microorganisms that result in the increased/decreased effectiveness of contaminants [38, 39].

Water is needed for microbial growth and the diffusion of nutrients and byproducts that occur through cell walls during the biodegradation process [33, 40]. A sufficient moisture content for microbial growth must be maintained. Low moisture can restrict bacterial movement [40]. Previous studies have confirmed that an appropriate soil humility facilitated hydrocarbon removal [41, 42]. Chaudhary et al. [33] showed that 11.2% of the hydrocarbons were

removed from the soil by spraying sterile water into the polluted soil. Wu et al. [31] indicated that about 16% of TPH removal rate when sterile water was added to soil. Sharma and Pandey [43] found that soil MC increased both microbial mobility and the petroleum biodegradation efficiency in soil. However, extra moisture may fill the small pores between soil particles and limit oxygen transport [34]. Table S5 provided some of the literature reports on the importance of an adequacy of moisture for bioremediation.

The inoculation of HDBF at 5% MC resulted in the reduction of the number of microorganisms and microbial diversities (Fig. S3), which affected soil microbial community stability and ecological function. After increasing soil moisture content to 15%, a minor reduction of the number and diversities of soil microbiomes was observed. In addition, the increment of soil moisture content avoided the loss of some bacterial species (Fig. 3) including *Mycobacterium*, *Sphingomonas*, and *Gemmatimonas*, which are considered to be the key species for petroleum hydrocarbon degradation [15, 34, 40, 42, 44, 45]. Soil moisture is contributed to bacterial activity, and a high soil water content favors the physiological requirements for microorganisms and reduces competition between foreign and indigenous microorganisms.

The soil bacterial communities in the soils with 5% MC were less similar in structure, resulting in more distinct and significantly different branches. It was also observed that bioaugmentation reduced soil biodiversity and altered key taxa linkages in the microbial network, thereby weakening the linkages of soil microbiomes. In addition, inoculation also reduced the availability of resources and food to soil native microbes, thereby weakening the soil microbial interactions. In contrast, the microorganisms in the soils with 15% MC were more tolerant and better adapted to environmental changes, which revealed greater similarities of soil microbial communities and greater synergy and microbial compactness (Fig. 4).

Our results also indicated that inoculation had a great impact on the classification of Proteobacteria and Actinomycetes in the soil. Almost all observed patterns of co-exclusion (90%) were attributed to bacterial taxa belonging to Proteobacteria and Actinomycetes. It was also found that enhanced moisture during inoculation reduced co-exclusion between foreign and native microorganisms (Fig. 5).

In 5% moisture soils, inoculation of degraders resulted in a significant reduction in metabolic pathways abundance, which is consistent with the trends of reduced species associated with petroleum hydrocarbon degradation (Figs. 6 and 7), and these bacteria are known to play an important role in bioremediation. In contrast, the same functional pathways were found in soils with 15% MC. There were fewer unique and overrepresented functional pathways due to the relatively high habitat similarity in the 15% MC soils, leading to overlapping functional capacities [36, 46].

In fact, the increment of soil moisture kept the stability of soil microbiota due to inoculation, which leading to a positive effect on petroleum removal. Soil moisture provided a suitable environment for petroleum degrading bacteria and increased bacterial density and activity [39, 42], which had promoted the growth of bacteria relating to petroleum degradation [36, 46]. Accordingly, the enhancement of petroleum remediation by increasing soil moisture was performed by improving the co-metabolism and petroleum degrading bacterial community structures.

Conclusions

This study demonstrated that petroleum degradation efficiency is promoted by enhancing soil moisture content during inoculation of the hydrocarbon-degraders since 15% of moisture content could strengthen the interaction of the bacterial community network and reduce the loss of some key bacteria species such as *Mycobacterium*, *Sphingomonas*, and *Gemmatimonas*. The results suggested that the dynamic balances of microbial communities and the metabolic interactions by 15% moisture content treatment are the driving forces for the enhancement of bioremediation in petroleum-contaminated soil. Therefore, in in situ petroleum-contaminated bioremediation, enhancement of soil moisture content may emerge as an important impact factor for petroleum removal. However, other soil moisture levels are needed to confirm these conclusions through analyzing more diverse microbiomes and petroleum-degrading genes using qPCR, DNA-SIP, and other advanced methods.

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Declarations

Conflict of Interest The authors declare no competing interests.

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