

# Petroleum-contamination drives the shift of microbiome through modifying soil metallome

Zhe Liu<sup>1</sup>, Meixia Gu<sup>2</sup>, Wei Zhu<sup>2</sup>, Mengru Zhang<sup>1</sup>, and Weizhi Zhou<sup>1</sup>

<sup>1</sup>Shandong University

<sup>2</sup>Sinopec Petroleum Engineering Design Co., Ltd

November 16, 2021

## Abstract

Soil oil-pollution is one of the most severe environmental issues at present. Shifts of soil metallome and microbiome are essential indicators for risk assessment and remediation of field soil pollutions, but not well studied undergoing the petroleum contamination. In this research, soil samples were collected from a short-term and long-term petroleum-contaminated oil field. The soil physicochemical properties, metallome, microbial community, and polluted and unpolluted soil network were testified. Results showed that the contents of soil total petroleum hydrocarbon, total carbon, total nitrogen, total sulfur, total phosphorus, calcium, copper, manganese, lead, and zinc were increased by petroleum contamination. In contrast, the soil pH was decreased by petroleum contamination regardless of the pollution duration. Petroleum-contamination also reduced bacterial and fungal  $\alpha$ -diversity indices. In contrast, bacterial  $\alpha$ -diversity was negatively correlated with soil TPH and EC, and fungal  $\alpha$ -diversity was negatively correlated with soil EC. Moreover, the relative abundances of Proteobacteria, Ascomycota, Oleibacter, and Fusarium in soil were increased by petroleum contamination. Network analysis showed that number of links, modules and the network invulnerability decreased in PS, followed by the OS group. These results demonstrate that short-term heavy petroleum contamination can cause shifts in soil physicochemical properties, metallome, and microbiome and assemble a less complex and vulnerable soil microbial network. Moreover, natural restoration can hardly amend soil properties and microbial network structure. This research emphasizes that the uncommonly studied soil metallome may play a vital part in the reaction of soil microbial communities to petroleum-contamination and potential application value of synthetic community in bioremediation.

## 1. Introduction

With the growth of the global population and the massive utilization of petroleum products, petrochemical production continues to develop, resulting in marine and land environmental pollution. This can happen during oil production, transportation, use and leakage (Ramadass et al., 2018; Baoune et al., 2019). Due to the complex composition, high biological toxicity, and low bioavailability of petroleum hydrocarbons, they are harmful to soil, groundwater, and the regional ecological environment (Dariush et al., 2007, Hassan and Al-Jawhari 2014). Moreover, the oil site soil is often accompanied by salinization, which makes its remediation extremely difficult.

The soil microbiota plays a vital role in the material cycles and energy flow in the soil system, such as the biogeochemical cycle of nitrogen, carbon and phosphorus (Liu et al., 2020). Furthermore, when soil is disturbed by pollutants, soil microorganisms react immediately to resist the invasion of pollutants. Many studies have used DNA-based technology to study the microbiota in petroleum hydrocarbons or organic pollutants polluted soil, water, and sediments (Sheng et al., 2016, Zhou et al., 2020, Hamdan and Salam 2020). As an oil-polluted lake in France, the profiles of the sedimentary bacterial community have been associated with the oil-contaminated gradient (Paisse et al., 2018). The complex pollution composition exerts selective pressure on these bacterial communities, and the oil degrading bacteria do not increase with the increase of oil pollutants. On the contrary, the study on the microbial community of an oil contaminated

site in China found that there were more oil-degrading microorganisms in polluted soils than in clean soils (Liu et al., 2019). However, few studies concentrated on the effects of oil contamination on soil microbial community in the long or short term, and few studies focused on culture-dependent microorganisms.

Microorganisms do not exist alone in the soil, but form a complex matrix of ecological interaction. With the exception of the environmental factors, community interactions also affect microbial behavior, including the interaction between prokaryotes and eukaryotes. The coexistence of bacteria and fungi in the same environment inevitably leads to material and energy exchange among them (De Boer et al., 2005; Benoit et al., 2015; Warmink et al., 2009). Further studies have been carried out in natural samples describing the coexistence between bacterial and fungal communities recently. The network study for limited-length polymorphism endpoint data showed that bacterial and fungal communities and soil properties were linked, as shown by a typical correlation module (De Menezes et al., 2015). The network study provides richer insights into microbial assemblages compared to simple indices of heterogeneity and structure. In addition, they supplement an important facet to our knowledge of the interactions among microbiota or between a known community and environmental parameters such as soil contamination. Up to now, though, few networks was assembled for soil microbial communities under petroleum contamination, especially under differing pollution duration.

Metals and metalloids are indispensable for agricultural products and land. In a previous study, Haraguchi (2004) described for the first time the “metalloidic” as an “integrated biometal science,” trying to supply a systematic perception of the absorption, change, function, and excretion of the metal within biological systems. Metallomics concentrates on a systematic investigation of metallomes and the interactions and functional associations of metals or metalloid species with gene, protein, metabolite, and other biomolecule in cells (Ge and Sun 2009). The examination of metals obtained from soil—the metallomes—can be detected through inductively coupled plasma–mass spectrometry (ICP–MS). Meanwhile, a previous work has shown that the shift of the soil ionome resulting from different fertilization can drive the assembly of the soil microbiome and alter microbial interaction and function (Liu et al., 2020). However, to date, little is known about how the soil metallome reacts to oil contamination in the long- or short-term.

To explore the short-term and long-term impacts of petroleum pollution on soil, the physicochemical properties, the contents of 18 metals, the diversity, composition, and structure of soil microbial communities were determined. In addition, three strains of bacteria with high petroleum degradation efficiency were isolated from long-term petroleum-contaminated soil, and the degradation efficiency of their synthetic community on petroleum hydrocarbons and the growth of maize seedlings under oil pollution stress were explored. We hypothesized that: 1) Petroleum pollution in different years caused significant differences in the diversity and composition of soil microbial community. 2) Petroleum pollution with different years will significantly change the topological parameters of the soil microbial network.

## 2. Methods and materials

### 2.1 Soil sampling and site description

Soil samples were collected from two oil production plants (Site I: 37°59'20"N, 118deg36'57"E and Site II: 37deg55'45"N, 118deg35'53"E) located in Shengli Oilfield, Hekou District, Dongying City, Shandong Province (**Fig. S1a**). In each site, three groups of soil samples were collected (0~20 cm): short-term contaminated soil (PS) within a one-meter radius near the working oil pumping machine (**Fig. S1b**), long-term contaminated soil (OS) within a one-meter radius near the abandoned oil pumping machine (Site I for eight years, site II for six years), and uncontaminated control soil (Control) nearby. There were five replicates for each sampling group, and ten soil cores thoroughly homogenized each replicate. Thus 30 soil samples were collected in total (two sites x three groups x five replicates). Soil samples were transported to the laboratory in sterilized plastic bags on drikold as soon as possible. After passing a 4-mm strainer, part of the soil samples was air-dried for physicochemical properties determination. The remaining soil samples were stored at -80 for DNA analyses.

### 2.2 Soil physicochemical properties analysis

Total petroleum hydrocarbon (TPH) concentrations in soil were detected using the Ultrasonic-Soxhlet gravimetric extraction method (Huesemann, 1995). Use a pH meter (PHS-3E; Shanghai) to measure soil pH at a soil-water ratio of 2:5. Soil total carbon (TC), total nitrogen (TN), and total sulfur (TS) were determined using an elemental analyzer (Elementar Vario EL III, Germany). Soil total phosphorus (TP) was digested with an  $\text{H}_2\text{SO}_4\text{-HClO}_4$  solution at 250 °C and evaluated by the molybdenum-blue colorimetric method (Walker and Adams, 1958). An electrical conductivity meter (DDSJ-318, Shanghai) was used to explore soil electrical conductivity (EC).

### 2.3 Soil metallomics analysis

In this study, the concentrations of Mg, Ca, Sc, Ti, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Ga, Ge, As, Se, Kr, and Pb in soil samples were detected by Inductively coupled plasma-mass spectrometry (ICP-MS). Shortly, 0.5 g of the soil samples were digested with 1 mL HCl, 1 mL HF, and 3 mL  $\text{HNO}_3$  at 150 °C for 8 hours, then raise the temperature to 200 °C to remove the acid. The remaining ash was resuspend using 10 mL 2% diluted  $\text{HNO}_3$  before using ICP-MS to detect metals concentrations.

### 2.4 Soil microbial community analysis

The whole genome DNA of soil was extracted using CTAB method. The concentration and purity of DNA were detected on 1% agarose gel. DNA was diluted to 1ng/ $\mu\text{L}$  using double distilled water according to the concentration.

16S rRNA genes from V4-V5 hypervariable region has been amplified with a primary (515F/806R) with barcode for bacteria. The internal transcribed spacer two regions (ITS2) were amplified using the primer set ITS7/ITS4. All PCR reactions have been carried out with 15  $\mu\text{L}$  of Phusion® High-Fidelity PCR Master Mix, two  $\mu\text{M}$  of forward and reverse primers, and about ten ng template DNA. The program consisted of initial denaturation at 95 °C for one minute, followed by 27 cycles of 95 °C for ten seconds, 57 °C for 30 seconds, and elongation at 72 °C for 30 seconds, final 72 °C for 10 minutes.

The same quantity of 1XTAE buffer was mixed with PCR products, and electrophoresis detection was operated on 2% agarose gel. PCR products were mixed in EU density ratios. Then, mixture PCR products were purified using DNA Purification Kit (Novo, Beijing). The methods for Illumina sequencing and Bioinformatics analysis see supplementary materials **X1**.

### 2.5 Microbial and plant growth assay

This study determined the degradation efficiency of petroleum hydrocarbons by three bacterial strains isolated from long-term petroleum-contaminated soil. In addition, the effects of three bacteria strains and their synthetic communities on the growth of maize seedlings under petroleum hydrocarbon stress were investigated. Briefly, the crude oil was evenly mixed with sterilized oilfield control soil at a proportion of 0.5% and stored for four weeks. Maize seeds were surface-sterilized with 70% ethanol and 50% bleach before germination in the hydroponics platform. All SynCom strains were grown to saturation in quarter-strength tryptic soy broth and washed using phosphate-buffered saline before root inoculation.

### 2.6 Statistical analysis

The normality and homogeneity of variances of the dataset in this study had been verified. The differences between three groups regarding soil physical, chemical properties and microbial  $\alpha$ -diversity indices had been obtained by one-way analysis of variance (ANOVA). While group means had been subjected to Duncan's test at  $p < 0.05$  using a DPS (Data Processing System) v.7.05. The concentrations of 18 soil metals among three sampling groups were shown by heatmap, generated by R software v.3.5.1 through the heatmap package. Principal Co-ordinates Analysis (PCoA) of soil microbial community based on ASVs level, analysis of similarities (ANOSIM), and variance partitioning analysis (VPA) for microbial community composition among groups were all using the vegan package in R software v.3.5.1.

A network analysis was conducted to reveal the relationship between bacterial and fungal taxa (relative abundance  $> 0.1\%$ ), soil metals, and physicochemical properties. We calculated the pairwise Spearman's

correlation coefficients ( $p < 0.05$ ) using psych package in R software v.3.5.1 and visualized the network using Gephi software v.0.9.2. Each node represented one microbial taxon or soil parameter. Red lines and blue lines represented strong positive and negative correlations, respectively. The destruction resistance of the network was generated using R software v.3.5.1 through randomly removing the nodes and calculating the natural connectivity and network degree values.

The direct or indirect effects of total petroleum hydrocarbons on soil parameters, bacterial and fungal diversity, and community compositions were generated through conducting the Spearman correlation or Mantel test between matrices ( $p < 0.05$ ).

### 3. Results

#### 3.1 Soil physicochemical properties

The physical and chemical properties of soil with different oil pollution duration showed significant differences (**Fig. 1**). The results showed that compared with the control, the contents of TPH, TC, TN, TS, and TP in soil were increased by oil pollution (one-way ANOVA,  $p < 0.05$ ), and the soil pH was significantly reduced. For soil electric conductivity, short-term contaminated soil is significantly lower than long-term oil-contaminated soil. In addition, the soil scanning electron microscope photos show that (**Fig. S2**) after the soil is polluted by oil, the soil is more likely to form a large block structure. At the same time, the uncontaminated soil is not only looser but also has a porous surface.

#### 3.2 Soil metallomes

ICP-MS determined the contents of 18 metal elements in soil samples. At the site I (**Fig. 2a**), compared with the control, short-term oil pollution increased the contents of Ca, Co, Cu, Fe, Mn, Ni, Pb, and Zn ( $p < 0.05$ ), and significantly reduced the contents of As, GA, Kr, SC, Ti and V. With the extension of pollution duration, soil Ca, Cu, Mn, Pb and Zn accumulated significantly. At site II (**Fig. 2b**), compared with the control, short-term oil pollution increased the contents of Ca, Co, Cu, Mg, Mn, Ni, and Zn ( $p < 0.05$ ), and significantly reduced the contents of As, Cr, Fe, GA, SC, Ti and V. With the extension of pollution years, soil Ca, Co, Cu, Kr, Mn, Pb and Zn accumulated significantly.

PCoA analysis and Spearman correlation analysis were based on soil physicochemical properties and metal datasets (**Fig. 3**). The results showed that soil samples from different sites and groups were significantly separated. Correlation analysis showed a strong positive correlation between the contents of Cu, Zn, Mn, Co, TS, and Pb ( $p < 0.05$ ), and there was a significant positive correlation between the contents of V, Cr, TP, Mg, and Ca. In addition, soil pH showed the most correlation with other indicators, followed by TPH.

#### 3.3 Soil microbial diversity

The soil bacterial and fungal  $\alpha$ -diversity index were differed among the three groups (**Fig. 4a, b, f, g**). Compared with Control, petroleum pollution led to a significant reduction in soil bacterial diversity ( $p < 0.05$ ). For fungi, soil fungal diversity had been significantly reduced by petroleum pollution regardless of the contamination duration. Moreover, correlations between microbial diversity index and soil physicochemical properties had been calculated (**Fig. S3**). The results showed that the Chao1 index was negatively correlated with soil TPH and EC ( $p < 0.05$ ) for bacteria. For fungi, the Chao1 index was negatively correlated with soil EC ( $p < 0.001$ ). The PCoA analysis of microbial community profiles proved that groups could separate soil bacterial and fungal communities (**Fig. 4c, d, h, i**). In addition, the results of ANOSIM uncovered that the total discrepancy among groups was more significant than within groups.

Through VPA analysis, the relative contributions of soil sampling points and soil groups, soil physical and chemical properties and metals to the composition of soil microbial community were revealed. The effects of soil sampling sites and groups, soil physical and chemical properties, metallomes and their interactions on the composition of soil microbial community were analyzed. For bacterial community (**Fig. 4e**), these variables collectively explained 53% of observed discrepancies, leaving 43% unexplained. Among those separate variables, soil physical and chemical properties explained the most significant part of the difference

in bacterial community profiles (12%,  $p < 0.05$ ), followed by soil metallome (8%,  $p < 0.05$ ), sampling location and sampling group (7%,  $p < 0.05$ ). Their interactions interpreted 36 percent of the total discrepancy in bacterial community composition ( $p < 0.05$ ).

For fungal communities (**Fig. 4j**), the same variable explained 60% of the observed discrepancies, and 40% of it was unexplained. Among these independent variables, sampling sites and sampling groups contributed the most to the change of fungal community composition (11%,  $p < 0.05$ ), while soil physicochemical properties only accounted for 6% of total variation ( $p < 0.05$ ). Interactions between soil physicochemical properties and soil metallomes explained 13% of the discrepancies in fungal community profiles, while the interactions between all variables explained 54% of the variation ( $p < 0.05$ ).

### 3.4 Soil Microbial community composition

In the whole data set, 1947305 bacterial sequences and 2631038 fungal sequences obtained from 30 soil samples were classified as 17295 and 1681 ASVs, respectively. On average, 98.11% of the sequences in each sample were classified as bacteria and only 1.89% were classified as archaea. For bacteria, Proteobacteria accounted for the highest proportion among the 30 samples, accounting for 77.79% of the total on average. Other dominant bacteria include Bacteroidetes (5.02%), Actinobacteria (3.95%), Firmicutes (3.04%), and Gemmatimonadetes (2.15%). Among the fungal sequence, the average is 100% fungi. Ascomycota predominated these samples, accounting for an average of 82.58% of the total sequence. The other prevalent phyla were Mortierellomycota (10.61%) and Basidiomycota (1.88%).

At the phylum level (**Fig. 5**), compared with the control, the relative abundance of Proteobacteria were increased, and that of Gemmatimonadetes and Bacteroidetes were decreased. In the fungal community, the relative abundance of Ascomycota and Chytridiomycota increased. In contrast, the relative abundances of Basidiomycota, Mortierellomycota, and Zoopagomycota decreased after short-term petroleum contamination ( $p < 0.05$ ) compared with Control. After long-term petroleum pollution, the relative abundance of Ascomycota was raised, while that of Chytridiomycota and Zoopagomycota were decreased ( $p < 0.05$ ).

Among the top 20 identified bacterial genera (**Fig. 5**), *Ralstonia*, *Pseudomonas*, *Sulfurimonas*, *Oleibacter*, and *Marinobacter* were most bounteous. Compared with Control, relative abundances of *Ralstonia*, *Acinetobacter*, *Lysobacter*, *Kaistobacter*, *Stenotrophomonas*, *Bacillus*, *Lactobacillus*, and *Sphingomonas* were significantly decreased in PS samples. Conversely, relative abundances of *Pseudomonas*, *Sulfurimonas*, *Oleibacter*, *Marinobacter*, *Marinobacterium*, *Arcobacter*, *Halomonas*, *Pseudidiomarina*, and *Shewanella* were increased ( $p < 0.05$ ); Relative abundances of *Ralstonia*, *Marinobacter*, *Acinetobacter*, *Lysinibacillus*, and *Lactobacillus* were significantly decreased in OS samples, conversely, relative abundances of *Oleibacter*, *Alcanivorax*, *Sediminibacter*, *Thiobacillus*, *Nitrosopumilus*, and *Candidatus Portiera* were increased ( $p < 0.05$ ). For fungi, *Fusarium*, *Mortierella*, *Pseudallescheria*, *Alternaria* and *Ochroconis* were most abundant. Compared with Control, relative abundances of *Mortierella*, *Alternaria*, *Cladosporium*, *Syncephalis* were decreased in PS samples. Conversely, relative abundances of *Fusarium*, *Ochroconis*, and *Nigrospora* were increased ( $p < 0.05$ ); Relative abundances of *Mortierella*, *Alternaria*, *Cladosporium*, *Syncephalis*, and *Aspergillus* were decreased in OS samples, whereas that of *Fusarium* and *Ochroconis* were increased ( $p < 0.05$ ).

### 3.5 Multiple drivers accounting for microbiome

A driving model is constructed based on the Spearman and Mantel test to explore how petroleum affects and drives the diversity and composition of soil physicochemical properties, metallome, and microbiome in the complex soil environment (**Fig. 6**). The results showed that after TPH entered the soil, the contents of TC and TN increased significantly, and the bacteria decreased  $\alpha$  diversity ( $p < 0.05$ ) significantly. In addition, petroleum hydrocarbons could directly change the soil metal group, bacterial and fungal community composition ( $p < 0.05$ ). In addition, it also indirectly affected the soil metal group through TC and TN ( $p < 0.05$ ), and the soil metal group indirectly affected the composition of bacterial community and fungi  $\alpha$  diversity was affected ( $p < 0.05$ ).

### 3.6 Microbial networks and topological properties

Based on the Spearman correlation ( $p < 0.05$ ) between soil physical and chemical properties, metal groups, bacteria, and fungi ASVs levels (relative abundance  $> 0.1\%$ ), microbial networks in three types of soils were constructed (**Fig. 7**). The results show that compared with control, the number of points, connections, average degree, modularity, and module books in PS and OS networks are gradually reduced. Interestingly, in the PS network, the proportion of positive connections decreased significantly to 55.4%, and in the OS network, the number of positive connections rebounded to 60.8%. In addition, the network survivability test results show that the stability of the three networks from high to low is Control, PS, and OS.

### 3.7 Microbiological and plant experiments

Three bacterial strains B1, B2, and B3 were isolated from OS soil samples and identified as *Pseudomonas stutzeri*, *Bacillus pumilus*, and *Pseudomonas anguilliseptica* (**Fig. 8a, b, c**), respectively. Simultaneously, *Pseudomonas* and *Bacillus* belong to keystone taxa in OS microbial network. A one-week petroleum hydrocarbon degradation experiment shows that the degradation efficiency for strain B1, B2, B3, and SynCom reached 32.4%, 37.1%, 40.2%, and 45.6%, respectively (**Fig. 8d**). Then SynCom was used to identify the effects of co-cultured bacterial strains B1, B2, and B3 on the growth of maize seedlings under petroleum pollution. The results showed that SynCom treatment significantly improves the seedlings' dry weight, shoot length, and root length (**Fig. 8e**).

## 4. Discussion

Soil pollution from petroleum is currently considered to be one of the most severe environmental problems. This type of pollution reduces or destroys soil fertility, modifies the elemental composition of soil and water cycles, results in losses in the aesthetic value of ecosystems, leads to secondary pollution of air and groundwater, and inhibits or eradicates soil organisms (Koshlaf and Ball 2017, Margesin et al., 2003, Haritash and Kaushik 2009, Mishra et al., 2001, Hu et al., 2013).

The content of soil carbon, nitrogen, and sulfur increased significantly. At the same time, the pH decreased significantly (**Fig. 1**) after petroleum pollution, which corresponds with former researches (Wang et al., 2010, Anikwe et al., 2017). This is mainly due to a large amount of carbon, nitrogen, and sulfur in petroleum, and when sulfur enters the soil, it forms sulfur oxides and reduces the soil pH. Scanning electron microscope photos visually describes oil pollution's adverse effects on soil particle structure (**Fig. S2**). The determined results of metalomics content in different soil samples show that petroleum pollution leads to the accumulation of soil Ca, Cu, Mn, Pb, and Zn (**Fig. 2**). In addition, the cluster analysis based on the data sets of physical and chemical properties and metal group content can well distinguish different samples (**Fig. 3**), which shows that the oil pollution of different years has a different degree of impact on the soil. The correlation analysis results show a positive coupling relationship between a large number of metal elements carried by petroleum.

Previous studies have found that petroleum pollution will lead to shifts in the soil microbial community. In this study, the microbiome of oil-contaminated soil in different years was studied. Results showed that petroleum pollution significantly reduced the soil bacterial and fungal diversity and shaped the microbial community (**Fig. 4**). Cheema et al. (2015) found that bacterial diversity in agricultural soil was significantly higher than in hydrocarbon-contaminated soil. Moreover, Wang et al. (2018) found that the toxicological effect of oil pollutants significantly decreased the soil fungal diversity.

In this study, the relative abundance of Proteobacteria and Ascomycota was increased by petroleum contamination (**Fig. 5**). A previous study (Zhang et al., 2012) had reported that Proteobacteria was proved to be the most easily cultivated bacteria in petroleum contaminated soils. Previous researches (Wu et al., 2019, Zhang et al., 2012) showed that part of the Proteobacteria can degrade petroleum hydrocarbons or PAHs in petroleum polluted soil. Kim et al. (2019) enriched microorganisms producing biosurfactants, which have the potential to degrade hydrocarbons in oil contaminated soil, and found that Proteobacteria has the highest relative abundance. Moreover, a previous study proved that Ascomycota was the most dominant

known petroleum-degrading fungal phyla (Ezekoye et al., 2018, de la Cruz-Izquierdo et al., 2021). In addition, results also showed that the relative abundance of *Oleibacter* and *Fusarium* was significantly increased in both PS and OS samples. *Oleibacter* was previously proved to be a high performance of HC-degrading bacteria species (Catania et al., 2015), while *Fusarium* (Hidayat and Tachibana 2012, Azin et al., 2018) was also used in petroleum degradation.

After petroleum hydrocarbon leakage into the soil, it will cause a series of changes in soil physicochemical properties, microbial diversity, and composition (**Fig. 6**). This study found that petroleum hydrocarbons can directly lead to the increase of soil carbon and nitrogen content, the decrease of bacterial diversity, and the change of metal group, bacterial and fungal community. The diversity of the soil fungal community was indirectly reduced by soil metallome. Carbon and nitrogen were vital factors limiting microbial populations in petroleum polluted soils. Previous research has proved that soil total petroleum hydrocarbon, total carbon, and total nitrogen were the most essential factors influencing the bacterial communities in oil polluted soils but varied at different contaminating levels (Feng et al., 2020). It was found that the best optimal Carbon: Nitrogen was between 20:1 and 50:1 for microorganism growth in petroleum polluted soil (Kaufmann et al., 2004).

The analysis of co-occurrence network may explain the structure and function of microbial community (Ma et al., 2020; Wagg et al., 2019). With the change of soil microbial community composition, microbial co-occurrence network consequentially changes. Our study showed that the number of nodes, links, and modules decreased in PS and OS samples compared to Control (**Fig. 7**). Functional cooperative behaviors have been reported to exist commonly in soil microbial communities (Cremer et al., 2019). The symbiotic network analysis identifies microbial taxa that have cooperative or co-dependent relationships, often representing a population, which usually indicate a mutual function (Deshpande et al., 2013). The modules have the function of minimizing the impact of environmental disorder (Kitano, 2004); hence, the fewer modules in the OS and PS network, the slower communication speed between modules, and the lower response efficiency to environmental stimulus.

Otherwise, fewer connections in petroleum-polluted soil samples represented inhibition in the interactions between soil microorganisms. This may be due to the decrease of microbial activity in soil caused by oil pollution (Guo et al., 2012, Labud et al., 2007). What's more, the proportion of positive links in PS was lower than that of OS samples. This indicates that the interaction between soil microorganisms is more competitive or antagonistic after receiving oil pollution quickly. After long-term natural recovery, the interaction mode between soil microorganisms becomes synergy or symbiosis. Meanwhile, the network invulnerability of Control was highest among all groups, followed by PS and OS, which means that the soil microbial network becomes less complex and vulnerable after petroleum contamination.

After long-term oil pollution, more petroleum hydrocarbon-degrading bacteria may be enriched in the soil. Thus, three bacterial strains with significant petroleum hydrocarbon degradation efficiency were isolated from OS samples (**Fig. 8**). *Pseudomonas stutzeri* (Li et al., 2020, Gałazka et al., 2012) and *Bacillus pumilus* (Sheeba et al., 2017, Patowary et al., 2015) are reported to be potential bacteria strains in petroleum and polyaromatic hydrocarbons degradation applications before. Niu et al. (2017) used an agar system and a SynCom composed of 7 bacterial strains cultured from corn roots to show that the absence of *Enterobacter cloacae* will lead to the whole collapse of microbial community profiles in the corn seedlings rhizosphere, proving the significance of microbial interactions and the presence of crucial taxa in the rhizosphere microbiota. Our study also found that the SynCom consisting of three bacterial strains could significantly improve the performance of maize seedlings under petroleum-pollution stress. This may provide a new strategy for petroleum-contaminated soil reuse.

## References

Anikwe, M., Ikenganyia, E., Egbonimale, J., Oputah, C., 2017. Assessment of some tropical plants for use in the phytoremediation of petroleum contaminated soil: effects of remediation on soil physical and chemical properties. *Int. J. Plant Soil Sci.* 14(2), 1–9.

- Azin, E., Moghimi, H., Heidarytabar, R., 2018. Petroleum degradation, biosurfactant and laccase production by *Fusarium neocosmosporiellum* RH-10: a microcosm study. *Soil Sediment Contam.* 27(4), 329–342.
- Baoune, H., Aparicio, J.D., Acuña, A., El Hadj-khelil, A.O., Sanchez, L., Polti, M.A., 2019. Effectiveness of the *Zea mays*-*Streptomyces* association for the phytoremediation of petroleum hydrocarbons impacted soils. *Ecotoxicol. Environ. Saf.* 184, 109591.
- Benoit, I., van den Esker, M.H., Patyshakuliyeva, A., Mattern, D.J., Blei, F., Zhou, M., Dijksterhuis, J., Brakhage, A.A., Kuipers, O.P., de Vries, R.P., Kovács, A.T., 2015. *Bacillus subtilis* attachment to *Aspergillus niger* hyphae results in mutually altered metabolism. *Environ. Microbiol.* 17, 2099–2113.
- Bokulich, N.A., Kaehler, B.D., Rideout, J.R., Dillon, M., Bolyen, E., Knight, R., Huttley, G.A., Caporaso, J.G., 2018. Optimizing taxonomic classification of marker-gene amplicon sequences with QIIME 2's q2-feature-classifier plugin. *Microbiome.* 6, 90.
- Callahan, B.J., McMurdie, P.J., Rosen, M.J., Han, A.W., Johnson, A.J.A., Holmes, S.P., 2016. DADA2: High-resolution sample inference from Illumina amplicon data. *Nat. Methods.* 13, 581.
- Catania, V., Santisi, S., Signa, G., Vizzini, S., Mazzola, A., Cappello, S., 2015. Intrinsic bioremediation potential of a chronically polluted marine coastal area. *Mar. Pollut. Bull.* 99, 138–49.
- Cheema, S., Lavania, M., Lal, B., 2015. Impact of petroleum hydrocarbon contamination on the indigenous soil microbial community. *Ann. Microbiol.* 65, 359–369.
- Cremer, J., Melbinger, A., Wienand, K., Henriquez, T., Jung, H., Frey, E., 2019. Cooperation in microbial populations: theory and experimental model systems. *J. Mol. Biol.* 431(23), 4599–4644.
- Dariush, M.T., Shahriari, M.H., Gholamareza, S.F., Kalantari, F., Azzi, M., 2007. Effect of light crude oil-contaminated soil on growth and germination of *Festuca arundinacea*. *J. Appl. Sci.* 7(18), 2623–2628.
- de Boer, W., Folman, L.B., Summerbell, R.C., Boddy, L., 2005. Living in a fungal world: impact of fungi on soil bacterial niche development. *FEMS Microbiol. Rev.* 29, 795–811.
- de la Cruz-Izquierdo, Rosa., Paz, A., Reyes, E., Francisco, V.J., Lenci, S.S., Martha, G.D.M., Rivera, G., 2021. Analysis of phenanthrene degradation by Ascomycota fungi isolated from contaminated soil from Reynosa, Mexico. *Lett. Appl. Microbiol.* 72.
- De Menezes, A.B., Prendergast-Miller, M.T., Richardson, A.E., Toscas, P., Farrell, M., Macdonald, L.M., Baker, G., Wark, T., Thrall, P.H., 2015. Network analysis reveals that bacteria and fungi form modules that correlate independently with soil parameters. *Environ. Microbiol.* 17, 2677–2689.
- Deshpande, R., VanderSluis, B., Myers, C.L., 2013. Comparison of profile similarity measures for genetic interaction networks. *PLoS One.* 8(7), e68664.
- Ezekoye, C.C., Chikere, C.B., Okpokwasili, G.C., 2018. Fungal diversity associated with crude oil-impacted soil undergoing in-situ bioremediation. *Sustain. Chem. Pharm.* 10, 148–152.
- Feng, X., Liu, Z., Jia, X., Lu, W., 2020. Distribution of bacterial communities in petroleum-contaminated soils from the Dagang oilfield, China. *Trans. Tianjin Univ.* 26, 22–32.
- Gałazka, A., Król, M., Perzyński, A., 2012. The Efficiency of rhizosphere bioremediation with *Azospirillum* sp. and *Pseudomonas stutzeri* in soils freshly contaminated with PAHs and diesel fuel. *Pol. J. Environ. Stud.* 21, 345–353.
- Ge, R., Sun, H., 2009. Metallomics: An integrated biometal science. *Sci. China, Ser. B: Chem.* 52 (12), 2055–2070.
- Guo, H., Yao, J., Cai, M., Qian, Y., Yue, G., Richnow, H.H., 2012. Effects of petroleum contamination on soil microbial numbers, metabolic activity and urease activity. *Chemosphere.* 87(11), 1273–1280.

- Hamdan, H.Z., Salam, D.A., 2020. Microbial community evolution during the aerobic biodegradation of petroleum hydrocarbons in marine sediment microcosms: effect of biostimulation and seasonal variations. *Environ. Pollut.* 265, 114858.
- Haraguchi, H., 2004. Metallomics as integrated biometal science. *J. Anal. At. Spectrom.* 19(1), 5-14.
- Haritash, A.K., Kaushik, C.P., 2009. Biodegradation aspects of polycyclic aromatic hydrocarbons (PAHs): A review. *J. Hazard. Mater.* 169, 1–15.
- Hassan, I., Ai-Jawhari, H., 2014. Ability of Some Soil Fungi in Biodegradation of Petroleum Hydrocarbon. *Appl. Environ. Microbiol.* 2, 46–52.
- Hidayat, A., Tachibana, S., 2012. Biodegradation of aliphatic hydrocarbon in three types of crude oil by *Fusarium* sp. F092 under stress with artificial sea water. *J. Environ. Sci. Technol.* 5(1), 64–73.
- Hu, G., Li, J., Zeng, G., 2013. Recent development in the treatment of oily sludge from petroleum industry: A review. *J. Hazard. Mater.* 261, 470–490.
- Huesemann, M.H., 1995. Predictive model for estimating the extent of petroleum hydrocarbon biodegradation in contaminated soils. *Environ. Sci. Technol.* 29, 7e18.
- Kaufmann, K., Christophersen, M., Buttler, A., Harms, H., HÄhener, P., 2004. Microbial community response to petroleum hydrocarbon contamination in the unsaturated zone at the experimental field site Værløse, Denmark. *FEMS Microbiol. Ecol.* 48, 387–399.
- Kim, T., Hong, J.K., Jho, E.H., Kang, G.Y., Lee, S.J., 2019. Sequential biowashing-biopile processes for remediation of crude oil contaminated soil in Kuwait. *J. Hazard. Mater.* 378, 120710.
- Kitano, H., 2004. Biological robustness. *Nat. Rev. Genet.* 5, 826–837.
- Koshlaf, E., Ball, S., 2017. Soil bioremediation approaches for petroleum hydrocarbon polluted environments. *AIMS Microbiol.* 3, 25–49.
- Labud, V., Garcia, C., Hernandez, T., 2007. Effect of hydrocarbon pollution on the microbial properties of a sandy and a clay soil. *Chemosphere.* 66(10), 1863–1871.
- Li, Q., Huang, Y., Wen, D., Fu, R., Feng, L., 2020. Application of alkyl polyglycosides for enhanced bioremediation of petroleum hydrocarbon-contaminated soil using *Sphingomonas changbaiensis* and *Pseudomonas stutzeri*. *Sci. Total Environ.* 719, 137456.
- Liu, R., Zhang, Y., Ding, R., Li, D., Gao, Y., Yang, M., 2009. Comparison of archaeal and bacterial community structures in heavily oil-contaminated and pristine soils. *J. Biosci. Bioeng.* 108, 400–407.
- Liu, Z., Guo, Q., Feng, Z.Y., Liu, Z.D., Li, H.Y., Sun, Y.F., Liu, C.S., Lai, H.X., 2020. Long-term organic fertilization improves the productivity of kiwifruit (*Actinidia chinensis* Planch.) through increasing rhizosphere microbial diversity and network complexity. *Appl. Soil Ecol.* 147, 103426.
- Liu, Z., Ma, X., He, N., Zhang, J., Wu, J., Liu, C., 2020. Shifts in microbial communities and networks are correlated with the soil ionome in a kiwifruit orchard under different fertilization regimes. *Appl. Soil Ecol.* 149, 103517.
- Ma, B., Wang, Y., Ye, S., Liu, S., Stirling, E., Gilbert, J.A., Xu, J., 2020. Earth microbial cooccurrence network reveals interconnection pattern across microbiomes. *Microbiome.* 8(1), 82.
- Margesin, R., Labbé, D., Schinner, F., Greer, C.W., Whyte, L.G., 2003. Characterization of hydrocarbon-degrading microbial populations in contaminated and pristine Alpine soils. *Appl. Environ. Microbiol.* 69, 3085–3092.
- Mishra, S., Jyot, J., Kuhad, R.C., Lal, B., 2001. In situ bioremediation potential of an oily sludge-degrading bacterial consortium. *Curr. Microbiol.* 43, 328–335.

- Nilsson, R.H., Larsson, K.H., Taylor, A.F.S., Bengtsson-Palme, J., Jeppesen, T.S., Schigel, D., Kennedy, P., Picard, K., Glöckner, F.O., Tedersoo, L., Saar, I., Kõljalg, U., Abarenkov, K., 2019. The UNITE database for molecular identification of fungi: handling dark taxa and parallel taxonomic classifications. *Nucleic Acids Res.* 47, 259-264.
- Niu, B., Paulson, J.N., Zheng, X., Kolter, R., 2017. Simplified and representative bacterial community of maize roots. *Proc. Natl. Acad. Sci. U S A.* 2017, 114, e2450.
- Paisse, S., Coulon, F., Goni-Urriza, M., Peperzak, L., McGenity, T., Duran, R., 2008. Structure of bacterial communities along a hydrocarbon contamination gradient in a coastal sediment. *FEMS Microbiol. Ecol.* 66, 295–305.
- Patowary, K., Saikia, R.R., Kalita, M.C., Deka, S., 2015. Degradation of polyaromatic hydrocarbons employing biosurfactant-producing *Bacillus pumilus* KS2. *Ann. Microbiol.* 65(1), 1–10.
- Ramadass, K., Mallavarapu, M., Kadiyalaand Naidu, R., 2018. Bioavailability of weathered hydrocarbons in engine oil-contaminated soil: impact of bioaugmentation mediated by *Pseudomonas spp.* on bioremediation. *Sci. Total Environ.* 636, 968–974.
- Sheeba, V.S., Brinda, L.M., Perumalsam, R., Manickam, V., 2017. Degradation of total petroleum hydrocarbon (tph) in contaminated soil using *Bacillus pumilus* MVSV3. *Biocontrol Sci.* 22(1), 17–23.
- Sheng, Y., Wang, G., Hao, C., Qian, X., Qian, Z., 2016. Microbial community structures in petroleum contaminated soils at an oil field, Hebei, China. *CLEAN–Soil, Air, Water.* 44(7), 829-839.
- Wagg, C., Schlaeppli, K., Banerjee, S., Kuramae, E.E., van der Heijden, M.G.A., 2019. Fungal-bacterial diversity and microbiome complexity predict ecosystem functioning. *Nat. Commun.* 10(1), 4841.
- Walker, T.W., Adams, A.F.R., 1958. Studies on soil organic matter: 1. Influence of phosphorus contents of parent materials on accumulations of carbon, nitrogen, sulfur, and organic phosphorus in grassland soils. *Soil Sci.* 85, 307–318.
- Wang, H.H., Kuang, S.P., Lang Q.L., Yu W.J., 2018. Effects of aged oil sludge on soil physicochemical properties and fungal diversity revealed by high-throughput sequencing analysis. *Archaea.* 8, 9264259.
- Wang, X.Y., Jiang, F., Zhao, J.M., 2010. Effects of crude oil residuals on soil chemical properties in oil sites, Momoge Wetland, China. *Environ. Monit. Assess.* 161, 271–280.
- Warmink, J.A., Nazir, R., Van Elsas, J.D., 2009. Universal and species-specific bacterial “fungiphiles” in the mycospheres of different basidiomycetous fungi. *Environ. Microbiol.* 11, 300–312.
- Wu, M.L., Wu, J.L., Zhang, X.H., Ye, X.Q., 2019. Effect of bioaugmentation and biostimulation on hydrocarbon degradation and microbial community composition in petroleum-contaminated loessal soil. *Chemosphere.* 237, 124456–124456.
- Zhang, D.C., Mortelmaier, C., Margesin, R., 2012. Characterization of the bacterial archaeal diversity in hydrocarbon-contaminated soil. *Sci. Total Environ.* 421–422, 184–196.
- Zhou, L., Lu, Y.W., Wang, D.W., Zhang, S.L., Tang, E.G., Qi, Z.Z., Xie, S.N., Wu, J., Liang, B., Liu, J.F., Yang, S.Z., Zhang, J., Gu, J.D., Mu, B.Z., 2020. Microbial community composition and diversity in production water of a high-temperature offshore oil reservoir assessed by DNA- and RNA-based analyses. *Int. Biodeter. Biodegr.* 151, 104970.

### Hosted file

Figures.docx available at <https://authorea.com/users/446411/articles/545652-petroleum-contamination-drives-the-shift-of-microbiome-through-modifying-soil-metallome>