



# Effect of plant waste addition as exogenous nutrients on microbial remediation of petroleum-contaminated soil

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## Abstract

**Purpose:** This study investigates the feasibility of bio-enhanced microbial remediation of petroleum-contaminated soil, and analyzes the effect of different plant wastes as exogenous stimulants on microbial remediation of petroleum-contaminated soil and the effect on soil microbial community structure, in order to guide the remediation of soil in long-term petroleum-contaminated areas with nutrient-poor soils.

**Methods:** The study was conducted in a representative oil extraction area in the Loess Hills, a typical ecologically fragile area in China. Through indoor simulated addition tests, combined with the determination of soil chemical and microbiological properties, the degradation efficiency of petroleum pollutants and the response characteristics of soil microbial community structure to the addition of different plant wastes in the area were comprehensively analyzed to obtain the optimal exogenous additive and explore the strengthening mechanism of plant wastes on microbial remediation of petroleum-contaminated soil.

**Results:** Compared with the naturally decaying petroleum-contaminated soil, the addition of plant waste increased the degradation rate of petroleum pollutants, that is, it strengthened the degradation power of indigenous degrading bacteria on petroleum pollutants, among which the highest degradation rate of petroleum pollutants was achieved when the exogenous additive was soybean straw; compared with the naturally decaying petroleum-contaminated soil, the addition of soybean straw and dead and fallen leaves of lemon mallow made the microbial species in the contaminated soil significantly reduced and the main dominant flora changed, but the flora capable of degrading petroleum pollutants increased significantly; the addition of exogenous nutrients had significant effects on soil microbial diversity and community structure.

**Conclusions:** Soybean straw can be added to the contaminated soil as the optimal exogenous organic nutrient system, which improves the physicochemical properties of the soil and gives a good living environment for indigenous microorganisms with the function of degrading petroleum pollutants, thus activating the indigenous degrading bacteria in the petroleum-contaminated soil and accelerating their growth and proliferation and new city metabolic activities, laying a foundation for further obtaining efficient, environmentally friendly and low-cost microbial enhanced remediation technology solutions. The foundation for further acquisition of efficient, environmentally friendly, and low-cost microbial-enhanced remediation technology solutions. It is important for improving soil remediation in areas with long-term oil contamination and nutrient-poor soils.

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**Keywords:** Loess hilly area, Petroleum hydrocarbons, Plant waste, Microbial remediation

## Introduction

Oil is one of the main energy sources for social and economic development nowadays, and is inseparable from people's production and life (Wang and Tang 2018). However, in the process of oil extraction, storage and transportation and use, oil spills and leaks and other accidents have led to increasingly serious oil pollution (Blumer et al. 1973). The entry of petroleum hydrocarbon compounds into the soil affects the soil structural composition, biochemical cycles and soil microbial community diversity, causing soil pollution, which in turn affects the ecological balance and leads to gradual environmental degradation (Rous et al. 1994; Zheng et al. 2021). The Loess Plateau area in northern Shaanxi Province, China, is an important oil and gas reserve and development area in China, and the relevant survey shows that the area of moderately contaminated soil is about 708.16 million m<sup>2</sup> (Ding and Liu 2020). Therefore, it is urgent to investigate and analyze the current situation of petroleum-contaminated soil in northern Shaanxi Province and explore the remediation methods suitable for petroleum-contaminated soil in the area (Lei et al. 2019; Wu et al. 2018; He et al. 2004).

At present, the remediation methods of petroleum-contaminated soil mainly include physical, chemical, and biological methods (Shi 2009; Zhong et al. 2021). Compared with the traditional physical and chemical methods, bioremediation is widely recommended because of its high efficiency, low cost and low pollution (Zhen LS, et al. 2015; Li et al. 2020; Yang et al. 2021). Bioremediation is a treatment technology that stabilizes the contaminants by degrading, absorbing or converting them into harmless forms such as CO<sub>2</sub> and H<sub>2</sub>O, mainly by remediating the soil organisms (Youngsook et al. 2001). In the process of soil petroleum pollutant treatment, in order to improve the bioremediation efficiency and remediation effect, many researchers also target soil environmental factors such as soil physicochemical properties (Long et al. 2020; Gao et al. 2019), pollution status and environmental conditions, and achieve the purpose of soil remediation by accelerating microbial metabolic activities through the use of fertilizers or other additives into oil-contaminated soil (Zhang et al. 2021; Xu et al. 2021)).

At present, most of the domestic and foreign research on oil-contaminated soil remediation technology focuses on how to improve the biochemical properties of soil after oil-contaminated soil (Zhang et al. 2018; Ames and Bethlenfalvay 1987; Eivazi et al. 2018), and

there is less research on improving oil-contaminated soil by using natural organic matter of agricultural waste (Struecker et al. 2015; Deng et al. 2008; Cheng 1987). Some researchers have studied the effect of different additives on the remediation of petroleum-contaminated soil by adding agricultural wastes such as hay, straw and leaves to the petroleum-contaminated soil (Wu et al. 2019; Ueno et al. 2010; Larkin et al. 2010). Therefore, in this study, to address the current situation of petroleum contamination in the soil of the petroleum extraction area of the Loess Plateau Land in northern Shaanxi Province, three common agricultural wastes in northern Shaanxi Province, such as soybean straw, corn cobs, and lemon leaves, were used as additives to study the effect on petroleum hydrocarbon contaminated soil and the improvement effect on it (Wichern et al. 2020; Macias-Benitez et al. 2020; Ibekwe et al. 2010). Through field sampling, indoor experiments, testing, and analytical measurements, we investigated the effects of different types of nutrient additives on soil microbial diversity, elucidated the structural characteristics of petroleum-contaminated soil microbial communities in response to different nutrient additives, and comprehensively analyzed the response mechanism of soil-microbial ecosystem to nutrient additives in this area, with a view to providing scientific basis for bioremediation and treatment of petroleum-contaminated soils on the Loess Plateau (Song et al. 2019).

## Materials and methods

### Sample collection

The soil tested in this study was collected from a typical Loess hilly area in China, namely Baota District, Yan'an City, Shaanxi Province, China. It is located in the middle reaches of the Yellow River and the central and southern part of the Loess Plateau. It belongs to the warm temperate semi-humid and drought-prone climate zone. Monsoon circulation, the annual average temperature is about 8.7 °C, and the annual average rainfall is about 500 mm. The main soil in the sample collection area is the calcareous beginning soil (Loess soil) developed from the Loess parent material, which is yellow, has no bedding, silty texture, loose, large voids, most of the soil nutrients are at a low level, and the soil is weakly alkaline as a whole (Wang BR, et al. 2018). The uncontaminated area was selected as the sample plot. Following the principles of randomness, equal amount and multi-point mixing, soil samples of 0–50 cm were collected by the S-spot method. The

**Table 1** Geographical information of sample collection sites

Sample number	Slope/(°)	Elevation (m)	Longitude	Latitude
YN1	22	1104	109° 58' 98"	36° 81' 02"
YN2	23.5	1156	109° 57' 32"	36° 25' 28"
YN1	26	957	109° 39' 02"	36° 55' 03"
YN1	25	1213	109° 59' 02"	36° 80' 15"

**Table 2** Test program

Additives	Sample no.		
CK	CK-1	CK-2	CK-3
Soybean straw (DD)	DD-1	DD-2	DD-3
Corn cob (YM)	YM-1	YM-2	YM-3
Lime leaf drop (NT)	NT-1	NT-2	NT-3

relevant geographic location information of the sample collection points is shown in Table 1 (Chen et al. 2008).

#### Indoor simulation test

Weigh crude oil with an oil content of 15 g/kg and dissolve it in petroleum ether, add it to clean soil, mix well, and after standing for 1 week, carry out an indoor simulated addition culture test, that is, according to the mass percentage of 30% (w/w) Addition amount The crushed and decomposed soybean straw (DD), corncob (YM), and caragana litter (NT) were added to the prepared oil-contaminated soil without exogenous addition treatment. (CK) is a blank control, cultivated for 120 days under constant conditions (environmental temperature controlled at  $25 \pm 2$  °C, soil moisture content maintained at 15%), with regular stirring and ventilation, a total of 4 treatments, each treatment setting 3 a parallel experiment. The specific experimental design is shown in Table 1. After the cultivation, soil samples were collected from eight different locations in each container, and after thorough mixing, a mixed sample of about 200 g was formed, which was used to determine the total petroleum hydrocarbon content and microbial diversity in the soil (Huang et al. 2008). The specific restoration protocols are shown in Table 2.

#### Testing indicators and measurement methods

The total amount of petroleum hydrocarbons in soil was determined by infrared spectroscopy, that is, an appropriate amount of soil samples were added to carbon tetrachloride and left to stand overnight.

Soil microbial diversity was determined by DNA extraction and high-throughput sequencing. The genomic DNA of the sample was extracted by CTAB or SDS method, and then the purity and concentration of the DNA were detected by agarose gel electrophoresis. Using the diluted genomic DNA as a template, according to the selection of the sequencing region, use specific primers with Barcode, Phusion® High-Fidelity PCR Master Mix with GC Buffer from New England Biolabs, and high-efficiency and high-fidelity enzymes to carry out PCR to ensure amplification Efficiency and accuracy. The corresponding regions of primers to identify bacterial diversity are 16S V4 primers (515F and 806R). The PCR products were detected by electrophoresis on agarose gel with a concentration of 2%; the qualified PCR products were purified by magnetic beads, quantified by enzyme labeling, and the samples were mixed in equal amounts according to the concentration of PCR products, and 2% agar was used after thorough mixing (Lusk et al. 2021; Liu et al. 2020). The PCR products were detected by gly-cogel electrophoresis, and the target bands were recovered using the gel recovery kit provided by Qiagen. The TruSeq® DNA PCR-Free Sample Preparation Kit was used for library construction. The constructed library was quantified by Qubit and Q-PCR. After the library was qualified, NovaSeq6000 was used for on-machine sequencing (Li et al. 2011).

#### Data statistics and analysis

One-way ANOVA was performed in SPSS 21.0, and Duncan's method was used for multiple comparisons among different treatments ( $P < 0.05$ ). Software R3.3.2 was used for data analysis and graph drawing. Shannon and Simpson indices were used to estimate species diversity, Chao 1 and ACE were used to calculate and estimate species richness, and Good's coverage reflected the coverage of community microorganisms by sequencing data. The UPGMA (unweighted pair-group method with arithmetic means) cluster analysis was performed by considering both species diversity and abundance, and the overall differences in microbial community structure were evaluated based on Bray-Curtis distances principal coordinate analysis (PCoA) (Liu and Peng 2001; Ali et al. 2020; Wagh GA, et al. 2020).

## Results and discussion

### Effects of different exogenous additions

#### on the degradation rate of petroleum pollutants

After 120 days of cultivation, the total amount of petroleum hydrocarbons in the oil-contaminated soil under the action of different exogenous additives was measured,

**Table 3** Degradation rate of petroleum pollutants under different treatment of exogenous substances

No.	Treatment	Petroleum hydrocarbon content before strengthening (g/kg)	Petroleum hydrocarbon content after strengthening (g/kg)	Degradation rate (%)
CK	Natural attenuation	15	8.63	42.46
YM	Addition of corncob	15	6.21	58.63
DD	Addition of soybean straw	15	3.89	74.06
NT	Addition of litter leaves of <i>Caragana korshinskii</i>	15	5.68	62.13

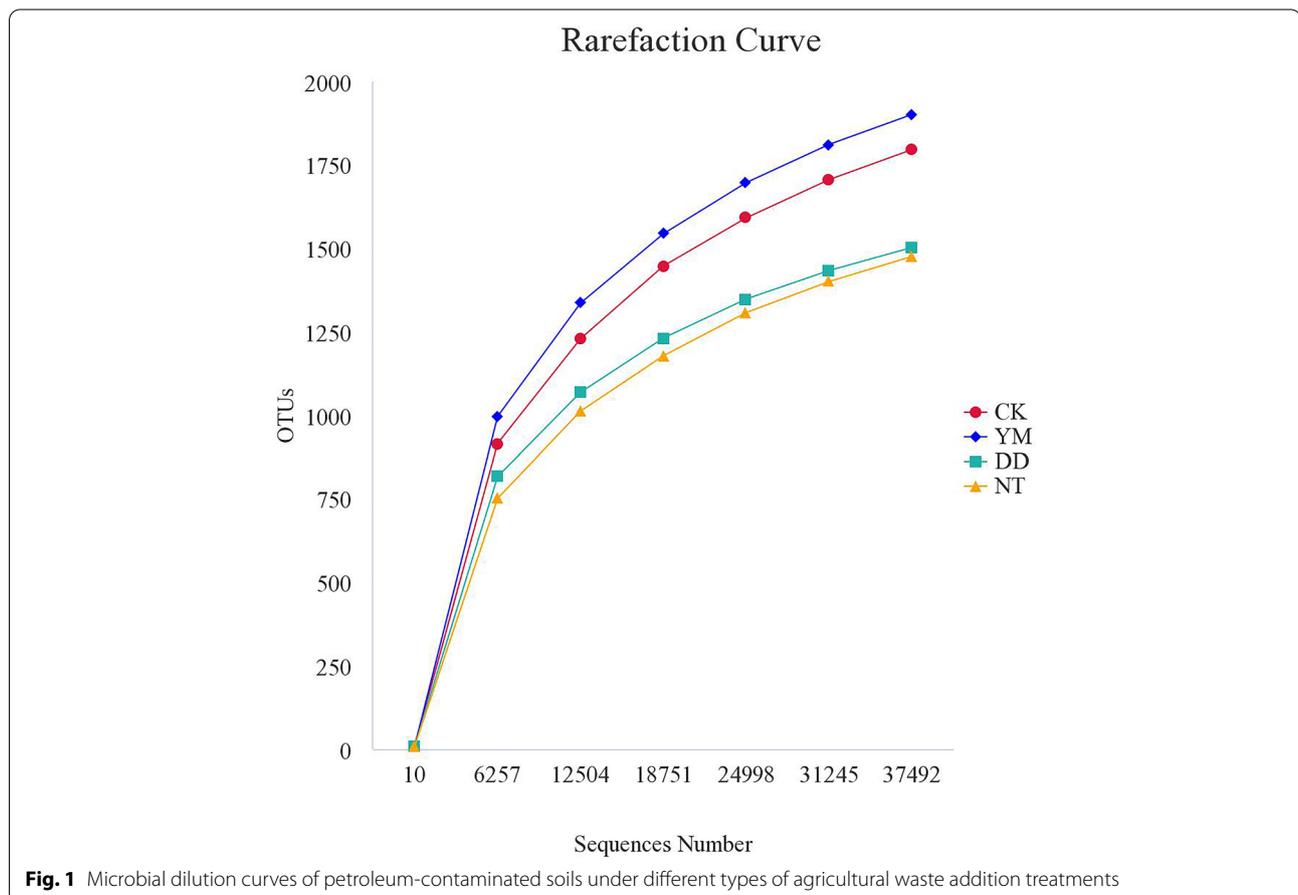
and the degradation rate was calculated from this (degradation rate = (initial oil content – final oil content)/initial oil content × 100)). From Table 3, it can be seen that the content of petroleum pollutants in soil decreased to different degrees. The degradation rates of petroleum pollutants in soil under CK, YM, DD, and NT treatments reached 42.46%, 58.63%, 74.06%, and 62.13%, respectively. Compared with the blank control group, the degradation rates of petroleum pollutants in the soil under the addition of exogenous nutrients were improved, and the degradation rate under the DD treatment was the highest, reaching 74.06%. It can be seen that adding corncob, soybean straw, and Caragana litter can improve the

degradation efficiency of petroleum pollutants in soil, and soybean straw has the best enhancement effect on pollutant degradation.

**Effects of different pollution concentrations on soil microbial diversity**

*Dilution curve*

The dilution curve is to randomly select a certain number of sequences from the sample, and count the Alpha diversity index of the samples corresponding to these sequences. Draw a curve and judge whether the amount of sequencing data is sufficient according to whether the curve is flat. It can be seen from Fig. 1 that the dilution



curve tends to be flat, indicating that the detection ratio of microbial communities in environmental samples is close to saturation, that is, the current sequencing volume can cover most of the species in the samples. In addition, the number of soil microbial OTUs under different treatments was  $YM > CK > DD > NT$ . It can be seen that the number of soil microbial species decreased significantly under DD and NT treatments, but the difference between the two was small; while the number of microbial species was slightly higher under YM treatment in CK (Chen et al. 2020).

### Species Venn diagram analysis

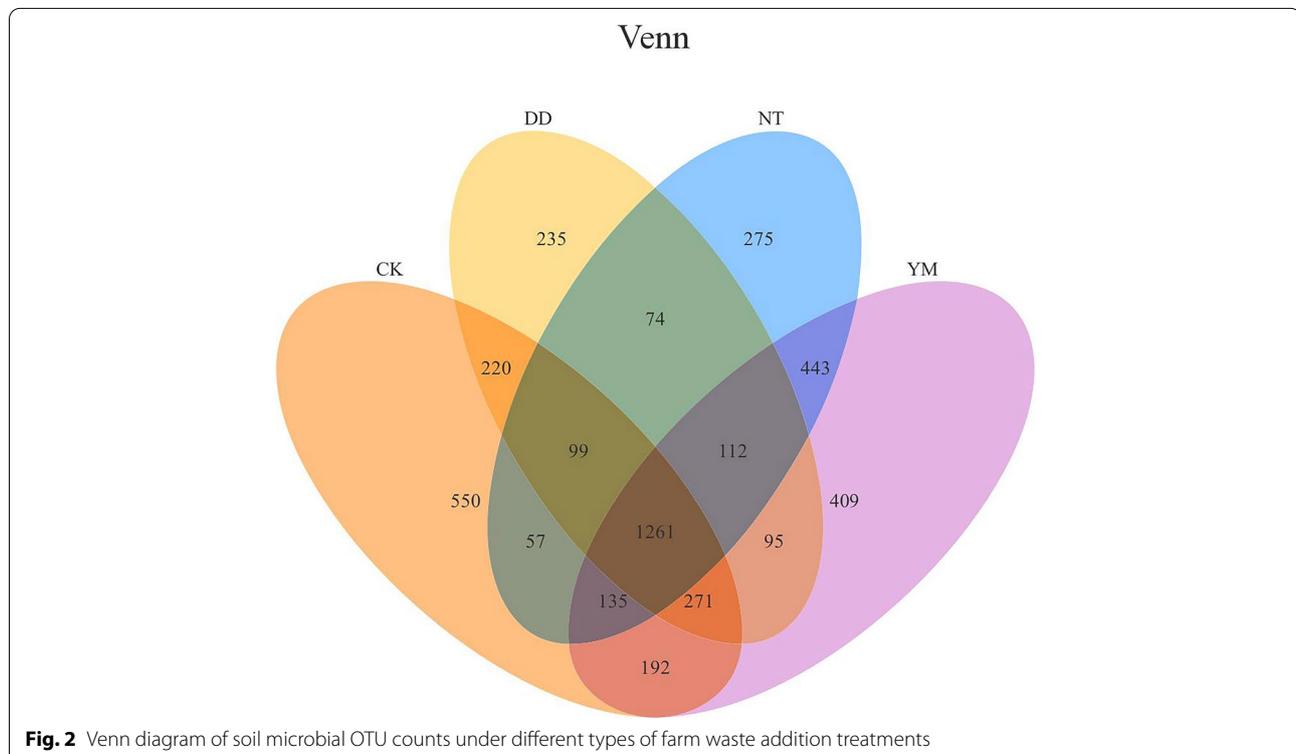
Venn diagrams can be used to count the number of common and unique species (OTUs) in multiple (group) samples, visually display the similarity and specificity of OTU composition in environmental samples, and perform bioinformatics statistics on OTUs classified by 97% similarity (Wang et al. 2019). After analysis, the results showed that each soil sample produced a total of 10526 OTUs, of which the total number of OTUs was 1261, accounting for 11.98% of the total number of OTUs (Fig. 2). The number of OTUs processed by DD and NT were 2367 and 2456, which were lower than CK (2785), and the number of OTUs processed by YM was 2918, slightly higher than that of CK. Compared with CK, the number of unique OTUs in YM, DD, and NT treatments were

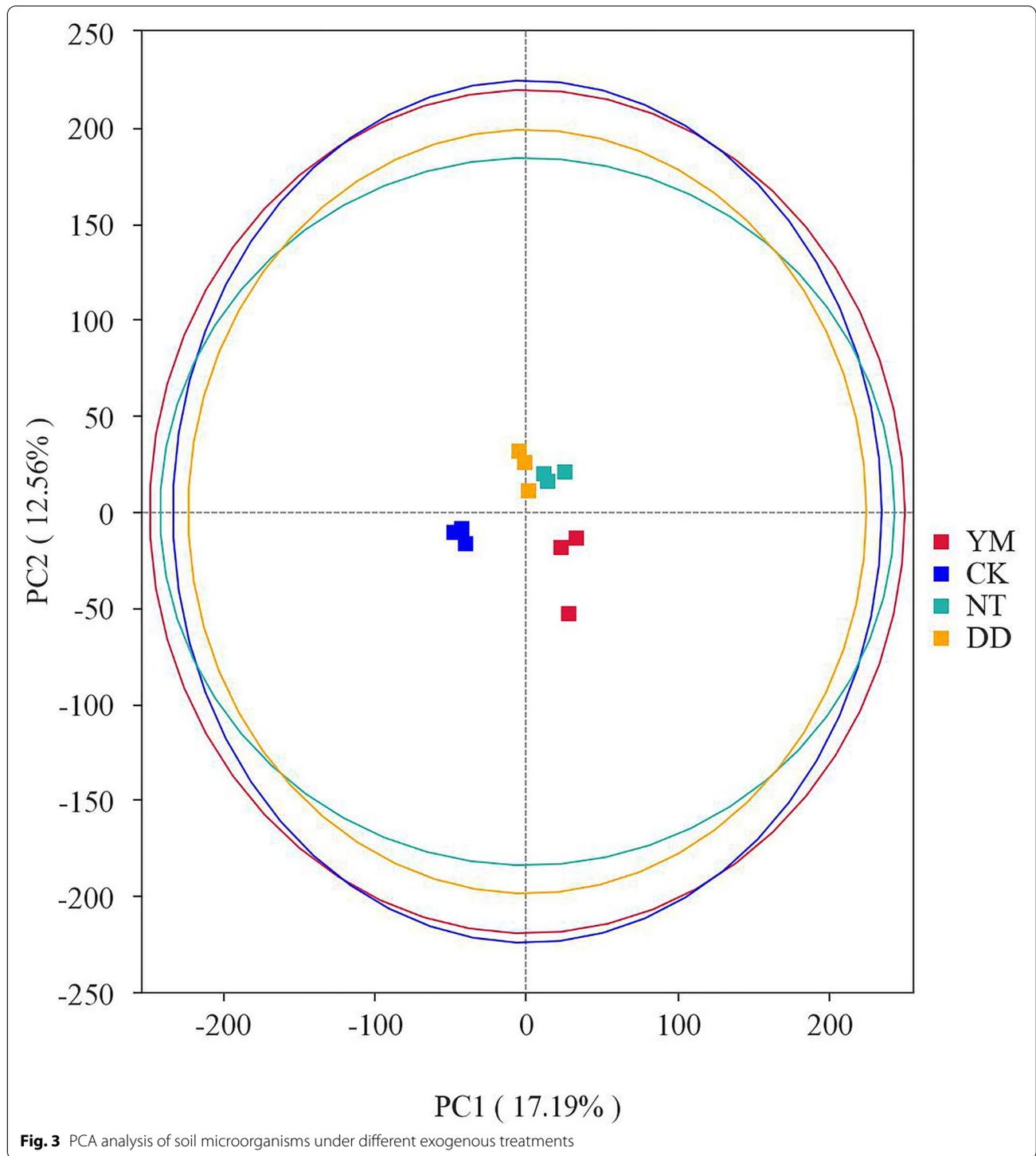
all reduced, and the reduction value of DD and NT was greater than 200. It can be seen that different exogenous nutrient addition treatments had a significant effect on the number of unique OTUs in soil. In addition, among the different treatments, the DD and NT treatments had the highest degree of overlap, and their bacterial community structures had a high similarity.

### Effects of different exogenous treatments on soil microbial community structure

#### *$\beta$ diversity-analysis of variability between groups*

In order to study the similarity or difference relationship of different sample community structures, clustering analysis can be performed on the sample community distance matrix, and a sample hierarchical clustering tree can be constructed. The different shape legends in Fig. 3 represent the control and soil samples treated with three different types of plant waste as exogenous nutrients, respectively. The differences in the microbial community composition of CK, YM, DD, and NT were analyzed by PCA. The results showed that the soil microbial community composition was different under different exogenous treatments. The difference between DD, NT, and CK was significant, but the difference between YM and CK was not significant. The PC1 axis and PC2 axis explained 12.56% and 17.19% of the results, respectively (Zhang et al. 2017).

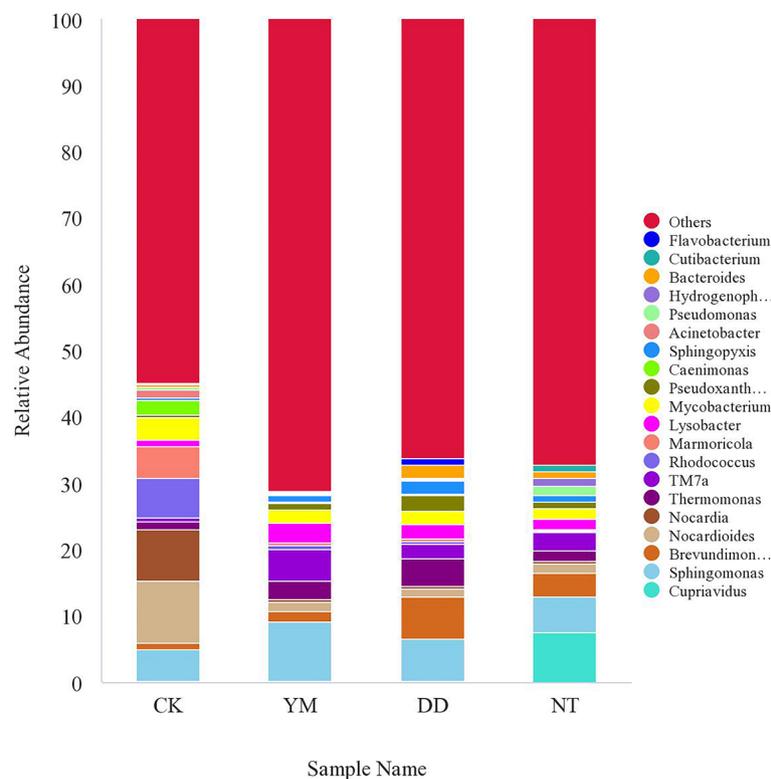




**Analysis of the variability of community composition**

The histogram can present the community composition and species abundance at different taxonomic levels (Zhu et al. 2020). In this study, community composition and species abundance were analyzed based on the genus

level. The dominant genera in petroleum-contaminated soil without the addition of exogenous substances (CK) mainly included *Nocardioides*, *Nocardia*, *Rhodococcus*, *Sphingomonas*, and *Marmoricola*. The abundance of *Sphingomonas*, *Thermomonas*, *TM7a*, and *Lysobacter*



**Fig. 4** Community composition characteristics of soil microorganisms at different genus levels

increased significantly under YM treatment. *Nocardioidea*, *Nocardia*, *Rhodococcus*, *Mycobacterium*, and *Marmoricola* significantly decreased in abundance under DD treatment, while *Sphingomonas*, *Brevundimonas*, *Sphingomonas*, *Thermomonas*, *TM7a*, *Lysobacter*, *Bacteroides*, *Pseudoxanthomonas*, *Sphingopyxis*, *Bacteroides*, and *Flavobacterium* were increased in abundance; *Cupriavidus*, *TM7a*, and *Brevundimonas* were significantly increased in abundance under NT treatment, and *Pseudomonas* and *Bacteroides* were increased in abundance but not significantly (Fig. 4).

## Conclusions

Using plant wastes as exogenous nutrients to biostimulate the remediation of contaminated soil will change the soil micro-ecological environment. In this study, by measuring the total amount of petroleum hydrocarbons in the soil under the addition of different exogenous substances, it can be seen that compared with the oil-contaminated soil with natural decay, the degradation rate of oil pollutants was improved after adding plant waste, that is to say, it strengthened the degradation ability of indigenous degrading bacteria to petroleum pollutants, in which the exogenous additive is soybean straw, the degradation rate

of petroleum pollutants reaches the highest, so it has the best enhancement effect on the degradation of petroleum pollutants by indigenous microorganisms, it may be because the carbon-nitrogen ratio of soybean straw is 20.4:1, and the phosphorus content is 0.07%, which is relatively high, which can acidify the soil, greatly promote soil respiration, and significantly increase invertase and hydrogen peroxide. Enzyme, alkaline phosphatase, and urease activity (Li et al. 2018).

Through high-throughput sequencing of soil microorganisms, the results show that, compared with the naturally attenuated oil-contaminated soil, the addition of soybean straw and Caragana litter leaves significantly reduced the types of microorganisms in the contaminated soil, but the main dominant flora changed, but the bacteria that can degrade petroleum pollutants increased significantly. The addition of exogenous substances has a significant impact on soil microbial diversity and community structure, which may be due to the application of organic nutrient systems to improve the physical and chemical properties of soil, giving indigenous microorganisms with the function of degrading petroleum pollutants. A good living environment, thereby activating the indigenous degrading bacteria and accelerating their growth, proliferation, and metabolic activities.

In this study, the methods of bio-enhanced microbial remediation of oil-contaminated soil were studied, and the strengthening effect of different plant wastes as exogenous stimuli on microbial remediation of oil-contaminated soil and the impact on soil microbial community structure were analyzed. The low-cost microbial-enhanced remediation technology scheme provides theoretical and data support, and is of great significance for improving soil remediation in areas that have been polluted by oil for a long time and have poor soil nutrients. Since this study is limited to the laboratory, further research and exploration are still needed on the enhancement effect of the in-situ remediation technology for oil-contaminated soils.

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#### Authors' contributions

LS guided the completion of this experiment, completed the first draft paper, and made revisions. WF guided the experimental methods and thesis writing. ZL performed experiments, recorded data, and data analysis. LY: data analysis. All authors read and approved the final manuscript.

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#### Availability of data and materials

The data were obtained by the authors.

#### Declarations

##### Ethics approval and consent to participate

I certify that this manuscript is original, has not been published, and will not be submitted elsewhere for publication while being considered by *Annals of Microbiology*. The study is not split up into several parts to increase the quantity of submissions and submitted to various journals or to one journal over time. No data have been fabricated or manipulated (including images) to support your conclusions. No data, text, or theories by others are presented as if they were our own.

The submission has been received explicitly from all co-authors, and authors whose names appear on the submission have contributed sufficiently to the scientific work and therefore share collective responsibility and accountability for the results.

The study did not violate ethics, and all participants agreed to publish the paper.

##### Consent for publication

Not applicable.

##### Competing interests

The authors declare that they have no competing interests.

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