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# Petroleum-contaminated soil extent recorded by $\delta^{15}N$ and $\delta^{13}C$ of plants and soils

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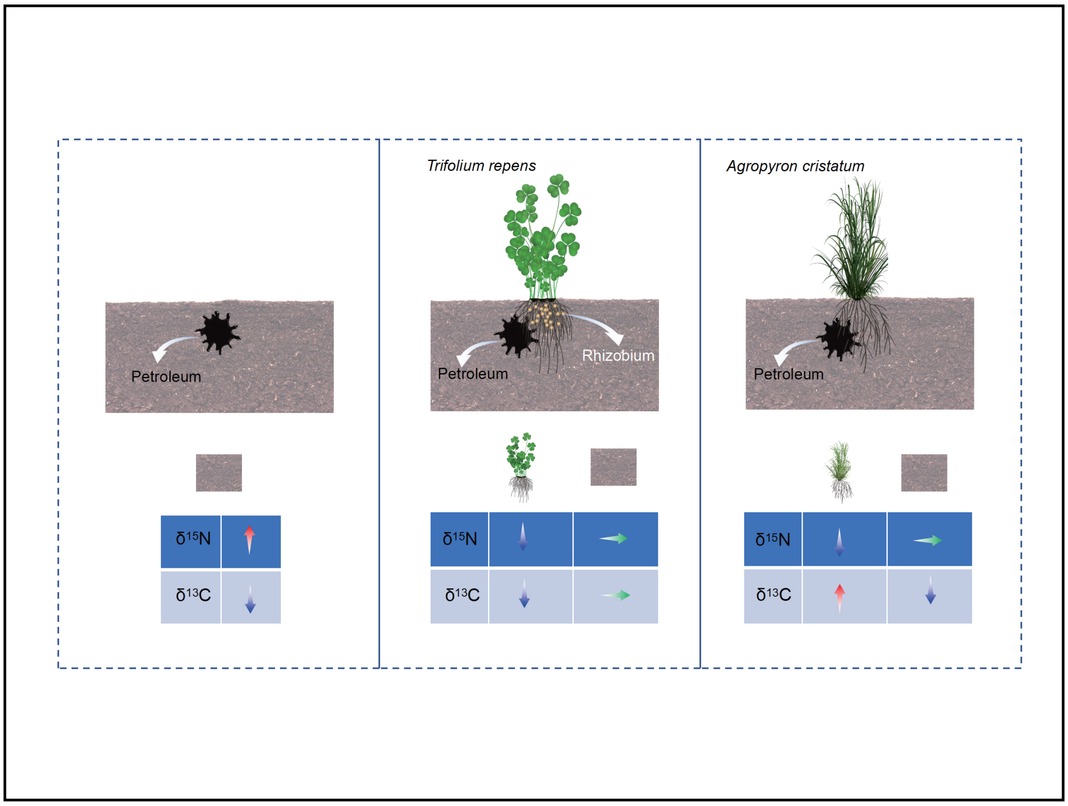
## Graphical abstract



*The $\delta^{13}C$ and $\delta^{13}N$ values of soil and plant changed with petroleum-contaminated soil concentration.*

## Public summary

- Petroleum-contaminated soil induced the soil $\delta^{15}N$ values to increase and $\delta^{13}C$ values to decrease.
- The characteristics of plants influence plant $\delta^{13}C$ and $\delta^{15}N$ values under petroleum-contaminated soil.
- The $\delta^{13}C$ and $\delta^{15}N$ values are useful proxies for monitoring petroleum-contaminated soil and evaluating the response of plants to the stress of petroleum contamination.

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# Petroleum-contaminated soil extent recorded by $\delta^{15}N$ and $\delta^{13}C$ of plants and soils

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**Abstract:** Petroleum contamination in terrestrial environments caused by industrial activities is a significant problem that has received considerable attention. Carbon and nitrogen isotopic compositions ($\delta^{13}C$ and $\delta^{15}N$) effectively describe the behavior of plants and soils under petroleum contamination stress. To better understand plant and soil responses to petroleum-contaminated soil, $\delta^{13}C$ and $\delta^{15}N$ values of the plants (*Trifolium repens*, Leguminosae with $C_3$ photosynthesis pathway, and *Agropyron cristatum* with $C_4$ photosynthesis pathway) and the soil samples under one-month exposure to different extents of petroleum contamination were measured. The results showed that petroleum contamination in the soil induced the soil $\delta^{15}N$ values to increase and $\delta^{13}C$ values to decrease; from 1.9‰ to 3.2‰ and from −23.6‰ to −26.8‰, respectively. However, the $\delta^{13}C$ values of *Agropyron cristatum* decreased from −29.8‰ to −31.6‰, and the $\delta^{13}C$ values of *Trifolium repens* remained relatively stable from −12.6‰ to −13.1‰, indicating that they have different coping strategies under petroleum-contaminated soil conditions. Moreover, the $\delta^{15}N$ values of *Trifolium repens* decreased from 5.6‰ to 0.8‰ near the air $\delta^{15}N$ values under petroleum-contaminated soil, which implies that their nitrogen fixation system works to reduce soil petroleum stress. The $\delta^{13}C$ and $\delta^{15}N$ values of *Agropyron cristatum* and *Trifolium repens* reflect changes in the metabolic system when they confront stressful environments. Therefore, stable isotopic compositions are useful proxies for monitoring petroleum-contaminated soil and evaluating the response of plants to petroleum contamination stress.

**Keywords:** petroleum contamination; stable nitrogen isotope; stable carbon isotope; soil; $C_3$ and $C_4$ plants

**CLC number:** Q946.91    **Document code:** A

## 1 Introduction

Contamination of soils and groundwater aquifers is a major problem in locations near industrial and urban areas[1–4]. An increasing number of sites are seriously contaminated by petroleum hydrocarbon pollutants due to industrial activities such as vehicle transit paths, pipes for petroleum transport, boat shoring sites, and oil tank replenishment and storage areas, as confirmed by academic and government organizations in the USA, Western Europe, Nigeria, China, etc.[5–8]. As a complex mixture of compounds, petroleum includes both branched and straight-chain alkanes and polycyclic aromatic hydrocarbons (PAHs)[9]. Petroleum compounds are carcinogenic, mutagenic, and teratogenic and are classified as priority environmental pollutants due to their persistence and recalcitrance[10–12]. In addition, petroleum compounds that enter soil and water adversely affect soil safety, plant growth and development, and human health [13, 14]. Therefore, accurately tracking complex petroleum pollutants in soil and plant systems is important for petroleum contamination assessment[14–16].

A series of biologically mediated chemical reactions occur when petroleum enters the soil, during which petroleum compounds can be converted into intermediate metabolites and subsequent end products via various enzymatic pathways without any long-term adverse effects on affected environments[17, 18]. For example, *Nocardia soli* Y48, a strain of Actinobacteria isolated from the Qinghai-Tibetan Plateau, can degrade nearly all components of crude oil through hydrocarbon degradation, biosurfactant synthesis, emulsification, and other related metabolic pathways[19]. Pereira et al.[20] found that *Bacillus methylotrophicus* and *Pseudomonas sihuiensis* can degrade medium ($C_8$–$C_{19}$) and long ($C_{20}$–$C_{33}$) chain aliphatic hydrocarbons, isoprenoids, anthracene, phenanthrene, and pyrene by producing biosurfactants that can reduce the surface tension and pH of aliphatic compounds and PAHs. Moreover, rhizoremediation is an attractive strategy that enhances microbial populations and activities of PAH-contaminated soils through plant root exudates[21]. The types of microorganisms[22, 23], soil physical and chemical features (such as pH, water content, oxygen availability, nutrient availability, salinity, etc.) [24, 25], and the surrounding environment affect the environmental behavior of petroleum compounds in soil[26–29], thus, further impacting plants, which depend on good

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soil quality for survival. However, an accurate description of petroleum environmental behavior and its impact in extreme environments (i.e., water scarcity, drought, and cold conditions) requires additional studies. A more useful monitoring index is required to monitor the response of plants and soil to petroleum contamination.

The stable or radioactive isotope-labeled techniques are useful in tracking sources and fates of organic compounds in the environment[30–32]. Substantial isotopic fractionation during the microbial degradation of the investigated compounds can help identify, qualify, and assess biodegradation[33–35]. Environmental conditions affect the fractionation of C isotopes of PAHs during the formation, transportation, and degradation processes[36]. Under anaerobic conditions, the $\delta^{13}$C values of methyl *tert*-butyl ether (MTBE) increased from −31.4‰ to −11.8‰, indicating MTBE degradation[37]. Generally, lighter isotopes exhibit faster reaction rates than heavier isotopologues during the biodegradation of organic compounds[38, 39]. Phase transfer (e.g., sorption, volatilization), transport processes (diffusion), chemical bond cleavage, or formation all result in isotopic fractionation[40]. Notably, $\delta^{2}$H and $\delta^{13}$C have been used to source the apportionment of polycyclic aromatic hydrocarbons[41, 42]. Nitrogen (N) is one of the essential nutrients for soil microorganisms and plants. However, soil pollution by organic or inorganic components affects the quantity and activity of soil microorganisms, which can influence nitrogen mineralization and nitrogen uptake by plants. As one of the important trace marks for nitrogen cycling, stable isotopic compositions of nitrogen have been used to trace nitrogen transfer in soil-plant systems. However, there have not been enough studies to assess $\delta^{15}$N changes under petroleum contamination conditions in soil and plants. Therefore, it is of interest to know whether $\delta^{13}$C and $\delta^{15}$N have potential as useful indices for plants and soil under petroleum-contaminated soil, which may provide some clues for soil and plants in polluted conditions.

In arid and semiarid areas, water scarcity and poor soil quality are unfavorable for microorganisms to degrade petroleum contaminants in the soil. In the Ordos Basin, an important energy and chemical industry base abundant in coal, gas, and oil in China, environmentally friendly and cost-efficient bioremediation techniques are needed for this fragile ecosystem[43, 44]. Two kinds of plants, *Agropyron cristatum* with the $C_4$ photosynthesis pathway and *Trifolium repens* with the $C_3$ photosynthesis pathway and nitrogen fixation ability, which adapt to arid and semi-arid environments, were used to compare plant responses to petroleum-contaminated soil. The major aims of this study were ① to identify whether $\delta^{13}$C and $\delta^{15}$N in soils and plants can be used as a specific index to trace petroleum-contaminated soil and ② to compare the different responses of plants to different soil petroleum concentrations.

## 2 Materials and methods

### 2.1 Study site

The field experiment was conducted at the Demonstration Field of Water and Environment (34°16′ N, 108°56′ E) at Chang'an University, Xi'an, China. The experimental site is located in the middle of the Guanzhong Basin, with an elevation of 400 m and a temperate continental climate. The annual mean temperature and precipitation are 13 °C and 600 mm, respectively[45].

### 2.2 Experimental design and sample collection

Silty loam soil used in this study was collected from the Loess Plateau of Gaoling, north of Xi'an City, Shaanxi Province, China. The chemical and physical properties of the soil were measured[46] (Table 1). According to the World Reference Base for Soil Resources (WRB), soil can be classified as Calciustepts and Haplic Calcisols[47].

Before the experiment, the soil was sieved with a 2 mm sieve to remove big rocks and other debris. Then the soil was put into 12 experiment quadrats made of brick and cement with 1 m length ×1 m width × 0.9 m height. To monitor the effects of petroleum contamination on soil and plants, crude oil taken from a petroleum extraction factory was added and mixed with 20 cm surface soil. The petroleum gradients were designed as 0 (control), 3000, 7000, and 10000 mg/kg (Table 2).

*Agropyron cristatum* and *Trifolium repens* were used as experimental plants to monitor the response and changes of $\delta^{13}$C and $\delta^{15}$N of the plant under different soil petroleum concentrations. The two kinds of experimental plants were planted on August 11, 2010. After one month of plant growing and contaminating exposure, soil and plant samples were collected from 12 quadrats for further analysis (Fig. 1). About 500 g of the petroleum-contaminated soil samples were collected

**Table 1.** General physical and chemical properties of experimental soil.

| Soil classification | Soil particle composition(%) | | | Soil chemical composition ($\omega$(B)/$10^{-2}$) | | | | | | | Oxidation reduction potential (mV) | Organic carbon(%) | Conductivity (μs/cm) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Sand | Silt | Clay | $SiO_2$ | $Al_2O_3$ | $Fe_2O_3$ | CaO | MgO | $K_2O$ | $Na_2O$ | | | |
| Calciustepts and Haplic Calcisols | – | 90.8 | 9.2 | 62.6 | 11.21 | 3.95 | 6.03 | 1.61 | 2.15 | 1.82 | 174 | 2.3 | 181 |

**Table 2.** Experimental design for petroleum concentration in the soil. Twelve quadrats were used in the experiment.

| Petroleum concentration in soil | 0 mg/kg | 3000 mg/kg | 7000 mg/kg | 10000 mg/kg |
|---|---|---|---|---|
| Treatments | *Agropyron cristatum*<br>*Trioflium repens*<br>Control | *Agropyron cristatum*<br>*Trioflium repens*<br>Control | *Agropyron cristatum*<br>*Trioflium repens*<br>Control | *Agropyron cristatum*<br>*Trioflium repens*<br>Control |

[Note] Control: soil without plant.

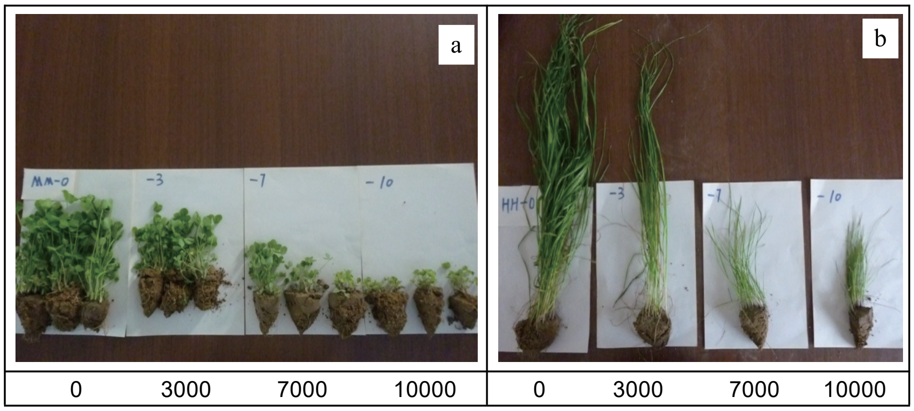

**Fig. 1.** The *Trifolium repens* (a) and *Agropyron cristatum* (b) were collected from the experimental field after one month of growth. 0, 3000, 7000, and 10000 in the picture means the petroleum contamination concentration in the soil increased from 0, 3000, 7000 to 10000 mg/kg, respectively.

using a shovel and oven-dried (40 ℃) for 72 h. Then the soils were ground in an agate mill and sieved with a 0.2 mm sieve for further analysis. For *Agropyron cristatum* and *Trifolium repens*, both the leaf and root were collected from the experimental field (Fig. 1). Then the plant was cleaned using deionized water and oven-dried (40 ℃) for 72 h. Three to five whole plants were pulled together as one testing sample, and all samples were ground in an agate mill for further analysis.

### 2.3 Nitrogen isotope ratio ($\delta^{15}$N) analysis

The $\delta^{15}$N values of the plants and soil were analyzed at the Isotope Laboratory of the Institute of Earth Environment, Chinese Academy of Sciences, Shaanxi Province, China. A CE FLASH 1112 elemental analyzer (CE Instruments, Rodano, Italy) equipped with an AS200 auto-sampler interfaced with a Delta PLUS isotope-ratio mass spectrometer via a Finnigan Conflo III interface (ThermoQuest, Finnigan, Germany) was used to determine the $\delta^{15}$N and nitrogen contents of the samples[48]. The $\delta^{15}$N values were calculated using the following equation:

$$\delta^{15}\mathrm{N}\,(‰) = \left(\frac{R_{\mathrm{sample}}}{R_{\mathrm{standard}}} - 1\right) \times 1000\,,$$

where $R_{\mathrm{sample}}$ and $R_{\mathrm{standard}}$ are the $^{15}$N/$^{14}$N ratios of the sample and standard, respectively. The $\delta^{15}$N values are reported relative to the atmospheric $N_2$ isotopic standard. During the laboratory measurements, soil produced by the laboratory with known $\delta^{15}$N values ($\delta^{15}\mathrm{N} = 5.46‰ \pm 0.16‰$) was used as an in-house standard to link the $\delta^{15}$N values to atmospheric $N_2$ and monitor analytical accuracy. Long-term analysis of the in-house standard showed stability and reliability. The standard deviation for duplicate analysis was less than 0.3‰ for $\delta^{15}$N. The difference between the $\delta^{15}$N of plants and soil is denoted as $\Delta^{15}\mathrm{N}_{\text{plant-soil}}$.

### 2.4 Carbon isotope ratio ($\delta^{13}$C) analysis

To remove the soil carbonate, approximately 3 g of soil samples were treated with 2 mol/L HCl for 24 h. Then, the samples were rinsed to pH > 4 with distilled water and dried at 60 ℃ [49].

The plant and soil samples were sealed using a quartz tube with copper oxide and silver foil under vacuum conditions. Consequently, the quartz tube was combusted for at least 4 h at 800–850 ℃. The $CO_2$ gas from the combustion tube was extracted and purified cryogenically for isotopic analysis[50].

Carbon isotope ratios ($\delta^{13}$C) were analyzed at the Isotope Laboratory of the Institute of Earth Environment, Chinese Academy of Sciences, Shaanxi Province, China. A MAT-251 gas mass spectrometer equipped with a dual-inlet system analyzed the soil and plant samples[49]. The carbon isotope results are expressed in delta ($\delta$) notation relative to the V-PDB standard, and the standard deviation for duplicate analysis was less than ±0.2‰.

### 2.5 Data analysis

One-way ANOVA statistical analyses and Duncan's multiple test range tests of the experimental data were used to determine whether there was a statistically significant difference between the medians of three or more independent groups; the different letters (a, b, or c) in figures indicate significant differences among petroleum contamination concentrations in the soil. The minimum level of significance of the results was set at $p < 0.05$.

## 3 Results

### 3.1 $\delta^{13}$C and $\delta^{15}$N variation in plants under different petroleum pollution concentration

Fig. 2 shows the variations in the $\delta^{13}$C values of soil with and without plants under different soil petroleum pollution concentrations. In petroleum-contaminated soil without plant disturbance, soil $\delta^{13}$C values linearly decreased as the soil petroleum concentration increased. The $\delta^{13}$C values of soil organic matter ranged from −23.6‰ to −26.8‰ with an average value of −25.2‰, which is lower than the $\delta^{13}$C values of the original soil (−21.3‰) but higher than the $\delta^{13}$C values of petroleum (−32‰). The soil $\delta^{13}$C values decreased as the soil petroleum concentration increased when *Agropyron cristatum* and *Trifolium repens* were planted. However, compared with *Trifolium repens* planted quadrats, the soil $\delta^{13}$C values in the *Agropyron cristatum* plantation changed significantly.

Fig. 3 shows the soil $\delta^{15}$N values with and without petroleum contamination. The soil $\delta^{15}$N values increased as soil petroleum concentration increased without plant conditions, and the soil $\delta^{15}$N values varied, ranging from 1.9‰ to 3.2‰ with an average value of 2.7‰. Under *Trifolium repens* plant-

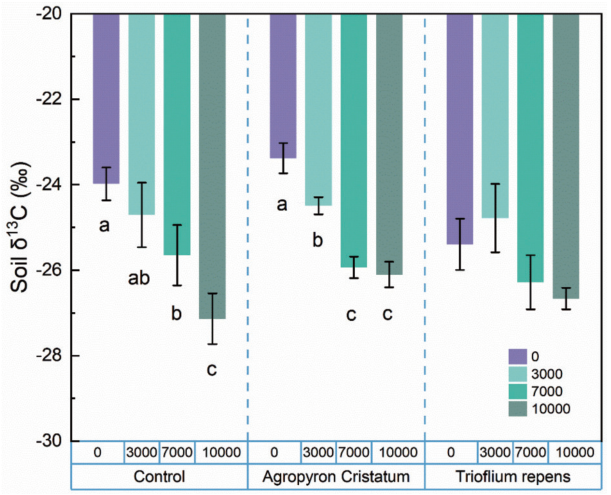

**Fig. 2.** Soil $\delta^{13}$C variations under different petroleum pollution conditions with and without plants. Error bars indicate the standard deviation ($n = 3$). Different letters indicate significant differences among petroleum-contaminated soil concentrations ( $p < 0.05$).

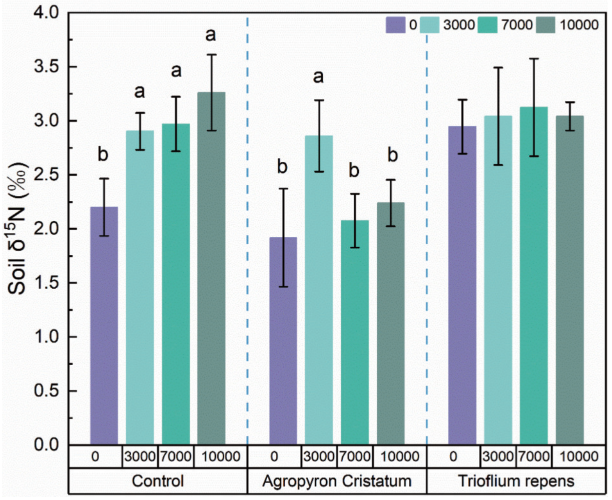

**Fig. 3.** Soil $\delta^{15}$N variations under different petroleum pollution conditions with and without plants. Error bars indicate the standard deviation ($n = 3$). Different letters indicate significant differences among petroleum-contaminated soil concentrations ($p < 0.05$).

ation, the soil $\delta^{15}$N values were close to 3.0‰. Except for 3000 mg/kg petroleum-contaminated soil with *Agropyron cristatum* planted, soil $\delta^{15}$N values were lower than petroleum pollution with *Trifolium repens* planted. The $\delta^{15}$N values of Loess and organic fertilizer in the experiment are 2.2‰ and 3.8‰, respectively.

## 3.2 $\delta^{13}$C and $\delta^{15}$N variation in plants under different petroleum pollution concentration

The $\delta^{13}$C values of *Trifolium repens* changed from −29.8‰ to −31.6‰, with an average of −30.5‰ (Fig. 4). A decreasing linear trend of the $\delta^{13}$C values was observed with increased petroleum contamination, where *Trifolium repens* was planted. The $\delta^{13}$C values of *Agropyron cristatum* have smoothly changed *Trifolium* from −12.6‰ to −13.1‰, with an average of −12.9‰, and the lowest values appeared for *Agropyron cristatum* without petroleum-contaminated soil (Fig. 4). Statistical analysis showed a significant difference among the different petroleum conditions for the $\delta^{13}$C values of *Agropyron cristatum* ($p < 0.05$). However, multiple comparison results showed that only the control and 3000 mg/kg petroleum-contaminated conditions differed from the $\delta^{13}$C values of *Agropyron cristatum*. Except for petroleum-contaminated soil conditions, a decreased linear trend of $\delta^{13}$C values was observed in the *Agropyron cristatum* plantation as petroleum contamination increased. The $\delta^{13}$C values show that *Trifolium repens* and *Agropyron cristatum* are $C_3$ and $C_4$ photosynthetic plants, respectively [51].

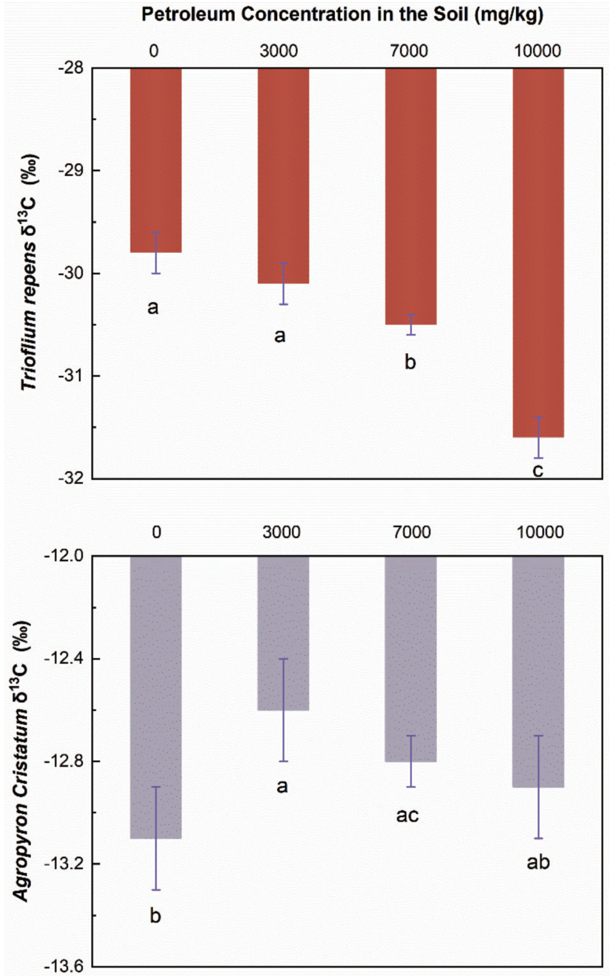

**Fig. 4.** $\delta^{13}$C changes of *Agropyron cristatum* and *Trifolium repens* under different soil petroleum pollution conditions. Error bars indicate the standard deviation ($n = 3$). Different letters indicate significant differences among petroleum-contaminated soil concentrations ($p < 0.05$).

Under different petroleum-contaminated soil concentrations conditions, $\delta^{15}$N values of *Agropyron cristatum* changed (Fig. 5). The $\delta^{15}$N values of *Agropyron cristatum* varied, ranging from 3.8‰ to 8.5‰ with an average value of 5.4‰. Except for control conditions, the $\delta^{15}$N values of *Agropyron cristatum* decreased as petroleum-contaminated soil concentration increased. However, there was no statistical difference between $\delta^{15}$N values without petroleum-contaminated soil and 10000 mg/kg petroleum-contaminated soil with *Agropyron cristatum.* The $\delta^{15}$N values of *Trifolium repens* ranged from 0.8‰ to 5.6‰ with an average of 3.7‰. $\delta^{15}$N values of *Trifolium repens* were at the same level without petroleum-contaminated soil, and 3000 mg/kg petroleum entered into the

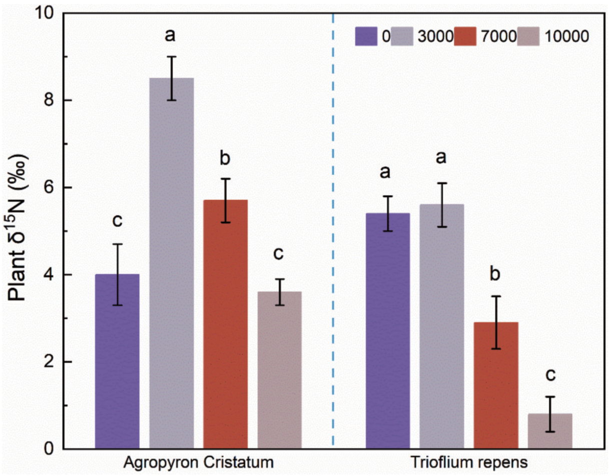

**Fig. 5.** $\delta^{15}$N changes of *Agropyron cristatum* and *Trifolium repens* under different soil petroleum pollution conditions. Error bars indicate the standard deviation ($n = 3$). Different letters indicate significant differences among petroleum-contaminated soil concentrations ($p < 0.05$).

soil. However, $\delta^{15}$N values of *Trifolium repens* changed significantly from 3000 to 10000 mg/kg of petroleum put into the soil (Fig. 5).

## 4 Discussion

In this study, we identified notable variations in $\delta^{13}$C and $\delta^{15}$N in plants and soil in 12 experimental quadrats exposed to different concentrations of petroleum-contaminated soil. The $\delta^{13}$C and $\delta^{15}$N values of the soil ranged from −23.6 ‰ to −26.8‰ and from 1.9‰ to 3.2‰, respectively. The $\delta^{13}$C and $\delta^{15}$N values of the $C_3$ plant of *Trifolium repens* ranged from −29.8‰ to −31.6‰ and from 0.8‰ to 5.6‰, respectively. In contrast, the $\delta^{13}$C and $\delta^{15}$N values of the $C_4$ plant *Agropyron cristatum* ranged from −12.6‰ to −13.1‰ and from 3.8‰ to 8.5 ‰, respectively. The $\delta^{13}$C values of plants and soil decreased with increasing petroleum concentration in the soil. However, as petroleum concentration in the soil increased, the $\delta^{15}$N values of the soil increased, but the $\delta^{15}$N values of plants decreased. Moreover, the $\delta^{13}$C and $\delta^{15}$N values of *Trifolium repens* and *Agropyron cristatum* differed under different soil petroleum concentrations.

The $\delta^{13}$C and $\delta^{15}$N values of soil organic matter correlate with soil organic matter decomposition and microbial processes[52]. When petroleum enters the soil, the carbon to nitrogen ratio increases, influencing the physical, chemical, and biological characteristics of the soil, such as soil porosity, permeability, and organic matter content[53]. Afterward, changes in microbial species and activities influence carbon assimilation and degradation in the soil and increase soil $\delta^{13}$C values[54]. Moreover, the large amount of different $\delta^{13}$C values of petroleum entering the soil is also the reason for soil $\delta^{13}$C values changes. In our study, the corresponding $\delta^{13}$C values decrease in the soil demonstrates that petroleum concentration induces these differences under control conditions. We applied the mixing model to calculate the initial $\delta^{13}$C values of the soil and petroleum compounds (Table 3). The $\delta^{13}$C values of the polluted soil decreased after one month of bioremediation. Presumably, the decreased $\delta^{13}$C values originated from microbial biomass production via the consumption of petroleum hydrocarbons and the production of organic acids via the partial oxidation of hydrocarbons[55, 56]. As the carbon chain length increases, the $\delta^{13}$C values of aliphatic petroleum hydrocarbons decrease with microbial oil degradation[57, 58]. Aromatic petroleum fractions show higher $\delta^{13}$C values than aliphatic hydrocarbons[57, 58]. Zyakun et al.[58] reported that the microbiota primarily consumed the $C_{12}$–$C_{18}$ aliphatic and aromatic hydrocarbon fractions in the petroleum-contaminated soil. Furthermore, degrading aromatic fractions in the soil increases the aliphatic hydrocarbon fractions and decreases the soil $\delta^{13}$C values[58]. The results of this study were consistent with these findings.

The plant also influences the soil $\delta^{13}$C values[59]. In this research, the $\delta^{13}$C values decreased when the *Trifolium repens* were planted, but lower $\delta^{13}$C values appeared when the *Agropyron cristatum* was planted. Usually, $\delta^{13}$C values of the $C_3$ pathway plant range from −20‰ to −32‰, whereas those of $C_4$ plants from −9‰ to −17‰[60]. The soil $\delta^{13}$C values were influenced by the percent of $C_3$ or $C_4$ plant remnants in the soils[49] and the soil microbial activity, such as microbial degradation reaction[32]. Consequently, the difference in soil $\delta^{13}$C values with plants in the soil comes from plant growth and soil microbial activity. Compared to the $C_4$ plant, the lower $\delta^{13}$C values of *Trifolium repens* caused lower $\delta^{13}$C values of carbohydrates into the soil. They decreased the soil $\delta^{13}$C values, which can also be testified by the relatively smaller $\delta^{13}$C value change of the *Agropyron cristatum* plantation (Fig. 4). However, this conclusion still needs to be testified because there has no clear statistical analysis to support it. This may also be related to the short duration of our experiment.

The $\delta^{13}$C values of *Trifolium repens* decreased as the petroleum-contaminated soil concentration increased (Fig. 4). However, the $\delta^{13}$C values of the *Agropyron cristatum* increased higher than those of the control. Usually, the $\delta^{13}$C values of plants increase under stress conditions (drought, nutrient limitation, saline conditions, sulfide toxicity, high temperature, and humidity deficit)[61, 62]. The petroleum added to the

**Table 3.** The calculated and determined values of petroleum-contaminated soil with or without plants; Δ indicates the difference of $\delta^{13}$C values between original soil and soil after one month of plant-microbial co-remediation.

| Soil petroleum concentration (mg/kg) | Initial value | Bare soil | | *Trioflium repens* | | *Agropyron cristatum* | |
|---|---|---|---|---|---|---|---|
| | $\delta^{13}C_{\text{original soil}}$(‰) | $\delta^{13}C_{\text{soil}}$(‰) | Δ(‰) | $\delta^{13}C_{\text{soil}}$(‰) | Δ(‰) | $\delta^{13}C_{\text{soil}}$(‰) | Δ(‰) |
| 0 | −21.9 | −23.6 | −1.7 | −25.4 | −3.5 | −23.4 | −1.5 |
| 3000 | −22.9 | −24.6 | −1.7 | −24.8 | −1.8 | −24.5 | −1.6 |
| 7000 | −24.0 | −25.6 | −1.6 | −26.1 | −2.1 | −25.9 | −1.9 |
| 10000 | −24.6 | −26.8 | −2.2 | −26.7 | −2.1 | −26.1 | −1.5 |

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soil reduces the soil porosity and gaseous exchange, which is toxic to plants and soil, and might be the same as the stress for a plant to uptake water, nutrients, and oxygen. In addition, petroleum entering the soil increases the soil organic carbon content. All of the reasons mentioned above stress the growth of plants and soil microorganisms. Thus, we can conclude that the increased δ$^{13}$C values of *Agropyron cristatum* are a response to environmental stress[62]. However, the δ$^{13}$C values decreased with increasing petroleum-contaminated soil concentrations for the *Trifolium repens* plantation, implying that this plant has higher adaptability to environmental stress. *Trifolium repens* is a leguminous plant that can fix nitrogen from the atmosphere. Therefore, although the increased soil carbon to nitrogen ratio stresses plant uptake of nitrogen from soil and soil microbial activities[63], the nitrogen fixation ability of *Trifolium repens* can ease petroleum contamination stress. When the nitrogen source comes from the air, the δ$^{15}$N values of nitrogen are close to zero[64]. The δ$^{15}$N values of *Trifolium repens* showed a decreasing tendency with increasing soil petroleum concentration, which may imply *Trifolium repens* increases its nitrogen fixation ability from the atmosphere to reduce the shortage of nitrogen from the soil. Moreover, the nitrogen fixation ability of *Trifolium repens* offsets nitrogen shortage for the plant itself and can even feed on soil microbes. This symbiotic relationship between nitrogen-fixing plants and soil microbes is a favorable condition for petroleum-contaminated soil.

In our experiment, only the nitrogen concentration was detected in the soil, and we did not detect the nitrogen from the petroleum used in the experiment. Thus, we can conclude that the nitrogen in the soil mainly originates from the sample soil and organic fertilizers. Laboratory analysis showed that the δ$^{15}$N values of the organic fertilizer and sample soil were 3.8‰ and 2.2‰, respectively. The δ$^{15}$N values increased with increasing petroleum-contaminated soil concentration under control conditions, implying that petroleum-contamination influences the soil microbial community and activity[65]. John et al. found that, compared with unpolluted soil, ammonium and nitrate concentrations decreased with increased oil pollution levels in a crude oil-contaminated wetland. Moreover, nitrite was not detected in the contaminated soil, probably due to a reduction in the number of nitrogen fixers. Nitrosomonas and Nitrobacter cannot grow well in oil-polluted soil[66]. Therefore, we cautiously speculate that changes in δ$^{15}$N values occur because Nitrosomonas and Nitrobacter activity and quantity are influenced by petroleum-contaminated soil.

Plants change soil δ$^{15}$N values under petroleum contamination conditions. Under the *Agropyron cristatum* planting condition, except for 3000 mg/kg petroleum contamination, the soil δ$^{15}$N values tended to comply with the control condition. However, the values of δ$^{15}$N were lower than that for the control condition, which may be due to the plant-microbial partnership. For example, in underwater or saline stress conditions, ammonium uptake by plants requires less water consumption[67, 68]. The higher values of soil δ$^{15}$N under 3000 mg/kg petroleum contamination with the *Agropyron cristatum* planted imply that a moderate concentration of soil oil pollution promotes soil and plant activity[3, 69, 70]. In the present study, higher concentrations of petroleum inhibited plant growth. However, *Trifolium repens* has lower δ$^{15}$N values than *Agropyron cristatum*, which implies that the nitrogen fixation system of *Trifolium repens* is activated during petroleum-contaminated soil.

## 5 Conclusions

In this study, δ$^{13}$C and δ$^{15}$N of plants and soil samples with varied soil petroleum concentrations were identified by the stable isotope-ratio mass spectrometer. Our in-situ field experiment shows that both δ$^{15}$N and δ$^{13}$C can be used as indices to trace the response and effect of petroleum-contaminated soil on plants and soil. The petroleum added to the soil induced the values of soil δ$^{13}$C to decrease and δ$^{15}$N to increase. *Trifolium repens,* and *Agropyron cristatum* can affect the soil values of δ$^{15}$N and δ$^{13}$C. However, the values of δ$^{15}$N and δ$^{13}$C for *Trifolium repens* and *Agropyron cristatum* showed different results with and without petroleum-contaminated soil, reflecting changes in the metabolic system when plants face a stressful environment. Our results suggest that δ$^{15}$N and δ$^{13}$C are useful proxies for monitoring soil petroleum contamination and evaluating plant stress response. The limitation of this study is that we only measured the whole plant and soil δ$^{13}$C and δ$^{15}$N values and only once during soil bioremediation processes, which impedes further detection of the mechanisms of plant and soil reactions in petroleum-contaminated soil. Future research efforts should focus on the mechanisms of petroleum component transformation, nitrogen mineralization, uptake assimilation, and transfer from the soil to plants under petroleum-contaminated soil.

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## Conflict of interest

The authors declare that they have no conflict of interest.

## Biographies

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