Shi and Liu *Annals of Microbiology* (2021) 71:29
https://doi.org/10.1186/s13213-021-01637-5

Annals of Microbiology

UNIVERSITÀ DEGLI STUDI DI MILANO

ORIGINAL ARTICLE Open Access

Stoichiometric characteristics of microbial biomass in oil-contaminated soil in the loess hilly region

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Abstract

Purpose: The present study envisaged the stoichiometry of microbial biomass in petroleum-contaminated soil, in order to study the influence of the petroleum-contaminated soil on the ecosystem stability.

Methods: A typical oil well area in the Northern Shaanxi was considered the research object and the oil pollution status was assessed by studying the physical, chemical, and microbiological characteristics of the soil in the area.

Results: From the measurement and analysis of the petroleum pollutants in the soil samples, it was observed that the concentration of the petroleum pollutants around all the oil well areas was higher than the critical value of 500 mg/kg. Furthermore, the C to N ratio of 8 soil samples around the oil wells (0.8:1~13.3:1) was lower than that of the control soil samples in most cases and could not reach the nutrient proportion level required by soil microorganisms. It was observed that the oil organic carbon content at 0~10 m from the wellhead was obviously higher than that in other areas, and decreased with an increase in the distance from the well. Based on the determination of soil organic carbon, total nitrogen, total phosphorus, and the soil microbial biomass carbon (SMBC), soil microbial biomass nitrogen (SMBN), and phosphorus content analysis, it was observed that only the soil organic carbon was significantly positively correlated to the oil pollutants in soil.

Conclusions: Imbalance in the C to N, SMBC, and SMBN ratio can lead to an acute shortage of the required nutrients than microorganisms, limit the soil microbial reproduction and growth, and thereby slow down the rate of indigenous microbial degradation of petroleum hydrocarbons, so as to reduce the impact of oil pollution on the stability of the entire ecosystem. Therefore, during the remediation of petroleum-contaminated soil in this study area, adequate nutrients need to be reasonably added to the soil.

Keywords: Oil pollution, Microbial biomass, Ecological stoichiometry, Northern Shaanxi

Introduction

Northern Shaanxi is known to possess rich oil reservoirs, and the petroleum resources are distributed in Yanchang, Ansai, Wuqi, Zhidan, and other counties and towns. With a long history of exploitation, it is an important base of China's petroleum industry. In recent years, with the expansion of the scale of oil exploitation, the fragile ecological environment in Northern Shaanxi has been severely tested, and the soil pollution and ecological destruction has become an increasingly serious issue. Investigation of soil oil pollution in Northern Shaanx has revealed that the area of soil oil pollution in Northern Shaanxi is 7.0816 million m^2, and the content of petroleum hydrocarbon in the soil is about 5~60 $g{\cdot}kg^{-1}$, so the oil pollution in the soil is very serious.

Oil is composed of hundreds of compounds, mainly hydrocarbon (above 95%) and non-hydrocarbon compounds and trace amounts of hydrogen, oxygen, sulfur,

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heavy metals, etc. Components with a high carbon content and organic composition exhibit strong viscosity, hydrophobicity, and low density than water. Petroleum hydrocarbons pollute the soil by changing the surface properties of the soil particles, composition of soil nutrient, and air composition. Furthermore, it influences the soil microbial community structure leading to crop failures by soil particles adhesion (Mashalash et al. 2007; Valeria et al. 2007; Wang et al. 2009), pore blockage, affecting the soil permeability, and hindering the crop root respiration, thereby causing root rot and killing the crop (Gong He et al. 2020; Xu et al. 2020; Liu Danqing 2020). The oil pollution increases the soil organic carbon and increases the soil original C/N ratio (C/N). Because petroleum carbon is different from normal soil organic matter and carbon fixed by plant photosynthesis, it easily causes an imbalance of the soil nutrient structure (Dong et al. 2019). Strong hydrophobicity of the petroleum products leads to the enhancement of soil hydrophobicity and the decrease in the field hydraulic capacity (Rowell and Hall 2007; Ko and Day 2004). Relevant studies have compared the effects of oil pollution on the soil properties and found that the soil with oil pollution has a higher total organic carbon content, C/N ratio, C/P ratio, and lower total nitrogen content, while the total phosphorus content and electrical conductivity exhibit no significant change (Mingyang et al. 2020; Wang and Wang 2010).

The change of element stoichiometry is the core that drives the flow of elements and energy in the ecosystem. Therefore, the ecological stoichiometry is mainly based on the element stoichiometry, and the threshold element proportion and internal stability of ecological stoichiometry is the research core of the present study. Studies have shown that the threshold element ratio is an important nutrient ratio, when the growth and element transfer of organisms are blocked (Yongming and Liangkang 1992). The degree of fluctuation of the microbial stoichiometry depends on the homeostasis mechanism of the ecological stoichiometry, which is an important balance standard to maintain the stability of ecosystem structure and function. For example, the strictly stationary microorganisms always maintain a constant stoichiometry, regardless of how their ecological stoichiometry changes. However, the elemental composition of the non-stationary organisms changes with the ecological stoichiometry of their living environment (Beck et al. 2021). The stoichiometry of autotrophs is usually malleable and changes with the ecological stoichiometry (Feng Huanying et al. 2019). On the contrary, changes in the ecological stoichiometry often have little influence on the elemental composition of heterotrophic objects (He et al. 2020). As important limiting nutrient factors in terrestrial ecosystems, C, N and P maintain a dynamic balance in the chemical cycle of the ecosystem and nutrient cycle in plants. At present, studies on ecological stoichiometry have mainly focused on the global environmental change and ecosystem evolution (Qianxin and Mendelssohn 1996; Xiaoli et al. 2009; Zhang et al. 2009), and the impact of oil pollution on soil ecosystem stoichiometry has not been thoroughly studied. Oil pollution has different effects on the primary productivity, nutrient cycle, and plant community composition of ecosystem, whereas the soil stoichiometry is an important index to reflect the soil quality. Through the study of soil stoichiometry, we can clarify the stability of the ecosystem and consider the effective remediation measures to control the soil stoichiometry induced by the oil pollution within a certain threshold range, so as to reduce the impact of oil pollution on the stability of the entire ecosystem.

This present research investigated the situation of the oil pollution, by studying the physical, chemical, and microbial characteristics of the soil in the typical oil well area in Northern Shaanxi. Through the field investigation, sampling, and determination of oil field regional characteristics of the petroleum hydrocarbon distribution in the soil, the present study analyzed the soil microbial ecosystem response to the oil pollution, and revealed the soil ecological system stability under different pollution levels. Our study can help to assess the soil environmental quality, pollutant emissions, and the bioremediation of contaminated soil in the region and can provide a scientific theoretical basis in the management for making discoveries in the ecological, social, and economic benefit maximization.

Materials and methods

Study area

The study area was located in the typical soil region of the Loess Plateau, and the sampling points were set at Liqu town (36° 39′ N, 109′ E) and Qiaogou town (36° 33′ N, 109′ E). The average altitude of the research site was about 1089 m in the typical hilly and gully region of the Loess Plateau in Northern Shaanxi. The region belonged to the arid and semi-arid continental monsoon climate of the warm temperate zone, with an annual average temperature of 8.8 °C. The average temperature of the coldest month (January) is about − 7.2 °C, the extreme minimum temperature is − 23.6 °C, the average temperature of the hottest month (July) is 22.8 °C, and the extreme maximum temperature is 36.8 °C. The annual accumulated temperature ≥ 10 °C is 3268.4 °C, the annual sunshine hours are 2397.3 h, the early frost starts in early October, the late frost ends in late April, and the frost-free period is 143~162 days. The annual average rainfall is about 328.4 mm, accounting for about 63% of the annual precipitation. In spring, there is little rain, while in summer, the temperature is hot and humid with droughts and floods. In autumn, it is cool and rainy, the temperature drops fast, frost and snow come early, and the winter is cold and dry. Natural disasters mainly include drought, frost, heavy rain, and hail. The main soil is loess soil developed on the parent material of loess after the erosion of loess soil. The soil fertility is low and the productivity is low.

Sample collection and processing

The soil samples for the test were collected on September 6, 2018, from the surrounding soil of 8 typical oil wells with different production ages in the Liqu Town and Qiaogou Town, Baota District, Yan'an City. The soil type was yellow soil, and the oil wells were numbered 1#, 2#, 3#, 4#, 5#, 6#, 7#, and 8#. The geographic locations of the eight wells are shown in Table 1.

Taking each well site as the central point, the sampling area was extended to the surrounding area, and the representative area was selected as the sampling area in 0~10 m, 10~30 m, and 30~50 m areas outside the center, and the control area was set up on the unpolluted abandoned land near the well site. Three quadrats were randomly selected from each sampling area, and soil samples in 0~30 cm depth were taken by soil drilling method (S or 3 to 5 points on the diagonal). After sampling, the soil samples were transported back to the laboratory, and the roots of the soil samples were removed. After mixing the soil, the soil samples were divided into dry samples and fresh samples by quartering method.

Sample determination and data processing

The total amount of petroleum hydrocarbon in soil was measured by an infrared oil meter. Soil organic matter and total nitrogen were measured by conventional methods in soil agrochemical analysis (Bao S D. 2000). The microbial biomass carbon, nitrogen, and phosphorus were determined by chloroform fumigation extraction method. Data were analyzed using one-way ANOVA. Multiple comparative tests were performed using LSD, and the significance level was $P < 0.05$. SPSS 20.0 software was used for statistical analysis and regression analysis, and Origin 9.0 was used to draw all the figures.

Results and discussion

Distribution characteristics of petroleum hydrocarbon pollution in soil

It can be seen from Fig. 1 that the petroleum hydrocarbons were detected in all the 8 soil sampling points around oil wells, in the range of 73 to 1090 mg/kg. Most of the sample points were higher than the critical value of soil oil pollution (500 mg/kg) (Bao S D. 2000). According to the evaluation standard of 500 mg/kg in the Technical Provisions for the Evaluation of Soil Pollution Status in China, except for the 8# oil well area, the soil at the sampling points around the oil wells in different production ages should be repaired. Among them, the sampling point in the 1# old oil well area exhibited the most serious pollution, which was more than two times higher than the critical value of petroleum hydrocarbon pollution in the soil. This was closely related to the extensive mining methods of the old oil wells in the past, the aging equipment, backward technology, and the frequent occurrence of running, leaking, dripping, and leakage near the oil wells.

In addition, the content of petroleum hydrocarbons in the soil around the 8 oil wells decreased with the increase of the distance from the oil wells. Previous researches on the content of petroleum hydrocarbons in the soil around the oil wells found that the basic rule of the content of petroleum hydrocarbons in the soil around the oil wells is that the farther away from the oil wells, the lower the content of petroleum hydrocarbons in the soil, which is consistent with the research results of this study (Wuxing et al. 2007). Production runs, drips, and leaks occur, which result in the surface runoff to long-distance transmission due to partial water solubility and low density, and emissions in the process of oil production accumulate a large amount of oily waste water near the mouth of the well in the soil, resulting in

Table 1 Sample collection location

Area	Serial number	Latitude and longitude	Condition
Qiao Gou town	1#	N 36° 39′ 55.32″, E 109° 29′ 06.05″	Running
	2#	N 36° 39′ 55.40″, E 109° 29′ 21.07″	Abandoned
	3#	N 36° 40′ 3.07″, E 109° 29′ 25.00″	Running
	4#	N 36° 40′ 25.39″, E 109° 29′ 10.83″	Abandoned
	5#	N 36° 40′ 14.19″, E 109° 28′ 51.10″	Abandoned
Li Qu town	6#	N 36° 33′ 47.19″, E 109° 32′ 34.65″	Running
	7#	N 36° 34′ 2.20″, E 109° 32′ 33.86″	Abandoned
	8#	N 36° 32′ 4.72″, E 109° 32′ 23.64″	Abandoned

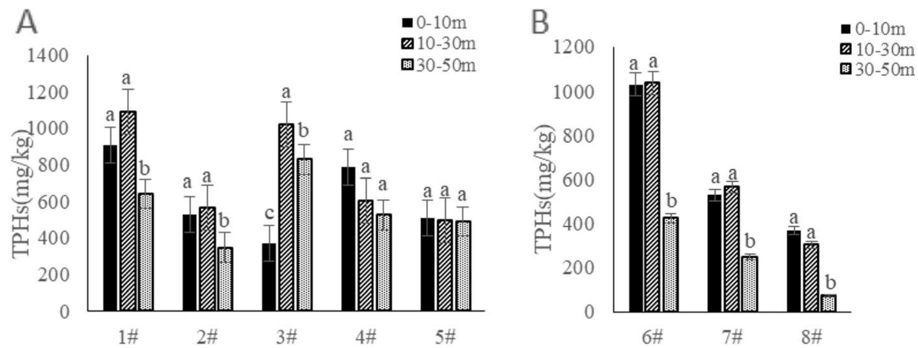

Fig. 1 Distribution characteristics of soil essential nutrients in different regions of oil well area

petroleum hydrocarbon concentration following the radius distribution pollution (Jian et al. 2014).

Effect of petroleum pollution on soil chemical properties

It was observed that the oil organic carbon content at 0~10 m from the wellhead was obviously higher than that in other areas and decreased with an increase in the distance from the well. As can be seen from Fig. 2, there was a significant positive correlation between the content of soil organic carbon and the content of petroleum hydrocarbons, i.e., with the aggravation of soil oil pollution, the content of soil organic carbon increased. Jia Jianli et al. investigated the oil-contaminated soil in the oil fields under different environmental conditions and found that the content of organic carbon in the oil-contaminated soil was significantly positively correlated with the content of petroleum hydrocarbons (Jia Jianli et al. 2009). This is because petroleum is a complex composed of naphthenes, chain burning light and aromatic diameter, and some petroleum hydrocarbon components are highly toxic to microorganisms and difficult to degrade, which persist in soil and become a part of soil organic carbon, resulting in the increase of the soil organic carbon content. There was no significant correlation between the content of petroleum hydrocarbons in soil and total nitrogen. Wang et al. studied some oil-

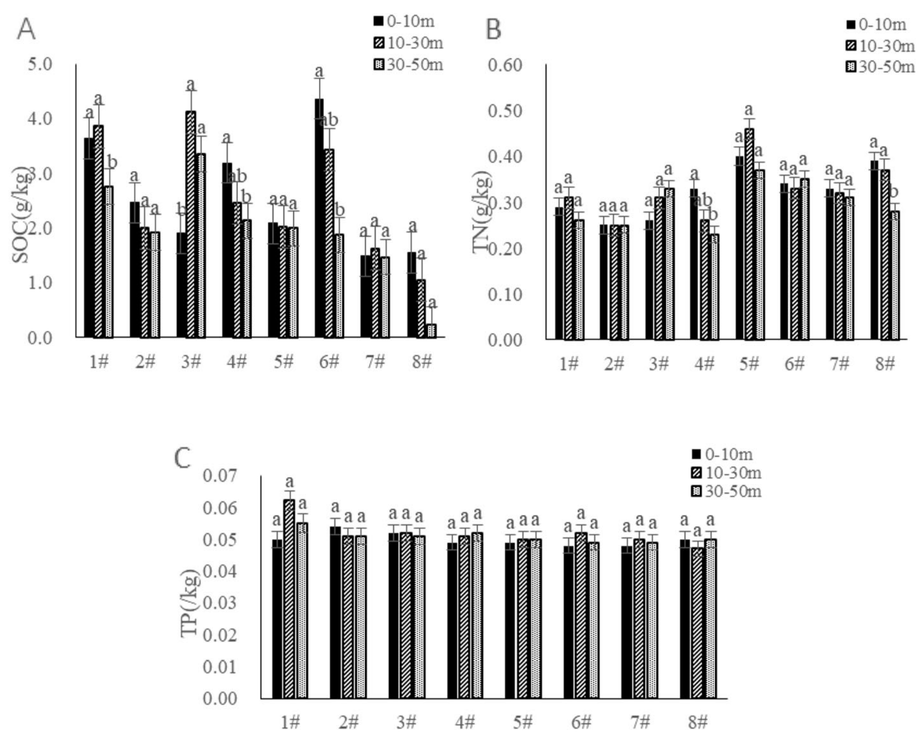

Fig. 2 Distribution characteristics of soil essential nutrients in different regions of oil well area

contaminated soils in the Yellow River Delta region and found that there was no significant correlation between the content of petroleum hydrocarbons and nitrogen in soil. This may be related to the chemical composition of the petroleum hydrocarbons themselves, which are mainly composed of carbon, hydrogen, sulfur, oxygen and other elements (Wang et al. 2010).

Response characteristics of soil microbial biomass stoichiometry to petroleum contamination

The C to N ratios of all the soil samples around the 8 oil wells were higher than those of the control soil samples, indicating that the proportion of soil nutrients was changed after oil entered the soil. Reasonable nutrient ratio is the key factor to improve the biodegradation rate of petroleum and the bioremediation effect of oil-contaminated soil. It can be seen from Fig. 3 that the C to N ratio of 8 soil samples around oil wells (0.8:1~13.3:1) was lower than that of the control soil samples in most cases and could not reach the nutrient proportion level required by soil microorganisms. Jia Jianli studied typical oilfield areas in northern China, wherein it was found that the nutrient element content of oily soil was far below the level of nutrient C to N ratio required by microorganisms (12:1) (Jia Jianli et al. 2009). The imbalance in the proportion of nutrients restricts the reproduction and growth of soil microorganisms, thus slowing down the degradation rate of the petroleum hydrocarbons (Du Y L.et al. 2012). Therefore, when carrying out remediation activities in this area, nutrient elements need to be reasonably added to the soil to improve the activity of microorganisms in the soil and accelerate the degradation of oil. The degradation effect of petroleum hydrocarbons by soil microorganisms is greatly affected by soil physical and chemical properties. The relationship between petroleum degradation bacteria and soil influencing factors need to be solved in practical application, which is also the key problem to be solved in the bioremediation of petroleum-contaminated soil. There was no significant correlation between the soil microbial biomass carbon (SMBC) and soil microbial biomass nitrogen (SMBN). The soil petroleum hydrocarbon content (SPHC) and the MBC to MBN ratio deviated from the soil background value, indicating that the petroleum pollution inhibited the soil microbial activity.

Conclusions

In the present study, the typical oil well areas with different mining years in the loess hilly region were selected as the research object, and each well site mouth was taken as the center point to expand to the surrounding area. Representative sections outside the center of 0–10 m, 10–30 m, and 30–50 m were selected as the sampling areas to collect soil samples and the total amount of petroleum hydrocarbons in the soil was measured. The results showed that the minimum amount of petroleum hydrocarbons in these soils was 73 mg/kg at 30–50 cm in abandoned oil well area No. 8, and the maximum amount was 1090 mg/kg at 10–30 m in

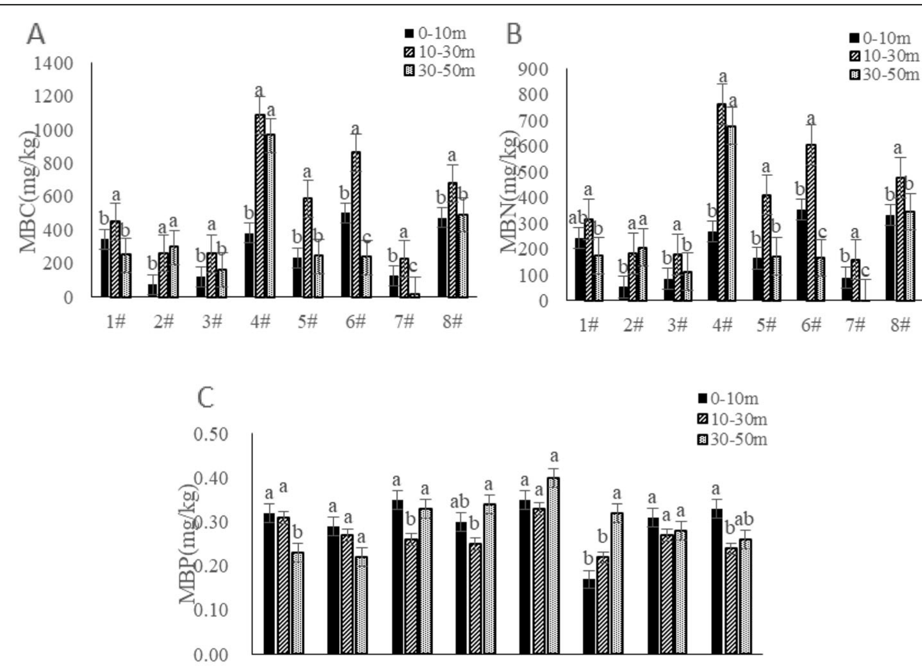

Fig. 3 Distribution characteristics of soil microbial biomass in different regions of oil well area

abandoned oil well area No. 1. Therefore, it was concluded that the range of oil pollution in the soil around the oil well was about 73–1090 mg/kg, and most of them were higher than the critical value of soil oil pollution 500 mg/kg. Furthermore, the petroleum hydrocarbon content in the soil was significantly positively correlated with organic carbon but not significantly correlated with total nitrogen, total phosphorus, microbial biomass carbon, microbial biomass nitrogen, and microbial biomass phosphorus. From the perspective of chemometrics of the soil and the microbial elements, long-term oil pollution can lead to the imbalance of soil C to N and SMBC to SMBN ratios. This can lead to an imbalance of the proportion of major nutrients in soil after oil pollution, and the destruction of the internal stability of the ecosystem, which is bound to affect the activity of indigenous microorganisms, limit the reproduction and growth of soil microorganisms, and slow down the degradation rate of petroleum hydrocarbons by indigenous microorganisms. Under the temperate monsoon climate in the loess hilly region, the reproduction rate and metabolic activity of indigenous microorganisms in the soil is not high. After being polluted by crude oil, the indigenous microorganisms metabolize the petroleum hydrocarbon as the carbon source, thereby reducing the concentration of pollutants in the soil. However, under natural conditions, the biodegradation rate of pollutants by indigenous microorganisms in contaminated soil is slow, which takes a long time. The cost of a single technology to improve the ambient temperature to accelerate the degradation of pollutants and then repair contaminated soil is high. Therefore, this study focused on revealing the limitation of pollution on indigenous microorganisms from the perspective of nutrient stoichiometry. In order to improve the activity of indigenous microorganisms and enhance the degradation efficiency of the indigenous microorganisms on petroleum pollutants, the negative impact of pollutants on the soil microorganisms needs to be reduced by adjusting the proportion of soil nutrients to balance. Therefore, during the remediation of petroleum-contaminated soil in this study area, adequate nutrients can be reasonably added to the soil.

Acknowledgements
This work was supported from the projects of Shaanxi Provincial Land Engineering Construction Group (DJNY2020-30, DJNY2021-33).

Ethical statements
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.

Authors' contributions
LS: guided the completion of this experiment, completed the first draft paper, and made revisions. ZL: performed experiments, recorded data, and data analysis. All authors read and approved the final manuscript.

Funding
This work was supported from the projects of Shaanxi Provincial Land Engineering Construction Group (DJNY2020-30、DJNY2021-33).

Availability of data and materials
The data were obtained by the authors.

Declarations

Ethics approval and consent to participate
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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Received: 16 April 2021 Accepted: 21 June 2021
Published online: 17 July 2021

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