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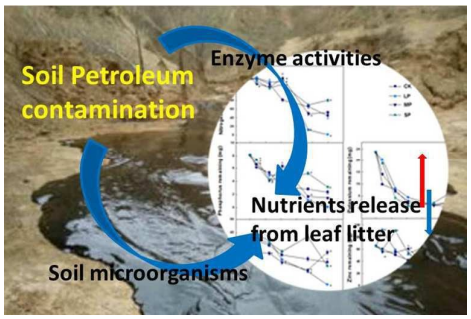

In this study, the impacts of soil petroleum contamination on nutrient release during litter decomposition were investigated.

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Impacts of soil petroleum contamination on nutrient release during litter decomposition of *Hippophae rhamnoides*

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Petroleum exploitation causes contamination of shrub lands close to oil wells. Soil petroleum contamination affects nutrient release during the litter decomposition of shrubs, which influences nutrient recycling and the maintenance of soil fertility. Hence, this contamination may reduce the long-term growth and stability of shrub communities and consequently, the effects of phytoremediation. Fresh foliar litter of *Hippophae rhamnoides*, a potential phytoremediating species, was collected for this study. The litter was placed in litterbags and then buried in different petroleum-polluted soil media (the petroleum concentrations were 15, 30, and 45 g kg^{-1} dry soil, which were considered as slightly, moderately and seriously polluted, respectively) for a decomposition test. The impacts of petroleum contamination on the release of nutrients (including N, P, K, Cu, Zn, Fe, Mn, Ca and Mg) were assessed. The results showed that (1) after one year of decomposition, the release of all nutrients was accelerated in the slightly polluted soil. In the moderately polluted soil, P release was accelerated while Cu, Zn and Mn release was inhibited. In the seriously polluted soil, Cu and Zn release was accelerated while the release of the other nutrients was inhibited. (2) The effect of the petroleum on the nutrient release from the litter differed in different periods during decomposition; this was mainly due to changes in the soil microorganisms and enzymes under the stress of petroleum contamination. (3) To maintain the nutrient cycling and the soil fertility of shrub lands, *H. rhamnoides* is only suitable for phytoremediation of soils containing less than 30 g kg^{-1} of petroleum.

Introduction

Petroleum is one of the most important energy sources. However, oil spills during petroleum exploitation, transport and storage can cause serious contamination of soil and water. Phytoremediation has been identified as a cost effective method for improving the ecological environment and restoration of contaminated areas. Merkl *et al.*[1] stated that *Brachiaria brizantha* shows strong tolerance to soil petroleum pollution and reduces the contents of saturated hydrocarbons and aromatic hydrocarbons. In addition, phytoremediating plants play an important role in stimulating the growth of petroleum-degrading microorganisms in rhizospheric soil, and thus stimulate the degradation of petroleum. For instance, Ribeiro *et al.*[2] suggested that *Juncus maritimus* and *Phragmites australis* exhibit potential to restore contaminated soil through their rhizosphere effects. Liu *et al.*[3] indicated that Fire Phoenix, a phytoremediating species, can increase the activity of dehydrogenase and polyphenol oxidase to decompose petroleum as a result of its rhizosphere effects. Moreover, plant litters can supply available nutrients, enhance soil enzyme activities, and accelerate the decomposition of petroleum, and thus recover the physical, chemical and biological properties of soil.[4] Because of its advantages, phytoremediation has become a promising method for the restoration of soil petroleum contamination.

The key work of phytoremediation research is to select suitable phytoremediating species. In recent decades, the tolerance of plants to petroleum (including the tolerance represented in the germination, growth and physiological properties) and their ability to remove petroleum pollutants were considered as the most important indicators for the selection of phytoremediating plants.[5-9] However, most of these studies did not determine the long-term stability of phytoremediating plant communities, especially the nutrients plant-soil cycling processes within the ecosystems. Nutrient release is a key link in material cycling in an ecosystem. It is not only affected by the litter quality, but also by the bio- and chemical properties of the soil.[10] Petroleum may influence the decomposition and nutrient release of litter because according to previous studies, petroleum pollution can cause significant decreases in soil available nutrient contents, and can lead to stimulation or inhibition of decomposers and alterations in the microflora. Petroleum pollution also inhibits

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the activity of urease, dehydrogenase, and protease, but stimulates the activity of cellulolytic enzymes and phosphatase.[11-13] Consequently, these alterations in soil will certainly influence the decomposition and nutrient release of litter. As an example, Zhang *et al*.[14] stated that petroleum pollution of soil at a concentration of 15 g kg^{-1} significantly accelerated the litter decomposition of *Hippophae rhamnoides* and other plants, whereas the litter decomposition was significantly inhibited by higher concentrations of petroleum. Mendelssohn and Slocum[15] also stated that petroleum reduced the decomposition rate of cellulose. If nutrient release from litter was inhibited, the supply of nutrients for phytoremediating plants would become limited after long-term growth, which may negatively affect these plant communities and consequently lead to an increase in the costs of restoration and ecosystem management.

Hippophae rhamnoides L. (Elaeagnaceae) is a shrub species that is widely distributed in the petroleum-contaminated areas in Northern Shaanxi, China. A previous study demonstrated that this species exhibits a strong tolerance to petroleum pollutants. Moreover, it also shows favourable abilities of bioaccumulation and bio-transference of petroleum hydrocarbons.[16] However, the impacts of petroleum contamination on the nutrient release of its litter have not been studied. Hence, we used *H. rhamnoides* litter as the object of this study, and the nutrient release dynamics of this litter were determined in soil contaminated with three concentrations of petroleum. The dynamics of the soil biological properties in the contaminated soil were also examined to determine the possible mechanisms underlying the nutrient release of *H. rhamnoides* litter in the presence of petroleum. We aimed to assess the possibility of establishing a stable community of *H. rhamnoides* over a long-term period to restore petroleum-contaminated soil.

Materials and methods

Sample collection

In late autumn of 2012, a waste grassland of the Yujiaping Oil Field (Northern Shaanxi, China) was chosen as the sampling area. Twenty quadrats (1 m×1 m) were established in the grassland. Plant litter and other material were removed from the soil surface and then the 0-10-cm humus layer was collected. Soil samples were mixed uniformly after the stones, roots and other plant and animal debris were removed. Homogenized fresh soil was passed through a sieve (5-mm pore size) and retained. The initial properties of the soil samples are presented in Table 1. Simultaneously, strongly growing communities of *H. rhamnoides* were selected in the same area, and their fresh litter was collected. Litter that had decayed or was infected by diseases or pests was discarded, and the remaining litter was rinsed rapidly and oven dried at 60 C to a constant weight after removing the water at 105 C for 30 min. Petroleum samples were collected from the Yujiaping Oil Field.

Preparation of petroleum-contaminated soil

After measuring the soil moisture using the oven-drying method, 12 fresh soil samples with a dry weight of 2.5 kg were prepared. Three soil samples were not treated with petroleum and were used as the control (CK). Next, petroleum was added to the remaining soil samples at concentrations of 15, 30, and 45 g kg^{-1} (petroleum: soil), resulting in three types of contaminated soil samples: slightly polluted soil (LP), moderately polluted soil (MP) and seriously polluted soil (SP). Each type of soil sample was prepared in three parts. The soil containing the petroleum was then uniformly mixed. In this process, no organic solvent was used to eliminate any possible effect on the soil properties. The mixed soil samples were stationary incubated for two days to obtain homogenized contaminated soil. The prepared soil samples were placed in 40 cm × 30 cm × 20 cm plastic box-type pots, and subsequently used as the medium for litter decomposition testing.

Litter decomposition testing

Sixty 5.00-g sub-samples of *H. rhamnoides* litter were prepared and placed into 14-cm× 20-cm nylon mesh litterbags (mesh diameter: 0.5 mm) and the bags were sealed. Each five of these bags were buried at an angle in one pot and uniformly separated from each other (to ensure sufficient contact with the soil); this comprised one treatment. Each treatment was repeated three times.

Next, distilled water was uniformly added to the soil medium to adjust the soil moisture to 50% of the saturated field water capacity, and the pots were weighed. The pots were covered with plastic film containing four air vents (1.5-cm Φ) to prevent excessive evaporation and to provide air for the microorganisms. The pots were weighed weekly, and distilled water was added using a sprayer according to the weight loss to maintain constant soil moisture during the incubation testing. On the basis of these methods, the litter was incubated for one year at room temperature (20-25 C).

Because litter decomposition was relatively slower in the later decomposition period, the litterbags were harvested in the 1st, 3rd, 5th, 9th and 12th month during the decomposition testing. When retrieving the litter, three litterbags were harvested from pots in the same treatment, and each bag was randomly selected from the five bags in one pot. The litter residues were then placed in sieves with a mesh size of 0.05 mm, and rinsed rapidly to remove the soil, hypha and other material. Cleaned litter residues were oven dried at 60 C and accurately weighed.

Determination of nutrients remaining in the litter

Nutrients concentration of litter or litter residues were determined by following methods. [17] First, the litter residues were digested with H_2SO_4 and H_2O_2. Then, the N concentration was measured using a continuous flow analytical system (Auto Analyser3, Bran Luebbe, Germany), the P concentration was determined by a phosphor-vanadic-molybdic colorimetric method using a UV-VIS spectrometer (UV-2450 Shimadzu Corporation, Kyoto, Japan) and the K concentration was measured using a flame photometer (BMB

Technologies UK LTD.). Next, the Cu, Zn, Fe, Mn, Ca and Mg concentrations were analysed using a polarized Zeeman atomic absorption spectrophotometer (Z-2000, Hitachi, Tokyo, Japan) after 1.0000 g-samples were carbonized using an electric stove, ashed at 550 C for 10 hours in a muffle furnace and dissolved using 1 mol L^{-1} HCl. The contents (total amounts) of each nutrient were obtained by multiplying the nutrient concentrations and corresponding dry weights of the litter or residues.

Determination of soil biological properties

Before the decomposition testing, the initial microorganism populations and the activities of the litter-decomposing enzymes in the soil were determined. As litter exhibits the highest decomposition rate in the earlier period of decomposition [14], while enzymes mainly participate in different stages during the decomposition process, we measured the microbial populations and the activities of the litter-decomposing enzymes three times, i.e., in the 1st, 6th and 12th month of the decomposition testing period. The soil samples were collected close to the litterbags. Simultaneously, the remaining soil from nearby the litterbags was used to cover the area surrounding the litterbags to maintain approximately constant conditions of the decomposition medium, and all litterbags remained completely buried in the soil. In addition, the soil samples used for the determination of the biological properties were collected from 3 pots in the same treatment, respectively and all biological property determination tests were replicated 3 times.

The microorganism populations were determined using a plate-count method (bacteria-beef extract peptone agar culture medium, fungi-potato dextrose agar culture medium, actinomyces-GAO 1st synthetic culture medium with 1% $K_2Cr_2O_7$ to prevent the growth of bacteria). [18]

Enzyme activities were measured using the following method suggested by Guan. [19] Protease activity was measured using ninhydrin colorimetry, and urease activity was measured using indophenol colorimetry. Sucrase, amylase and carboxymethyl cellulose activities were measured using 3,5-dinitrosalicylic acid colorimetry, β-1,4-glucosidase was measured using nitrophenol colorimetry, and xylanase was measured using iodometry. Alkaline phosphatase activity was measured using disodium phenyl phosphate colorimetry, and catalase was measured using titrimetry. Peroxidase and polyphenol oxidase activities were measured using pyrogallol colorimetry, and dehydrogenase activity was measured using triphenyltetrazolium chloride colorimetry.

Data processing

The treatment and CK data (nutrients remaining in the litter, enzyme activities, and soil microorganism populations) were subjected to t-tests using IBM SPSS 19.0 software. The level of significance used was $\alpha = 0.05$, and SigmaPlot 12.5 software was used for drawing the graphs.

Table 1 Initial properties of the soil samples

FAO Taxonomy	Granulometric composition [%]			Initial content of available nutrients [mg kg^{-1}]			pH	CEC [cmol kg^{-1}]
	1-0.05 mm	0.05-0.01 mm	< 0.01 mm	N	P	K		
Calcic Cambisols	29.6	48.3	22.1	20.22	8.92	114.30	8.60	12.91

Results

Nutrients release from *H. rhamnoides* litter in petroleum contaminated soil

The nutrient release was obviously altered in the petroleum-contaminated soil (Fig. 1). In general, the impacts of petroleum contamination on nutrient release were variable during months 1-5 of the decomposition experiments, while the mass loss of the litter was rarely affected during this period (Fig. 2). These results indicated that the nutrient release was not synchronized with the litter decomposition in the early and intermediate periods. However, at the end of decomposition (9-12 months), LP exhibited significant acceleratory effects on the nutrient release of *H. rhamnoides* litter, MP exhibited approximately equal accelerating and inhibitory impacts, while SP exhibited significant overall inhibitory impacts, which were in line with the effects of petroleum contamination on the mass loss (Fig. 2). That is, during the late stages of decomposition, the nutrient release was controlled by the mass loss of litter.

Specifically, in the LP soil, the release of P, K, Cu, Zn and Ca was inhibited while the release of Mn was accelerated in the 1st month. The release of Fe, Mn and Ca was accelerated in the 3rd month. In the 5th month, the release of N, P and K was inhibited while the release of Fe, Mn and Ca was accelerated. In the 9th month, the release of all nutrients except for K was accelerated, while at the end of decomposition, the release of all nutrients was accelerated.

In the MP soil, the release of P and K was inhibited while the release of N, Cu, Zn, Fe, Mn, Mg and Ca was accelerated in the 1st month. The release of P, Cu, Fe, Mn and Ca was accelerated in the 3rd month. The release of N, P, K and Zn was inhibited while the release of Cu was accelerated in the 5th month. In the 9th month, the release of Mn was inhibited while the release of N, P, Cu and Zn was accelerated. At the end of decomposition, the release of Cu, Zn and Mn was inhibited while the release of P was accelerated.

In the SP soil, the release of P and K was inhibited while the release of Fe, Mn, Mg and Ca was accelerated in the 1st month. The release of Zn and Mn was inhibited while the release of P, K, Fe and Ca was accelerated in the 3rd month. In the 5th month, the release of N, P, Zn, Fe, Mg and Ca was inhibited while the release of K was accelerated. In the 9th month, the release of all nutrients except N was inhibited. At the end of decomposition, the release of Cu and Zn was

accelerated while the release of the other nutrients was inhibited.

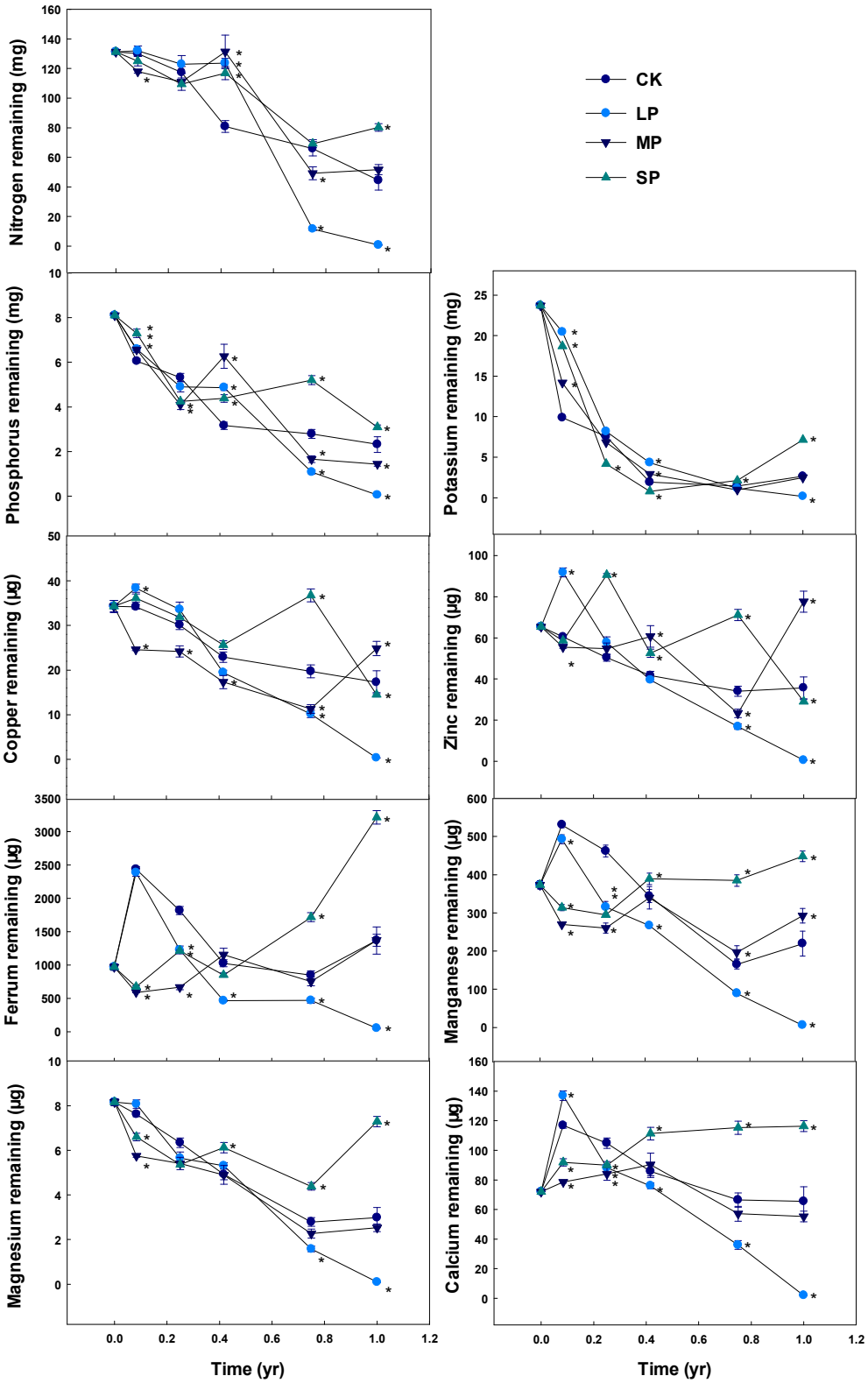

Fig. 1 Nutrient dynamics during litter decomposition of *H. rhamnoides* in petroleum-contaminated soil.
Note: *, there was a significant difference between the treatment and CK values at the 0.05 level, the same below.

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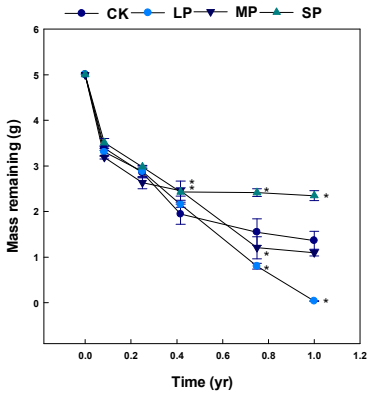

Fig. 2 Mass loss during the decomposition of *H. rhamnoides* litter.

Impacts of petroleum contamination on soil biological properties during the litter decomposition process of *H. rhamnoides*

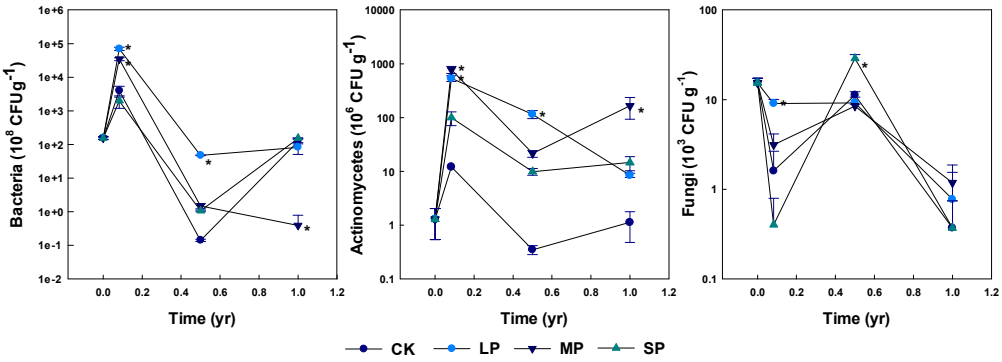

Fig. 3 Population of microorganisms in petroleum-contaminated soil.
Note: CFU is the colony forming unit. The points that overlap with each other (0.0 yr on the X-axis) indicate the initial microbial populations in uncontaminated soil.

Enzyme activities. Soil enzymes catalyse the decomposition of macromolecules in litter, and their activities are usually used to determine the litter decomposition rate. Litter-decomposing enzymes can be classified into four groups according to the substrate characteristics: cellulose decomposing enzymes, lignin decomposing enzymes, protein hydrolysing enzymes and phosphatases. [20] Enzymes are extremely sensitive to environmental factors and thus their activities were significantly affected in the contaminated soil (Fig. 4). In general, LP caused increases in the activities of xylanase and alkaline phosphatase during the decomposition process (1-12 months). Furthermore, LP significantly increased the overall activities of the cellulose/lignin decomposing enzymes and significantly decreased the amylase activity in the intermediate and late periods of decomposition (6th and 12th month). However, the protease activity was significantly inhibited in the early and late periods (1st and 12th month).

MP caused significant decreases in the activities of alkaline phosphatase and dehydrogenase at the early and intermediate periods but stimulated their activities in the late period. MP also significantly increased the activities of some cellulose/lignin decomposing enzymes in the intermediate period. However, MP caused significant overall decreases in the lignin decomposing enzymes in the early period, and significant decreases in the activities of xylanase, amylase and protease in the late period of decomposition.

SP significantly increased the activities of some cellulose/lignin decomposing enzymes but decreased the activity of protease in the early period. In the intermediate period, SP caused significant decreases in the activities of

Populations of microorganisms. The population and community structures of soil microorganisms directly influence the release of nutrients from litter. In general (Fig. 3), LP caused significant increases in bacterial (including *Actinomyces*) and fungal populations in the early and intermediate periods, but from 6-12 months, LP caused an abnormal decrease in *Actinomyces* populations relative to the CK soil. MP caused significant increases in bacterial (including *Actinomyces*) populations in the 1st month, and a significant increase in *Actinomyces* populations in the 12th month. However, from 6-12 months, MP caused an abnormal decrease in the bacterial populations, leading to significantly lower bacterial populations relative to CK in the 12th month. In the SP soil, only fungal growth was accelerated in the intermediate period.

xylanase and sucrase and significant increases in the overall activities of the lignin decomposing enzymes and alkaline phosphatase. In the late period, SP caused significant increases in the activities of alkaline phosphatase and carboxymethyl cellulase, but also significantly decreased the activities of the lignin decomposing enzyme (polyphenol oxidase), protease and low-molecular carbohydrate decomposing enzymes.

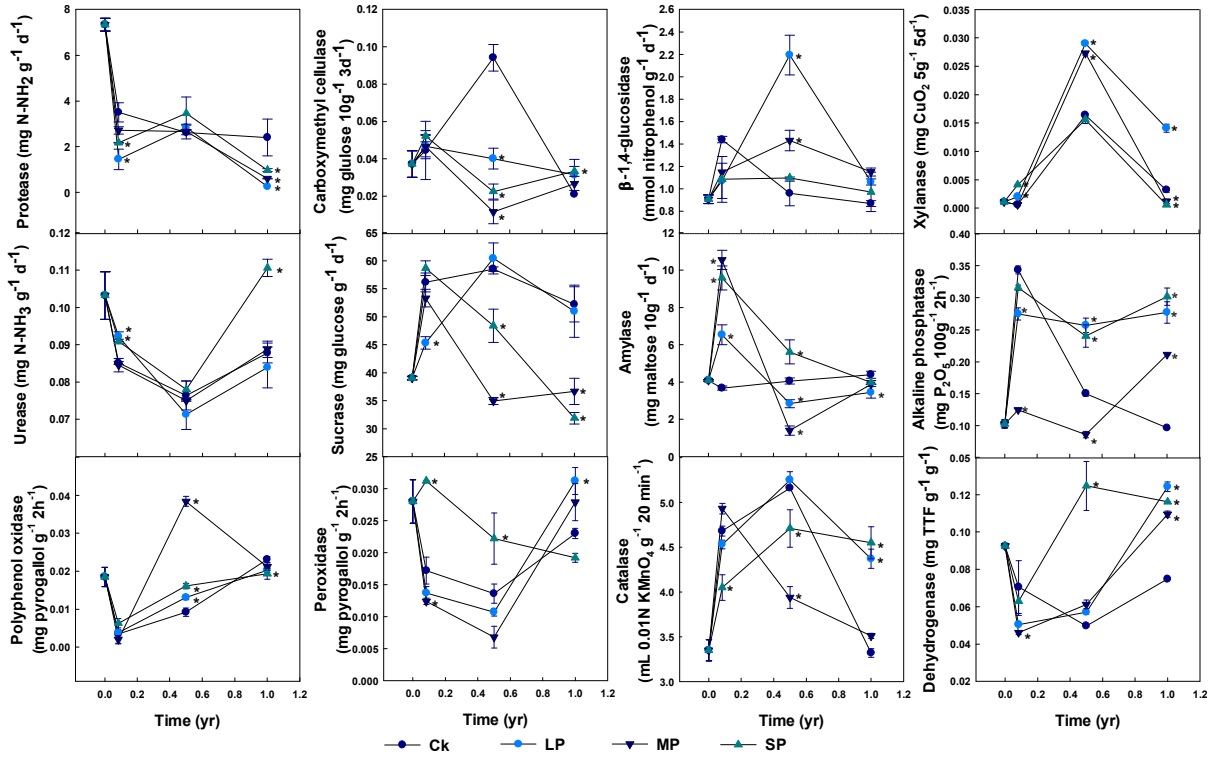

Fig. 4 Activities of enzymes in petroleum-contaminated soil.
Note: The points that overlap with each other (0.0 yr on the X-axis) indicate the initial enzyme activities in uncontaminated soil.

Discussion and conclusion

Overall impacts of petroleum contamination on nutrient release ratio of litter

In general, after decomposing for one year, petroleum showed similar effects on the release of N, P, K, Fe, Mn, Ca and Mg. The release of these nutrients was significantly accelerated in the LP soil, not significantly affected in the MP soil, and significantly inhibited in the SP soil. These results support our previous study, which indicated that the decomposition rate of *H. rhamnoides* litter was significantly stimulated in LP soil, not significantly affected in MP soil, and significantly inhibited in SP soil. [14] The effects of petroleum on soil microorganisms and enzymes might be responsible for these phenomena.

Our results demonstrated that in the early and intermediate periods of decomposition, LP significantly stimulated the growth of soil bacteria (including *Actinomyces*), and also stimulated the growth of fungi in the early period. In the late period, these microbial groups were not remarkably affected by petroleum pollutants. These findings are in agreement with those of Blakely *et al.* [21] Indigenous microorganisms usually exhibit tolerance to pollutants and thus small amounts of petroleum may not obviously inhibit their reproduction and activity, or may only exert short-term toxicity effects on these organisms. [12, 22] Furthermore, as the pollutants increase the carbon sources for microorganisms, they may even accelerate the growth of microorganisms and increase their activities. [21] In addition, petroleum contamination would change the community structure of microorganisms, which directly influences their functional diversity and enzyme secretion characteristics. [23] Our results indicated that LP increased the activities of the main enzymes that participated in specific litter decomposition stages (Fig. 4), for example, amylase (participates in the early decomposition of litter), β-1,4-glucosidase and xylanase (intermediate stage) and peroxidase (late stage). [24] Qasemian *et al.*[12] stated that after soil was polluted by polycyclic aromatic hydrocarbons for three months, the activities of lignocellulolytic enzymes were significantly stimulated. We obtained similar results in our experiments. Based on these changes in soil, LP would accelerate the overall decomposition of litter and therefore increase the amounts of nutrients released.

The growth of bacteria and actinomyces are also accelerated in the early period in the MP soil, and the growth of fungi was stimulated in the intermediate period in SP soil. However, previous study stated that the components of petroleum exhibited strong bio-toxicity, and large amounts of petroleum thus can lead to a sharp decrease in microbial activity. [13] In addition, in contaminated soil, petroleum-decomposing microorganisms would become the dominant groups, and the microorganisms that mainly decompose litter are thus relatively inhibited. [25-27] Furthermore, we observed a decrease in the activities of litter-decomposing enzymes such as phosphatase, sucrase, amylase, carboxymethyl cellulase and xylanase, etc. in the MP and SP soil (Fig. 4), which is in line with the findings of Guo *et al*.[11], Lv *et al*.[28] and Ma *et al*.[29] These phenomena might explain the inhibitions in nutrient release. The possible reasons for the decrease in enzyme activities might be the pH changes after oil pollution, which might lead to variable impacts on enzymes. In addition, large doses of petroleum may cover the cells, hindering the expression of enzyme activities, and may simultaneously decrease enzyme activities by inhibiting microorganism growth and/or complexing the enzyme active centers with the heavy metal ions contained in the petroleum. [30] Certainly, the following points still need further clarification: (1) There were many types of microorganisms and enzymes that participated in litter decomposition and nutrient release. However, petroleum usually increased or decreased the activities of several types of enzymes simultaneously. For example, MP exhibited stimulatory effects on β-1,4-glucosidase activity in the intermediate period of decomposition, but significantly inhibited the activity of carboxymethyl cellulase at the same time. Therefore, the impacts of the petroleum on nutrient release were simultaneously controlled by its impacts on several types of enzymes. (2) Oxidoreductases, such as polyphenol oxidase, dehydrogenase and peroxidase, participated in the decomposition of both the litter and the petroleum components; an increase in their activities did not inevitably lead to a higher decomposition rate of recalcitrant substances. It could also be caused by the inducing effects of pollutants. [3, 20, 27, 31] (3) Because petroleum pollutants showed high C/N and C/P ratios, the microorganisms might secrete large amounts of enzymes (such as urease, protease, alkaline phosphatase, etc.) to decompose the litter to obtain N and P, resulting in an increase in the enzyme activities. However, the nutrients released from litter might be directly immobilized by the microorganisms colonizing the litter, which might decrease the quantity of nutrients released.

Interestingly, the overall release of Cu and Zn was significantly stimulated in the LP and SP soil, but inhibited in the MP soil. Because the dominant microbial species differed in the 3 types of contaminated soils[12], the microorganisms showed differences in their nutrient requirements. We speculated that the dominant species in the MP soil might require more Cu and Zn, and thus result in Cu and Zn retention. Certainly, the specific mechanism needs to be studied further.

Dynamics of nutrient release of litter in petroleum-contaminated soil

Our results indicated that LP significantly inhibited the release of N, P, K, Cu, Zn and Ca in the early and intermediate period of decomposition (or part of these stages), but significantly accelerated their release in the later decomposition period. LP continuously and significantly accelerated the release of Fe, Mn and Mg in the initial period or after decomposition had occurred for a period of time. According to the nutrients mineralization theory, microorganisms tend to retain nutrients initially for their own physiological actions, and thus inhibit the release of nutrients from litter. [32] In the present study, the populations of bacteria (including *Actinomyces*) and fungi were increased to some extent in the LP soil in the initial and intermediate periods of decomposition (Fig. 3). The sharp increase in the microbial population might result in the consumption of large amounts of nutrients and thus lead to an obvious inhibitory effect on the release of N and the 5 other elements. However, our previous research indicated that LP significantly accelerated the overall decomposition of *H. rhamnoides* litter. [14] Simultaneously, in the late decomposition period, the microorganism population decreased, as did the nutrient requirement, and the nutrients in the microorganisms were released. Consequently, greater nutrient release from the litter occurred in the late decomposition period.

MP exhibited alternating "acceleratory-inhibitory" effects on the release of N and Zn, and inhibited the overall release of these two elements during the decomposition process. On the contrary, MP showed alternating "inhibitory-acceleratory" effects on the release of P, but accelerated its overall release. MP accelerated the release of K, Fe, Mg and Ca in the early and intermediate periods but inhibited the release of these nutrients in the late period. By contrast, MP accelerated the release of Cu and Mn in the early period but inhibited their release in the late period. The acceleratory effects of petroleum on N release in the initial period might be caused by the consumption of litter nutrients by microorganisms as they simultaneously participated in the degradation of the low quality pollutants (high C/N and C/P ratios). A previous study demonstrated that nutrients could be transferred from nutrient-rich to nutrient-poor litter during the decomposition of mixed litter as a result of microbial activity. [33] Similarly, the N from litter may have been rapidly released and used in the petroleum degradation in some stages of decomposition because the C/N ratio of the litter was considerably lower than that of the pollutants (hydrocarbons). The rapid release of K, Cu, Zn, Fe, Mn, Mg and Ca might be caused by the same mechanism. In the late period of decomposition, the main factors that control litter decomposition are the contents of lignin and other recalcitrant materials, and the microorganisms that colonize litter tend to retain nutrients for their physiological requirements and thus cause inhibitory effects on N release. [34] The impact of petroleum on P release were contrary to that of N release, which may have been caused by the differences

in the N and P requirement of the microorganisms. In the early period of decomposition, the microorganisms may have been limited by P supply and thus tended to retain P, followed by an acceleration in P release as they participated in the degradation of petroleum components with a high C/P ratio. [35] In addition, the alteration in the alkaline phosphatase and protease activities in the contaminated soil also affected the N and P release. Our results revealed that protease activity was significantly inhibited in the MP soil at the end of the decomposition process (Fig. 4). Similarly, the alkaline phosphatase activity was increased when P release was stimulated, and vice versa (Fig. 4). The inhibitory effect of petroleum on Cu, Zn and Mn in the late period of decomposition may have been caused by potential absorption by the litter. The litter might absorb the heavy metal elements contained in petroleum, and thus lead to an accumulation of these nutrients. [4]

SP inhibited N release in the intermediate and final periods of decomposition, and exhibited inhibitory-acceleratory -inhibitory effects on P release. SP initially accelerated the release of K, Fe, Mn, Mg and Ca, which was followed by inhibition. In addition, with respect to Cu and Zn release, SP exhibited inhibitory effects after decomposition had occurred for a period of time, followed by acceleratory effects at the end of the decomposition process. As mentioned above, the impacts of petroleum on the release of N and P were controlled by the nutrient requirements in both the litter decomposition and petroleum degradation processes, and the N-, P-related enzyme activities also influenced the release of these nutrients. K, Fe, Mn, Mg and Ca might participate in the microbial degradation of petroleum. In addition, SP significantly inhibited the decomposition of *H. rhamnoides* litter[14], and thus the release of these seven elements was ultimately inhibited. In contrast to our expectations, Cu and Zn release was only transitorily inhibited but significantly accelerated at the end of decomposition. Further research is needed to clarify the mechanisms of these phenomena.

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Environmental impact

Phytoremediation is a promising approach to restore the petroleum contaminated soil. Numerous studies focus on the selection of plants for phytoremediation by determining their germination, growth and physiological tolerance in petroleum contaminated soil. However, few researches took the stability of plants-contaminated soil ecosystem into consideration. Litter decomposition and nutrient release plays an important role in maintaining the stability of ecosystem and these processes might be influenced by petroleum contamination. Thus to investigate the impacts of petroleum contamination on litter decomposition and nutrient release is helpful for the selecting of suitable plant to form stable community for phytoremediation.