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RESEARCH ARTICLE

Dynamics of the biological properties of soil and the nutrient release of *Amorpha fruticosa* L. litter in soil polluted by crude oil

Xiaoxi Zhang[1] · **Zengwen Liu**[2,3] · **Nhu Trung Luc**[1,4] · **Xiao Liang**[1] · **Xiaobo Liu**[5]

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Abstract Litter from *Amorpha fruticosa*, a potential phytoremediating plant, was collected and used in a decomposition experiment that involved the litterbag in soil polluted by crude oil. The dynamics of the biological properties of soil and the nutrient release of the litter were detected. The results indicated that (1) in lightly polluted soil (LP, petroleum concentration was 15 g kg^{-1}), the bacteria (including actinomycetes), and fungi populations were significant higher than those in unpolluted soil (CK) at the 1st month after pollution, and the bacteria (including actinomycetes) populations were higher than those in the CK at the 6th and 12th months. In moderately polluted soil (MP, 30 g kg^{-1}), the bacteria (including actinomycetes) populations were higher than those in the CK at the 1st and 6th months, whereas only the actinomycetes population was greater than that in the CK at the 12th month. In seriously polluted soil (SP, 45 g kg^{-1}), only the fungi population was higher than that in the CK at the 6th month. (2) The activities of soil protease, carboxymethyl cellulase, and sucrase were generally inhibited in polluted soil. Peroxidase activity was generally inhibited in the LP and MP soil, and polyphenol oxidase activity was inhibited in the SP soil at 6–12 months. (3) At the end of litter decomposition, the LP soil significantly increased the release rate of all nutrients, except for K. The MP soil reduced the release rate of Fe and Mn, whereas it increased that of C and Cu. The SP soil decreased the release rate of all nutrients except for Cu and Zn. In conclusion, SP by crude oil would lead to limitations in the release of nutrients from the litter and to decreases in the community stability of a phytoremediating plant. *A. fruticosa* could only be used in phytoremediation of polluted soil at concentrations below 45 g kg^{-1} (crude).

Keywords Crude polluted soil · Enzyme activity · Microbes · Litter · Nutrient release

Responsible editor: Zhihong Xu

✉ Zengwen Liu
zengwenliu2003@aliyun.com

Xiaoxi Zhang
zhangxiaoxi712100@gmail.com

[1] Institute of Soil and Water Conservation, Northwest A&F University, Yangling 712100, Shaanxi, China

[2] College of Natural Resources and Environment, Northwest A&F University, Yangling 712100, Shaanxi, China

[3] Key Laboratory of Plant Nutrition and the Agri-environment in Northwest China, Ministry of Agriculture, Yangling 712100, Shaanxi, China

[4] Department of Agriculture and Rural Development of Lao Cai, Lao Cai City 330100, Vietnam

[5] College of Forestry, Northwest A&F University, Yangling 712100, Shaanxi, China

Introduction

Soil pollution caused by the spilling of crude oil during exploitation and transportation has become a severe environmental problem in recent decades and threatens the ecological safety of producing areas. Crude oil can cause serious disturbances in soil properties, such as blocking soil pores, a decrease in permeability, and alterations of the composition and structure of organic matter (Andrade et al. 2004). Simultaneously, crude oil can cause a remarkable decrease in the availability of soil nutrients by its combination with nitrogen (N) and phosphorus (P) and its inhibitory effects on nitrification and dephosphorylation, which led to obvious imbalances in the soil C/N and C/P ratios (Ogboghodo et al. 2004; Wang et al. 2010). In addition, soil microbes and

enzymes that are directly exposed to crude pollutants are not only directly affected by the toxic effects of the crude but also secondarily stressed by alterations in the physical and chemical properties of the soil. Because the population, activity, and community structure of microbes and soil enzyme activities are highly sensitive to the changes in the environment and because they play key roles in the self-restoration ability of soil, they have been extensively studied. For instance, Kirk et al. (2005) reported that the rhizosphere of *Lolium perenne* can accelerate the growth of petroleum degrading microbes. Liu et al. (2014) investigated the promotional effect of Fire Phoenix grass on soil dehydrogenase and polyphenol oxidase activities and recommended this species as a phytoremediating plant for soil polluted by crude oil. Maliszewska-Kordybach and Smreczak (2003) and Kirk et al. (2005) detected the responses of dehydrogenase and urease activities, soil respiration, and microbial biomass to petroleum components and used them to estimate the degrees of soil contamination. Guo et al. (2012) suggested that the populations and activities of cultivable microbes and soil urease activity can be used as indicators to assess the self-restoration ability of the soil. Similarly, Ma et al. (2014) reported that soil urease, sucrase, and polyphenol oxidase activities can be used in such assessments.

Most of the aforementioned studies focused on the determination of microbial and enzymatic properties in petroleum contaminated soil and analyzing their relations with petroleum degradation. The results were applied to evaluate the environmental effects of crude oil pollution and the effectiveness of restoration technologies, and potential restoration species (including microbes and plants) were subsequently screened based on these evaluations (Andreoni et al. 2004; Blakely et al. 2002; Joshi et al. 2010). However, few studies evaluate the long-term influence of crude oil pollution on ecological processes and ecological functions, such as the nutrient cycle of ecosystems.

Changes in soil properties might result in the destruction of ecological functions of forest/grassland ecosystems and may cause more severe consequences. As mentioned above, the decreases in the microbe population and enzyme activities (Rahn 2012) and the alteration of microbial functional diversity (Thavamani et al. 2012) might hinder litter decomposition and nutrient release processes, which were strongly controlled by microbial and enzymatic properties. These would consequently lead to a slow down or interruption of the nutrient cycle in the plant–soil system and threaten the stability of the ecosystem. Many studies have investigated the adaptability and restoration ability of plants in soil contaminated by petroleum (Bento et al. 2012; Bramley-Alves et al. 2014), though the influences of petroleum on litter decomposition and nutrient release are still unclear. Thus, the long-term availability and stability of phytoremediation needs to be further studied.

A. fruticosa L. is a main shrub species that is distributed in the crude oil-producing area of North Shaanxi, China. It also has been proved as an available phytoremediating species for its benign resistance, enrichment, and translocation abilities for crude oil in polluted soil (Zhang 2013). With the poor nutrient content of the local soil, litter was the main source of nutrients for the plant community. Hence, whether the *A. fruticosa* could form stable communities and have long-term eco-environment benefits depended greatly on the influences of soil pollution on the decomposition of plant litter and nutrient release processes. In this study, the enzyme activities that decompose litter, microbe populations, and nutrient release from the litter of *A. fruticosa* were determined. We aimed to assess the damaging risks of crude oil pollution to the biological properties of soil and the stability of the phytoremediating plant community.

Materials and methods

Sampling

In the late autumn of 2012, ten quadrats (1 m × 1 m size) were established within a wasteland of the Yujiaping Oil Field, Northern Shaanxi, China. Plant litter and other sundries were removed from the ground surface, and a 0–10-cm layer of soil was collected. The soil samples were homogenized after removing the stones and animal/plant debris and were then passed through a 5-mm sieve for storage. Fresh litter of well grown *A. fruticosa* was gathered in the sampling area. The litter that was decayed or infected by diseases or pests was discarded, and the remaining litter was rinsed rapidly and oven dried at 60 °C. The crude samples were bought from the Yujiaping Oil Field.

Preparation of crude oil contaminated soil

Twelve subsamples of the soil samples (2.5 kg dry weight per part) were prepared. Three of them were used as the control (CK). Next, crude oil was added to the remaining samples at concentrations of 15, 30, and 45 g kg^{-1}, respectively. Contaminated soils were then repeatedly kneaded (to avoid their influences on soil properties, no organic solvent was used) and were incubated in place for two days to acquire homogenized slightly polluted (LP, petroleum concentration was 15 g kg^{-1}), moderately polluted (MP, 30 g kg^{-1}), and seriously polluted (SP, 45 g kg^{-1}) media. Each type of medium was prepared for three replications, placed in 40 cm × 30 cm × 20 cm plastic box-type pots, respectively, and was used for the testing of litter decomposition.

Litter decomposition

Sixty samples of litter from *A. fruticosa* with a weight of 5.00 g were prepared and placed into nylon mesh litterbags (size, 14 cm×20 cm; mesh diameter, 0.5 mm). Five of these bags were buried in one pot and uniformly separated from each other (to obtain a sufficient connection with the soil) to form a treatment. Every treatment had three replications (three pots containing the same media, with five litter bags buried in each pot).

Second, distilled water was uniformly added to the soil media to adjust the soil moisture to 50 % of the saturated field water capacity and weighed. Plastic films with four vents (Φ1.5 cm) covered the pots to control excessive evaporation and provide air for the microorganisms. To keep the soil moisture constant, distilled water was added weekly according to the weight loss of each pot. Based on these methods, the litter was incubated for 1 year at room temperature (20–25 °C).

Litterbags were harvested in the 1st, 3rd, 5th, 9th, and 12th months during the decomposition testing (because the litters presented relatively fast decomposition in the early stages, the litterbags were harvested more frequently in the first half-year). When retrieving the litter, one litterbag was harvested from each of the three pots with same treatment, and every litterbag was randomly selected from the five within the same pot. The litter residues were then placed in sieves (mesh size, 0.05 mm) and rinsed rapidly to remove the soil, hypha, and other sundries. Cleaned litter residues were oven dried at 60 °C and accurately weighed.

Determination of soil biological properties

The microbe populations and activities of litter-decomposing enzymes were determined at the 1st, 6th, and 12th months of the decomposition testing. The soil samples used for these determinations were collected from near the litterbags. Simultaneously, the remaining soil near the litterbags was used to cover the litterbags to keep the decomposition medium properties approximately constant.

Microorganism populations were determined using a plate-count technique (bacteria-beef extract peptone agar culture medium, fungi-potato dextrose agar culture medium, actinomyces-GAO 1st synthetic culture medium with 1 % $K_2Cr_2O_7$; Nanjing Institute of Soil Science 1985).

The soil protease activity was determined by ninhydrin colorimetry; urease by indophenol colorimetry; sucrase, amylase, and carboxymethyl cellulose by 3,5-dinitrosalicylic acid colorimetry; β-1,4-glucosidase by nitrophenol colorimetry; xylanase by iodometry; alkaline phosphatase by disodium phenyl phosphate colorimetry; catalase by titrimetry; peroxidase and polyphenol oxidase by pyrogallol colorimetry; and dehydrogenase by triphenyltetrazolium chloride colorimetry (Guan 1986).

Determination of litter nutrient contents

First, undecomposed litter (reserved after sampling) and litter residues were digested using a mixture of H_2SO_4 and H_2O_2. Then, the N concentration of the litter or litter residues was determined using an AA3 continuous flow analytical system (Bran Luebbe, Germany), P was determined by the phosphovanadicmolybdic colorimetric method using a UV-2450 UV–VIS spectrophotometer (Shimadzu Corporation, Japan), and K was determined by a flame photometer (BMB Technologies UK LTD.). Second, the concentrations of micro-elements (Cu, Zn, Fe, Mn, Ca, and Mg) were analyzed by a Z-2000 polarized Zeeman atomic absorption spectrophotometer (Hitachi, Tokyo, Japan) after the samples were ashed and dissolved by 1 mol/L HCl. The nutrient content was calculated by the nutrient concentrations and the dry weights of the litter or residues (Bao 1996).

Data analysis

The IBM SPSS 19.0 software was employed to analyze the significance of the differences between the CK and treatments using the one-way analysis of variance method (the least significant difference method was employed for post hoc analysis), and the level of significance testing was $P<0.05$. The SigmaPlot 12.5 software was used for drafting.

Results

Population dynamics of microbes in soil polluted by crude oil

Soil microorganisms are the most important decomposers of organic debris and control the nutrient release from the litter. The populations of soil cultivable bacteria (including actinomycetes) and fungi were noticeably affected in soil polluted by crude oil during litter decomposition (Fig. 1). The LP soil significantly accelerated the growth of bacteria (including actinomycetes) and fungi at the 1st month after pollution, and it caused an increase in the populations of bacteria and actinomycetes at the 6th and 12th months, respectively. The MP soil significantly accelerated the growth of bacteria (including actinomycetes) at the 1st and 6th months after pollution, but it only promoted the reproduction of actinomycetes at the 12th month. The SP soil only led to a significant increase in the fungi population at the 6th month. However, the promoting effects of crude oil on microbes weakened with the increases in duration. Within the stages in which the microbial populations were significantly affected by crude contamination, the promoting effects of petroleum on bacteria were generally weakened with the increase in the concentration of crude oil in early stages of decomposition, which even changed to non-

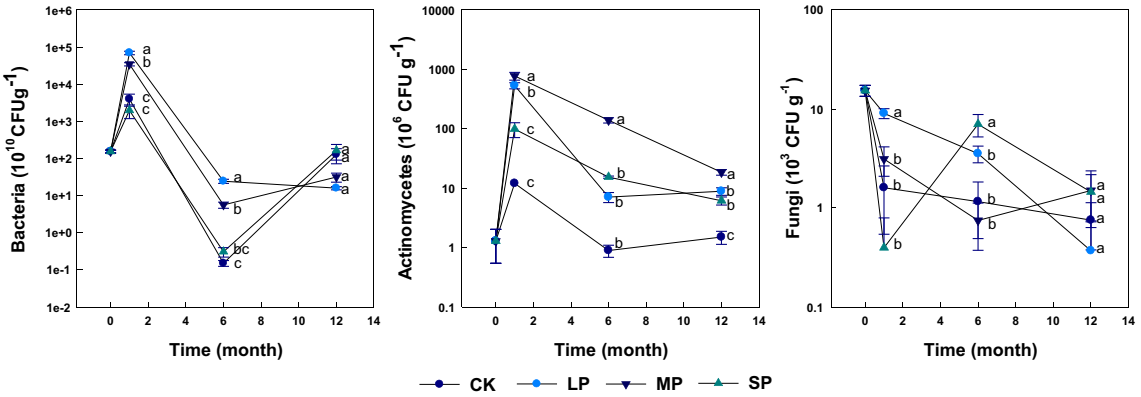

Fig. 1 Dynamics of microbe populations in soil polluted by crude oil. Note: *CK* control, *LP* lightly polluted, *MP* moderately polluted, *SP* seriously polluted. *Different letters in the same column* indicate significant differences between treatments and CK at the 0.05 level, the same below

significant inhibition. Actinomycetes and fungi did not show regular responses to the increase in crude concentration: The MP soil always showed the most significant promoting effects on actinomycetes, whereas the LP and SP soils only showed weaker promotions, and their impacts did not show significant differences in general (except for the early stages of decomposition). For fungi, the LP and SP soils showed significant positive effects at the 1st and 6th months, respectively, whereas the MP soil did not affect it remarkably.

Dynamics of enzyme activity dynamics in soil polluted by crude oil

Soil enzymes play key roles in both litter decomposition and crude oil degradation processes, and they are quite sensitive to environmental changes in the soil. After pollution, the enzymes associated with litter decomposition and nutrient cycling were variably affected (Fig. 2). The activities of protease, sucrase, and alkaline phosphatase were significantly inhibited ($P<0.05$, the same below), whereas those of urease, amylase and xylanase were accelerated in LP soil at the 1st month after pollution. Soil protease, carboxymethyl cellulase, sucrase, and peroxidase activities were inhibited, whereas xylanase activity was promoted at the 6th month after pollution. At the 12th month after pollution, soil protease, carboxymethyl cellulase, sucrase, polyphenol oxidase, and peroxidase activities were significantly decreased, whereas the activities of alkaline phosphatase and catalase were accelerated.

In the MP soil, significant increases in alkaline phosphatase, peroxidase, and dehydrogenase activities were observed at the 1st month, though the amylase activity was the opposite. The soil carboxymethyl cellulase, sucrase, peroxidase, and catalase activities were remarkably inhibited at the 6th month, though the polyphenol oxidase activity was weakened. At the end of pollution (12th month), the protease, urease, carboxymethyl cellulase, sucrase, and xylanase activities were noticeably reduced, and the β-1,4-glucosidase and catalase activities were increased.

Significant reductions in protease and catalase activities and increases in urease, amylase, xylanase, and peroxidase activities were observed at the 1st month in the SP soil. At the 6th month, the soil sucrase, polyphenol oxidase, and carboxymethyl cellulase activities were significantly inhibited. At the end of the pollution duration, protease, carboxymethyl cellulase, sucrase, polyphenol oxidase, and dehydrogenase activities were inhibited, whereas the activities of xylanase, alkaline phosphatase, and catalase were increased.

Compared with microbial populations, the impacts of crude on soil enzyme activities showed unpredictable trends as the crude oil concentration increased. The activities of neither a single enzyme nor the enzyme groups that participated in a particular decomposition stage showed an obvious alteration trend.

Dynamics of litter nutrient release in soil polluted by crude oil

Crude oil pollution represented remarkable and complex impacts on the nutrient release process of *A. fruticosa* litter (Fig. 3). During decomposition, a small dose of crude oil significantly decreased the K release at the 1st month, but accelerated the release of Cu, Zn, and Fe. At the 3rd month, the LP soil hindered the release of C, K, Mn, and Mg, but accelerated the release of P and N. At the 5th month, the release of P, Cu, Zn, and Mg were promoted, and all of the nutrient release rates were remarkably increased at the 9th month ($P<0.05$). After 1 year of decomposition, the LP soil significantly increased the final release rates of all nutrients except for K.

During the decomposition, the MP soil only significantly decreased the release of K and N at the 1st month, but accelerated the release of Zn and Fe. At the 3rd month, the MP soil

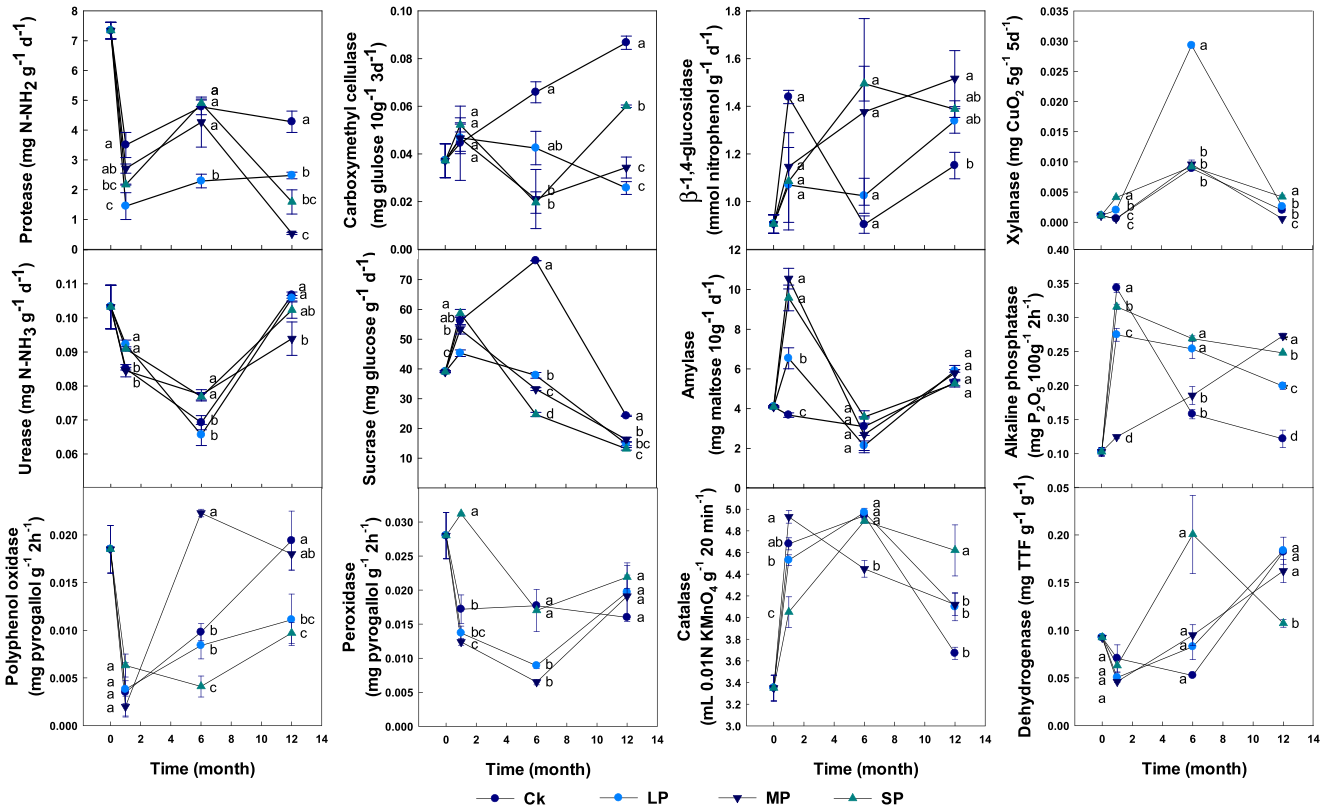

Fig. 2 Dynamics of enzyme activities in soil polluted by crude oil

hindered the release of K, P, and Mg, but accelerated the release of C, N, Cu, Zn, Fe, and Ca. At the 5th month, only the release of Mn was inhibited, whereas the release of P and N was promoted. At the 9th month, significant reductions in the release of K, Fe, and Mn and increases in the release of P, N, Cu, and Zn were observed. However, the MP soil significantly reduced the final release rate of Fe and Mn, while it increased the release rates of C and Cu after 1 year of decomposition.

In the SP soil, crude oil only showed obvious inhibitory effects on the release of K, but it strongly promoted the release of C, Cu, Zn, Fe, and Mn. However, a large dose of crude oil represented extensive and noticeable inhibitions. The release rates of C, K, N, Fe, Mn, and Mg were significantly reduced, though only the release of Cu was promoted in the early period of decomposition (3rd month). At the 5th month, the release rates of C, Fe, Mn and Ca were significantly reduced, but the release rates of P, Zn, and Mg were increased. At the late stage of decomposition (9th month), all of the nutrient releases were remarkably decreased. Generally, the final release rates of all elements except Cu and Zn were significantly decreased at the end of the decomposition process.

The impacts of petroleum contamination on nutrient release were irregular as the petroleum concentration increased in the early stages (1–5 months). However, in the late stages (9–12 months), petroleum showed an obvious trend of changing from a significant acceleration to inhibitory effects on the final litter nutrient release rates: the LP soil primarily showed significant accelerating effects, the MP soil rarely showed significant effects; its integrated effects were not obvious (inhibited two types of nutrient release while promoted another 2 types), and the SP soil significantly reduced the nutrients release rates as a whole.

Discussion and conclusion

Effects of crude oil pollution on the biological properties of soil

Microbes

Many studies have demonstrated that microbial properties are inhibited in soil polluted by crude oil (Eze et al. 2013; Serrano et al. 2009) because they were not only stressed by the toxicity of the crude oil in their direct exposure to pollutants but also indirectly influenced by changes in soil aeration, moisture conditions, nutrient availability, and pH after pollution. However, our results demonstrated that during 1 year of pollution, crude oil did not significantly inhibit microbes. In contrast, it accelerated the reproduction of some or all of the three groups of microorganism at different stages, which was

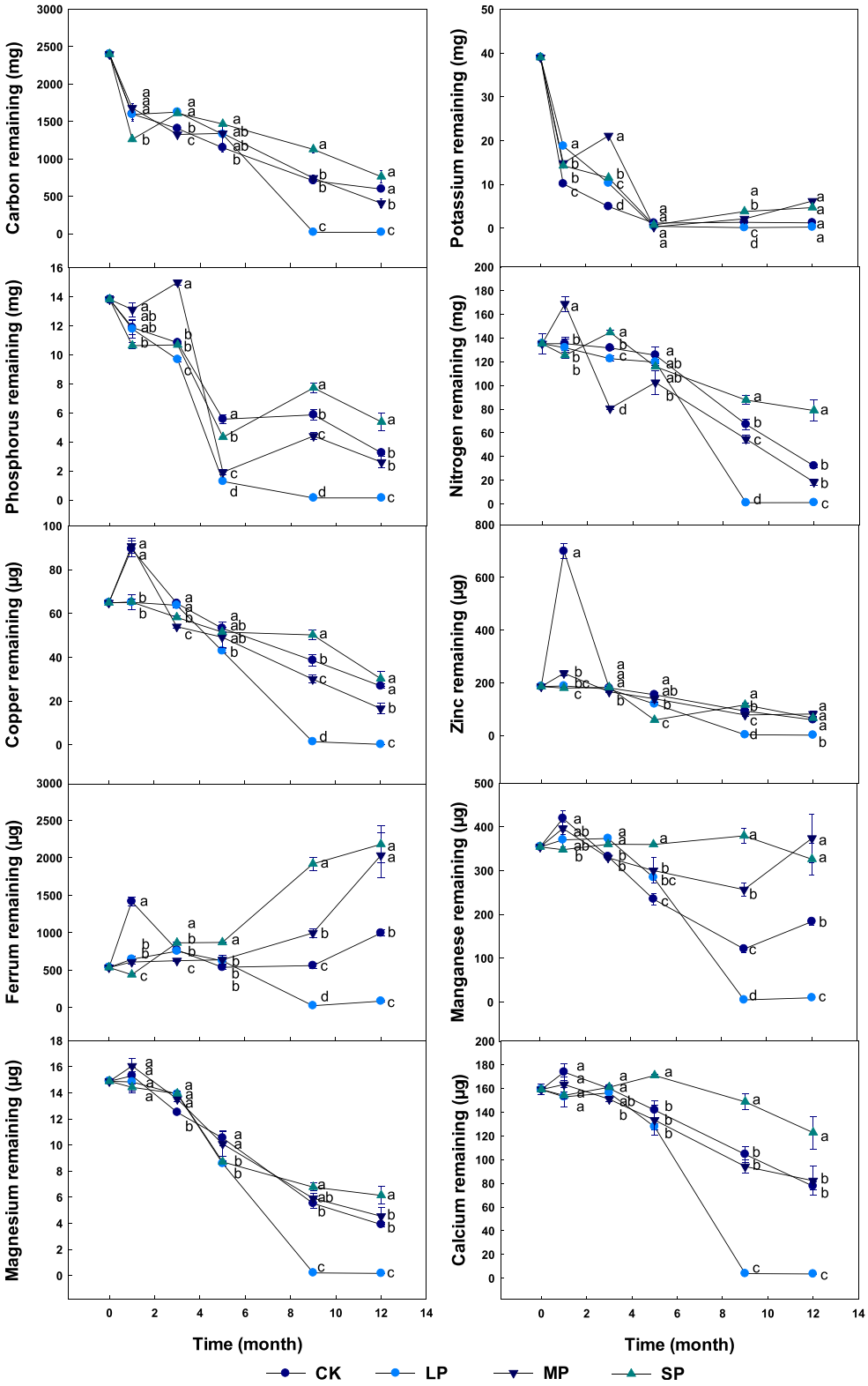

Fig. 3 Nutrient release dynamics during decomposition in soil polluted by crude oil

contrary to the findings of Eze et al. (2013) but agreed with those reported by Franco et al. (2004), Liu et al. (2007), and Joshi et al. (2010). Because microorganisms have benign environmental adaptability, their populations can quickly recover in the short term (Robertson et al. 2010). As an example, McKinley et al. (2003) stated that the energy metabolism can be restored to the former level or higher in only 4–8 h after petroleum contamination. It might be due to the carbon

sources provided by the petroleum contamination. These various carbon sources in large doses can accelerate the growth of microbes, especially petroleum-degrading species (Blakely et al. 2002; Tejada and González 2007; Trevors 2010).

We observed a decrease in the accelerating effects of crude oil on microbes over time. This effect might be caused by the decrease in the easily utilized components in crude oil after long-term degradation (Zhou et al. 2012). For instance, Yu et al. (2015) stated that after being incubated for six months, the concentration of petroleum hydrocarbons in the polluted soil with added leaf litter can be reduced by 34.99–67.57 %. Microbes had difficulty in using the more toxic residues, and the accelerating effects of crude on microbes thus decreased (Pan et al. 2011; Zhou et al. 2012). Our results revealed that only fungi populations increased in the SP soil at the 6th month after pollution, whereas fungi was observed as the main microbial group degrading the recalcitrant, pentacyclic, or polycyclic toxic hydrocarbons (Blakely et al. 2002). Thus, our results proved the mentioned hypothesis.

The accelerating effects of crude oil on bacteria weakened as the crude concentration increased, which is similar to that reported by Eze et al. (2013). As the crude concentration increased, the bio-toxicity also increased. Though it was still within the resistance threshold of the indigenous tolerant microbe groups, crude oil might have affected the speed and viability of reproduction and caused decreases in their populations (Serrano et al. 2009). Simultaneously, a large dose of crude oil can remarkably change the physical and chemical environment of the soil, hinder the oxygen, water, and nutrient acquisition of microbes and thus inhibit their growth (Robertson et al. 2007). However, the responses of the other two microbial groups to the increasing crude concentration were quite different from bacteria. For instance, the MP soil showed the most significant acceleration during the entire incubation period, whereas fungi were only significantly accelerated in the LP and SP soils. This might be caused by the differences in the biological properties among the microbial species (Qasemian et al. 2012a, b). The variable environmental conditions in the LP, MP and SP soils might thus lead to the alteration of dominance in the microbial community and the bloom of some microbe species. Interestingly, the population of microbes at times did not show significant differences among the three types of soil polluted by crude oil, and this phenomenon always occurred in the late incubation stages (12th month for bacteria and fungi). The degradation of pollutants might be responsible for this phenomenon, which indicates that the dynamics of soil microbial properties were simultaneously controlled by the concentration of crude oil and the duration of the pollution.

Enzyme activities

Our results demonstrated that soil enzyme activities were considerably affected by crude oil, but that the influences showed obvious randomness as the crude oil concentration and incubation duration increased. In the three levels of pollution, protease, carboxymethyl cellulase, and sucrase were generally inhibited. Peroxidase was mainly inhibited in the LP and MP soil, and polyphenol oxidase was mainly inhibited in the SP soil in the moderate and late stages. Ma et al. (2014) and Lv et al. (1997) stated that after being exposed to petroleum in the environment, soil urease, protease, sucrase, and dehydrogenase activities were obviously reduced, whereas the polyphenol oxidase activity was increased. Our results were in line with their findings but were in contrast with those of Shen et al. (2005) and Qasemian et al. (2012a, b). Shen et al. (2005) stated that pollutants only show temporary inhibitory effects on soil enzymes, and the enzyme activities can recover after a period of time as the microbial populations and activity recuperate. Qasemian et al. (2012a, b) suggested that petroleum pollutants can increase the activity of the lignocellulolytic enzymes of fungi. However, our results illustrated that crude pollution showed a significant and lasting negative effects on soil enzymes, including lignocellulolytic enzymes (hydrolase and oxidordeuctase). This might be caused by the inhibitory effects of crude oil on the microbes that participate in the cycling of soil nutrients. Crude can cover enzyme–mineral complexes and isolate them from the decomposition substrate (Serrano et al. 2009). In addition, pollutants can change the molecular structure of enzymes and destroy their functions (Wyszkowska and Wyszkowski 2010). Furthermore, alterations to the pH caused by pollution might exceed the optimum range of enzymes and thus inhibit the expression of their activities. In contrast to the aforementioned enzymes, xylanase activity was generally promoted in the LP and MP soil. Amylase activity was generally promoted in all soil, and alkaline phosphatase and catalase activities were generally promoted at the late stages of the pollution duration, which were supported by the investigation of Qasemian et al. (2012a, b). We hypothesized that in the early stages of pollution, microbes might first utilize the easily obtained carbon source from the litter and thus cause an increase in amylase activity. In the late stages, however, P availability sharply decreased, and the strong toxicity of crude oil pollutants caused reactive oxygen damage to microbes. Thus, the microbes were forced to secrete alkaline phosphatase and catalase to obtain P sources and mitigate the damage. Although a few studies have indicated that lignocellulolytic enzymes and oxidordeuctase (such as polyphenol oxidase, peroxidase, catalase, and dehydrogenase) can catalyze the degradation of petroleum, we did not detect obvious increases in these enzymes' activities (except for xylanase). The high crude concentration might be liable for this.

Effects of crude oil pollution on the release of nutrients from the litter

Our results showed that the effects of crude oil on the release of nutrients from *A. fruticosa* litter were variable as the

concentration of crude oil and the duration of decomposition increased from the 1st to the 5th month. This finding indicated that the impacts of crude oil on litter decomposition were not constant because crude oil would noticeably disturb the biological properties of the soil (such as dominant species of microbes and enzyme activities, Serrano et al. 2009; Eze et al. 2013, Rahn 2012). However, in the late stages of decomposition (9–12 months), the impacts of crude oil on the release of nutrients showed an obvious trend. In general, the LP soil showed a significant promoting effect on the release of nutrients as a whole; the MP soil did not noticeably affect the overall release of nutrients; and in the SP soil, crude pollution tended to substantially inhibit the release of nutrients and it hindered almost all nutrient release. A correlation analysis indicated the final release rates of several nutrients had a significant positive relationship with the bacteria and fungi populations (populations detected at the 1st month, Pearson's correlation coefficients ranged from 0.958 to 0.986, $P<0.05$, unpresented data). Thus, the low final nutrient release rates of litter in the MP and SP soil (see Fig. 3) might be partly caused by the lower population of bacteria and fungi at the 1st month of decomposition (see Fig. 1).

To be specific, in LP soil, there was only a low concentration of toxic components, and the growth of microbes was accelerated. However, microbes might tend to directly utilize the nutrients released from litter for their physiological needs but not let the nutrients be released (Schimel and Hattenschwiler 2007). Thus, in the early stages of decomposition, crude oil pollution only led to an increase in microelements (which was relatively not heavy demanded) but did not obviously influence the release of macro elements. At the moderate or late stages of decomposition, the available carbon source was lacking (a small dose of crude oil was degraded and litter decomposition was approximately completed), the population of microbes was reduced sharply, and the nutrients that were immobilized in the microbes that colonized the litter were released. Consequently, the nutrient release in the moderate and late stages was relatively promoted. However, these accelerating effects would be weakened as the crude oil concentration increased. Compared with a small dose of crude oil, a large dose showed lasting heavy toxicity and environmental stresses (or screening effects) to microbes and was more likely to cause a decrease in microbial activity and the alteration of microbial community structure (Serrano et al. 2009). For instance, Thavamani et al. (2012) stated that the microbes mainly utilizing hydrocarbons become the main species in petroleum contaminated soil. In addition, our results revealed that many types of litter-decomposing enzyme activities were considerably inhibited in the SP soil, such as sucrase (mainly participate in moderate stage of decomposition), protease (moderate and late stages), and carboxymethyl cellulase, dehydrogenase, and polyphenol oxidase (late stage). These might be responsible for the inhibitions that occurred at the moderate and late stages of the release of litter nutrients. Certainly, there were also some types of enzymes whose activities were significantly accelerated. However, in these enzymes, oxidordeuctase, e.g., polyphenol oxidase and catalase, participates in both the decomposition of litter and the degradation of crude oil (Berg and McClaugherty 2014; Qasemian et al. 2011), and thus, the increases in their activities did not inevitably mean that the decomposition of the recalcitrant portion of the litter could be promoted, and it could just be a result caused by the induction of crude oil pollutants (Cajthaml et al. 2008; Liu et al. 2014; Ge et al. 2013). In addition, the decomposition and nutrient release were controlled by a variety of enzymes and microorganisms, whereas crude pollution usually showed inhibitory and promoting effects on them. These enzymes could participate in the same litter decomposition stages. For example, carboxymethyl cellulase, polyphenol oxidase, and dehydrogenase activities were significantly reduced in the late stage in the SP soil, whereas xylanase and catalase activities were simultaneously accelerated. Hence, the final effect of crude oil pollution was due to the affected degrees of a variety of enzymes. Furthermore, microbes need large amounts of N, P, and other nutrients from the litter for degradation of crude oil pollutants which had high C/N and C/P ratios, and thus, would secrete a large number of enzymes related to nutrient cycling and promote their activities (e.g., alkaline phosphatase activity was increased in the late stage of decomposition). However, the nutrients could be directly utilized by the microbes colonizing the litter but not be released. Based on the aforementioned facts, increases in the activity of a few types of enzyme might only show quite limited promoting effects on processes of litter nutrient release.

References

Andrade M, Covelo E, Vega F, Marcet P (2004) Effect of the prestige oil spill on salt marsh soils on the coast of Galicia (northwestern Spain). J Environ Qual 33:2103–2110

Andreoni V, Cavalca L, Rao MA, Nocerino G, Bernasconi S, Dell'Amico E, Colombo M, Gianfreda L (2004) Bacterial communities and enzyme activities of PAHs polluted soils. Chemosphere 57:401–412

Bao S (1996) Soil agro-chemistrical analysis. China Agriculture Press, Beijing **(in Chinese)**

Bento RA, Saggin-Júnior OJ, Pitard RM, Straliotto R, Da Silva EMR, de Lucena Tavares SR, Volpon AGT (2012) Selection of leguminous trees associated with symbiont microorganisms for phytoremediation of petroleum-contaminated soil. Water Air Soil Pollut 223:5659–5671

Berg B, McClaugherty C (2014) Plant litter decomposition, humus formation, carbon sequestration. Springer, Berlin

Blakely J, Neher D, Spongberg A (2002) Soil invertebrate and microbial communities, and decomposition as indicators of polycyclic aromatic hydrocarbon contamination. Appl Soil Ecol 21:71–88

Bramley-Alves J, Wasley J, King CK, Powell S, Robinson SA (2014) Phytoremediation of hydrocarbon contaminants in subantarctic soils: an effective management option. J Environ Manag 142:60–69

Cajthaml T, Erbanová P, Kollmann A, Novotný Č, Šašek V, Mougin C (2008) Degradation of PAHs by ligninolytic enzymes of Irpex lacteus. Folia Microbiol 53:289–294

Eze CN, Ogbonna JC, Anyanwu CU, Eze EA (2013) Determination of the relative abundance and distribution of bacteria and fungi in Bonny light crude oil-contaminated sandy loam soil. Sci Res Essays 8:375–381

Franco I, Contin M, Bragato G, De Nobili M (2004) Microbiological resilience of soils contaminated with crude oil. Geoderma 121:17–30

Ge GF, Zheng B, Wang J, Gao HJ (2013) Effect of phenanthrene pollution with one-time and cumulative patterns on soil enzymatic activity. J Nucl Agric Sci 27:1560–1566 **(in Chinese)**

Guan SY (1986) Soil enzyme and research technology. Agriculture Press, Beijing **(in Chinese)**

Guo H, Yao J, Cai M, Qian Y, Guo Y, Richnow HH, Blake RE, Doni S, Ceccanti B (2012) Effects of petroleum contamination on soil microbial numbers, metabolic activity and urease activity. Chemosphere 87:1273–1280

Joshi SR, Kumar R, Saikia P, Bhagobaty RK, Thokchom S (2010) Impact of roadside pollution on microbial activities in sub-tropical forest soil of North East India. Res J Environ Sci 4:280–287

Kirk JL, Klironomos JN, Lee H (2005) The effects of perennial ryegrass and alfalfa on microbial abundance and diversity in petroleum contaminated soil. Environ Pollut 133:455–465

Liu WX, Luo YM, Teng Y, Li ZG, Wu LH (2007) Eco-risk assessment and bioremediation of petroleum contaminated soil. II. Changes in physico-chemical properties and microbial ecology of petroleum contaminated soil. Acta Pedologica Sin 44:848–853 **(in Chinese)**

Liu R, Xiao N, Wei S, Zhao L, An J (2014) Rhizosphere effects of PAH-contaminated soil phytoremediation using a special plant named Fire Phoenix. Sci Total Environ 473:350–358

Lv GF, Zhao J, Zhao L, Liao YN (1997) Primary study on the evaluation of raw oil pollution using soil enzyme activities in typical steppe region. Acta Sci Nat Univ NeiMongol 28:687–691 **(in Chinese)**

Ma J, Shen J, Liu Q, Fang F, Cai H, Guo C (2014) Risk assessment of petroleum-contaminated soil using soil enzyme activities and genotoxicity to Vicia faba. Ecotoxicology 23:665–673

Maliszewska-Kordybach B, Smreczak B (2003) Habitat function of agricultural soils as affected by heavy metals and polycyclic aromatic hydrocarbons contamination. Environ Int 28:719–728

McKinley VL, Federle TW, Vestal JR (2003) Effects of petroleum hydrocarbons on plant litter microbiota in an arctic lake. Appl Environ Microbial 43:129–135

Nanjing Institute of Soil Science CAS (1985) Analysis of soil microorganism. Science Press, Beijing **(in Chinese)**

Ogboghodo I, Iruaga E, Osemwota I, Chokor J (2004) An assessment of the effects of crude oil pollution on soil properties, germination and growth of maize (Zea mays) using two crude types-Forcados light and Escravos light. Environ Monit Assess 96:143152

Pan F, Geng QJ, Chu HJ, Wang LL (2011) Analysis of polycyclic aromatic hydrocarbons in petroleum contaminated soils and its ecological risk assessment. J Ecol Rural Environ 27:42–47 **(in Chinese)**

Qasemian L, Guiral D, Belghazi M, Ferre E, Gros R, Farnet A (2011) Identification of various laccases induced by anthracene and contribution to its degradation in a Mediterranean coastal pine litter. Chemosphere 84:1321–1328

Qasemian L, Guiral D, Ziarelli F, Ruaudel F, Farnet A (2012a) Does anthracene affect microbial activities and organic matter decomposition? A comparative study in *Pinus halepensis* litters from Mediterranean coastal and inland areas. Chemosphere 89:548–555

Qasemian L, Guiral D, Ziarelli F, Van Dang TK, Farnet A (2012b) Effects of anthracene on microbial activities and organic matter decomposition in a *Pinus halepensis* litter from a Mediterranean coastal area. Soil Biol Biochem 46:148–154

Rahn JH (2012) A test method for the evaluation of soil microbial health in a petroleum hydrocarbon contaminated boreal forest soil. Dissertation, The University of Guelph

Robertson SJ, McGill WB, Massicotte HB, Rutherford PM (2007) Petroleum hydrocarbon contamination in boreal forest soils: a mycorrhizal ecosystems perspective. Biol Rev Camb Philos Soc 82: 213–240

Robertson SJ, Kennedy NM, Massicotte HB, Rutherford PM (2010) Enhanced biodegradation of petroleum hydrocarbons in the mycorrhizosphere of sub-boreal forest soils. Environ Microbiol Rep 2:587–593

Schimel J, Hattenschwiler S (2007) Nitrogen transfer between decomposing leaves of different N status. Soil Biol Biochem 39: 1428–1436

Serrano A, Tejada M, Gallego M, Gonzalez JL (2009) Evaluation of soil biological activity after a diesel fuel spill. Sci Total Environ 407: 4056–4061

Shen GQ, Lu YT, Zhou QX, Hong JB (2005) Interaction of polycyclic aromatic hydrocarbons and heavy metals on soil enzyme. Chemosphere 61:1175–1182

Tejada M, González J (2007) Application of different organic wastes on soil properties and wheat yield. Agron J 99:1597–1606

Thavamani P, Malik S, Beer M, Megharaj M, Naidu R (2012) Microbial activity and diversity in long-term mixed contaminated soils with respect to polyaromatic hydrocarbons and heavy metals. J Environ Manag 99:10–17

Trevors JA (2010) Microbial genomics, DNA, and pollution: what's next? Water Air Soil Pollut 205:119

Wang XY, Feng J, Zhao JM (2010) Effects of crude oil residuals on soil chemical properties in oil sites, Momoge Wetland, China. Environ Monit Assess 161:271–280

Wyszkowska J, Wyszkowski M (2010) Activity of soil dehydrogenases, urease, and acid and alkaline phosphatases in soil polluted with petroleum. J Toxicol Environ Health 73:1202–1210

Yu Q, Zhang XX, Liu ZW, Wang WX, Zhang ZY, Wang N (2015) Remediation effects of urban greening-tree litters on petroleum-contaminated soil in oil producing region of northern Shaanxi. J Agro-Environ Sci 34:50–57 **(in Chinese)**

Zhang LJ (2013) The selection of tree and grass species to tolerate soil contamination in the Loess Plateau in Northern Shaanxi. Dissertation, Northwest A&F University (in Chinese)

Zhou F, Bai J, Li YY (2012) Relationship of microbial activity and petroleum pollution of Liaohe river delta reed wetland. Trans Oceanol Limnol 35:159–166 **(in Chinese)**