

# Effect of petroleum on decomposition of shrub-grass litters in soil in Northern Shaanxi of China

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### ABSTRACT

The impacts of petroleum contamination on the litter decomposition of shrub-grass land would directly influence nutrient cycling, and the stability and function of ecosystem. Ten common shrub and grass species from Yujiaping oil deposits were studied. Litters from these species were placed into litterbags and buried in petroleum-contaminated soil with 3 levels of contamination (slight, moderate and serious pollution with petroleum concentrations of 15, 30 and 45 g/kg, respectively). A decomposition experiment was then conducted in the lab to investigate the impacts of petroleum contamination on litter decomposition rates. Slight pollution did not inhibit the decomposition of any litters and significantly promoted the litter decomposition of Hippophae rhamnoides, Caragana korshinskii, Amorpha fruticosa, Ziziphus jujuba var. spinosa, Periploca sepium, Medicago sativa and Bothriochloa ischaemum. Moderate pollution significantly inhibited litter decomposition of M. sativa, Coronilla varia, Artemisia vestita and Trrifolium repens and significantly promoted the litter decomposition of C. korshinskii, Z. jujuba var. spinosa and P. sepium. Serious pollution significantly inhibited the litter decomposition of H. rhamnoides, A. fruticosa, B. ischaemum and A. vestita and significantly promoted the litter decomposition of Z. jujuba var. spinosa, P. sepium and M. sativa. In addition, the impacts of petroleum contamination did not exhibit a uniform increase or decrease as petroleum concentration increased. Inhibitory effects of petroleum on litter decomposition may hinder the substance cycling and result in the degradation of plant communities in contaminated areas.

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## Introduction

The leakage of petroleum during its exploitation, transportation and storage can cause serious environmental disruption (Eze et al., 2013). The hilly gullied area of Northern Shaanxi is a key source of petroleum energy resources for China. The fragile ecosystem there – especially the shrub–grass land ecosystem – has been directly threatened by petroleum

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contamination. Petroleum contamination influences not only nutrient cycling but also the stability and function of the ecosystem. Among existing methods for the restoration of petroleum contamination, phytoremediation has become a favored method because of its low cost, low levels of secondary pollution, large biomass and improvements for the eco-environment. Numerous studies focus on selecting plants for phytoremediation by determining their germination, growth, and tolerance of physiological activities in petroleum contaminated soil. The restoration effects of living plants or their litter on soil biological and chemical properties, and their ability to remove petroleum hydrocarbons have also been widely investigated for selecting suitable plants (Brandt et al., 2006; Ghazisaeedi et al., 2014; Cui et al., 2014; Xu et al., 2013; Pérez-Hernández et al., 2013; Wei and Pan, 2010). However, little research has considered the stability of plant communities. If plants are not able to form stable communities in the long term in contaminated soil and need to be replanted repeatedly, the cost of restoration would increase sharply, making management a more difficult enterprise. Thus, investigating the stability of the plant-contaminated soil ecosystem is a necessity.

Litters are important sources of nutrients for grass-shrub land, and their rate of decomposition and release of nutrients control substance cycling. Consequently, litter plays an important role in maintaining the stability of ecosystems (Hättenschwiler and Jørgensen, 2010). In any given climate conditions, soil biological and chemical properties are the most important factors affecting litter decomposition aside from the substrate quality of litter (such as contents of N, P and recalcitrant matters, Liu et al., 2010). Numerous studies have demonstrated that petroleum contamination can cause significant alteration of soil stoichiometric ratios and microflora. The activities of soil enzymes can be either inhibited or accelerated (Andreoni et al., 2004; Rahn, 2012; Abed et al., 2015). These alterations influence litter decomposition and nutrient release rates. Grasses and shrubs are the most adaptable plants in the hilly gullied area with its dry climate and poor soil fertility. Previous studies have indicated that Hippophae rhamnoides, Artemisia vestita and Medicago sativa grow well in contaminated soil, and they can significantly decrease the petroleum concentration in soil (Zhang, 2013). The rhizosphere effect of Trrifolium repens accelerates the removal of persistent organic pollutants, such as benzopyrene (Xu et al., 2009). However, impacts of petroleum contamination on the decomposition of litter in these plants have not been studied.

Therefore, considering that grass–shrub lands have been seriously contaminated by petroleum in the hilly gullied area, 10 grass or shrub species commonly used for phytoremediation were chosen as objects. Their litters were collected and buried into petroleum contaminated soil with 3 levels of contamination degrees (slight, moderate and serious pollution, the petroleum concentration were 15, 30 and 45 g/kg, respectively) for simulating decomposition testing. A double-exponential model was used to accurately simulate decomposition processes. In addition, the impacts of soil petroleum contamination on litter decomposition rates were assessed. The results may provide a scientific basis for selecting plants used for phytoremediation.

### 1. Materials and methods

#### 1.1. Study region

The study region is located in the Yujiaping oil deposits, Zichang County in Northern Shaanxi, China. This region belongs to the hilly gullied area of the Loess Plateau. Its altitude is 930–1562 m and the climate of this location is classified as a warm temperate semiarid territoriality monsoon climate, with an annual average precipitation of 514.7 mm. It also has an annual average evaporation of 1573.4 mm and a non-frost period of 175 days. The soil here is classified as Ustochnept soil (USDA Soil Taxonomy system). The common vegetation here is grass and shrub land.

# 1.2. Collection and treatment of soil and grass and shrub litter samples

In late autumn of 2012, a typical wasteland close to oil deposits in the study region was chosen. Twenty quadrats with a size of 1 m  $\times$  1 m were randomly established within it. After removing weeds and other sundries from the ground, the surface soil from the 0–10 cm layer was gathered. Soil samples were mixed uniformly after the stones, roots and other debris of plants and animals were removed. Homogenized fresh soil was passed through a sieve (pore size was 5 mm) to reserve.

Ten types of well grown grass and shrub species were chosen for study: H. rhamnoides, Caragana korshinskii, Amorpha fruticosa, Ziziphus jujuba var. spinosa, Periploca sepium, M. sativa, Coronilla varia, Bothriochloa ischaemum, A. vestita, and T. repens. Current-year litters (dead stands of grass or foliar litter of shrubs) from these plants were collected and the litters which decayed or were infected by diseases and insect pests were removed. Selected litters were washed quickly and oven dried at 65 °C to a constant weight.

Petroleum used for testing was purchased from local oil producing wells.

#### 1.3. Preparation of petroleum contaminated soil

A total of 120 fresh soil samples with a dry weight of 2.5 kg were prepared. Thirty of them were not treated with petroleum to serve as control samples. According to the range of soil petroleum concentration (12.493–45.370 g/kg) in this region, petroleum was added into the remaining 90 parts of soil samples with 3 contamination degrees: slight pollution (LP, the ratio of petroleum to dry soil was 15 g/kg, the same below), moderate pollution (MP, 30 g/kg), and serious pollution (SP, 45 g/kg). Each type of contaminated soil was divided into 30 parts. Soil samples with petroleum were kneaded repeatedly and stationary cultured for 2 days to obtain homogenized contaminated samples. The control samples were treated with the same methods. Prepared soil samples were placed into 40 cm  $\times$  30 cm  $\times$  20 cm plastic cultivation pots and used as a decomposition medium.

#### 1.4. Litter decomposition experiments

First, 5.00 g of litter from each plant was weighed and placed into nylon mesh litterbags (the size was 14 cm  $\times$  20 cm, and

the mesh size was 0.5 mm) and then sealed. A total of 60 bags of each type of litter were prepared. Five litterbags containing the same litter were buried into soil medium, canted at 45°, and uniformly spaced to keep sufficient contact with the soil. Treatments of the same litter and degree of contamination were repeated 3 times (that is, 3 pots and 5 litterbags within

Second, distilled water was uniformly added into the soil medium to adjust the soil moisture to 50% of the saturated field water capacity. Plastic firms with 4 air vents (the size was approximately 1 cm<sup>2</sup>, to provide air for the respiration of microorganisms) were used as covers for each pot to prevent excessive evaporation. During the experimental period, each pot was weighed weekly, and distilled water was added according to the amount of weight lost to maintain soil moisture constant. Shrub foliar litters were incubated for 1 year at constant moisture at 20–25 °C, and grass dead standings were incubated for six months.

As there was only little alteration of litter weights at the later stages of decomposition, during the incubation period, shrub foliar litters were harvested after decaying for 1, 3, 5, 9 and 12 months. Meanwhile grass dead standings were harvested on the 1st, 2nd, 3rd, 4th and 6th months. When harvesting litterbags, 3 litterbags were retrieved from 3 pots (i.e., 1 litterbag per pot) with the same treatment, and each litterbag was retrieved randomly from the 5 litterbags within the same pot. Harvested litter residues were placed in 0.25 mm soil sieves, and then rinsed rapidly to remove sundries such as soil and mycelium. Cleaned litters were oven dried at 60 °C to a constant weight.

#### 1.5. Determining the initial substrate quality of the litter

Initial litter C content was measured using titrimetry with  $K_2Cr_2O_7$ -FeSO<sub>4</sub>. After the samples were digested by  $H_2SO_4$ - $H_2O_2$ , N content was measured using a continuous flow analytical system (Auto Analyzer3, Bran Luebbe, Germany). P content was measured by the phosphovanadicmolybdic colorimetric method using a UV-Vis spectrophotometer (UV-2450 Shimadzu Corporation, Kyoto, Japan), and K content was measured by a flame photometer (BMB Technologies UK Ltd., Halstead, Essex, United Kingdom) (Jones, 2001). Initial polyphenol content was measured using the folin-phenol colorimetric method with a UV-Vis spectrophotometer. Lignin content was measured using the acidic detergent method (Raiskila et al., 2006).

#### 1.6. Data analysis

each pot).

Indicators of initial chemical composition of litter were analyzed with SPSS 19.0 using an integrated principal component analysis (IPCA). The integrated principal component value *F* obtained by IPCA was used as an indicator for the resistance of litter to decomposition, that is, poor substrate quality (indicators of accelerating decomposition, such as N and P contents, were transformed for analysis; hence, greater *F* values demonstrate poorer substrate quality).

Litter residues harvested from the same pot (5 times) were weighed. Their weights were then converted into residual ratios. Each set of ratios was sent to Origin Pro 8.50 software and fitted by the model (Eq. (1)) proposed by Berg and McClaugherty (2014). The turnover period (defined as the time when the litter residual ratio was 5%,  $T_{0.95}$ ) and the half-life period (defined as the time when the litter residual ratio was 50%,  $T_{0.50}$ ) of decomposition were also calculated using Origin Pro 8.50 software.

$$R = a e^{-k_1 t} + b e^{-k_2 t} \tag{1}$$

where, R is the litter residual ratio, while a, b,  $k_1$  and  $k_2$  are the parameters of the model, and t is the duration of decomposition (year).

The turnover periods (or half-life periods) of decomposition for litters under different contamination degree treatments were submitted to SPSS 19.0 software for a one-way analysis of variance (ANOVA). The least significant difference (LSD) method was employed for multiple comparison analysis ( $\alpha = 0.05$ ).

### 2. Results

#### 2.1. Initial litter quality of grass and shrub species

In the given environmental conditions, initial substrate quality was the primary factor controlling litter decomposition. Low contents of N and P, high contests of polyphenols and lignin and high ratios of lignin/N, C/N and C/P always exhibited inhibitory effects on litter decomposition (Ball et al., 2008, 2009; Gnankambary et al., 2008; Hättenschwiler and Jørgensen, 2010). Besides C content, the other indicators of substrate quality previously mentioned were significantly different among the 10 types of tested litters (Table 1). The integrated principle component value F of the substrate quality of litters indicated that the litter of H. rhamnoides, Z. jujuba var. spinosa, P. sepium, B. ischaemum, C. korshinskii and A. fruticosa had relative poor initial substrate quality. These litters usually had some, or all of the characteristics, including high contents of polyphenols and lignin and high ratios of lignin/N, C/N and C/P, and low content of N and P. Among them, the litter of H. rhamnoides had the highest levels of polyphenols and lignin (31.59 and 275.77 g/kg, respectively) and a high lignin/N ratio (10.51), but the lowest P content (1.62 g/kg). The litter of Z. jujuba var. spinosa had the highest C/P ratio (283.16), a high polyphenol content and C/N ratio (25.13 g/kg and 20.37, respectively) and low contents of N and P (23.44 and 1.69 g/kg, respectively). The litter of P. sepium had the highest polyphenol content (29.86 g/kg), high ratios of lignin/N, C/N and C/P (11.08, 23.14 and 227.60, respectively) and low contents of N and P (18.60 and 1.89 g/kg, respectively). The litter of B. ischaemum had the highest lignin content (283.13 g/kg) and ratios of lignin/N and C/N (19.67 and 31.81, respectively) and the lowest N content (14.40 g/kg). The litter of C. korshinskii had the highest lignin content (286.20 g/kg). The litter of A. fruticosa had the highest lignin content and a high lignin/N ratio (276.93 g/kg and 10.33, respectively).

Litter of M. sativa, C. varia, A. vestita and T. repens had benign initial substrate quality. All of these litters had the highest P content, high N content, low polyphenol and lignin contents, and low ratios of lignin/N, C/N and C/P. Among these species, the litter of M. sativa had the highest N content

Table 1 – Initial litter quality of 10 grass and shrub species.	quality of 10 gras	ss and shrub spe	cies.						
Litter species	C (g/kg)	N (g/kg)	P (g/kg)	Polyphenols (g/kg)	Lignin (g/kg)	Lignin/N	C/N	C/P	F
H. rhamnoides	482.24 ± 20.48a	26.24 ± 0.08d	$1.62 \pm 0.01d$	31.59 ± 0.02a	275.77 ± 2.51a	$10.51 \pm 0.12b$	18.38 ± 0.75 cd	297.51 ± 11.49a	1.7878
C. korshinskii	480.06 ± 7.16a	$32.89 \pm 0.13b$	$2.35 \pm 0.01c$	$12.92 \pm 0.16d$	286.20 ± 5.07a	8.70 ± 0.13c	14.59 ± 0.16ef	203.93 ± 3.55c	0.3269
A. fruticosa	479.49 ± 9.81a	$27.04 \pm 1.73d$	$2.76 \pm 0.04b$	$11.95 \pm 0.48 de$	276.93 ± 2.92a	$10.33 \pm 0.73b$	$17.87 \pm 1.11d$	$173.53 \pm 4.07d$	0.0817
Z. jujuba var. spinosa	477.38 ± 7.84a	23.44 ± 0.45e	$1.69 \pm 0.04d$	$25.13 \pm 0.17b$	193.83 ± 5.22c	8.27 ± 0.22c	20.37 ± 0.23c	283.16 ± 4.34a	1.1785
P. sepium	430.20 ± 16.20a	$18.60 \pm 0.23f$	$1.89 \pm 0.02 d$	$29.86 \pm 0.11a$	$206.00 \pm 2.55c$	$11.08 \pm 0.13b$	$23.14 \pm 0.94b$	$227.60 \pm 9.26b$	0.5741
M. sativa	492.02 ± 43.63a	40.25 ± 0.71a	3.43 ± 0.10a	$10.24 \pm 0.05e$	$235.13 \pm 5.43b$	$5.84 \pm 0.03d$	12.20 ± 0.87 fg	143.18 ± 8.79e	-0.5787
C. varia	453.58 ± 22.33a	39.40 ± 0.36a	3.20 ± 0.22a	9.89 ± 0.42ef	102.97 ± 2.09e	2.61 ± 0.07e	11.52 ± 0.67 g	$142.14 \pm 3.11e$	-1.4640
B. ischaemum	457.97 ± 4.31a	14.40 ± 0.13 g	$2.66 \pm 0.05b$	$7.75 \pm 0.16f$	283.13 ± 3.12a	19.67 ± 0.28a	$31.81 \pm 0.43a$	$172.39 \pm 3.93d$	0.4747
A. vestita	441.11 ± 3.00a	$26.25 \pm 0.15d$	3.16 ± 0.13a	$22.26 \pm 2.48c$	49.90 ± 0.75f	1.90 ± 0.03e	16.80 ± 0.03de	$140.01 \pm 4.75e$	-1.4234
T. repens	468.83 ± 49.49a	30.73 ± 0.58c	3.39 ± 0.13a	$13.72 \pm 0.53d$	$157.87 \pm 7.65d$	5.15 ± 0.32d	$15.26 \pm 1.61e$	138.61 ± 14.90e	-0.9575
Data are presented as average $\pm$ SE, different letters after the data in the	verage ± SE, differen	it letters after the d:		same column indicate significant differences, $\alpha = 0.05$ .	differences, $\alpha = 0.05$ .				
F is the Integrated principal component value of the substrate quality of	ripal component valı	ue of the substrate o	quality of litters.						

(40.25 g/kg) and the lowest ratios of C/N and C/P (12.20 and 143.18, respectively). The litter of C. varia had the highest N content (39.40 g/kg), low lignin content (102.97 g/kg), the lowest polyphenol content (9.89 g/kg) and low ratios of lignin/N, C/N and C/P (2.61, 11.52 and 142.14, respectively). The litter of A. vestita had the lowest lignin content (16.80 g/kg) and lignin/N ratio (1.90) and a low C/P ratio (140.01). The litter of T. repens had low lignin content (157.87 g/kg), and the lowest C/P ratio (138.16).

# 2.2. Impacts of soil petroleum contamination on the decomposition rate of litters

The results of the decomposition experiment (Table 2) indicated that litter from *P. sepium* decomposed the most slowly, with a turnover period of 8.9515 years (which was significantly slower than other litters), followed by litters from *C. korshinskii*, *M. sativa*, *Z. jujuba* var. spinosa, *A. fruticosa*, *H. rhamnoides*, *B. ischaemum* and *T. repens*. The decomposition of litters from *A. vestita* and *C. varia* was the fastest, with turnover periods of only 1.1205 and 0.9636 years, respectively.

The decomposition turnover period  $(T_{0.95})$  and half-life period (T<sub>0.50</sub>) of litters were obviously altered after being contaminated by petroleum (Table 2). Slight pollution significantly (P < 0.05) shortened the decomposition  $T_{0.95}$  of litters from H. rhamnoides, C. korshinskii, A. fruticosa, Z. jujuba var. spinosa, P. sepium, M. sativa and B. ischaemum by 42.39%-89.72%. However, the  $T_{0.50}$  of the former 6 types of litter were not influenced, while that of litter of B. ischaemum was significantly (P < 0.05) shortened by 94.20%. These data indicated that a small amount of petroleum mainly accelerated the later decomposition of these 6 litter species, and also may accelerate the previous decomposition of the litter from B. ischaemum. Slight pollution did not significantly impact the T<sub>0.95</sub> of litters from C. varia, A. vestita and T. repens, whereas the  $T_{0.50}$  of C. varia was significantly (P < 0.05) shortened by 81.77%. This indicated that the small amount of petroleum accelerated the previous decomposition but inhibited the decomposition of litter from C. varia in the later stages. Meanwhile, the previous and later decomposition of A. vestita and T. repens were both unaffected by the slightly contaminated soil.

In moderately contaminated soil, the  $T_{0.95}$  of litters from C. korshinskii, Z. jujuba var. spinosa and P. sepium were significantly (P < 0.05) shortened by 51.09%–83.90%. The  $T_{\rm 0.50}$  of litters from C. korshinskii and P. sepium were not altered. On the other hand, the  $T_{0.50}$  of litters from Z. jujuba var. spinosa were significantly extended by 99.02% (P < 0.05), which indicated that a moderate amount of petroleum might accelerate the later decomposition of H. rhamnoides, A. fruticosa and B. ischaemum. The  $T_{0.95}$  of litters from H. rhamnoides, A. fruticosa and B. is chaemum changed a little. The  $T_{0.50}$  of litter from B. ischaemum was significantly extended by 36.02% (P < 0.05), which indicated that a moderate amount of petroleum might inhibit the previous decomposition but accelerate the later decomposition of this litter species. Moderate pollution significantly inhibited the decomposition of litters from M. sativa, C. varia, A. vestita and T. repens, but extended their  $T_{0.95}$  by 44.72%–516.04% (P < 0.05). Among these litters, the  $T_{0.50}$  of litter from M. sativa was not altered, whereas that of the other 3 litter

# Table 2 – Decomposition turnover period ( $T_{0.95}$ ) and half-life period ( $T_{0.50}$ ) of litter decomposition under different soil petroleum contamination degrees.

Litter species	Contamination degrees	Model of decomposition	R <sup>2</sup>	T <sub>0.95</sub> (year)	T <sub>0.50</sub> (year)
H. rhamnoides	СК	$R = 0.324e^{-20.02t} + 0.676e^{-1.01t}$	0.9626**	2.2027 ± 0.1242bCD	0.2913 ± 0.0357b
	LP	$R = 0.472e^{-2.31t} + 0.472e^{-2.32t}$	0.8771*	1.2689 ± 0.0180c	0.2748 ± 0.0076b
	MP	$R = 0.723e^{-1.22t} + 0.278e^{-20982.54t}$	0.9639**	2.2310 ± 0.1239b	0.2935 ± 0.0224b
	SP	$R = 0.562e^{-0.20t} + 0.435e^{-11.86t}$	0.9652**	12.8177 ± 0.0528a	0.6236 ± 0.1327a
C. korshinskii	CK	$R = 0.699e^{-23.45t} + 0.301e^{-0.29t}$	0.9994**	4.7135 ± 0.6734aB	0.0528 ± 0.0036a
	LP	$R = 0.379e^{-2.02t} + 0.621e^{-25.65t}$	0.9976**	0.9965 ± 0.0414c	0.0524 ± 0.0030a
	MP	$R = 0.379e^{-1.04t} + 0.621e^{-26.71t}$	0.9976**	2.3056 ± 0.6288b	0.0536 ± 0.0040a
	SP	$R = 0.572e^{-25.10t} + 0.428e^{-0.96t}$	0.9953**	2.8626 ± 1.0628ab	0.0639 ± 0.0128a
A. fruticosa	CK	$R = 0.798e^{-1.08t} + 0.202e^{-22349.23t}$	0.9922**	2.5821 ± 0.0998bC	0.4314 ± 0.0152b
-	LP	$R = 0.486e^{-2.46t} + 0.486e^{-2.46t}$	0.7973*	1.2063 ± 0.0204c	0.2699 ± 0.0078b
	MP	$R = 0.264 e^{-10.08t} + 0.737 e^{-0.98t}$	0.9947**	2.7741 ± 0.1042b	0.4094 ± 0.0233b
	SP	$R = 0.738e^{-0.50t} + 0.262e^{-1.79E8t}$	0.9472**	5.8224 ± 1.2601a	0.7858 ± 0.0965a
Z. jujuba var. spinosa	CK	$R = 0.292e^{-0.67t} + 0.713e^{-10.49t}$	0.9954**	2.6786 ± 0.0356aC	0.1021 ± 0.0037c
	LP	$R = 0.512e^{-7.04t} + 0.512e^{-7.04t}$	0.9668*	0.4270 ± 0.0226d	0.1017 ± 0.0068c
	MP	$R = 0.467e^{-3.06t} + 0.467e^{-3.06t}$	0.8819*	1.0389 ± 0.1018c	0.2032 ± 0.0019a
	SP	$R = 0.305e^{-2.80E13t} + 0.262e^{-1.85t}$	0.9501*	1.5002 ± 0.1786b	0.1770 ± 0.0021b
P. sepium	CK	$R = 0.693e^{-14.41t} + 0.307e^{-0.19t}$	0.9982**	8.9515 ± 0.1746aA	0.0872 ± 0.0023a
-	LP	$R = 0.545e^{-2.60t} + 0.455e^{-23.49t}$	0.9966**	0.9201 ± 0.0253d	0.0834 ± 0.0185a
	MP	$R = 0.605e^{-1.77t} + 0.395e^{-1235.09t}$	0.9570*	1.4415 ± 0.1683c	0.1052 ± 0.0155a
	SP	$R = 0.530e^{-19.65t} + 0.470e^{-0.74t}$	0.9604**	2.9118 ± 0.1881b	$0.1100 \pm 0.0054a$
M. sativa	CK	$R = 0.803e^{-20.91t} + 0.197e^{-0.27t}$	0.9995**	4.2674 ± 0.5602bB	$0.0460 \pm 0.0009a$
	LP	$R = 0.640e^{-23.18t} + 0.360e^{-1.49t}$	0.9995**	1.3067 ± 0.2453c	0.0472 ± 0.0028a
	MP	$R = 0.738e^{-25.03t} + 0.263e^{-0.15t}$	0.9999**	10.9414 ± 0.4145a	0.0499 ± 0.0049a
	SP	$R = 0.355e^{-1.21t} + 0.645e^{-3.30E8t}$	0.9976**	1.6805 ± 0.2676c	0.0045 ± 0.0001b
C. varia	CK	$R = 0.247e^{-1.67t} + 0.753e^{-26.91t}$	0.9995**	0.9636 ± 0.0029bcE	0.0417 ± 0.0022b
	LP	$R = 0.499e^{-3.13t} + 0.501e^{-506.29t}$	0.9848**	0.7483 ± 0.1065c	0.0076 ± 0.0002c
	MP	$R = 0.603e^{-21.30t} + 0.397e^{-1.56t}$	0.9931**	1.3945 ± 0.1601a	0.0671 ± 0.0088a
	SP	$R = 0.791e^{-791.44t} + 0.209e^{-1.27t}$	0.9886**	1.1152 ± 0.0577ab	0.0344 ± 0.0028b
B. ischaemum	CK	$R = 0.616e^{-36.04t} + 0.384e^{-1.04t}$	0.9958**	2.1409 ± 0.3992bCD	0.0483 ± 0.0061b
	LP	$R = 0.506e^{-1.76E6t} + 0.494e^{-2.75t}$	0.9661*	0.8558 ± 0.1386c	0.0028 ± 0.0006c
	MP	$R = 0.567e^{-2.04t} + 0.433e^{-664.76t}$	0.9920**	1.2658 ± 0.1025bc	0.0657 ± 0.0327a
	SP	$R = 0.590e^{-1.01E7t} + 0.410e^{-0.71t}$	0.9932**	3.0903 ± 0.5798a	0.0020 ± 0.0014c
A. vestita	CK	$R = 0.547e^{-2.07t} + 0.453e^{-13.73t}$	0.9908**	1.1205 ± 0.0033cDE	0.1316 ± 0.0160c
	LP	$R = 0.740e^{-2.79t} + 0.260e^{-740.57t}$	0.7926*	0.9675 ± 0.0409c	0.1408 ± 0.0044c
	MP	$R = 0.592e^{-0.37t} + 0.408e^{-26.05t}$	0.9999**	6.9027 ± 1.1273a	0.4964 ± 0.1385a
	SP	$R = 0.661e^{-1.03t} + 0.339e^{-1009.95t}$	0.9544**	2.7784 ± 0.5876b	0.2908 ± 0.0478b
T. repens	CK	$R = 0.770e^{-3306.40t} + 0.230e^{-1.22t}$	0.9968**	1.8920 ± 0.721bCDE	$0.0100 \pm 0.0004b$
-	LP	$R = 0.325e^{-2.12t} + 0.675e^{-5.36E6t}$	0.9964**	1.1005 ± 0.3841b	0.0094 ± 0.0000b
	MP	$R = 0.811e^{-17.96t} + 0.190e^{-0.44t}$	0.9984**	3.0860 ± 0.0928a	0.0471 ± 0.0029a
	SP	$R = 0.261e^{-1.19t} + 0.739e^{-1.13E5t}$	0.9993**	1.4800 ± 0.2118b	0.0096 ± 0.0002b

Different lowercase and capital letters after the values of  $T_{0.95}$  and  $T_{0.50}$  indicated significant differences in decomposition rates among different contamination degrees of the same litter and among 10 species in the uncontaminated soil medium, respectively,  $\alpha = 0.05$ .

CK: control testing (petroleum concentration is 0 g/kg, petroleum/dry soil), LP: slight pollution (15 g/kg), MP: moderate pollution (30 g/kg), SP: serious pollution (45 g/kg).

Listed models were fitted from the average mass remind rate. The turnover period and half-life period were obtained by 3 independent models, which were fitted using the data of each repeat experiment, respectively. Values are represented as average  $\pm$  SE.

\*, \*\*, fitted equations were significant at the 0.05 level (Prob > F value was smaller than 0.05) and at the 0.01 level (Prob > F value was smaller than 0.01), respectively.

species was significantly extended by 60.91%–371.00% (P < 0.05). These data showed that moderate levels of pollution mainly inhibited the later decomposition of litter from *M. sativa*, and also inhibited the previous decomposition of litters from *C. varia*, *A. vestita* and *T. repens*.

Finally, for soils with high levels of petroleum contamination, the  $T_{0.95}$  of litters from *Z. jujuba* var. spinosa, *P. sepium* and *M. sativa* were significantly shortened by 43.99%–67.47% (P < 0.05). The  $T_{0.50}$  of litter from *M. sativa* was significantly shortened by 90.22% (P < 0.05), while that of litter from *Z. jujuba* var. spinosa was significantly extended by 73.36% (P < 0.05). These data indicated that a large amount of petroleum accelerated the overall decomposition of litter from *M*. sativa, and the previous and later decomposition of litter from *Z*. *jujuba var*. spinosa was significantly inhibited and promoted, respectively. The  $T_{0.95}$  and  $T_{0.50}$  of litters from *C*. korshinskii, *C*. varia and *T*. repens were not influenced, while the  $T_{0.95}$  of litters from *H*. rhamnoides, *A*. fruticosa, *B*. ischaemum and *A*. vestita was significantly (P < 0.05) extended by 44.35%–481.56%. In these 4 litter species, the  $T_{0.50}$  of litter from *B*. ischaemum was significantly shortened by 95.86% (P < 0.05), while that of the other 3 litter species was significantly extended by 82.15%–120.97% (P < 0.05). This result demonstrates

that serious pollution accelerated the previous decomposition but strongly inhibited the later decomposition of the litter from *B. ischaemum.* For the other 3 litter species, serious pollution inhibited decomposition overall.

According to the results, the impacts of petroleum on litter decomposition did not monotonically increase or decrease as the degree of contamination increased. There were 2 main response patterns. First (Pattern I), there was a tendency from weakening acceleration to inhibition (including "acceleration– slight acceleration–no significant impact (NS)" for litter from *C. korshinskii*, "acceleration–NS–inhibition" for litters from *H. rhamnoides*, *B. ischaemum* and *A. fruticosa*, and "weakening acceleration" for litter from *Z. jujuba var. spinosa* and *P. sepium*). Second (Pattern II), there was an inhibitory tendency under moderate pollution (including "acceleration–inhibition–acceleration" for litter from *M. sativa*, "NS–inhibition–NS" for litters from *C. varia* and *T. repens* and "NS to weakening inhibition" for litter from *A. vestita*).

# 2.3. Relationship between initial substrate quality and litter decomposition rate under petroleum contamination

To assess the relationship between initial substrate quality and litter decomposition under contaminated conditions, the individual and integrated substrate quality indicators (including their integrated principal component value F) and the corresponding decomposition turnover periods, or the expanded rates ( $R_e$ ) of the turnover periods under petroleum contaminated conditions (Eq. (2)), were submitted to SPSS software for Pearson correlation analysis.

$$R_{\rm e} = (T_{0.95\rm p} - T_{0.95\rm CK}) / T_{0.95\rm CK} \times 100\%$$
<sup>(2)</sup>

where,  $T_{0.95P}$  (year) was the turnover periods of litter decomposition under polluted conditions.  $T_{0.95CK}$  (year) was the turnover period represented in control testing.

The results demonstrated that individual substrate quality indicators did not show significant correlations with decomposition turnover periods at any treatment (not presented here). However, their integrated principal component value *F* showed significant negative correlation (P < 0.05) with expanded rates of turnover periods under moderate pollution conditions (Table 3), which means better initial substrate quality would cause obvious inhibitory effects on litter decomposition under conditions of moderate pollution. Indeed, we noticed that poor quality litter decomposition proceeded according to pattern II (Tables 1 and 2).

### 3. Discussion

# 3.1. Responses of litter decomposition to different levels of petroleum contamination

For litters from H. rhamnoides, C. korshinskii, A. fruticosa, Z. jujuba var. spinosa, P. sepium and B. ischaemum, slight pollution accelerated their decomposition. This finding is consistent with the studies of Mendelssohn and Slocum (2004) and Lü (1998). Because petroleum contains diversiform components, slight pollution could increase the quantity and species of carbon sources which, in turn, stimulates the reproduction of microbes, and increases their activity and induces their utilization of carbon sources and enzyme secretion to litter substance-utilization type (Blakely et al., 2002; Li et al., 2012; Cajthaml et al., 2008). For instance, McKinley et al. (1982) reported that the populations of cellulose-decomposing microorganisms increased after crude oil pollution. Qasemian et al. (2011, 2012) indicated that the activities of lignocellulolytic enzymes such as cellulose and β-glucosidase increased significantly after being contaminated by polycyclic aromatic hydrocarbon for 3 months. Cellulose was the main component of litter, and lignin was one of the main substances that inhibited the later decomposition of litter (Fujii and Takeda, 2010). Hence, the mentioned increases of microbe biomass and enzyme activities might accelerate litter decomposition.

However, as the concentration of petroleum increases, the activities of microorganisms would sharply decrease due to the accumulation of toxic petroleum components, limitations in nutrient supply and adverse environmental conditions (Serrano et al., 2009; Liu et al., 2014). For instance, for the H. rhamnoides litter, the impact of moderate pollution on its decomposition was not significant, while serious pollution significantly inhibited its decomposition. In addition, as petroleum concentration increased, the main communities of microbes would change to hydrocarbon degradation groups, such as Deltaproteobacteria and phyla Bacteroidetes (Thavamani et al., 2012; Rahn, 2012; Zhou et al., 2012), causing a relative decrease in the populations of litter-utilization species. Moreover, large amounts of oil would cover the surface of microbe cells, which would inhibit the activity of litter-decomposing enzymes (e.g., urease, invertase, protease, dehydrogenase and phosphatase) (Guo et al., 2012; Lü et al., 1997; Ma et al., 2014; Andreoni et al., 2004; Serrano et al., 2009). These would consequently weaken the accelerating effects of petroleum and even represent inhibition of litter decomposition.

Table 3 – Pearson correlation coefficients between expanded rates of the turnover periods (relative to control) and initial substrate quality indicators.

Initial substrate quality indicators							
Ν	Р	Polyphenols	Lignin	Lignin/N	C/N	C/P	F
0.286	0.533	-0.157	-0.622	-0.496	-0.322	-0.474	-0.605
0.236	0.553	-0.012	-0.689	-0.602	-0.323	-0.512	-0.637*
-0.143	-0.321	0.434	0.142	0.097	0.054	0.406	0.374
	0.286 0.236	0.286 0.533 0.236 0.553	N         P         Polyphenols           0.286         0.533         -0.157           0.236         0.553         -0.012	N         P         Polyphenols         Lignin           0.286         0.533         -0.157         -0.622           0.236         0.553         -0.012         -0.689	N         P         Polyphenols         Lignin         Lignin/N           0.286         0.533         -0.157         -0.622         -0.496           0.236         0.553         -0.012         -0.689         -0.602	N         P         Polyphenols         Lignin         Lignin/N         C/N           0.286         0.533         -0.157         -0.622         -0.496         -0.322           0.236         0.553         -0.012         -0.689         -0.602         -0.323	N         P         Polyphenols         Lignin         Lignin/N         C/N         C/P           0.286         0.533         -0.157         -0.622         -0.496         -0.322         -0.474           0.236         0.553         -0.012         -0.689         -0.602         -0.323         -0.512

In some special cases, slight pollution did not significantly influence litter decomposition (e.g., litters from C. varia, T. repens, and A. vestita) because few toxic components may only show weak and short-term effects on microbe biomass and activities. For instance, McKinley et al. (1982) stated that after the contamination of soil by crude oil, the ATP level of soil microbe communities could restore to normal levels in a short period of 4-8 hr. On the contrary, serious pollution significantly accelerated the litter decomposition of some species. For instance, the decomposition of Z. jujuba var. spinosa, P. sepium and M. sativa were significantly promoted at high contamination levels. Such effects might be caused by the co-metabolism (Antizar-ladislao et al., 2004) and co-oxidation (Werner et al., 1984a, 1984b) of microbes on litter aryl-compounds and petroleum, which may have circumvented the inhibitory effects of petroleum on decomposition. Interestingly, our results revealed that decomposition of high-quality litters was clearly inhibited by moderate levels of pollution. This finding indicated that litter quality also influenced the response of litter decomposition to petroleum. All four of these litter species came from grasses (C. varia, T. repens, A. vestita and M. sativa) and might have increased the activities of certain allelochemicals inhibiting the activities of microorganisms or enzymes, as these litters contained more nutrients. Because previous studies indicated that the activity of allelochemicals might be activated by sufficient nutrients, allelochemical activity may also be affected by the bio-chemical conditions of soil (Timsina et al., 2011). We hypothesized that these allelochemicals were activated the most in soil conditions under moderate levels of pollution where sufficient nutrients are supplied from the litter. In addition, the particular microbe species that colonize the litter depend greatly on litter quality, while microbes show distinct responses to petroleum at different concentrations (Qasemian et al., 2012). Thus, microbes that mainly colonize high quality litters might be primarily inhibited by moderate amounts of petroleum. These hypotheses might explain why high quality litter decomposition was inhibited mainly under moderately contaminated conditions. Certainly, further research is needed for accurate verification.

# 3.2. Responses of different litter decomposition periods to certain degrees of petroleum contamination

Petroleum contamination did not show monotonically inhibitory or promotional effects on different decomposition periods. The decrease of petroleum concentration and the unique substrate quality of litters might be the primary reasons for this pattern. Some petroleum compounds were harmless, even stimulative for microbe activity during short periods of time. However, in the long term, recalcitrant petroleum components with high biological toxicity, such as alkane isomers, benzo[k]uoranthene, indeno[1,2,3-cd]pyrene and benzo[q,h,i]pyrene, were concentrated (Coulon et al., 2005; Pan et al., 2011), and the easy-utilized organic matters were reduced, which results in a sharp decrease in microbe populations (Zhou et al., 2012). For instance, we observed a 10<sup>2</sup>–10<sup>3</sup> times increase of bacteria and actinomycete quantities during the first month after contamination, and they fell to CK and lower levels within 2-12 months. During this period, fungus quantities were usually represented as lasting decreases (unpublished data). Zhao et al. (2014) reported that the quantities of bacteria declined rapidly in oil-contaminated soil. Consequently, the litter decomposition was promoted during previous periods, but inhibited during later periods (*e.g.*, slight pollution accelerated previous decomposition, but inhibited the later decomposition of litter from *C. varia*).

On the other hand, the structural change of soil or organic matter would cause aging or immobilization of petroleum compounds (e.g., Nam et al. (2003) stated that soil aggregation could reduce the toxicity of phenanthrene), while some compounds with high acute toxicity, such as acenaphthylene, were usually decomposed or volatilized rapidly (Pan et al., 2011). Our results also demonstrated that the total content of petroleum hydrocarbons decreased by 27.10% to 63.33% after being incubated for 6 months (unpublished data). Thus, the decomposition of litter might be inhibited during previous periods, but promoted in later periods as the community of decomposers recovered (e.g., moderate pollution inhibited previous decomposition, but accelerated the later decomposition of litter from B. ischaemum). In addition, different periods of litter decomposition were controlled by different enzymes (Zhang et al., 2006). Petroleum contamination usually decreased or stimulated the activities of litter-decomposing enzymes, and thus influenced different periods of litter decomposition. For example, Serrano et al. (2009) stated that after polluting the soil with heavy diesel, the activities of soil dehydrogenase, urease, protease, *β*-glucosidase, phosphatase and aryl-sulfatase were inhibited at first, but then significantly promoted, which consequently accelerated litter decomposition during later periods.

For some types of litter, their decomposition was influenced only during previous or later periods. For example, moderate pollution levels primarily inhibited the later decomposition of litter from *M. sativa*. There are two possible reasons for this delayed inhibition: (1) the content of recalcitrant substances was different during both stages (Table 1), thereby generating inhibitory effects on decomposition at different stages, or (2) the species, contents, release properties and transformation processes of the allelochemicals from 10 tested litters were variable, thereby generating variable impacts on the physiological activity of decomposers, which might stimulate or inhibit litter decomposition during the given stages.

### 4. Conclusions

Slight pollution did not inhibit the decomposition of any litters. Instead, it significantly promoted litter decomposition of H. rhamnoides, C. korshinskii, A. fruticosa, Z. jujuba var. spinosa, P. sepium, M. sativa and B. ischaemum. Moderate pollution significantly inhibited litter decomposition of M. sativa, C. varia, A. vestita and T. repens and significantly promoted litter decomposition of C. korshinskii, Z. jujuba var. spinosa and P. sepium. Serious pollution significantly inhibited litter decomposition of H. rhamnoides, A. fruticosa, B. ischaemum and A. vestita and significantly promoted litter decomposition of Z. jujuba var. spinosa, P. sepium and M. sativa. The impacts of soil petroleum contamination on litter decomposition were controlled by numerous factors including the degradation of petroleum, the tolerance and physiological properties of decomposers, litter quality, and the interactions between litter decomposition and soil properties. Consequently, the impacts of soil petroleum contamination were usually more complex and less predictable. In future investigations, the dynamics of soil biological and chemical properties and nutrient release from litter decomposition in petroleum contaminated soil need to be clarified.

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