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Insight into the indirect function of isopods in litter decomposition in mixed subtropical forests in China

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ABSTRACT

Aboveground consumers can shape belowground processes in brown-food webs of forests by converting litter into faeces. Woodlice are one of the important aboveground consumer groups driving the dynamics of belowground soil. Although considerable data on litter decomposition exists, the indirect effects of woodlice (different qualities of faeces) on litter decomposition need to be explored further. In this study, we assessed the influence of isopods (*Armadillidium vulgare*) and its by-products (three contrasting qualities of faeces) on the decomposition of broad-leaf and needle litter via soil incubation. Litter mass loss, soil microbial biomass and soil extracellular enzyme activities treated with different isopod by-products were determined in six-month laboratory incubations.

The results showed that after incubation, the indirect effect of fauna (faeces) did not significantly increase broad-leaf litter decomposition. However, faeces of isopods fed on high-quality legume litter significantly increased needle litter decomposition. The effects of isopod faeces on microbial activities were significant: most of the soil microbial biomass and extracellular enzyme activities increased significantly compared with non-faeces treatments in the soil of broad-leaved and coniferous forests; and high-quality faeces strengthened the correlation of litter mass loss with peroxidase and phenol oxidase in coniferous forest. The result suggests that faeces with higher concentrations of nitrogen and labile carbon can mediate the decomposition of refractory materials like lignin and phenolic compounds, which might be highly relevant for the litter decomposition process in ecosystems with high macrofauna abundance. This study infers that the indirect effect of isopods (faeces) on litter decomposition depends on the quality of the litter they consumed and the type of decomposing litter. Isopods indirectly accelerate the nutrient-poor litter decomposition process by converting high-quality litter into faeces. As such, the negative effects of litter recalcitrance on microbial decomposition in mixed forests are indirectly alleviated.

1. Introduction

Litter decomposition is a major process that drives the release of nutrients from its source (detritus) in terrestrial ecosystems. In the brown-food web, over 80% of the carbon fixed via photosynthesis in the forest falls as litter, which is then decomposed by microbes, detritivores, and their predators (Kaspari and Yanoviak, 2009; Wardle et al., 2011). Among these decomposers, microbes and soil fauna are of great importance in releasing carbon (C) into the atmosphere, recycling nutrients, and sustaining much of the forest's biodiversity (Lummer et al., 2012; Clay et al., 2013). Thus, studying their function in the litter decomposition process is necessary to elucidate the fluxes of nutrients in the brown-food web (Freschet et al., 2013).

Litter and consumers' faeces, which complexly interact through macro-detritivores, are two important intermediates that link above- and below-ground ecological processes (Wardle et al., 2004; Vos et al., 2013). In litter decomposition, biochemical fluxes and concentrated bioavailable nutrients are transmitted to detrital decomposers and microbes (Vauramo et al., 2006; Kagata and Ohgushi, 2013; Kaneda et al., 2013). Macro-detritivores convert large quantities of consumed litter into faeces, which could reach a critical level of energy and nutrient input for soil microorganisms (Hunter, 2001; Clark et al., 2010). Macro-detritivore faeces, with higher concentrations of nitrogen (N) and labile C compared with leaf litter (Madritch et al., 2007), were observed to increase

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bacterial counts (Suzuki et al., 2013), change the microbial community structure (Coulis et al., 2013), and accelerate decomposition (Coleman et al., 2004).

The quality of faeces (such as C:N ratio and tannin content) produced by macro-detritivores mostly depends on the litter they consume (Madritch et al., 2007; Kagata and Ohgushi, 2013). A mature forest is often composed of mixed arbor, in which detritivores preferentially feed on litter species with high quality, so with high N concentrations (Hättenschwiler and Jørgensen, 2010) and/or low concentrations of lignins, polyphenols, or tannin (Hättenschwiler and Gasser, 2005). Their different food selection makes them produce different qualities of faeces. These different-quality faeces provide different bioavailable nutrients for detrital microorganisms, which may enhance the average decomposition of litter mixtures. Thus, macro-detritivores can influence litter decomposition rates through indirectly providing nutrients for microorganisms. Although the indirect effects of macro-detritivores have been studied in much detail before (Lavelle and Spain, 2001; David and Gillon, 2002; Hunter et al., 2007; Bardgett and Wardle, 2010; Coulis et al., 2013; Suzuki et al., 2013), the effects of different qualities of faeces on litter decomposition need to be studied thoroughly.

Microorganisms are major decomposers in the litter decomposition process and provide nutrition for the entire soil food web (Enowashu et al., 2009; Allison et al., 2013). They produce extracellular enzymes that specifically degrade C, N, or phosphorus (P)-containing complex organic compounds into small utilizable molecules that are then assimilated by the microbes. Extracellular enzymes have been recommended as the most appropriate indicator of microbial decomposition, soil fertility, and ecological stability (Freeman et al., 2001; Lv et al., 2014). Thus, changes in soil extracellular enzymes and microbial biomass during treatments with different qualities of faeces can explain the ecological effects of macro-detritivore faeces on litter decomposition in forest ecosystems.

Isopods are saprophagous invertebrates that often are dominant members of soil fauna communities (David and Handa, 2010). They are voracious macro-detritivores that convert large amounts of dead and decaying organic matter (like litter) into faeces (Hunter, 2001; Clark et al., 2010). Thus, in this study, Isopoda (*Armadillidium vulgare*, Armadillidiidae) was chosen to assess its indirect role (faeces) in the decomposition of broadleaf (*Quercus acutissima*) and needle (*Pinus massoniana*) litter in a six-month laboratory experiment. Additionally, soil microbial biomass and enzymatic activities were measured to directly monitor the functional responses of microorganisms to isopods and/or their indirect faeces-derived effects in subtropical forest ecosystems. We hypothesize that by converting different-quality litter into faeces soil macro-detritivores might indirectly (1) have a positive effect on the decomposition of low-quality litter after ingesting high-quality litter and (2) have a negative effect on the high-quality litter decomposition after consuming low-quality litter.

2. Materials and methods

2.1. Collection of soil, leaf litter, and isopods

Soil and leaf litter for the experiments were collected from two forests of Zijin Mountain (32°5′ N, 118°48′ E), Nanjing, China; a broad-leaved forest dominated by *Q. acutissima* and a coniferous forest dominated by *P. massoniana*. The mountain has an altitude of 447.1 m and a subtropical monsoon climate. The area has an annual mean temperature of 15.4 °C with a monthly mean temperature reaching a maximum of 28.2 °C in July and a minimum of 1.9 °C in January. The rainy season is from June to July, and the average annual precipitation is 1106.5 mm. The coverage degree of litter reaches as high as 90%. The soil is classified as slightly acidic Humic Cambisol with a pH of about 5.0 (FAO-UNESCO., 1987). The bedrock materials are sandstone and shale, and a considerable amount of nutrients and organic matter is accumulated in the humus layer.

Four discrete sites (2 m × 2 m), which are approximately 10 m apart, were chosen in both broad-leaved forest and coniferous forest. In October 2012, freshly fallen leaves of *P. massoniana* and *Q. acutissima* were collected at each of the four sites and then mixed, separately. Furthermore, freshly fallen leaves of legumes were also collected under *Robinia pseudoacacia* trees of the four forest sites. *R. pseudoacacia* is a tree with litter rich in N and low lignin and polyphenol levels in the Zijin Mountain and is sparsely distributed in the two forests. In our experiment, fallen leaves of *R. pseudoacacia* were considered as high-quality litter. Leaf litter of *P. massoniana,* with high levels of lignin and phenolic compounds, was defined as low-quality litter. All litter samples were taken back to the laboratory and oven-dried at 55 °C for 24 h to obtain a constant weight to be used in a subsequent study (the initial litter quality is shown in Table 1). Two soil samples from each forest were collected from the top layers (0–5 cm) of each site, sieved through a 2-mm mesh, and kept in a refrigerator at 4 °C until incubation.

A. vulgare is one of the most numerous species of arthropods in the forest (Chen, 2000), and the most extensively investigated terrestrial isopod species (Zimmer, 2002). It is reported to have prevailing densities as high as 10,000 individuals per m^2 in the USA (Frouz et al., 2004) and 100–500 individuals per m^2 in Nanjing (Tang and Gui, 1994). Adult samples (>1 cm) were hand-collected in broad-leaved forest and coniferous forest of Zijin Mountain in April 2013. The isopods were cultured in 1 L clear plastic containers with a medium of 95% plaster and 5% activated charcoal. All containers were then stored in the dark at 20 °C and moistened weekly using deionized water. All the isopods were starved for 24 h in pots before being introduced into the experimental microcosms. The isopods were fed weekly with three different moistened leaves. Faeces were collected every 3 days and added to the treatments representing the broad-leaved forest and coniferous forest.

2.2. Decomposition experiment in the laboratory

The decomposition of leaf litter was determined in a laboratory microcosm. Oven-dried litter (0.5 g ± 0.02 g) was mixed with 40 g of soil in 240 mL plastic boxes with a surface area of 95 cm^2 and covered with a lid. The soil samples derived from broad-leaved forest and coniferous forest were selected as the sources of

Table 1
Initial characteristics of three different qualities of leaf litter and isopod (*A. vulgare*) faeces used for the litter decomposition experiement. Different superscript letters in a transverse row denote significant differences ($p < 0.05$) among different types of litter and faeces.

Composition	Litter			Faeces		
	PL	QL	RL	PF	QF	RF
Total C (%)	51.1^a	48.8^b	46.3^c	32.4^f	40.3^e	43.7^d
Total N (%)	0.78^d	0.66^d	3.26^a	1.46^c	2.15^b	3.02^a
Lignin (%)	41.2^a	30.9^b	27.5^c			
Total polyphenol (mg gallic acid equivalent g^{-1})	270^a	193^b	79.4^c			
C:N	65.2^b	73.6^a	14.2^e	22.2^c	18.8^d	14.5^e
Lignin:N	52.6^a	46.5^b	8.46^c			

Abbreviations: PL – *Pinus massoniana* litter; QL – *Quercus acutissima* litter; RL – *Robinia pseudoacacia* litter; PF – faeces of isopods fed on *Pinus massoniana* litter; QF – faeces of isopods fed on *Quercus acutissima* litter; RF – faeces of isopods fed on *Robinia pseudoacacia* litter.

microbes. *Q. acutissima* and *P. massoniana* leaves represented litter from broad-leaved forest and coniferous forest soil, respectively.

The experiment was designed to have five treatments consisting of only adding deionized water (control, CK), adding isopods (I), faeces from isopods fed on *P. massoniana* leaves (PF), faeces from isopods fed on *Q. acutissima* leaves (QF), and faeces from isopods fed on *R. pseudoacacia* (RF) for the two types of litter (*Q. acutissima* leaves and *P. massoniana* leaves), which accounts to a total of ten treatment combinations. All treatments were conducted with four replicates and six collection times, so a total 240 microcosms were prepared.

In the isopod treatments, every microcosm received two adult *A. vulgare* based on the low density of 200 individuals per m^2 in Zijin Mountain (Tang and Gui, 1994). The microcosms were checked twice a week, and dead isopods were replaced by similar-sized ones from the container with a corresponding food source and were then tagged. For faeces treatments, the faeces quantity added to the chambers was equal to the faeces production rate of the two *A. vulgare* individuals. The faeces production rates of one *A. vulgare* fed on *P. massoniana*,*Q. acutissima*, and *R. pseudoacacia* leaves were 0.47, 0.54, and 0.69 mg day^{-1} (our unpublished data), respectively. On average, 4.0 mg of PF, QF, and RF were added to the broad-leaved forest (*Q. acutissima* leaves with broad-leaved forest soil) and coniferous forest (*P. massoniana* leaves with coniferous forest soil) incubations every third day. Faeces were added as a suspension in 2.5 mL of deionized water. The microcosms were incubated in the dark at 25 °C for six months. During the incubation, soil moisture content was monitored and maintained between 50% and 60% of the gravimetric moisture content by spraying deionized water to the soil samples.

Four replicate samples per treatment were systematically harvested every month. The soil adhering to the litter was carefully removed, and the litter samples were oven-dried at 60 °C for one week to a constant weight for subsequent mass loss determination. The inoculated soil samples were also harvested, kept in sealed bags, and stored in a refrigerator at 4 °C, for no more than one week, for analyses of soil microbial biomass, soil pH, and enzyme activity.

2.3. Chemical analysis, soil pH, soil microbial biomass, and enzyme activity determination

At the beginning of the experiment, 2 g collected leaf litter and soil samples were oven-dried at 60 °C for 48 h to determine their initial chemical properties. Total C and N concentrations were determined using an elemental analyzer (Elemental Vario MICRO, Germany). The lignin concentration of the litter samples was measured using a gravimeter applying hot sulphuric acid digestion (Osono and Takeda, 2002). Soil moisture content was determined after oven-drying the samples at 105 °C overnight.

Soil microbial biomass was measured via the substrate-induced respiration method. Soil (1 g) was placed in a glass vial (100 mL). Each soil sample was controlled to have equal moisture content (60% dry weight basis) to remove any potential water limitation. Glucose was then added at 10 mg glucose g^{-1} soil dry weight for each sample. The samples were then sealed and incubated at 25 °C for 1 h. CO_2 evolution was determined using an infrared gas analyzer twice to assess difference between the final and initial CO_2 concentrations (Bailey et al., 2002). Soil pH was measured using a glass electrode at a ratio of 1:2.5 (soil/water) after shaking the equilibration for approximately 30 min (Dick et al., 2000).

Enzyme assays began within 24 h of sample collection. Enzyme activities involved in cycling of C (cellulase, cellobiohydrolase, β-glucosidase, β-xylosidase), N (nitrate reductase, urease), P (acid phosphatase, alkaline phosphatase), and polyphenol metabolism (phenol oxidase, peroxidase) in litter decomposition were determined spectrophotometrically with little modification. Cellulase (E.C. 3.2.1.4) activity was determined using 1% carboxymethylcellulose solution as substrate with incubation at 50 °C for 30 min (pH 5.5; glucose concentration was determined with a spectrophotometer at 540 nm; Ghose, 1987). Activities of cellobiohydrolase (E.C. 3.2.1.91), β-1,4-glucosidase (E.C. 3.2.1.21), and β-1,4-xylosidase (E.C. 3.2.1.37) were determined using 1.2 mM 4-nitrophenyl-β-D-linked substrates (cellobioside, glucopyranoside, xylopyranoside) with incubation in the dark at 40 °C for 1.5 h (pH 5.0; 0.2 M Na_2CO_3 was used to stop the reaction. 4-Nitrophenyl concentrations were quantified by measuring absorbance at 400 nm using a microplate spectrophotometer in 96-well plates; Vepsäläinen et al., 2001). Phenol oxidase (E.C. 1.10.3.2) and peroxidase (E.C. 1.11.1.7) activities were measured spectrophotometrically using 50 μL of 25 mM 1-3,4-dihydroxyphenylalanine (L-DOPA) as the substrate with incubation at 28 °C for 1 h (pH 5.5; peroxidase assays plus 10 μL of 0.3% H_2O_2 before measurement. Activity was quantified by measuring absorbance at 450 nm using a microplate spectrophotometer in 96-well plates; Saiya-Cork et al., 2002). Nitrate reductase (E.C. 1.7.99.4) activity was determined using 200 mM KNO_3 solution as substrate with incubation at room temperature for 30 min (pH 7.5; NO_2^- concentration was determined with a spectrophotometer at a wavelength of 520 nm; Daniel and Curran 1981). Urease (E.C. 3.5.1.5) activity was determined using 10% urea solution as substrate with incubation at 37 °C for 24 h (pH 6.7; NH_4^+-N concentration was determined with a spectrophotometer at a wavelength of 578 nm; Nannipieri et al., 1980). Acid phosphatase (E.C. 3.1.3.2) and alkaline phosphatase (E.C. 3.1.3.1) activities were determined using 0.5% disodium phenyl phosphate solution as substrate with incubation at 37 °C for 24 h (pH 5.0 for acid phosphatase; pH 10.0 for alkaline phosphatase; phenol concentration was determined with a spectrophotometer at 570 nm; Kandeler et al., 1999).

2.4. Data analyses

The litter mass loss rate was determined using an exponential equation (Olson, 1963):

$$x_t = x_0 e^{-kt}$$

where x_0 is the original mass of litter, x_t is the amount of litter remaining after time t (month), and k is the litter decomposition rate constant ($month^{-1}$).

Data were checked for deviations from normality and homogeneity of variance before analysis by Shapiro–Wilk test and Quantile–Quantile Plot. An analysis of variance (ANOVA) and Tukey's honestly significant differences test were applied to assess significant differences between the various treatments. With two-way ANOVA, the effects of faeces added and forest type on soil enzyme activities and microbial biomass were tested. To test the correlation patterns of litter mass losses and soil enzyme activities, Principal Component Analysis (PCA) was used. All statistical analyses were performed by SPSS program (version 17.0).

3. Results

3.1. Litter decomposition

The cumulative mass loss of the two types of litter showed a similar trend during the six-month period with a rapid increase of litter decomposition during the first three months and slow acceleration in the latter three months in all treatments (Fig. 1). In most cases, the cumulative mass loss in the soil taken from the broad-leaved forest was higher than that in the soil taken

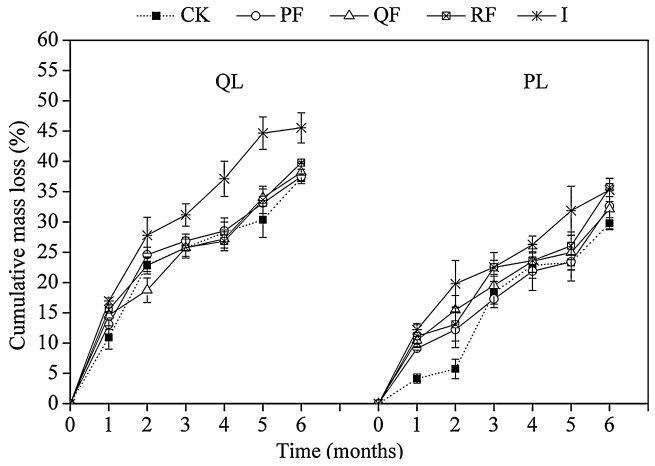

Fig. 1. Effects of isopods (*A. vulgare*) and different qualities of isopod faeces on cumulative mass loss of *Q. acutissima* litter and *P. massoniana* litter during a six-month incubation period. Error bars indicate standard deviation (SD, $n = 4$).
Abbreviations: QL – *Quercus acutissima* litter; PL – *Pinus massoniana* litter; CK – control; PF – faeces of isopods fed on *Pinus massoniana* litter; QF – faeces of isopods fed on *Quercus acutissima*; RF – faeces of isopods fed on *Robinia pseudoacacia*; I – isopod treatment.

from the coniferous forest (Fig. 1). In the isopod treatment, total litter mass loss over the six-month period was higher for the broad-leaved forest soil than for the coniferous forest soil (Fig. 1).

In the faeces treatments, litter mass losses in the soil taken from the two forests were higher than in the control. In the broad-leaved forest, litter mass losses in the QF and RF treatments were, on average, 2.43%, and 6.63% higher than that in the control (Fig. 1). In the coniferous forest, litter mass losses in the PF, QF, and RF treatments were, on average, 15.7, 17.3, and 18.8% higher than in the control treatment, respectively (Fig. 1).

In general, litter decomposition rate constants (k) were higher for the soil taken from the broad-leaved forest than from the coniferous forest (Fig. 2). Isopod treatments significantly (Tukey test, $p < 0.05$) increased the litter decomposition rates in the soil taken from the broad-leaved forest compared with QF treatment

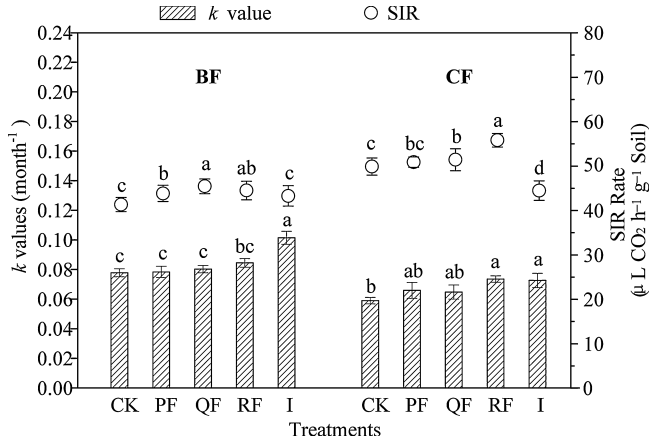

Fig. 2. Changes in decomposition rate constant (mean k values, month^{-1}, Y1) and mean substrate-induced respiration (SIR Rate, μL CO_2 h^{-1} g^{-1} Soil, Y2) in the two forests under isopod (*A. vulgare*) and isopod faeces treatments after six-months of litter decomposition. White circles indicate the mean substrate-induced respiration and bars show decomposition rate constants. Data with different lowercase letters indicates a significant difference ($p < 0.05$). Error bars indicate standard deviation (SD, $n = 4$).
Abbreviations: BF – broad-leaved forest; CF – coniferous forest; k – decomposition rate constant; CK – control; PF – faeces of isopods fed on *Pinus massoniana* litter; QF – faeces of isopods fed on *Quercus acutissima* litter; RF – faeces of isopods fed on *Robinia pseudoacacia* litter; I – isopod treatment.

whereas RF treatments significantly (Tukey test, $p < 0.05$) increased the litter decomposition rates only in the soil taken from coniferous forest (Fig. 2). The rates of litter decomposition in the PF treatment were the lowest compared with the other faeces treatments in the soils from both forests (Fig. 2).

3.2. Soil pH

After the six-month incubation period, most of the soil pH values in all treatments were higher than those of the control and increased compared with the initial soil pH values. The soil pH-H_2O values of broad-leaved forest increased from an initial value of 4.17 to 4.22, 4.41, and 4.41 in the PF, QF, and RF treatments, respectively. Moreover, the pH-H_2O of the coniferous forest soil increased from an initial value of 4.48 to 4.80, 4.78, and 4.82 in the PF, QF, and RF treatments, respectively. In the isopod treatments, however, the pH-H_2O of the broad-leaved and coniferous forest soils decreased to 3.95 and 4.03, respectively. In all treatments, the pH of the coniferous forest soil increased more rapidly than that of broad-leaved forest soil (data not shown).

3.3. Soil microbial biomass

Substrate-induced respiration was used to estimate the soil microbial biomass. After the six-month decomposition period, the average substrate-induced respiration in all faeces (PF, QF, and RF) treatments was significantly (Tukey test, $p < 0.05$) higher compared to the control in the broad-leaved forest soil (Fig. 2). A similar trend was observed in the QF and RF treatments in the coniferous forest soil. For the broad-leaved forest soil, the highest substrate-induced respiration was found in the QF treatment, whereas in the coniferous forest soil respiration was highest in the RF treatment. In the isopod treatment the substrate-induced respiration was significantly (Tukey test, $p < 0.05$) lower compared with the PF and QF treatments (Fig. 2), in both the broad-leaved and coniferous forest soils. In the latter respiration was also lower compared to the control.

3.4. Enzyme activities

After the six-month incubation period, most soil extracellular enzyme activities involved in C and N cycling were significantly enhanced compared to the control in most of the treatments in both forest soils except for β-1, 4-glucosidase in the coniferous forest soil (Table 2). Soil extracellular enzyme activities involved in C cycling were significantly (Tukey test, $p < 0.05$) higher in the coniferous forest than in the broad-leaved forest, whereas soil extracellular enzyme activities involved in N cycling were significantly lower in the coniferous forest than in the broad-leaved forest (Table 2). Faeces fertilization significantly accelerated nitrate reductase and urease activity compared to the control in the broad-leaved forest soil. In the coniferous forest soil, β-1, 4-xylosidase, urease, alkaline phosphatase, and phenol oxidase activities significantly (Tukey test, $p < 0.05$) increased compared to the control in most treatments (Table 2). The results of the two-way ANOVA showed that activities of most of the soil enzymes responded significantly ($p < 0.05$) to forest type and isopod faeces fertilization.

The correlation patterns between litter mass loss and soil extracellular enzyme activities were examined via PCA (Fig. 3). In general, faeces fertilization strengthened the correlation of litter mass loss with acid phosphatase, nitrate reductase, and phenol oxidase activities in the broad-leaved forest. In the coniferous forest, QF and RF treatments strengthened the correlation of litter mass loss with peroxidase, and phenol oxidase activities (Fig. 3). Isopod treatments strengthened the correlation of litter mass loss

Table 2
Effects of isopods (*A. vulgare*) and isopod faeces on extracellular enzyme activities after six-month incubation in two forest types. Data represent mean values of six sampling during the period of litter decomposition and standard error (SE, $n = 4$). Different superscript letters in a vertical row denote significant differences ($p < 0.05$) among treatments.

	Cellulase (IU)	CBHI (IU)	BG (IU)	BX (IU)	NR (IU)	Urease (IU)	ACP (IU)	ALP (IU)	Pero (IU)	PhOx (IU)
BF										
CK	0.84 ± 0.01a	1.77 ± 0.02bc	2.01 ± 0.02c	1.81 ± 0.03cd	12.6 ± 0.27c	5.50 ± 0.06b	15.5 ± 0.14a	22.8 ± 0.24b	0.219 ± 0.001c	0.124 ± 0.001ab
PF	0.84 ± 0.02a	1.73 ± 0.01cd	2.04 ± 0.02bc	1.79 ± 0.01d	14.6 ± 0.06a	5.29 ± 0.03c	15.3 ± 0.26b	22.9 ± 0.13ab	0.240 ± 0.009b	0.121 ± 0.004b
QF	0.85 ± 0.01a	1.71 ± 0.01d	2.05 ± 0.01bc	1.82 ± 0.02cd	13.4 ± 0.15b	6.04 ± 0.04a	14.9 ± 0.26b	22.8 ± 0.22b	0.233 ± 0.005bc	0.129 ± 0.001a
RF	0.83 ± 0.004a	1.86 ± 0.02a	2.04 ± 0.02bc	1.93 ± 0.04a	12.7 ± 0.21c	5.99 ± 0.01a	15.0 ± 0.26b	23.2 ± 0.26ab	0.270 ± 0.008a	0.131 ± 0.001a
I	0.83 ± 0.01a	1.78 ± 0.01bc	2.10 ± 0.02ab	1.88 ± 0.02b	13.7 ± 0.04a	5.49 ± 0.06b	14.7 ± 0.08b	23.5 ± 0.11a	0.270 ± 0.003a	0.133 ± 0.002a
CF										
CK	1.18 ± 0.01a	1.85 ± 0.03c	2.13 ± 0.02ab	1.82 ± 0.02b	9.85 ± 0.14a	4.85 ± 0.07c	15.8 ± 0.30a	33.8 ± 0.13c	0.176 ± 0.005b	0.127 ± 0.002d
PF	1.12 ± 0.01bc	1.90 ± 0.03bc	2.09 ± 0.03b	1.96 ± 0.03a	8.84 ± 0.10b	4.90 ± 0.03c	15.4 ± 0.55a	35.1 ± 0.18b	0.158 ± 0.001c	0.147 ± 0.003b
QF	1.09 ± 0.01c	1.96 ± 0.02ab	2.07 ± 0.01b	1.91 ± 0.03a	9.86 ± 0.08a	5.26 ± 0.01b	15.2 ± 0.32a	35.2 ± 0.40b	0.161 ± 0.003c	0.135 ± 0.001c
RF	1.08 ± 0.02d	1.96 ± 0.03a	2.06 ± 0.01b	1.90 ± 0.03a	9.70 ± 0.05a	5.29 ± 0.08b	15.6 ± 0.28a	35.2 ± 0.41b	0.179 ± 0.003b	0.146 ± 0.001b
I	1.14 ± 0.02ab	1.99 ± 0.01a	2.21 ± 0.04a	1.92 ± 0.01a	9.90 ± 0.13a	5.47 ± 0.03a	15.5 ± 0.13a	36.6 ± 0.53a	0.199 ± 0.004a	0.159 ± 0.002a

Abbreviations and active international unit (IU) definition of Soil enzyme activities: cellulase (1 mg glucose released $min^{-1}\,g^{-1}$ soil); CBHI – cellobiohydrolase; BG – β-1,4-glucosidase; BX – β-1,4-xylosidase (1 μmol PNP released $h^{-1}\,g^{-1}$ soil); NR – nitrate reductase (1 μg NO_2^- released $min^{-1}\,g^{-1}$ soil); Urease (1 mg NH_3-N released $h^{-1}\,g^{-1}$ soil); ACP – acid phosphatase; ALP – alkaline phosphatase (1 mg P released $h^{-1}\,g^{-1}$ soil); Pero – peroxidase; PhOx – phenol oxidase (1 μmol L-DOPA $h^{-1}\,g^{-1}$ soil); BF – broad-leaved forest; CF – coniferous forest; CK – control; PF – faeces of isopods fed on *Pinus massoniana* litter; QF – faeces of isopods fed on *Quercus acutissima*; RF – faeces of isopods fed on *Robinia pseudoacacia*; I – isopod treatment.

with β-1,4-glucosidase, acid phosphatase, and phenol oxidase and attenuated their correlation with β-1,4-xylosidase and alkaline phosphatase activities compared with that of control in the two forest soils.

4. Discussion

Aboveground soil macro-detritivores can influence belowground processes by affecting the organic matter decomposition process in brown-food webs of subtropical forests (Bardgett and Wardle, 2010; Toyota and Kaneko, 2012). Macroarthropods, as fragmenters, generally have direct (via digestion) and indirect (via faeces) effects on litter decomposition. The indirect effect is more important for litter decomposition compared with the direct effect (Lavelle and Spain, 2001; Coulis et al., 2013). This study provides an insight into the indirect effect of isopods during litter decomposition process in subtropical forest ecosystems.

Our study showed that faeces treatments significantly stimulated most of the microbial activities in both broad-leaved and coniferous forests. Contrary to our initial hypothesis, faeces of isopods fed on any of three qualities of food did not significantly affect broad leaf litter decomposition, whereas the faeces from isopods fed on high-quality litter *R. pseudoacacia* significantly increased needle litter decomposition. Most investigators mentioned that macroarthropods indirectly stimulate microbial activity through their faeces and subsequently significantly accelerate litter decomposition (Coleman et al., 2004; Bardgett and Wardle, 2010). Coulis et al. (2013) found that millipede faeces changed the microbial community structure but did not increase organic matter decomposition. Suzuki et al. (2013) reported that the effect of litter-transforming fauna (such as millipedes) on leaf litter decomposition depends on litter type. Our results imply that in the responses of litter decomposition to isopods indirect (faeces-derived) effects are dependent on the quality of the food offered to the isopods and on the decomposing litter type.

Two reasons could explain this phenomena. First, coniferous forest litter contains many refractory compounds such as lignin and phenolic compounds, which are key functional traits for predicting litter decomposition (Chomel et al., 2014). With high levels of lignin and phenolic compounds in needle litter, the nutrient limitations on microbial biomass are much more mitigated in coniferous forest soil compared with broad-leaved forest soil after fertilization with faeces from isopods fed on high-quality litter. Therefore the stimulation is more effective in the coniferous forest soil system. Second, Suzuki et al. (2013) showed that leaf litter and millipede faeces were dominated by gram-negative bacteria, which preferred more labile C sources. So, the high-quality faeces could provide a large amount of gram-negative bacteria to the litter decomposer community. *R. pseudoacacia* litter had a markedly higher quality than the other two leaf litters used in our experiment. The nutrients transferred from this litter to faeces increased the soil microbial biomass and soil enzyme activities and subsequently promoted the decomposition of the nutrient-poor litter.

Extracellular enzymes play a crucial function in litter decomposition and provide useful information on microbial activities. In this study, most soil extracellular enzyme activities were significantly enhanced in the QF and RF treatments during the decomposition of two types of litter. This may be due to the external supply of nitrogen (N) and labile C to the microbial community by the faeces of the isopods. Subsequently, soil microbial biomass increased significantly, and the metabolic activities of soil microorganisms, especially the excretion of exo-enzymes, were enhanced (Lv et al., 2013). The result implies that isopods fed with high-quality litter indirectly increase soil microbial activities and accelerate the litter-decomposing process. The PCA results showed that in the coniferous forest, compared with the control, QF and RF treatments strengthened the correlation of litter mass loss with peroxidase and phenol oxidase. Coulis et al. (2009) reported that detritivores could reduce the level of condensed tannins in litter to near zero in their faeces. The transformation of such significant amounts of leaf litter into faeces fundamentally changes either the quantity or quality or both of the condensed tannins at the litter–soil interface. Our results suggest that faeces with higher concentrations of N and labile C can mediate the decomposition of refractory materials like lignin and phenolic compounds, which might be highly relevant for the litter decomposition process in ecosystems with high macrofauna abundance.

Isopods are soil invertebrate grazers that shred dead and decaying organic matter and regulate fungal abundance, community composition, and diversity in natural and agricultural systems (Mitschunas et al., 2006; Crowther et al., 2011). In our study, isopod treatment significantly decreased the soil microbial biomass compared with the PF and QF treatments in coniferous forest and broad-leaved forest, respectively. However, their faeces (needle-isopod faeces, PF treatment; broad-leaved isopod faeces, QF) had positive effects on soil microbial biomass. This implies that

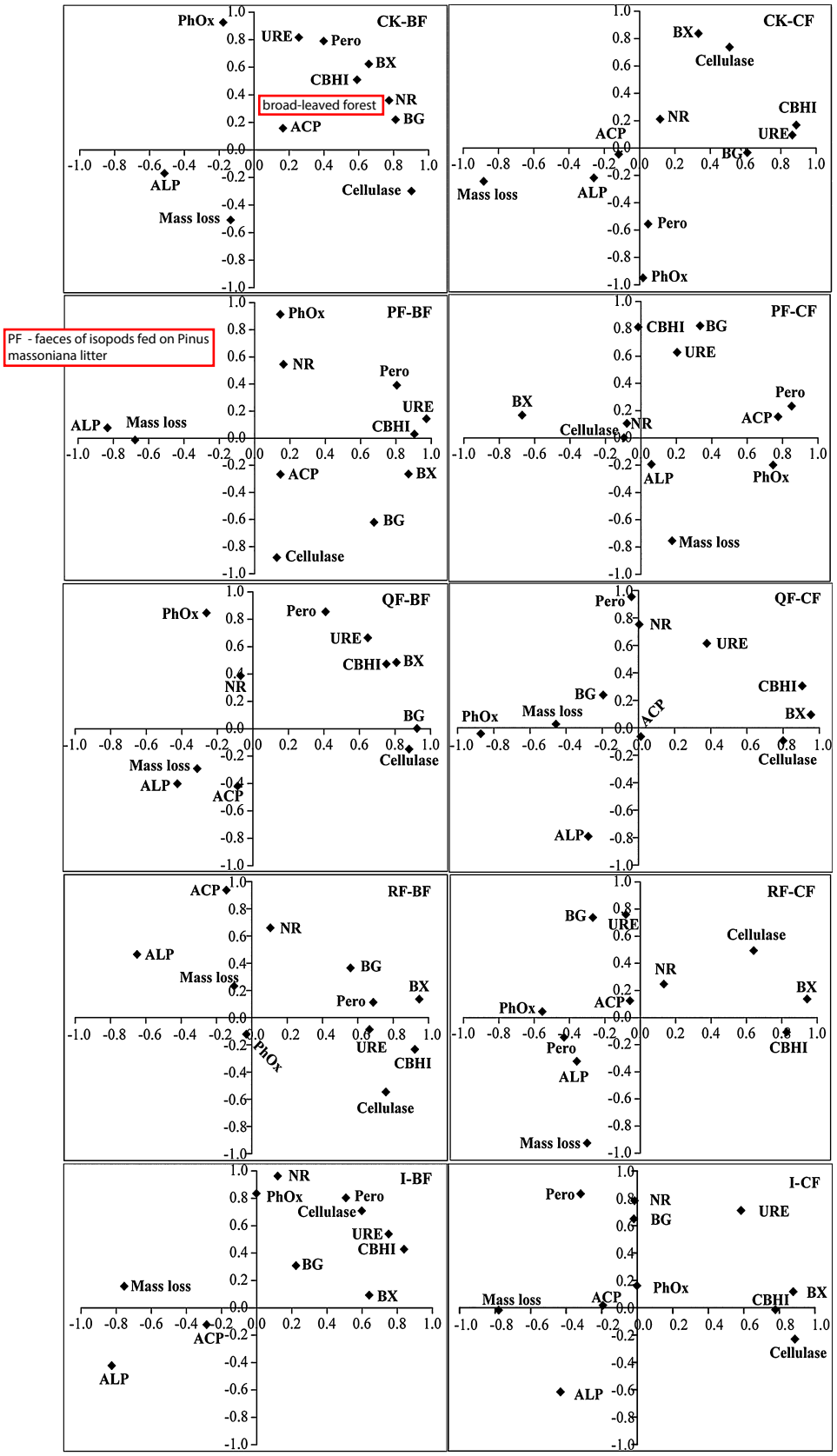

Fig. 3. PCA of the correlation patterns of litter mass loss with soil extracellular enzymatic activities in the two forests under isopod (*A. vulgare*) and isopod faeces treatments after six-months of litter decomposition. The *X* axis accounts for 34.5, 40.4, 33.6, 35.6, 37.3,25.2, 21.2, 30.9, 23.3, and 27.9% of the total variation, and the *Y* axis accounts for 24.4, 21.3, 21.9, 19.1 26.0; 23.5, 20.8, 21.6, 22.1 and 19.9% of the total variation in CK-BF, PF-BF, QF-BF, RF-BF, I-BF, CK-CF, PF-CF, QF-CF, RF-CF and I-CF treatments, respectively.
Abbreviations: BF – broad-leaved forest; CF – coniferous forest; CBHI – cellobiohydrolase; NR – nitrate reductase; URE – urease; ACP – acid phosphatase; ALP – alkaline phosphatase; Pero – peroxidase; PhOx – phenol oxidase; CK – control; PF – faeces of isopods fed on *Pinus massoniana* litter; QF – faeces of isopods fed on *Quercus acutissima* litter; RF – faeces of isopods fed on *Robinia pseudoacacia* litter; I – isopod treatment.

A. vulgare might consume part of the soil microbes when feeding litter in forests. Although the isopods also produced some faeces after consuming litter in the isopod treatments, the accelerated effect of their faeces is not enough to compensate for the negative regulating (feeding) effect of isopods on soil microbes. However, how isopods and/or their faeces regulate the microbial community structure during litter decomposition needs further investigation.

The majority of the forests in China are mixed forests, which consist of various kinds of plants. However, pure forests, especially plantation forests, are also commonly found (about 6 km^2), most of which are coniferous (such as *Pinus* plantation forests). These pure-species forests generally lack the structural and habitat complexity compared with mixed forests. Litters, especially needle litters, are very difficult to decompose, which then results in the formation of a deep layer of litters in coniferous forest floors. The most reasonable explanation for this phenomenon is the lack of micro-decomposers (Gartner and Cardon, 2004). Our study suggests that in the mixed broad-leaved – coniferous forest, macro-detritivores choose to consume high-quality broad leaves, and their excreta randomly fall to the mixed litter surface. This process could indirectly fertilize the litter micro-decomposer and accelerate decomposition of the low-quality litter (such as needles). Our research provided an explanation for the positive effect of litter diversity on needle litter decomposition in terms of macro-detritivores metabolism, which may be useful to understand nutrient cycling, competition, resource partitioning and co-existence between the invertebrate and microbial components in plantation forest (Wu et al., 2013).

In conclusion, our study showed that in the brown-food webs of terrestrial ecosystem, the indirect (faeces-derived) effects of isopods on litter decomposition are related with its food (litter) and the decomposing litter type. Through converting high-quality litter into faeces, soil micro-detritivores could indirectly accelerate low-quality litter decomposition in mixed forests. The effect of macro-detritivores faeces on litter decomposition indicates its unique function of connecting above- and below-ground nutrient cycling processes in brown-food webs of subtropical forest ecosystems. Factors affecting the soil litter transformer community (e.g. drying, land cover change, and the lower-quality litter because of global change) may have multiple effects on the balance of ecosystem nutrient cycling. As such, these effects need to be further explored.

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