Soil Biology & Biochemistry 101 (2016) 207–216

Contents lists available at ScienceDirect

Soil Biology & Biochemistry

journal homepage: www.elsevier.com/locate/soilbio

Biological transfer of dietary cadmium in relation to nitrogen transfer and ^{15}N fractionation in a soil collembolan-predatory mite food chain

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ARTICLE INFO

Article history:
Received 9 April 2016
Received in revised form
25 July 2016
Accepted 28 July 2016
Available online 4 August 2016

Keywords:
Cadmium pollution
Soil invertebrate food chain
Cadmium trophic transfer
Nitrogen isotope fractionation
Nitrogen transfer

ABSTRACT

Effects of cadmium (Cd) on collembolans and predatory mites have been well studied but Cd accumulation and biological transfer and the relationship between Cd trophic transfer and nitrogen (N) isotope fractionation and transfer in soil micro-arthropod food chains remain poorly understood. Here, we investigated the biological transfer and trophic toxicity of Cd and the effects of dietary Cd exposure on ^{15}N fractionation in animal tissues and N transfer from food in a soil collembolan (*Folsomia candida*)-predatory mite (*Hypoaspis aculeifer*) food chain using experiments conducted in plate and soil systems. Synchronized *F. candida* were fed yeast or wheat spiked with 0 (Control), 50 (Cd50), 100 (Cd100) or 200 (Cd200) μg Cd g^{-1} food for two weeks and were then offered to synchronized *H. aculeifer* as prey for two weeks. Cadmium concentrations in tissues of both the collembolan and its predator increased as the Cd concentrations in the food yeast or wheat increased in both the plate and soil systems, and the Cd concentrations in the food and the animals were all significantly and positively correlated. Except for the controls, the Cd bioaccumulation factor (BAF) between adults of predatory mites and consumer collembolans (0.20–1.13) was 2.5–14 times higher than that from food yeast/wheat to consumer collembolans (0.02–0.08). Cadmium transfer has not altered the reproduction of the filial generation collembolans but the reproduction of the predatory mite declined by 34.3% with the Cd concentration in its juveniles reaching 5.94 μg g^{-1}, suggesting that Cd transfer from the spiked food can present a higher toxicity to the predator *H. aculeifer*. Food spiked with Cd decreased the N content in the collembolan tissues assimilated from the food and enriched the ^{15}N fraction ($\delta^{15}N$) in their tissues by affecting their metabolic turnover and growth rates. The Cd trophic transfer between collembolans and predatory mites from spiked food can directly increase the $\delta^{15}N$ value of predatory mites and restrict the movement of N from collembolans to predatory mites. The $\delta^{15}N$ of soil animals has considerable potential as a biomarker of Cd stress, ^{15}N isotope application may be limited in Cd polluted soil ecosystems, and the N transfer function of soil food webs may be more vulnerable in polluted ecosystems.

1. Introduction

Cadmium (Cd) is a major non-essential biological pollutant element with high potential toxicity, high mobility, high recalcitrance and long half-lives in the environment (Mann et al., 2007; Qiu, 2015). Anthropogenic processes have greatly increased Cd concentrations in the environment and this has aroused public concern (Gall et al., 2015). Because Cd represents a high risk of accumulation in living organisms by consumption or inhalation, further transfer to higher trophic levels can eventually threaten human health (Gall et al., 2015). Research on trophic transfer of Cd in food chains is therefore important and is urgently required (Ding et al., 2013). However, the majority of previous studies have focused on aquatic and aboveground terrestrial food chains (Ng and Wood, 2008; Li et al., 2015), and Cd trophic transfer in belowground terrestrial food chains which are direct receptors of soil Cd pollution is poorly understood.

Collembolans and predatory mites are pivotal within belowground terrestrial food chains and their typical trophic levels play

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http://dx.doi.org/10.1016/j.soilbio.2016.07.026
0038-0717/

an important role in the soil ecosystem (e.g. organic decomposition and carbon-nitrogen cycles) (Deyn et al., 2003; Pey et al., 2014). Several species of collembolans and predatory mites, for example, *Folsomia candida* and *Hypoaspis aculeifer*, have been recognized for many years as valuable models for single species eco-toxicological studies (OECD, 2008, 2009). Although Cd concentrations in collembolans have been studied following exposure to Cd-spiked diets (Fountain and Hopkin, 2001; Ardestani et al., 2014), internal body Cd concentrations of predatory mites are poorly understood after exposure to Cd, and some studies have indicated that Cd can accumulate only in oribatid mites (Skubala and Kafel, 2004; Skubala and Zaleski, 2012). Studies on other animals suggest that Cd in the body tissue pool of prey is generally highly bioavailable to predators (Hispard et al., 2008; Ng and Wood, 2008; Xie et al., 2010). Janssen et al. (1991) also showed that the Cd assimilation efficiency of other soil arthropod predators was higher than that in consumers. However, only a few studies have been reported in which pollutants influenced population variation (e.g. reproduction and survival) in the collembolan-predatory mite food chain (Hamers and Krogh, 1997; Schnug et al., 2014) in stark contrast to the large number of snail food chain studies (Scheifler et al., 2002; Gimbert et al., 2008; Li et al., 2015). So far, no experiment has quantitatively investigated Cd transfer and its effects on the collembolan-predatory mite food chain, especially effects on high trophic level predatory mites.

Nitrogen isotope fractionation ($\delta^{15}N$) is often used as a powerful tool by ecologists and eco-toxicologists to investigate trophic levels of animals and biomagnification of contaminants in food webs (Vanderklift and Ponsard, 2003; Brose and Scheu, 2014; Ek et al., 2015). Many studies involving trophic transfer of heavy metals have adopted $\delta^{15}N$ to quantify the trophic positions of animals in aquatic and aboveground terrestrial food webs (Jara-Marini et al., 2009; McMeans et al., 2015). It has been stated that the difference in average $\delta^{15}N$ between trophic levels is 3.4 (Ek et al., 2015). Nevertheless, the $\delta^{15}N$ value in animals varies with biotic and abiotic factors (Haubert et al., 2004, 2005). Moreover, only a few studies have been conducted on the effects of pollutants in the diet on N isotope fractionation in animal tissues. Banas et al. (2009) observed no effect on the value of $\delta^{15}N$ in the tissues of fish fed DDT-spiked food due to DDT exposure. In contrast, Ek et al. (2015) showed that lindane elevated $\delta^{15}N$ values to overestimate trophic positions by growth inhibition and changes in metabolism related to detoxification. In line with this aquatic system, the $\delta^{15}N$ values in snowy egrets fed Hg-contaminated diets were higher than in those fed control diets due to increasing the ratio of protein breakdown to synthesis (catabolic processes) (Shaw-Allen et al., 2005). This implies that the relationship between $\delta^{15}N$ values and trophic position in consumers in polluted environments can potentially change, but more studies are required because of the conflicting evidence. Since soil is an opaque system and soil food webs are notoriously difficult to assess, the recent use of $\delta^{15}N$ in soil food webs has received much attention and continues to expand (Brose and Scheu, 2014). However, the relationships between pollution and N isotope fractionation are poorly understood in soil food webs. One study has been performed regarding effects of fungal toxins on stable isotope fractionation of collembolans and shows that although fungal toxin production and N isotope fractionation were not closely correlated, the $\delta^{15}N$ in collembolans fed toxin-producing fungi often differed from the control (Staaden et al., 2010). Furthermore, so far no study has revealed relationships between pollutants in the food and ^{15}N isotope fractionation of higher trophic level predators and consumers.

Nitrogen transfer in soil food webs plays a key role in the N biogeochemical cycle and N transfer is a vital ecological function of soil food webs (Hättenschwiler and Field, 2005). Evidence has increased recently that heavy metal stress may result in a shift of N transferred from the diet in soil animals. In general, animals exhibit food avoidance behaviour and/or increased excretion on exposure to heavy metal contaminated food (Fountain and Hopkin, 2001; Ding et al., 2013), suggesting a reduction in ingestion with food. Several studies report that when animals were fed less heavy metal contaminated food, the food remained in the gut for a longer period so that more nutrients could be incorporated (Drobne and Hopkin, 1995; Loureiro et al., 2006). However, whether the total N content in animal body tissues absorbed from heavy metal contaminated food has changed remains unclear, especially in the case of higher trophic level predators rather than the consumers.

The present study sought to explore Cd transfer between trophic levels and its effect on high trophic level predatory mites and to reveal whether Cd transfer influences N transfer from the diet and ^{15}N fractionation of animal tissues in a typical soil detritus food chain. We hypothesized that (I) Cd occurring in spiked food may accumulate and transfer to higher trophic level predatory mites mediated by the consumer collembolans and produce a risk of secondary toxicity to the predatory mites; (II) the presence of Cd in food will enrich ^{15}N fractionation of the consumer collembolan tissues, and Cd transfer between collembolans and predatory mites from spiked food may increase the $\delta^{15}N$ value of predatory mites, since dietary Cd exposure can alter metabolic turnover and growth rates which affect stable isotope fractionation; and (III) the incorporated N content of Cd stressed collembolans from food will decrease, and Cd transfer will restrict N transfer from the diet in predatory mites. The collembolan *Folsomia candida* and the predatory mite *Hypoaspis aculeifer* were used to establish a typical soil detritus food chain. Ingestion is a crucial exposure pathway to soil contaminants in collembolans and predatory mites (Nakamori and Kaneko, 2013; Pereira et al., 2015) and therefore dietary exposure was used. The ^{15}N natural abundance (δX) in the body tissues at each trophic level with exposure to Cd-spiked food yeast was compared to show ^{15}N fractionation in the animals, and the ^{15}N abundance (atom%) with exposure to ^{15}N-labelled Cd-spiked food wheat was determined to reflect N trophic transfer.

2. Materials and methods

2.1. Collembolan and predatory mite

Stock cultures of *Folsomia candida* (collembolan) and *Hypoaspis aculeifer* (predatory mite) were obtained from Aarhus University in Denmark. They were reared on Petri dishes with a layer of moist plaster of Paris mixed with activated charcoal (9:1 w/w) at 20 ± 1 °C and 75% relative humidity (RH) with a 16:8 h (dark:light, 800 lux) light regime and have been cultured in our laboratory for more than five years. Twice a week *F. candida* was fed dried baker's yeast (produced by Angel Yeast Co., Ltd, China) and *H. aculeifer* was reared with juveniles of *F. candida*. Distilled water was added weekly to maintain the moisture content. Age-synchronized *F. candida* and *H. aculeifer* were obtained according to the standardized methods of the Organization for Economic Co-operation and Development (OECD, 2008, 2009). The age of *F. candida* exposed to Cd spiked food was 10–12 days and that of *H. aculeifer* exposed to Cd-contaminated *F. candida* was 32–35 days.

2.2. Exposure food for collembolan

Baker's yeast (N content: 6.86 ± 0.12%, Cd concentration: 0.08 ± 0.05 μg g^{-1}) and ^{15}N-labelled (5.54 ± 0.07 atom %) dried wheat litter material were used as food for *F. candida*. The wheat litter material (N content: 2.18 ± 0.05%, Cd concentration: 0.41 ± 0.12 μg g^{-1}) was obtained by growing wheat (cultivar

Yangmai 158) in a phytotron from seed. The plant growth cabinet (length: 62 cm, width: 48 cm, height: 18 cm) contained 20 kg clay loam soil (OM: 12.3 g kg^{-1}, pH $_{water}$: 5.13, CEC: 15.2 cmol (+) kg^{-1}, total N: 0.77 g kg^{-1}, total Cd: 0.04 mg kg^{-1}) and was used to grow wheat for 8 weeks. A ^{15}N-enriched solution of $^{15}NH_4^{15}NO_3$ (10.09 atom%; SRICI, China) was applied as fertilizer to the cabinet (Ke and Scheu, 2008). After good seedling emergence occurred, 1 g $^{15}NH_4^{15}NO_3$ was added to the cabinet every week for 6 weeks. The harvested wheat litter material was washed with distilled water, dried at 60 °C and ground with a stainless steel mill.

A series of cadmium nitrate solutions were mixed with the yeast and the ^{15}N-enriched wheat to obtain nominal concentrations of 0 (Control), 50 (Cd50), 100 (Cd100) and 200 (Cd200) μg Cd g^{-1} dry weight food. The Cd-spiked yeast and wheat were dried at 60 °C, ground and stored in desiccators at 4 °C. The Cd concentrations were selected by reference to Cd concentrations in the field and the literature (Fountain and Hopkin, 2001; Scheifler et al., 2002; Nakamori and Kaneko, 2013).

2.3. Cd exposure via the diet

The experiments were conducted in both plates without soil and soil systems (Fig. 1) because the plate system gives good control of variables (Fountain and Hopkin, 2001) and the soil system is more closely related to actual field conditions. Four replicates of each exposure treatment were set up. All the experiments were conducted in the culture environment.

In the plate system (Fig. 1), experiments were established in plastic containers (inner diameter, 12.5 cm; height, 6.5 cm) with a layer (thickness: 1 cm) of moist plaster of Paris mixed with activated charcoal (9:1 w/w). Three hundred synchronized *F. candida* (10–12 days old) were transferred by vacuum into each plate. They were exposed to the two types of Cd-spiked food and the four Cd concentrations (yeast and wheat: control, Cd50, Cd100, and Cd200) for two weeks, respectively. Ample food was supplied three times a week and distilled water was added once a week to maintain humidity. After two weeks of the exposure treatments, fifty individuals of *F. candida* were extracted for analysis of Cd concentrations and ^{15}N abundance in the body tissues. The surplus *F. candida* were transferred to humid filter papers and starved for three days and then introduced into new plates as prey items of *H. aculeifer* according to the same treatments and replicates as above. Ten individuals of *H. aculeifer* (adult females) at an age of 32–35 days old were added to each plate for exposure to the Cd-contaminated *F. candida* by feeding the *F. candida* for two weeks. Two weeks later the Cd concentrations and ^{15}N abundance in the *H. aculeifer* (adult) body tissues were determined.

Soil system experiments (Fig. 1) were conducted in plastic cylinders (5 cm high, inner diameter 5.5 cm) containing 30 g clay loam soil (WHC: 50%, OM: 12.3 g kg^{-1}, pH $_{water}$: 5.1, CEC: 15.2 cmol (+) kg^{-1}, total N: 0.77 g kg^{-1}, Cd: 0.04 mg kg^{-1}). The treatments with yeast or wheat as *F. candida* foods contaminated with different Cd concentrations and the replication were the same as in the plate system described above. Three hundred synchronized *F. candida* (10–12 days) as the parental generation (F_0) were transferred into each cylinder with soil and incubated for two weeks. The Cd-contaminated foods were also refreshed three times and water was added once per week. After two weeks, the F_0 generation collembolans were extracted in a controlled temperature gradient extractor from 25 to 45 °C for two days from the soils in cylinders into plastic containers with a bottom layer of plaster-activated charcoal substrate (Fountain and Hopkin, 2001). The extracted *F. candida* individuals (F_0) were starved for one day on humid filter papers. Forty of the F_0 generation *F. candida* were used to measure Cd concentrations and ^{15}N abundance in the body tissues and 10 F_0 were transferred into new plastic cylinders with 30 g clean clay loam soil to assess the effect of Cd dietary exposure on reproduction of collembolans (F_0) according to the test methods of the OECD. Approximately 30 mg clean yeast was placed on the soil surface in each cylinder and refreshed once a week. After exposure for 23 days, the animals were extracted using the same method as described above. The extracted juveniles were regarded as the filial (F_1) generation of collembolans. Ten of the F_1 generation *F. candida* (the largest were 8–12 days old) were added to new plastic cylinders with 30 g clear clay loam soil for 28 days again to detect the effect of Cd transfer on reproduction of the F_1 generation

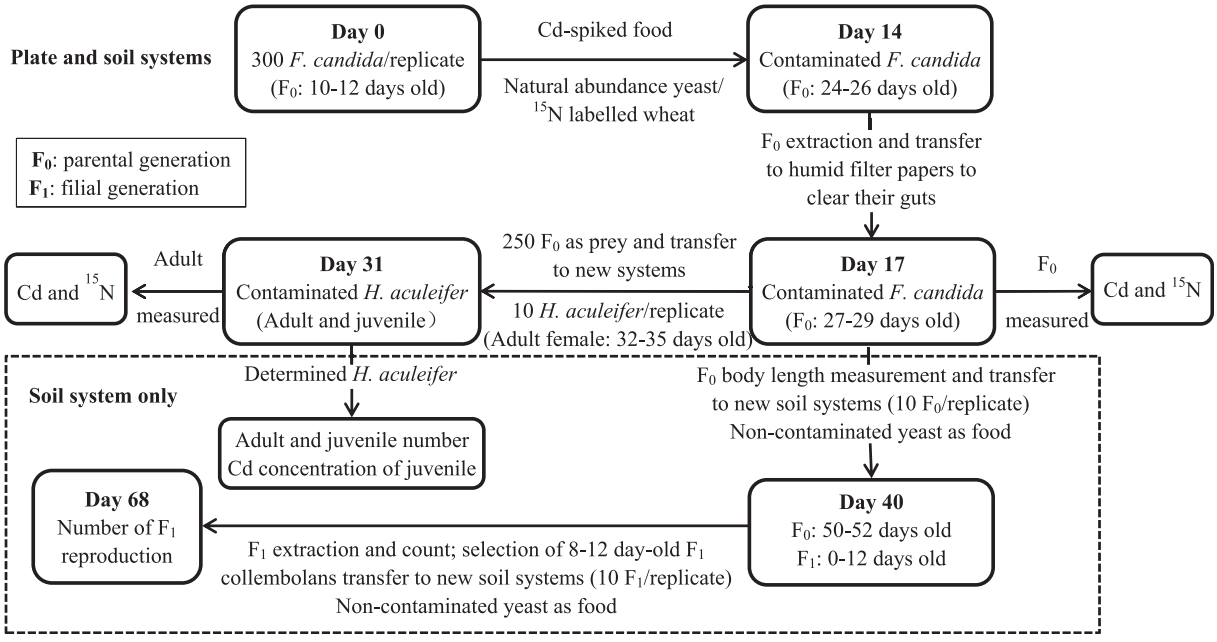

Fig. 1. Flow scheme of the dietary cadmium (Cd) exposure experiments (n = 4 replicates per treatment); Cd-spiked food level: control, Cd50, Cd100 and Cd200) in plate and soil systems. The experimental steps within the dotted line were conducted in the soil system only.

collembolans. The remainder of the F_0 generation *F. candida* were used as prey items of *H. aculeifer* and transferred into new cylinders with clean soil and ten individuals of *H. aculeifer* (adult females, 32–35 days old) in each cylinder corresponding to the same treatments and replicates as described above. The cylinders were incubated for two weeks and the animals were extracted. Cadmium concentrations in both the extracted adults and juveniles and ^{15}N abundance in the adults of *H. aculeifer* body tissues were determined together with the body length of the F_0 generation *F. candida* after Cd dietary exposure for two weeks, reproduction of the F_0 and F_1 generations of *F. candida*, and survival and reproduction of *H. aculeifer*.

Ten mL distilled water together with a drop of blue ink was supplied to each container to suspend the animals and the water surface was then photographed. The pictures were used to count the number of adults and juveniles and to measure their body lengths using the ImageJ 1.47a software package.

2.4. Chemical analysis

The extracted (live) *F. candida* and *H. aculeifer* individuals from both plate and soil systems were transferred to plastic containers with pieces of humid filter paper for three days to remove their gut contents. The animals were then washed with ultrapure water, killed by freezing (−72 °C), dried at 60 °C to constant dry mass, weighed using a Mettler Toledo XS3DU automatic electronic microbalance (precision ± 1 μg) and stored in a desiccator at 4 °C until analysis.

2.4.1. Cadmium analysis of body tissues and quality control

The animal body tissues were analysed for Cd using the procedures of Zhu et al. (in press). Briefly, the weighed animals were transferred into small glass test tubes (Pyrex, 4.2 mm i.d., 25.0 mm long, 1.3 mm thick) which had been cleaned with ultrapure reagent grade nitric acid (69%, Nanjing Chemical Reagent Co., Ltd.) and ultrapure water five times. The glass test tubes were inserted into a high pressure digestion vessel. The animals were digested at 105 °C for 3 h with 50 μL 3:1 mixture of ultrapure nitric acid and hydrogen peroxide (20%, Nanjing Chemical Reagent Co., Ltd.) and the Cd concentrations in the digests were determined using a graphite furnace atomic absorption spectrophotometer (Varian 220FS, 220Z, Palo Alto, CA). The limits of detection (0.0023 μg L^{-1}) and quantification (0.0076 μg L^{-1}) were much lower than the Cd concentrations in the animal digests.

The quality of the animal body tissue Cd analysis was controlled using blank samples and a certified reference material (pork liver, IGGE, China). Measured Cd concentrations in the blank samples were always below the detection limit and the recoveries of Cd in the reference material were always >85%.

2.4.2. ^{15}N isotope analysis

The weighed animals were analysed for ^{15}N isotope signatures using a Flash EA 2000 Series Elemental Analyser connected via a Conflo IV to a DeltaV Advantage isotope ratio mass spectrometer (FLASH-EA-DELTA-V, Thermo Finnigan, Waltham, MA) (Ek et al., 2015). An internal reference (fish muscle tissue) was determined after each batch of 10 samples for quality control. Precision of the ^{15}N measurement was <0.10‰.

The study used the δX notation which denotes the deviation from the reference in parts per thousand (‰) to express ^{15}N isotope natural abundance and was calculated using the following formula (Banas et al., 2009; Staaden et al., 2010):

$$\delta X = ((R_{sample} - R_{reference})/R_{reference}) \times 1000$$

where R_{sample}, mean $^{15}N/^{14}N$ of the sample, and $R_{reference} = {}^{15}N/{}^{14}N$ of the reference. The atmospheric N_2 (air) was used as the reference value for ^{15}N.

The labelled abundance of the ^{15}N isotope was expressed directly using atom% ^{15}N.

2.5. Calculation of bioaccumulation factor (BAF)

The BAF values of *F. candida* and *H. aculeifer* (adults and juveniles) were calculated using the following formula (Scheifler et al., 2002):

$$BAF = C_c/C_f$$

where C_c represents the Cd concentration of consumer tissue and C_f the mean Cd concentration in the food.

2.6. Statistical analysis

The effects of the Cd exposure treatments (four levels: Control, Cd50, Cd100 and Cd200) on Cd concentrations in *F. candida* and *H. aculeifer*, ^{15}N fractionation and enriched abundance, body length and reproduction of the F_0 generation *F. candida* and reproduction of *H. aculeifer* were subjected to tests of single factor variance (ANOVA). If the differences were significant, Fisher's Least Significant Difference test (LSD) was used to compare pairs of treatments. The generalized linear model (GLM) was used to evaluate the effects of multiple factors on Cd concentration, total N, $\delta^{15}N$, atom% ^{15}N and reproduction of F_0 generation *F. candida* and *H. aculeifer* and the F_0 generation *F. candida* body length (details in Appendix 1.). All analysis was performed using the IBM SPSS statistics software package version 21 and the significant difference was set at the 5% level. If the variance of data was not homogeneous the data were log-transformed prior to statistical analysis. Cadmium concentrations in food/prey and consumer/predator tissues via different Cd concentration exposure treatments were analysed by linear fitting and regression using OriginPro 9.1. Cadmium concentrations in *F. candida* and adult *H. aculeifer* are expressed as mean ± standard deviation (SD) and all other data are presented as mean ± standard error (SE).

3. Results

3.1. Survival, growth and reproduction in the soil system

After two weeks of exposure to Cd-enriched food, the body length of the F_0 generation *F. candida* fed yeast ($F_{3.119} = 6.72$, $P < 0.001$) and fed wheat ($F_{3.149} = 16.52$, $P < 0.001$) (Fig. 2a) and reproduction of those fed yeast ($F_{3,\ 12} = 6.97$, $P = 0.006$) and fed wheat ($F_{3,\ 12} = 8.69$, $P = 0.002$) (Fig. 2c) showed that these differences were significant among treatments, but reproduction of the F_1 generation collembolans showed no significant changes (Fig. S1). In the Cd200 treatment of the F_0 generation *F. candida* fed yeast, significant inhibition of body length (inhibition ratio: 10.1%) occurred compared to the control, but no such inhibition was observed in the other treatments (Fig. 2a). Within the treatments fed wheat, body length of the F_0 generation *F. candida* decreased significantly with increasing Cd concentration in the dietary wheat, and that in the Cd200 treatment was lower (by 12.2%) than the control but higher (by 11.9%) than the Cd100 treatment (Fig. 2a). Reproduction of the F_0 generation *F. candida* in the Cd200 treatment decreased by 24.5% fed yeast and by 26.7% fed wheat compared to the control (Fig. 2c).

After two weeks of exposure to increasing Cd concentrations via the prey animals (*F. candida*) fed wheat, reproduction of *H. aculeifer*

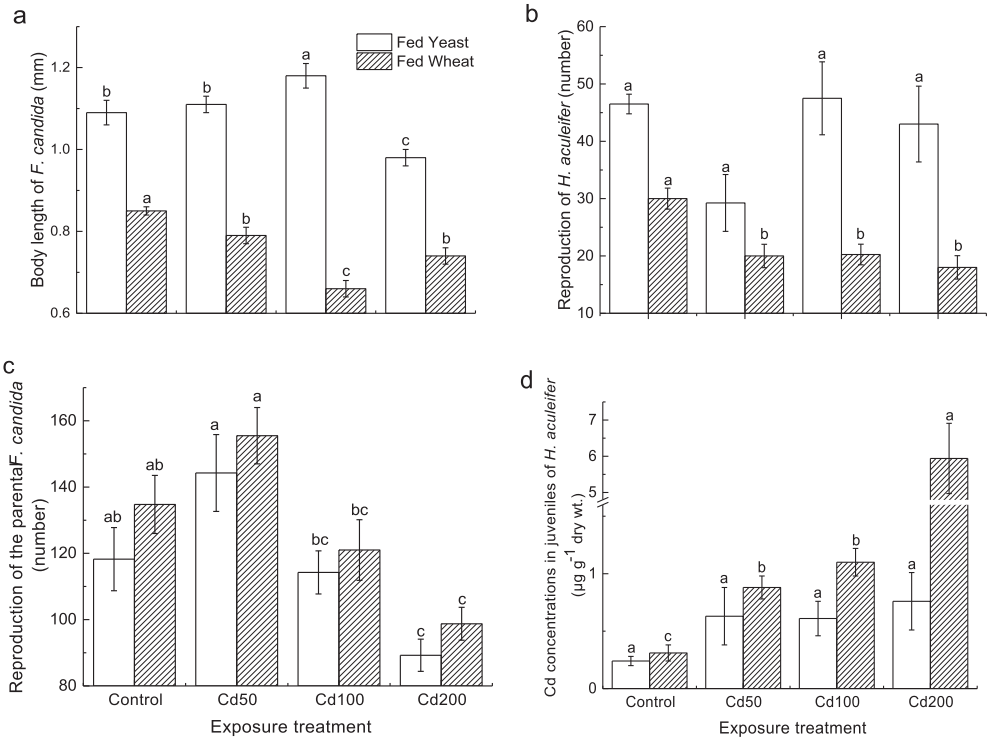

Fig. 2. After two weeks in the soil system, body length (a) (mean ± SE, n > 30) and reproduction (c) (mean ± SE, n = 4) of the parental (F_0) generation *F. candida* exposed to Cd-spiked food and reproduction (b) (mean ± SE, n = 4) of predatory mite *H. aculeifer* and Cd concentration (d) (mean ± SE, n = 4) in juveniles of *H. aculeifer* exposed to Cd-enriched *F. candida*. Fed Yeast and Fed Wheat indicate the F_0 generation *F. candida* were fed Cd spiked yeast and wheat (control: spiked with a trace of Cd, Cd50: 50 μg g^{-1}, Cd100: 100 μg g^{-1}, and Cd200: 200 μg g^{-1}, respectively) for two weeks. The Cd contaminated *F. candida* obtained were supplied to *H. aculeifer* as prey. Different letters indicate significant differences ($P < 0.05$) among exposure treatments (LSD test) when fed the same food.

decreased significantly in treatment Cd50 by 33.3%, Cd100 by 27.8% and Cd200 by 40.0% compared to the control ($F_{3,\ 12} = 7.79$, $P = 0.004$), but there was no effect via the prey *F. candida* fed yeast ($F_{3,\ 12} = 2.55$, $P = 0.105$) (Fig. 2b). The survival of *H. aculeifer* was not significantly affected via prey fed either yeast or wheat (Fig. S2). The Cd concentrations (5.94 ± 0.96 μg g^{-1}) in juveniles of *H. aculeifer* in Cd200 were significantly higher than in the control by approximately 15 times via prey fed wheat ($F_{3,\ 12} = 10.16$, $P < 0.001$, Fig. 2d).

3.2. Cadmium concentrations in different trophic levels (F. candida and H. aculeifer)

Cadmium concentrations in the body tissues of both the collembolan and the predatory mite increased as the Cd concentrations in the food yeast or wheat increased in both the plate and soil systems, and the food Cd concentrations were significantly and positively correlated with those in the animals (Fig. 3). In the plate system the Cd concentrations in *F. candida* and *H. aculeifer* with wheat were overall higher than those with yeast (Fig. 3a, b) but in the soil system the Cd concentrations in *F. candida* were also generally higher with wheat than with yeast but those in *H. aculeifer* with wheat were lower than those with yeast (Fig. 3c, d). The Cd concentrations in *F. candida* and *H. aculeifer* in the soil system were significantly higher than those in the plate system (Fig. 3, Table 1).

3.3. Bioaccumulation factors (BAF) of Cd in the collembolan–predatory mite food chain

Except for the controls, the BAF of Cd from yeast/wheat to *F. candida* were 0.02–0.08 in the plate system and 0.04–0.06 in the soil system and from *F. candida* to its predator were 0.20–0.28 in the plates and 0.49–1.13 in adults and 0.09–0.51 in juveniles in the soil system (Table 2). In the soil system, the BAF values of Cd from *F. candida* to *H. aculeifer* adults were approximately 2–6 times higher than those in the plate system. BAF of Cd from *F. candida* to *H. aculeifer* adults were much higher than those from yeast/wheat to *F. candida*, by 2.5–14 times in the plate system and 8.5–23 times in the soil system. The highest BAF value of Cd in the collembolan–predatory mite food chain was 1.13 (from *F. candida* to *H. aculeifer* adults) in the Cd50 treatment in the soil system. In the soil system the BAF of Cd from *F. candida* fed yeast to *H. aculeifer* adults were higher than those fed wheat, but to *H. aculeifer* juveniles showed the opposite trend.

3.4. ^{15}N fractionation ($\delta^{15}N$) and enriched abundance in F. candida and H. aculeifer

^{15}N signatures in the collembolan and the predatory mite body tissues were significantly different among the four treatments after two weeks of exposure to Cd-enriched food (Fig. 4a, c; Table 1). When feeding on yeast nominally containing 50, 100, and 200 μg Cd g^{-1} the $\delta^{15}N$ in *F. candida* tissues exceeded that of prey fed control yeast by 58.2, 79.3 and 73.2% in the plate system, and the corresponding values in the soil system were 65.2, 74.9 and 79.7%, respectively. Apart from *H. aculeifer* in the Cd100 treatment in the plate system, the $\delta^{15}N$ of *H. aculeifer* with collembolan fed yeast as prey in the control was lower than in the experimental treatments (Cd50 and Cd200) by 34.5 and 33.9%, respectively, in the plate system and the other treatments (Cd50, Cd100 and Cd200) by 13.6, 14.5 and 25.4%, respectively, in the soil system.

In the case of treatments with ^{15}N labelled wheat litter,

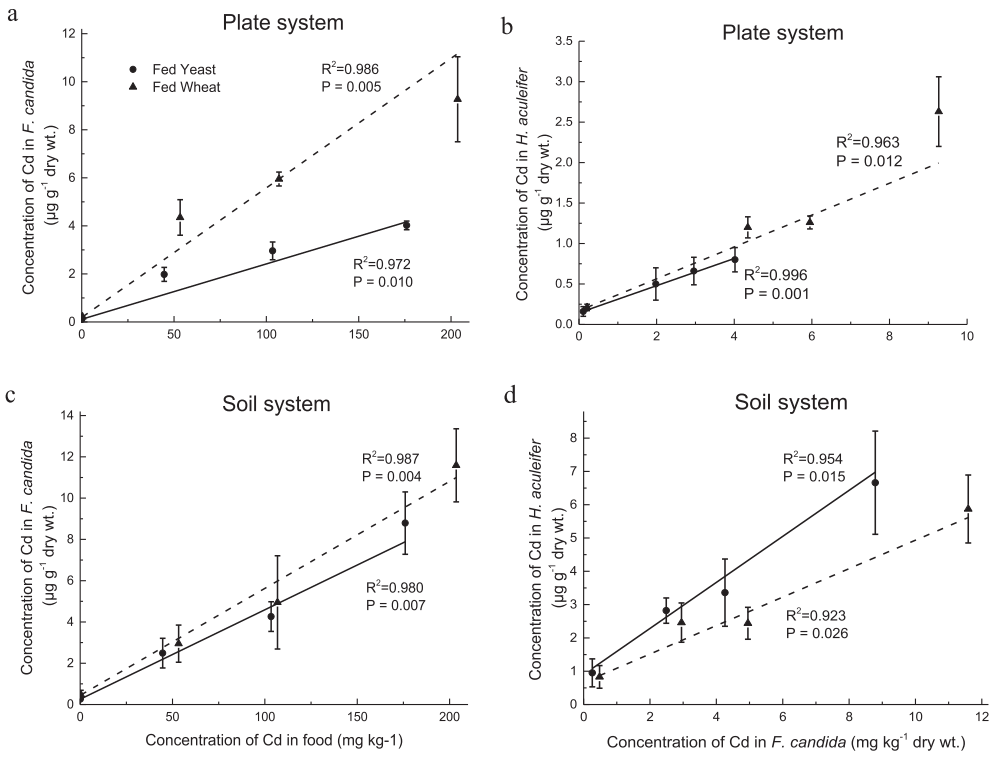

Fig. 3. Cadmium concentrations (mean ± SD, n = 4) in the collembolan *F. candida* (a, c) and the predator mite *H. aculeifer* (b, d) tissues after two weeks of exposure to Cd-enriched food. Fed Yeast indicates *F. candida* were fed Cd-spiked yeast (measured Cd: 0.08, 44.66, 103.4, 176.0 μg g^{-1}). Fed Wheat indicates *F. candida* were fed Cd spiked wheat (measured Cd: 0.41, 53.29, 106.8, 203.6 μg g^{-1}). The *H. aculeifer* were fed Cd-contaminated *F. candida* (a, c) pre-exposed to Cd spiked yeast or wheat. R^2: coefficient of determination, and significance (P) of the correlation was tested by linear regression analysis.

abundance of ^{15}N (atom% ^{15}N) in *F. candida* and *H. aculeifer* body tissues declined significantly as the Cd concentration in the dietary wheat increased (Fig. 4b, d, Table 1). Apart from *F. candida* in the Cd50 treatment in the plate system, the atom% ^{15}N values of *F. candida* and *H. aculeifer* in the control surpassed those in the experimental treatments (Cd100 and Cd200) by 59.0 and 95.4% in *F. candida* and in Cd50, Cd100 and Cd200 by 21.7, 52.0 and 66.7% in *H. aculeifer* in the plate system, with corresponding values in the soil system of 20.7, 34.2 and 48.4% in *F. candida* and 19.4, 22.9 and 37.9% in *H. aculeifer*. Atom% ^{15}N values in *F. candida* and *H. aculeifer* in the soil system were significantly higher than in the plate system (around twice as high) (Table 1).

4. Discussion

As hypothesized, Cd can be absorbed by detritus collembolans through their Cd-spiked food fungi/litter in contaminated environments and can accumulate in their body tissues. The Cd that has accumulated in the detritus consumer collembolans can be transferred to their predators (predatory mites) and accumulate in the predator tissues with higher BFA values (0.20–1.13). Dietary exposure of Cd significantly inhibited the growth and reproduction of the F_0 generation collembolan, enriched ^{15}N fractionation of collembolan tissues and decreased N content from litters in collembolans. Furthermore, Cd trophic transfer significantly increased the Cd concentrations in juvenile tissues of predatory mites, reduced the reproduction of predatory mites, promoted ^{15}N fractionation of predatory mite tissues and restricted the movement of N from collembolans to predatory mites. These results suggest that Cd food exposure can produce a risk of secondary toxicity to predators mediated by consumers, and soil food webs in Cd contaminated ecosystems may be more vulnerable in N transfer function and variable with higher $\delta^{15}N$ values on average in animals at higher trophic levels.

4.1. Cadmium bioaccumulation, trophic transfer and toxic response

Bioaccumulation is an important sensitive indicator of the exposure of animals to heavy metals in contaminated environments (Ardestani et al., 2014), and the efficiency of Cd trophic transfer in food chains can reflect the Cd risk to animals in higher trophic levels. Cadmium concentrations in both *F. candida* and *H. aculeifer* tissues showed maximum values on exposure to the highest concentration of Cd-spiked food (Cd200) in our study, and the highest Cd concentrations accumulated were 11.6 ± 1.02 and 6.7 ± 0.89 μg Cd g^{-1}, respectively. Unlike our results without biomagnification, body tissues of the snail *Helix aspersa* fed Cd-contaminated food (83.2 ± 6.53 μg g^{-1}) for two weeks contained 282.3 μg Cd g^{-1} with bio-magnification but tissues of its predatory carabid beetle, *Chrysocarabus splendens*, contained 0.53 ± 0.08 μg Cd g^{-1} (Scheifler et al., 2002), showing that Cd accumulation of predatory mites is much higher than that of predatory carabid beetles. Moreover, a significant negative correlation between Cd concentrations in juveniles of *H. aculeifer* (prey: fed *F. candida* with wheat) tissues and reproduction of the *H. aculeifer* was observed and reproduction of *F. candida* was inhibited only at the Cd200 treatment (Fig. 1). The BAF values of adults and juveniles of *H. aculeifer* were 0.20–1.13 and 0.09–0.51 except in the control in our study. The BAF values of predatory mites were higher than those of other similar predators such as the snail *H. aspersa* to the carabid beetle *Chrysocarabus splendens* at only 0.002 (Scheifler et al., 2002), from *H. aspersa* to the *Wistar* rat at 0.024 (Hispard et al., 2008) and from the oligochaete *Lumbriculus variegatus* to the rainbow trout *Oncorhynchus mykiss* at

Table 1
Analysis of variance of the Cd concentration, total nitrogen (N), $\delta^{15}N$, atom% ^{15}N and reproduction of the parental (F_0) generation *F. candida* (F_0 *F. can*) and *H. aculeifer* (*H. acu*) and the F_0 generation *F. candida* body length using the general linear model.

	df	F_0 *F. can.*		*H. acu.*	
		SS	*F*	SS	*F*
Cd concentration					
Sys.	1	11.78	11.48**	79.71	313.5***
Food	1	54.90	53.52***	0.35	1.37
Tre.	3	186.4	181.8***	32.86	129.2***
Sys. × Food	1	10.62	10.35**	7.70	30.27***
Sys. × Tre.	3	12.97	12.65***	10.77	42.34***
Food × Tre.	3	10.27	10.01***	0.30	1.18
Sys. × Food × Tre.	3	1.40	1.36	1.06	4.18*
$\delta^{15}N$					
Sys.	1	25.35	86.12***	0.07	0.10
Tre.	3	8.71	29.61***	14.30	19.67***
Sys. × Tre.	3	0.42	1.44	2.99	4.11*
Atom% ^{15}N					
Sys.	1	24.43	1970***	1.06	1145***
Tre.	3	1.47	118.2***	0.08	85.21***
Sys. × Tre.	3	0.26	21.22***	0.002	2.54
Total N					
Sys.	1	136.5	316.5***	6.58	15.01***
Food	1	25.28	58.59***	2.24	5.12*
Tre.	3	0.15	0.34	0.29	0.67
Sys. × Food	1	5.77	13.38**	0.17	0.39
Sys. × Tre.	3	0.02	0.06	0.99	2.27
Food × Tre.	3	0.68	1.57	0.80	1.81
Sys. × Food × Tre.	3	0.84	1.95	1.01	2.30
Body length					
Food	1	6.36	330.2***		
Tre.	3	0.11	5.51**		
Food × Tre.	3	0.28	14.61***		
Reproduction					
Food	1	957.0	3.50	3042	48.11***
Tre.	3	4233	15.44***	264.2	4.18*
Food × Tre.	3	32.37	0.12	136.3	2.16

Cadmium concentration and total N data were analysed using three-way analysis of variance including system (sys.) (plate and soil; n = 2), food (yeast and wheat; n = 2) and Cd treatment (tre.) (control: spiked with a trace of Cd, Cd50: 50 μg g^{-1}, Cd100: 100 μg g^{-1}, and Cd200: 200 μg g^{-1}; n = 4). Repeated-measures analysis of variance for $\delta^{15}N$ and atom% ^{15}N data included system and Cd treatment and was used to analyse body length and reproduction data, with food and Cd treatment as between-subject factors. Abbreviations: df indicates degrees of freedom and SS expresses sum of squares. (Significant differences: *P < 0.05; **P < 0.01; ***P < 0.001.)

0.009–0.064 (Ng and Wood, 2008). These results indicate that Cd transfer from Cd-spiked food mediated by *F. candida* can present a higher ecological risk for the predator *H. aculeifer*.

The collembolan two-generation test is a valuable tool which makes the risk assessment of pollutants more efficient (Ernst et al., 2015). Notably, no effect was observed in reproduction of the F_1 generation *F. candida* after any of the Cd exposure treatments in our study (Fig. S1), suggesting that the effect of Cd transfer from the F_0 to the F_1 generation on collembolans was not clear. Cadmium accumulation, ^{15}N fractionation and N trophic transfer of animal tissues in the soil system were higher than those in the plate system. The explanation may be that *F. candida* and *H. aculeifer* foraged for more food to obtain more energy in the soil system. Soil is a complex ecosystem with multiple interfaces and numerous factors (e.g. pH, OM, CEC and fungi) in the soil that can affect the metabolism of the soil fauna. However, the tendencies of change were accordant overall in the plate and soil systems.

4.2. Stable nitrogen isotope fractionation of animal tissues

One of the most important limitations in the use of N isotope fractionation ($\delta^{15}N$) in soil detritus animals is that $\delta^{15}N$ values may vary with natural (e.g. diet, temperature and life stage) and anthropogenic (contaminant exposure) factors (Vanderklift and Ponsard, 2003; Ek et al., 2015). Compared to the effects of natural factors on ^{15}N fractionation, effects of anthropogenic factors have been much less understood, especially in soil ecosystems (Staaden et al., 2010). In our study the fractionation of ^{15}N in the tissues of the collembolan *F. candida* was significantly enriched in all Cd-contaminated food treatments compared to the control, indicating that Cd-spiked diets can increase the $\delta^{15}N$ value of consumer collembolans. Although the underlying mechanisms of the shift of $\delta^{15}N$ value are not well understood, it is known that metabolic reworking and growth dilution are major factors driving ^{15}N fractionation (Carleton and Del Rio, 2010; Ek et al., 2015). Isotopic incorporation per unit of synthesized biomass will increase with increasing metabolic turnover (Tarboush et al., 2006; Ek et al., 2015). In the Cd exposure environment the storage of Cd is mainly in the midgut epithelium of collembolans (Vijver et al., 2004), and they have a greater tolerance of Cd in the diet through intestinal exfoliation during moulting (Ardestani and van Gestel, 2013; Ardestani et al., 2014), suggesting that metabolic turnover of Cd stressed collembolans will quicken to form new epithelial cells (Vijver et al., 2004). In a Cd-exposed *F. candida* study, Maria et al. (2014) also showed that lipids were strongly damaged and antioxidant enzymes were significantly affected in relation to metabolic detoxification. Thus, the shift in collembolan metabolic turnover necessary for detoxification is an important mechanism leading to the increase in the $\delta^{15}N$ of *F. candida* by exposure to Cd-spiked food. Within a certain time, changes in body length are generally considered to reflect animal growth rates. Our study found that the body length of *F. candida* increased at exposure to relatively low Cd treatments (Cd50 and Cd100) fed yeast compared to the control in the soil system (Fig. 2a), indicating that intermediate Cd exposure can increase the growth rate of collembolans due to hormesis. Previous studies indicate that when the growth rate is higher, the $\delta^{15}N$ value may be lower due to growth dilution (Ek

Table 2
Bioaccumulation factors (BAF) for the transfer of Cd between different trophic levels of the fungus (yeast)/litter (wheat)–collembolan *F. candida* (*F. can.*)–predatory mite *H. aculeifer* (*H. acu.*) food chain.

Exposure treatment	Food	Plate system BAF		Soil system BAF		
		F. can.	Adult *H. acu.*	*F. can.*	Adult *H. acu.*	Juvenile *H. acu.*
Control	Yeast	1.38	1.45	3.25	3.65	0.92
	Wheat	0.51	1.02	1.17	1.73	0.65
Cd50	Yeast	0.04	0.25	0.06	1.13	0.25
	Wheat	0.08	0.28	0.06	0.83	0.30
Cd100	Yeast	0.03	0.22	0.04	0.79	0.14
	Wheat	0.06	0.21	0.05	0.49	0.22
Cd200	Yeast	0.02	0.20	0.05	0.76	0.09
	Wheat	0.05	0.28	0.06	0.51	0.51

F. candida were exposed to Cd spiked food (yeast/wheat) (control: spiked with a trace of Cd, Cd50: 50 μg g^{-1}, Cd100: 100 μg g^{-1}, and Cd200: 200 μg g^{-1}), and *H. aculeifer* were exposed to increasing Cd concentrations via the prey *F. candida* after two weeks of exposure.

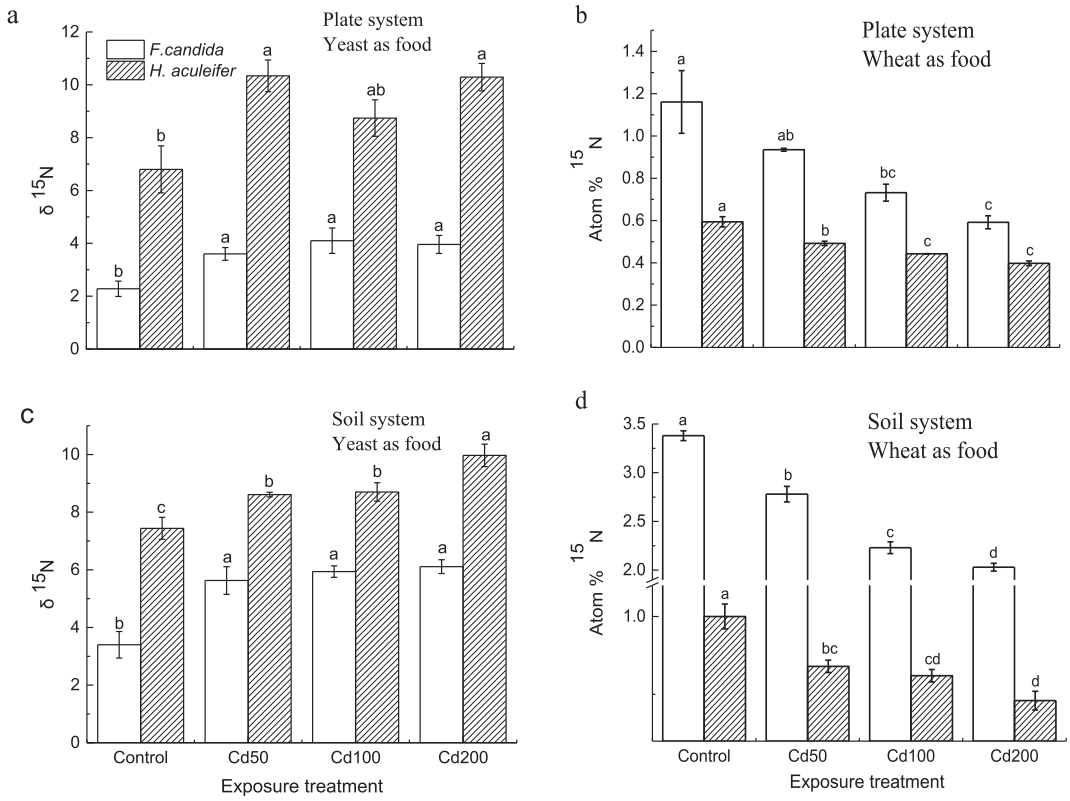

Fig. 4. $\delta^{15}N$ (a, c) and atom% ^{15}N (b, d) (mean ± SE, n = 4) in collembolan *F. candida* and predator mite *H. aculeifer* tissues after two weeks of exposure to Cd-enriched food. The *F. candida* (a, c) were exposed to control yeast (spiked with a trace of Cd), 50 μg g^{-1} (Cd50), 100 μg g^{-1} (Cd100), and 200 μg g^{-1} (Cd200). After two weeks, the *H. aculeifer* (a, c) were fed the Cd-contaminated *F. candida* (a, c) pre-exposed to Cd spiked yeast. The ^{15}N enriched wheat (control: spiked with a trace of Cd, Cd50: 50 μg g^{-1}, Cd100: 100 μg g^{-1}, and Cd200: 200 μg g^{-1}) were offered to the *F. candida* (b, d). Then the *H. aculeifer* (b, d) were fed the Cd-contaminated *F. candida* (b, d) pre-exposed to Cd spiked wheat. Different letters indicate significant differences ($P < 0.05$) among exposure treatments (by LSD) in *F. candida* or *H. aculeifer*.

et al., 2015). However, there was a positive effect of growth rate on $\delta^{15}N$ enrichment at a Cd exposure concentration range of 0–100 mg Cd kg^{-1} (relatively low Cd treatments) in our study. Since more ^{15}N is incorporated by metabolic turnover at the low Cd exposure range, growth dilution will be comparatively low, leading to overall $\delta^{15}N$ enrichment in the collembolans at low Cd treatments. This might also imply that metabolic turnover makes more contribution to $\delta^{15}N$ variation than growth dilution in low Cd treatments. In the present study, at relatively high Cd treatment (Cd200) the lower growth of collembolans was associated with higher $\delta^{15}N$, in contrast to the low Cd treatments, suggesting that the contribution of growth mediation increased to $\delta^{15}N$ enrichment of collembolans at high Cd exposure. Hence, at low Cd exposure (Cd50 and Cd100) the $\delta^{15}N$ enrichment of collembolans can result from a higher contribution of metabolic turnover than of growth dilution, and the contribution of growth-mediated incorporation to the variation in $\delta^{15}N$ will increase at high Cd exposure (Cd200).

Moreover, different diets may significantly alter the fractionation of ^{15}N in collembolan tissues (Chahartaghi et al., 2005; Staaden et al., 2010). When the food yeast is spiked with Cd, collembolans can avoid the contaminated food and may ingest other resources to maintain their metabolism (Fountain and Hopkin, 2001; Pfeffer et al., 2010). In this way, collembolans might be feeding on something different and acquiring their ^{15}N from other sources due to Cd exposure, which may also make a contribution to the difference in $\delta^{15}N$ of the collembolans. Resources fed to the collembolans are much richer in the soil system than in the plate system. The ^{15}N fractionation of collembolan tissues in the soil system was higher than that in the plate system in the present study, which further supports our suggestion.

In the soil system the $\delta^{15}N$ of predatory mite *H. aculeifer* tissues increased significantly by preying on the consumer *F. candida* exposed to Cd-spiked yeast compared to the control, which was in line with our hypothesis, indicating that the presence of Cd in the decomposer can indirectly enrich the ^{15}N fractionation of the predator mediated by the consumer. By calculating the differences in $\delta^{15}N$ between the predatory mite and the collembolan in all treatments we further found that the difference values were constant overall. The $\delta^{15}N$ of predators is known to increase with increasing $\delta^{15}N$ of their prey in unpolluted systems (Chahartaghi et al., 2005; Klarner et al., 2013). The $\delta^{15}N$ enrichment of the predatory mite may result mainly from higher ^{15}N fractionation in Cd stressed collembolans in our study. Moreover, apart from the Cd200 treatment in the soil system, the Cd concentration in the collembolan prey in all other treatments with the predatory mite as predator was much lower (the highest value was 4.26 ± 0.73 μg g^{-1}) than in *F. candida* with Cd-spiked yeast, and the survival and reproduction of the predatory mite showed no significant difference in all yeast-fed Cd exposure treatments relative to the collembolans. This may suggest that relatively low Cd exposure does not directly alter the $\delta^{15}N$ of the predatory mite through the prey. Interestingly, the $\delta^{15}N$ values of collembolans were constant in all treatments other than the control in the soil system but at Cd200 the $\delta^{15}N$ of the predatory mite was significantly higher than at Cd50 or Cd100 (Fig. 4c). This indicates that Cd enriched prey (8.79 ± 0.76 μg g^{-1}) exposure might also increase the ^{15}N fractionation of the predatory mite at relatively high Cd additions. Thus, the presence of Cd in yeasts can indirectly increase the ^{15}N fractionation of the predatory mite through the collembolan and can also directly enrich the $\delta^{15}N$ of the predatory mite by Cd

transfer in the yeast-collembolan-predatory mite food chain.

The present study suggests that when the $\delta^{15}N$ value of animals is used to analyse soil food web architectures, if the effect of Cd stress on the $\delta^{15}N$ values of collembolans and predatory mites is ignored this might lead to overestimates and give erroneous conclusions regarding the trophic levels of animals in Cd-contaminated environments. $\delta^{15}N$ shifts in the collembolan and predatory mite were observed at Cd concentrations below the toxic responses of classic indicators such as survival, reproduction and body length and the results in both plate and soil systems showed that the response of $\delta^{15}N$ to Cd exposure was comparatively stable and the $\delta^{15}N$ in soil animals also has great potential as a biomarker of Cd stress.

4.3. Nitrogen transfer from food

There are complex interactions among elements in biogeochemical cycles. For example, an iron-nitrogen coupling process is well known in natural soil ecosystems (Ding et al., 2015). However, the relationship between trophic transfer of pollutant elements (i.e. heavy metals) and biogeochemical cycles of nutrient elements such as N remain poorly understood in contaminated soil ecosystems, especially in the soil detritus food chain which plays a key role in the biogeochemical cycling of elements due to the presence of producers, decomposers and predators. In the present study, Cd trophic transfer significantly decreased N transfer from litters in the soil detritus food chain with exposure to Cd-spiked food, suggesting a negative effect of Cd transfer on the biogeochemical cycling rate of N mediated by the soil detritus food chain. Collembola can identify and avoid feeding on heavy metal contaminated food, and the excretion rate of collembolans will strengthen for detoxification exposure to Cd-spiked food (Fountain and Hopkin, 2001; Pfeffer et al., 2010). This might explain the reduction in N content in collembolans assimilated from Cd-spiked litters. In contrast, it has been reported that although animals consume less food when exposed to food highly contaminated with Cd, the assimilation efficiency of the food increases compared to the control (Drobne and Hopkin, 1995; Loureiro et al., 2006). Our study is the first to employ quantitative data via ^{15}N isotope tracer techniques revealing that the N content decreased in animals derived from the diet by exposure to Cd-contaminated food. Regarding the shift of N transfer in predatory mites from the litters, the rate of N transfer reduction in the predatory mite was higher than that in the collembolan (Fig. 4b, d), suggesting that Cd trophic transfer from collembolans to predatory mites might decrease the movement of N through them. Thus, the decline in movement of N in Cd stressed collembolans may result from both avoidance behaviour and excretion processes and in predatory mites apart from the effect of the prey collembolan is ascribed to other mechanisms resulting from Cd transfer but the specific mechanism also requires further investigation. Moreover, the present study suggests that a Cd-N coupling process might exist in the Cd-contaminated soil detritus food chain.

The shift in N transfer can directly reflect changes in the functioning of food webs (Hättenschwiler and Field, 2005). The present results reveal that Cd trophic transfer severely impaired the function of the soil detritus food chain in relation to N transfer. The change in N transfer in collembolans and predatory mites can be used as a functional ecological indicator in soil ecotoxicology studies, which contributes to elevating soil micro-arthropod indicator systems mainly on the basis of single species tests or isolated biological indicators. Moreover, the sensitivity of N transfer as an indicator of Cd-spiked food exposure was higher than that of classic indicators such as survival, reproduction and body length in our study.

5. Conclusions

In Cd-contaminated ecosystems, the Cd that has accumulated in the consumer collembolans from spiked food fungi/litter can be transferred to their predators (predatory mites) and accumulate in the predator body tissues with decline in reproduction of predatory mites, suggesting that Cd trophic transfer can produce a risk of secondary toxicity to higher trophic level predatory mites. The presence of Cd in the environment introduced by food will enrich ^{15}N fractionation of the consumer collembolan tissues, and Cd trophic transfer between collembolans and predatory mites from spiked food can also directly increase the $\delta^{15}N$ value of predatory mite tissues. Incorporated N content of Cd stressed collembolans from food decreased, and Cd trophic transfer restricted N transfer from food between consumer collembolans and predatory mites. These results suggest that the effect of Cd on the $\delta^{15}N$ shift should be considered in ^{15}N isotope application studies and the N transfer function of soil food webs may be more vulnerable in polluted ecosystems.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (Nos. 41271264 and 41325003). We thank the anonymous reviewers for helpful comments to improve the manuscript.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.soilbio.2016.07.026.

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