Ecotoxicity of cadmium in a soil collembolan-predatory mite food chain: Can we use the ^{15}N labeled litter addition method to assess soil functional change?[☆]

Dong Zhu [a, b], Xin Ke [c, **], Longhua Wu [a, *], Zhu Li [a], Peter Christie [a], Yongming Luo [d]

[a] Key Laboratory of Soil Environment and Pollution Remediation, Institute of Soil Science, Chinese Academy of Sciences, Nanjing 210008, China
[b] University of the Chinese Academy of Sciences, Beijing 100049, China
[c] Institute of Plant Physiology and Ecology, Shanghai Institutes of Biological Sciences, Chinese Academy of Sciences, Shanghai 200032, China
[d] Key Laboratory of Coastal Zone Environmental Processes, Yantai Institute of Coastal Zone Research, Chinese Academy of Sciences, Yantai 264003, China

A R T I C L E   I N F O

A B S T R A C T

Effects of cadmium (Cd) on predator-prey relationships and soil ecological function are poorly understood and there are few methods available to measure soil functional change. Thus, we structured a soil-dwelling food chain containing the predatory mite *Hypoaspis aculeifer* and its collembolan prey *Folsomia candida* to study the effects of Cd exposure for eight weeks in a spiked soil aged for five years. The ^{15}N labeled litter was added as food to analyze the change in nitrogen (N) transfer content. *H. aculeifer* reproduction and growth and the survival and reproduction of *F. candida* were all negatively affected by Cd exposure, and *H. aculeifer* reproduction was the most sensitive parameter. The sensitivity responses of *F. candida* and *H. aculeifer* were different from those using the previous single species test. The results suggest that predator—prey interactions might influence the toxicity of Cd by predation and food restriction. Cadmium lethal body concentrations of adults and juveniles of *F. candida* and *H. aculeifer* juveniles were 500—600, 180—270 and 8—10 µg g^{-1}, respectively. The content of N transfer from litter to animals in the food chain decreased significantly with increasing soil Cd concentration between 100 and 400 mg kg^{-1}. The results suggest that the ^{15}N labeled litter addition method is potentially useful for quantitative assessment of soil functional change for further risk assessment purposes.

1. Introduction

Soil microarthropods constitute one of the most species-rich components of the soil fauna and play an important role in soil ecosystems (Coleman et al., 2004; Lavelle et al., 2006; Soong et al., 2016). Thus, they have often served as important ecological receptors in soil ecological risk assessment and their value as indicators of environmental pollution has long been investigated (van Gestel, 2012; Zhao et al., 2013; Stankovic et al., 2014). So far, indicator systems for soil microarthropods have been built mainly on the basis of single species tests (van Gestel, 2012). However, through participating in soil ecological processes (e.g. the biogeochemical cycling of carbon and nitrogen), soil organisms are

tightly related with each other as a whole and interact intimately in realistic soil ecosystems (Sechi et al., 2014). When chemical substances produce adverse influence on a species they will likely also affect the other species and soil ecological processes indirectly. It is therefore vital to enhance our knowledge of how chemical substances affect interactions such as predator-prey relationships, competition for resources, beneficial mutualistic interactions and commensalism in the soil food chain/web and soil ecological function (Coleman et al., 2004). However, only a few studies have involved the effects of chemical substances on the interactions between species and soil ecological function (Scott-Fordsmand et al., 2008; Jensen and Scott-Fordsmand, 2012; Schnug et al., 2014; Sechi et al., 2014).

Several types of semi-field systems have been used to simulate the processes and interactions of natural situations under controlled conditions to improve our evaluation and understanding of the toxicities of pollutants on the above-mentioned soil faunal species interactions (Schnug et al., 2014). van Voris et al. (1985)

firstly presented a Terrestrial Model Ecosystem (TME) test which exposes an indigenous soil-dwelling species assemblage or community by collecting intact field soil cores to an additional toxicant to evaluate the effects of pollutants under indoor or outdoor conditions. Subsequently, numerous studies have further developed and improved the TME by exposing a mixture of indigenous and added animals or entirely added animals extracted from field soil cores to the contaminated soil (Burrows and Edwards, 2002; Cortet et al., 2006; Scott-Fordsmand et al., 2008; Sechi et al., 2014). These systems, by employing an indigenous pool of organisms in field intact soil cores, closely resemble the real field ecosystem, but they will inevitably produce large variation and low stability between replicates due to the spatio-temporal heterogeneity of natural soil ecosystems (Heemsbergen et al., 2004). This may reduce the ability of the statistical tests to discriminate between treatment differences and the repeatability of the results, especially at relatively low concentrations of contaminants. To remedy the gap between gaining more real ecosystem information and lower variability, studies have increasingly focused on soil-multi-species (SMS) test systems which construct a model soil food chain/web (Scott-Fordsmand et al., 2008; Jensen and Scott-Fordsmand, 2012; Schnug et al., 2014; Sechi et al., 2014). The constructed systems do not reflect the complete information about organisms in real soil ecosystems but they contain different functional groups of specific organisms representing the soil ecosystem in a simplified way and the basic ecological interactions (e.g. predation, competition). Moreover, the species composition of the test systems can be homogenous and adjusted for different research purposes and concerns and they also enable high reproducibility of tests.

Soil faunal communities make a strong contribution to the functioning of soil ecosystems (e.g. organic matter decomposition, carbon and nitrogen cycling) (Hättenschwiler and Field, 2005). However, toxicity tests of soil animals have rarely included relevant functional endpoints due to the availability of only two methods to evaluate soil ecosystem function (André et al., 2009; Römbke, 2014). The most popular classic, well-known, and frequently used method is the litter-bag method mass loss of litter in soil ecosystems is detected (Crossley and Hoglund, 1962; Alchami et al., 2016; Mackintosh et al., 2016). Nevertheless, this method is relatively time-consuming and laborious, and it is difficult to identify the contribution of the soil fauna or microbial community. An alternative approach is the bait-lamina method (Törne, 1990) and its frequency of use is currently increasing (Römbke, 2014; Klimek et al., 2015). It is a very simple and rapid means of measuring the feeding activity (and small-scale distribution) of soil fauna to assess changes in function. Compared with the litter-bag method, however, the link between its endpoint (feeding rate) and soil function remains controversial (Römbke, 2014). Hence, there is a pressing need to develop and improve relevant functional methods and endpoints which can be included in toxicity tests of soil animals.

Cadmium (Cd) is a major heavy metal pollutant element in many soils and exhibits high toxicity and non-biodegradability (Nordberg et al., 2015). The present study constructs a typical soil food chain as the model system to test the ecotoxicity of Cd. The model food chain consists of the collembolan *Folsomia candida* and the predatory mite *Hypoaspis aculeifer*. Collembolans are important secondary decomposers in soil ecosystems and *H. aculeifer* is a pivotal predator of collembolans (Geisen et al., 2015). The model system was therefore driven by predator-prey relationships. Cadmium cannot exert direct toxicity to the collembolan and predatory mite but may affect them indirectly by altering the numbers of prey or predator. In addition, we used wheat materials isotopically labeled with nitrogen (^{15}N) as food to simulate litter and trace N element transfer. Changes in N recycling can provide direct evidence of changes in soil function (Hättenschwiler and Field, 2005). Finally,

we compared the differences in sensitivity between the previous single-species and the model food chain test systems.

The present study sought to incorporate classic and functional species endpoints based on the model food chain and to develop a new method to assess soil function. We hypothesized (1) that the predator-prey relationship would influence species sensitivities to soil Cd pollution and (2) that Cd pollution might restrict N nutrient transfer in the model food chain.

2. Materials and methods

2.1. Collembolan and predatory mite

The collembolan *Folsomia candida* is a widespread parthenogenetic microarthropod (Fountain and Hopkin, 2005) and the gamasid mite *Hypoaspis aculeifer* is a relevant representative and hemiedaphic/eu-edaphic polyphagous predatory mite (Jensen and Scott-Fordsmand, 2012). The procedures for culturing and synchronizing animals are consistent with the methods of Zhu et al. (2016a). The ages of *F. candida* and *H. aculeifer* were 10–12 and 32–35 days, respectively, in our tests.

2.2. Soil and food

A light clay soil (udic-ferrosols) was collected from the top 15 cm of the soil profile in a forest at Yingtan city, Jiangxi province, southeast China. Before use the soil was air dried at ambient temperature in the shade and sieved through a 2-mm mesh. Selected physico-chemical properties of the soil were as follows: pH $_{water}$ = 4.9, organic matter = 5.77 g kg^{-1}, cation exchange capacity = 9.76 cmol (+) kg^{-1}, total N = 1.04 g kg^{-1}, Cd = 0.18 mg kg^{-1}, Pb = 36.5 mg kg^{-1}, Zn = 108 mg kg^{-1}, Cu = 17.1 mg kg^{-1}. Cadmium nitrate was dissolved in deionized water to obtain a stock solution of Cd (10 g Cd kg^{-1}). 0, 0.625, 1.25, 2.5, 5, 10 and 20 mL Cd stock solution were mixed with 500 g dry soil to produce a series of Cd spiked soils. Nominal concentrations in the soil were accordingly 0, 12.5, 25, 50, 100, 200 and 400 mg Cd kg^{-1} dry soil. The moisture content of the spiked soil was adjusted to 50% of water holding capacity (WHC) and the spiked soil was aged in sealed plastic containers at 20 ± 2 °C over five years. During the aging of the soil deionized water was added at monthly intervals to maintain the moisture content.

The study employed ^{15}N-labeled (5.54 ± 0.07 atom %) dried wheat-straw powder N content 2.18 ± 0.05%, Cd concentration 0.41 ± 0.12 mg kg^{-1}. The procedure of Zhu et al. (2016a) using ^{15}N-labeled wheat as food was followed to simulate litter.

2.3. Cadmium exposure assay

The ecotoxicity test was performed in plastic containers (5 cm high, 5.5 cm inner diameter) and each container contained 45 g moist soil (Fig. 1). Four replicates of each treatment were established and a total of 15 mg labeled wheat-straw powder was added as food to each replicate throughout the exposure test. At the start of exposure, 15 synchronized *F. candida* individuals (10–12 days) were carefully transferred into each container and 10 mg ^{15}N-labeled wheat-straw powder was scattered evenly on each soil surface as food. On the seventh day each container was replenished with 5 mg ^{15}N-labeled wheat straw powder. After exposure for 14 days, ten individuals of *H. aculeifer* (adult females, 32–35 days) were introduced into each container to construct a collembolan-predatory mite food chain test system. The exposure of *F. candida* prior to *H. aculeifer* aimed at producing sufficient food for the predatory mite (Jensen and Scott-Fordsmand, 2012) and ensuring the comparability of *F. candida* testing as generally 10 or 15
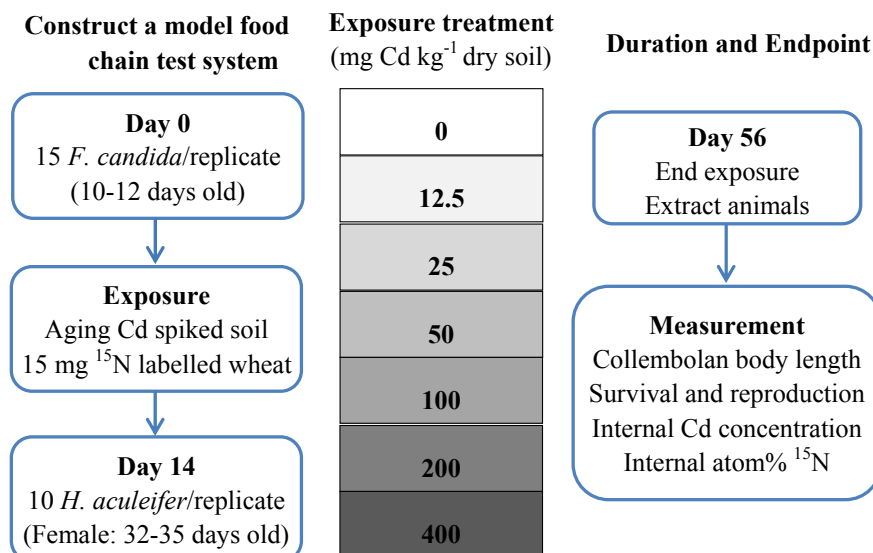
Construct a model food chain test system | **Exposure treatment** (mg Cd kg^{-1} dry soil) | **Duration and Endpoint**

Fig. 1. Experimental design scheme of the model food chain test system for cadmium (Cd) exposure (four replicates per treatment).

individuals of *F. candida* were added in its single species test (Bur et al., 2010). The provision of ^{15}N-labeled wheat-straw powder halted after addition of the predatory mite. Replace of lost water continued by dripping deionized water onto the soil surface twice a week. The total duration of the test exposure was 56 days.

After exposure the containers were placed in a controlled temperature gradient extractor and ramped from 25 to 45 °C for two days to extract the animals from the soil (Fountain and Hopkin, 2001). The extracted animals were suspended on the surface of water by adding 10 mL deionized water mixed with a drop of blue ink (Specification: 232, Hero Fountain Pen Factory Co., Ltd, Shanghai, China). Subsequently, the suspended animals were photographed to count and measure body lengths of *F. candida* using the Image J 1.47a software package (National Institutes of Health, Bethesda, MD). Finally, the extracted animals were divided into four classes, namely *F. candida* juveniles, *F. candida* adults, *H. aculeifer* juveniles and *H. aculeifer* adults. They were then placed on humid filter paper to remove interfering substances from their guts. The animals were ultrasonically cleaned for 15 min, dried at 60 °C for 2 days, weighed in a Model XS3DU automatic electronic balance (precision ± 1 μg, Mettler Toledo, Columbus, USA) and stored in a desiccator at 4 °C prior to analysis.

2.4. Chemical analysis

Cadmium concentrations of the animal tissues were determined according to the method of Zhu et al. (2016b). Briefly, the weighed animals were digested at 105 °C for 4 h with 50 μL of a 3:1 mixture of ultrapure nitric acid and hydrogen peroxide (Nanjing Chemical Reagent Co., Ltd., Nanjing, China) in small glass test tubes which were inserted into a high pressure digestion vessel.

Approximately 0.20-g air-dried soil samples were digested with 10 mL mixed acid (HCl: HNO$_3$) to determine total Cd concentrations (Luo et al., 2014b). The aging soil was extracted using 0.01 M CaCl$_2$ to evaluate Cd availability. Air-dried soil (2 g) was extracted with 0.01MCaCl$_2$ (20 mL) by shaking for 2 h at 20 °C, then centrifuging at 3000 rpm for 10 min and filtering through a 0.45 μm membrane filter to obtain the exchangeable solution.

Cadmium concentrations were determined by graphite furnace atomic absorption spectrophotometry (Varian 220FS, 220Z, Palo Alto, CA). The quality of the animal and soil Cd determinations was

controlled with replicate samples, blanks, and certified reference materials (GBW10051, pork liver and GBW07401, a dark brown podzolic soil, provided by the Institute of Geophysical and Geochemical Exploration, Langfang, Hebei, China). The Cd recoveries of the reference materials were always between 85 and 115%.

^{15}N isotope signatures of *F. candida* and *H. aculeifer* adult tissues were determined with a Flash EA 2000 Series Elemental Analyzer connected via a Conflo IV to a Delta V Advantage isotope ratio mass spectrometer (Thermo Finnigan, Waltham, MA) (EK et al., 2015). Atom% ^{15}N directly indicates the values of the ^{15}N isotope signature measurements in animal tissues. The quality of ^{15}N isotope signature measurements was controlled using an internal reference (fish muscle tissue) after each batch of 10 samples. The precision of the ^{15}N analysis was <0.10‰. The reference value of ^{15}N was based on that of the atmospheric N$_2$ (air).

2.5. Statistical analysis

Analysis of variance (ANOVA), Student's t-test and least significant difference (LSD) test were performed using the IBM SPSS v. 21 statistics software package at the 5% level and data are expressed as mean ± standard error (SE). The LC values for *F. candida* survival were calculated by a generalized linear model with a binomial distribution and logit link function fit, and significant effects of Cd pollution on adult survival were undertaken by the generalized linear model with a binomial distribution that employs the chi-square test to analyze significance (P < 0.05) in R version 3.3.1. The ECx values were computed with a dose-effective curve based on a four-parameter Weibull model for *F. candida* and a Cedergreen-Ritz-Streibig model for *H. aculeifer* using the package 'drc' version 2.5—12 of R version 3.3.1, and the lowest observed effect concentration (LOEC) was calculated by comparison of the differences between treatments and the control. Significance analysis between EC values employed the t-test based on the EC value obtained and its standard deviation (P < 0.05). For frequency analysis of *F. candida* body lengths, the width of body length sub-range was set to 0.1 mm and the total *F. candida* individual number of each treatment in each sub-range was counted and is presented using OriginPro 9.1.

3. Results

3.1. Body length of F. candida

The body lengths of *F. candida* adults ($F_{6, 137} = 59.03$, P < 0.01) and juveniles ($F_{6, 555} = 5.39$, P < 0.01) changed significantly after exposure to Cd (Fig. 2). Mean values of adult length increased with increasing soil Cd concentration from 0 to 50 mg Cd kg^{-1} and decreased between 50 and 400 mg Cd kg^{-1} (Fig. 2b), and mean values of juvenile length showed a downward trend overall (Fig. 2d). Compared with 0 mg Cd kg^{-1} (Control), adult length was significantly lower (by 21 and 24%) at 200 and 400 mg Cd kg^{-1}, respectively, and juvenile length initially declined significantly (by 10%) at 25 mg Cd kg^{-1}, (*t*-test, P < 0.01).

The size distribution of adult body length shows that individual adult lengths ranging between 0.8 and 1.0 mm occurred only at 200 and 400 mg Cd kg^{-1}, and their percentage was >46% (Fig. 2a). The percentage of adults with length >1.5 mm was highest at 50 mg Cd kg^{-1} (>22%). With the sole exception of the 100 mg Cd kg^{-1} treatment the percentage of juveniles in the range 0.2–0.5 mm increased with increasing Cd concentration (Fig. 2c).

3.2. Survival and reproduction

The number of *F. candida* adults decreased significantly except at 50 mg kg^{-1} ($F_{6, 21} = 17.95$, P < 0.01) with increasing soil Cd concentration and the number of *H. aculeifer* adults, >7 individuals, showed no significant change (Fig. 3a). Notably, the survival of *F. candida* adults was significantly lower than at 0 mg Cd kg^{-1}, by 22% between 12.5 and 25 mg Cd kg^{-1} (*t*-test, P < 0.05). The number of adults showed an upward trend at 50 mg Cd kg^{-1}.

Soil Cd concentration and reproduction showed a good dose-response relationship (Fig. 3b). Reproduction of *F. candida* and *H. aculeifer* did not change significantly between 0 and 50 mg Cd kg^{-1} or between 12.5 and 50 mg Cd kg^{-1}, respectively. Compared to 0 mg kg^{-1}, however, reproduction of *H. aculeifer* declined significantly by 45% at 12.5 mg Cd kg^{-1} (*t*-test, P < 0.05).

3.3. Cadmium toxicity threshold

The EC10, EC50 and LOEC values of reproduction of *H. aculeifer* were lower than those of survival or reproduction of *F. candida* (*t*-test, P < 0.05), and the differences in sensitivity among indicators (survival, reproduction and body length) on the basis of total soil Cd were consistent with those based on extractable Cd (Table 1). Taking EC50 as the toxicity threshold, the five indicators followed the sequence in sensitivity *H. aculeifer* reproduction > *F. candida* reproduction = *F. candida* survival > *F. candida* adult length = *F. candida* juvenile length and the values based on soil Cd concentration were 35.0, 91.6, 136, 513 and 524 mg Cd kg^{-1}, respectively.

3.4. Soil exchangeable Cd concentration

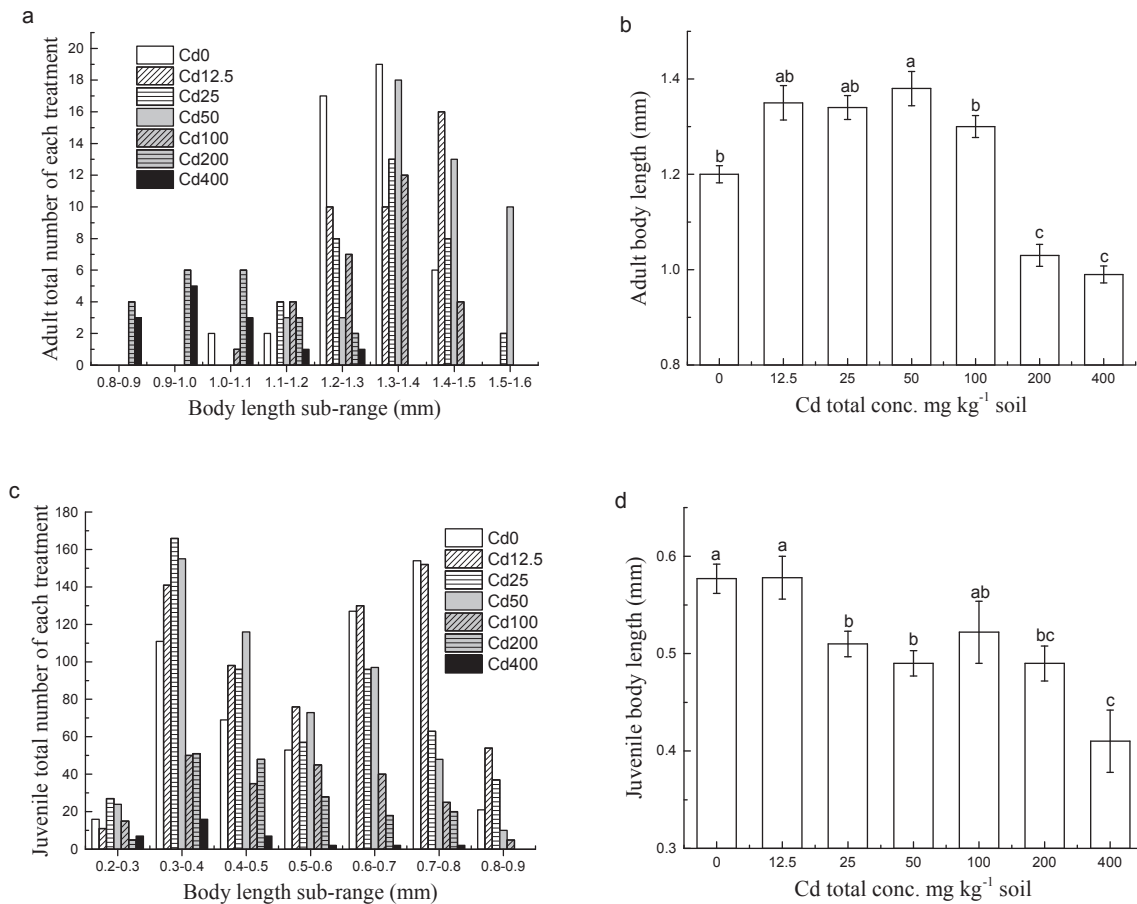The CaCl$_2$-extractable Cd concentration increased with the soil

Fig. 2. Total number of (a) *F. candida* adults and (c) juveniles for each length class (mm) of each treatment in the Cd spiked soil aged for more than five years, and body lengths (mean ± SE) of (b) *F. candida* adults and (d) juveniles in each treatment based on Cd total nominal concentration (conc.). Different letters (b, d) indicate significant differences (*p* < 0.05) among Cd exposure treatments (LSD test).
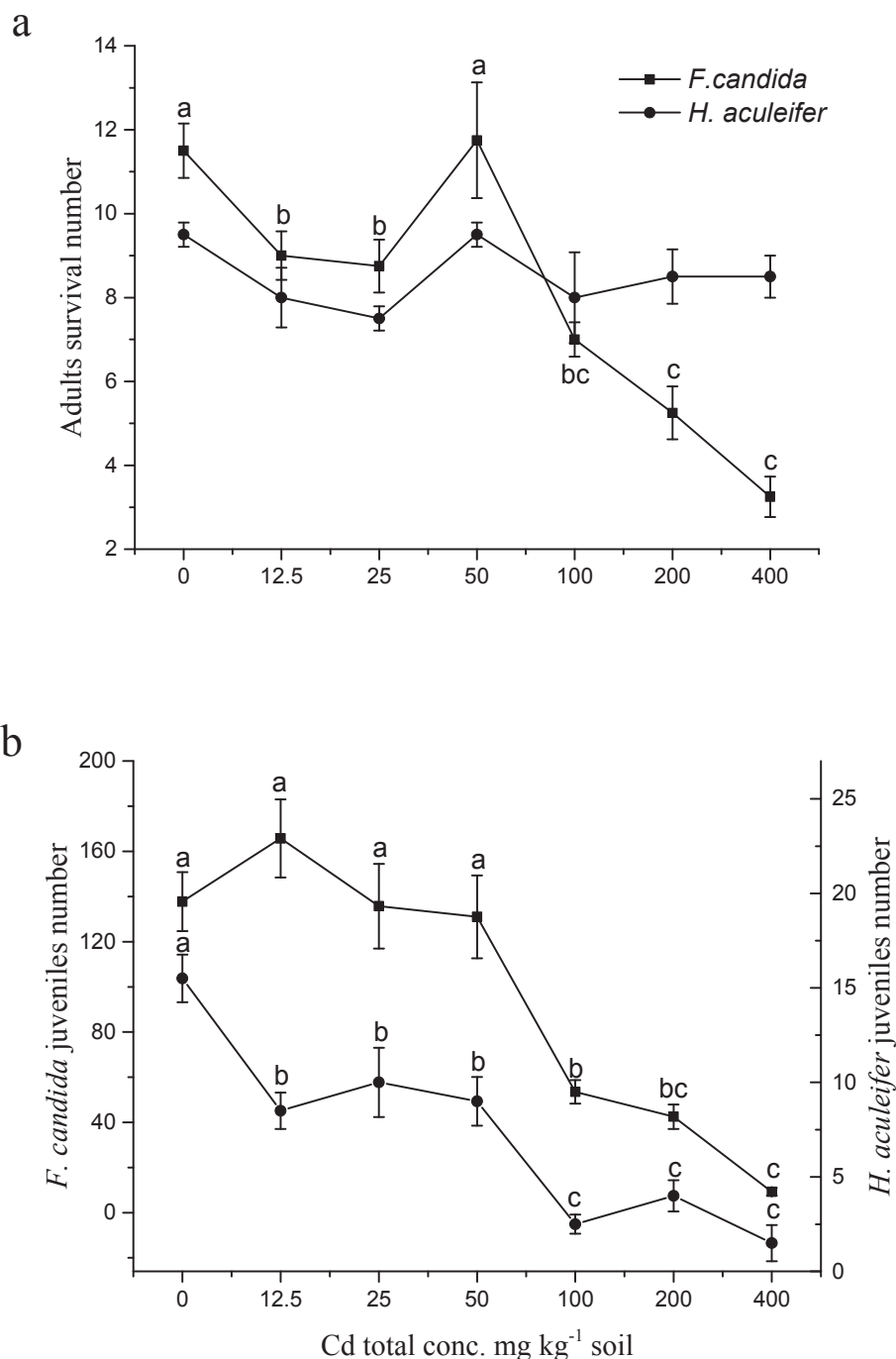
Fig. 3. Dose-response relationship of the survival ((a) mean number of adults ± SE, n = 4) and the reproduction ((b) mean number of juveniles ± SE, n = 4) of *F. candida* and *H. aculeifer* exposed to Cd contaminated soil (based on nominal concentrations), respectively. Different letters indicate significant differences ($p < 0.05$) among Cd exposure treatments (the chi-square test for survival data and LSD test for reproduction data) in *F. candida* or *H. aculeifer*.

Cd nominal concentrations (Table 2). The CaCl$_2$-extraction efficiency was 0.28–0.52 and increased with increasing soil Cd concentration.

3.5. Cadmium concentrations of animal tissues

Cadmium concentrations in animal tissues increased highly significantly (F-test, P < 0.01) with increasing soil Cd concentration (Fig. 4). Cadmium concentrations of *F. candida* adults reached maximum values (500–600 μg Cd g^{-1}) within the range 100–400 mg Cd kg^{-1} (Fig. 4a) and the highest Cd concentrations (50–60 μg Cd g^{-1}) in *H. aculeifer* adults were observed between 200 and 400 mg Cd kg^{-1} (Fig. 4c). Under the same soil Cd concentration conditions the Cd concentration of *H. aculeifer* juveniles was higher than that of the adults and, in contrast, the Cd concentration in *F. candida* juveniles was lower than in the adults. Omitting 0 mg Cd kg^{-1}, the bioconcentration factors of *F. candida* (adults: 1.54–6.52, juveniles: 2.12–2.89) were higher than those of *H. aculeifer* (adults: 0.28–0.65, juveniles: 0.56–0.75) on the basis of soil Cd concentration.

Table 1
The LC10, LC50 and LOEC values (with corresponding 95% confidence intervals) for survival (adult numbers) of the collembolan *Folsomia candida* (exposure for 8 weeks), and the EC10, EC50 and LOEC values (with corresponding 95% confidence intervals) for reproduction (juvenile numbers) and growth (body length) of the collembolan *F. candida* and reproduction (juvenile numbers) of the predatory mite *Hypoaspis aculeifer* (exposure for 6 weeks) in a Cd spiked soil aged for more than five years.

	EC10 (LC10)	EC50 (LC50)	LOEC
F. candida			
Adults			
Total	–	136 (71.2–208)	11.6 ($p < 0.05$)
CaCl$_2$ extractable	–	58.4 (20.4–96.5)	4.12 ($p < 0.05$)
Juveniles			
Total	20.9 (3.08–38.7)	91.6 (59.9–123)	94.1 ($p < 0.01$)
CaCl$_2$ extractable	15.3 (2.84–27.7)	29.4(20.5–38.2)	38.1 ($p < 0.01$)
Body length of adults			
Total	178 (123–206)	513(412–635)	183 ($p < 0.01$)
CaCl$_2$ extractable	71.2 (45.6–93.3)	256(135–342)	86.9 ($p < 0.01$)
Body length of juveniles			
Total	14.3 (8.12–38.5)	524 (387–713)	24.5 ($p < 0.01$)
CaCl$_2$ extractable	6.23 (3.84–11.2)	271 (154–468)	9.37 ($p < 0.01$)
H. aculeifer			
Juveniles			
Total	0.41 (0.11–0.72)	35.0 (4.74–65.3)	11.6 ($p < 0.01$)
CaCl$_2$ extractable	0.37 (0.08–0.64)	15.1 (1.87–29.6)	4.12 ($p < 0.01$)

*NB: Responses of Cd toxicity are related to total Cd (measured concentration) and 0.01 M CaCl$_2$-exchangeable Cd in the soil (mg kg^{-1}). The LCx values were calculated by a generalized linear model with a binomial distribution and logit link function fit, the ECx values of *F. candida* and *H. aculeifer* were estimated, respectively, through a four-parameter Weibull model and a Cedergreen-Ritz-Streibig model, and the LOEC values were calculated using analysis of variance (significant differences: $p < 0.05$). The symbol (–) indicates that the value ranges are unavailable.

Table 2
Cadmium concentrations in the soil aged for more than five years (nominal and measured values) and corresponding concentrations in the 0.01 M CaCl$_2$-exchangeable fraction (mean ± SE, n = 3, mg kg^{-1}).

Nominal	Measured	CaCl$_2$-exchangeable
0	0.18 ± 0.03	0.05 ± 0.01
12.5	11.6 ± 0.4	4.12 ± 0.04
25	24.5 ± 1.2	9.37 ± 0.36
50	42.2 ± 1.1	18.2 ± 0.1
100	94.1 ± 2.0	38.1 ± 0.2
200	183 ± 4	86.9 ± 2.7
400	348 ± 5	180 ± 3

3.6. Atom% ^{15}N of animal tissues

With the exception of 100 mg Cd kg^{-1}, a significant dose-related reduction in the ^{15}N abundance of animal tissues was observed (F-test, P < 0.01) in Cd-contaminated soils (Fig. 5). Compared to the control (0 mg Cd kg^{-1}), the ^{15}N abundance of *H. aculeifer* tissues decreased significantly by 24% (*t*-test, P < 0.01) at 12.5 mg Cd kg^{-1}. From 0 to 100 mg Cd kg^{-1} the ^{15}N abundance in *F. candida* tissues did not show any significant change. The ^{15}N abundance of *F. candida* tissues was significantly higher than that of *H. aculeifer*.

4. Discussion

4.1. Classic indicators

To our knowledge, the present study is the first to report the EC50 value (35.0 mg Cd kg^{-1}) of *H. aculeifer* reproduction in terms of Cd toxicity. It also indicates, somewhat surprisingly, that the sensitivity of *H. aculeifer* reproduction is higher than that of *F. candida* towards soil Cd pollution (*t*-test, P < 0.05). *H. aculeifer* has a continuous and thick exoskeleton and has been generally considered to be a tolerant species (Jansch et al., 2005). Most

studies have indicated that *H. aculeifer* has lower sensitivity to inorganic chemical substances (exposure duration: 14 days) than most other soil invertebrates and especially collembolans (exposure duration: 28 days) (Smit et al., 2012; Owojori et al., 2014). For instance, in a previous study the *H. aculeifer* reproduction EC50 value (2459 mg kg^{-1}) for Cu in OECD soil with 5% OM was approximately four times that (751 mg kg^{-1}) of *F. candida* in OECD soil with 10% OM (Owojori et al., 2014). Moreover, in seawater or a gradient of NaCl-spiked soil *F. candida* was found to have higher salt sensitivity than *H. aculeifer* (Pereira et al., 2015) and phosphogypsum was more toxic to *F. candida* than to *H. aculeifer* (Hentati et al., 2015). A comparison of Cd toxicity between *H. aculeifer* and *F. candida* could not be made due to a lack of available data in the literature on single species tests. There is only one report of studies on the effects of Cd contaminated soils collected from a former iron mine on *H. aculeifer*, which also showed that *H. aculeifer* had a low sensitivity to Cd polluted soils in the single species test (Madani et al., 2015). These results suggest that the difference in reproduction sensitivity between *H. aculeifer* and *F. candida* for Cd might produce a change in our model food chain test system due to predator-prey interactions (Fig. 6). A similar study with a soil multi-species test system also indicates that the EC10 value for Cu (240 mg kg^{-1}) of the predatory mite *H. aculeifer* was slightly lower than that (375 mg kg^{-1}) of the collembolan *Folsomia fimetaria* (exposure duration: 56 days) (Scott-Fordsmand et al., 2008). In our model food chain and previous soil multi-species test systems, the amount of prey available to the predatory mite has been restricted and the prey has been introduced only prior to the predatory mite. However, in the single species test the predatory mite is supplied with sufficient prey and fresh prey individuals are added regularly (OECD, 2008). In Cd polluted soil there are two pathways of exposure to the predatory mite, namely ingestion and contact (Heckmann et al., 2005; Pereira et al., 2015). The limited supply of prey might lead to starvation of the predatory mite and starvation would retard the locomotory behavior of the predatory mite due to energy depletion (Baatrup et al., 2005). This could extend the contact time between the predatory mite and the soil solution and thus increase the adverse effect of the Cd. On the other hand, a large amount of Cd accumulated in the prey tissues during the long exposure time in our test system, and this would contribute to the sensitivity of the predatory mite (Fig. 6). Clearly, with increasing soil Cd concentration the mass mortality of the prey collembolan would indirectly bring about the death of the predator mite. A study of two-species toxicity test systems also suggests that a decrease in the availability of prey can make an important contribution to the decline in *H. aculeifer* reproduction under exposure to high concentrations of the insecticide dimethoate (Hamers and Krogh, 1997). Cadmium would exert more toxicity to higher trophic levels through predator-prey interactions (Fig. 6). The present model food chain test system was also closer to conditions in the natural environment in which the numbers of predators are restricted largely by the prey in real soil food webs.

Reproduction of *F. candida* (EC50 value 91.6 mg Cd kg^{-1} on the basis of the soil total Cd concentration) exhibited greater sensitivity to Cd toxicity than the survival (136 mg Cd kg^{-1}) or growth (adult, 513 mg Cd kg^{-1}; juvenile, 524 mg Cd kg^{-1}) in our model food chain test system. This result is in good agreement with published results (EC50 of *F. candida* reproduction 28–780 mg Cd kg^{-1}) using the single species test (Crommentuijn et al., 1997; van Gestel and Mol, 2003; Bur et al., 2010; Ardestani et al., 2013). The above-mentioned data show that EC50 values of *F. candida* reproduction based on total Cd vary widely in different studies and differences in soil properties may be an important contributory factor. Animals suffer from an adverse effect because of exposure to the bioavailable fraction of metal in the soil (Smith et al., 2012). Soil metal
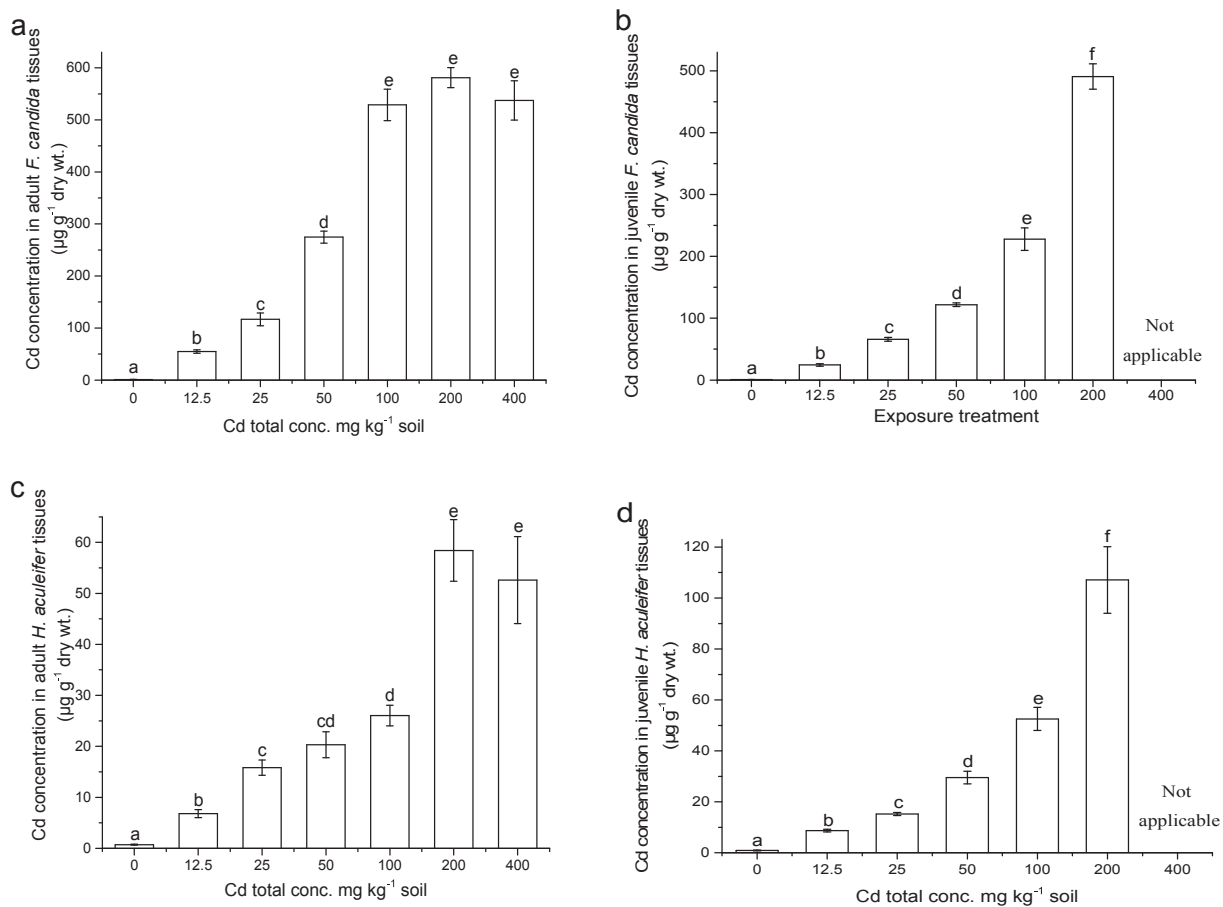
Fig. 4. Cd concentrations (mean ± SE, n = 4) of animal body tissues exposed to Cd contaminated soils. Different letters indicate significant differences ($p < 0.05$) among Cd exposure treatments (LSD test) in each species, and Cd concentration data were ln-transformed prior to statistical analysis; "not applicable" indicates that insufficient animal juveniles were available to determine Cd concentration in their tissues at the 400 mg Cd kg^{-1} exposure treatment.
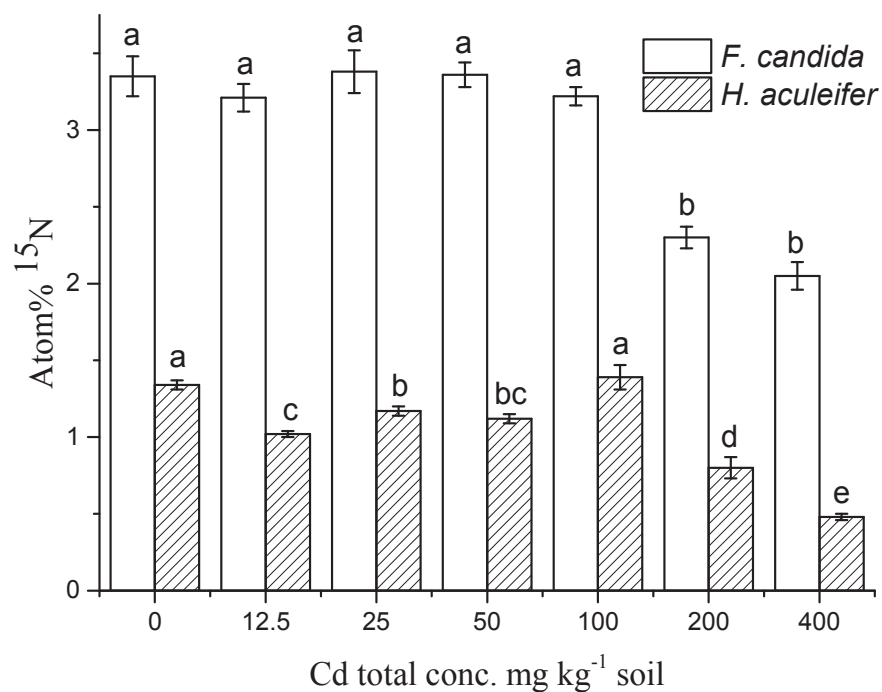

Fig. 5. Atom% ^{15}N (mean ± SE, n = 4) in *F. candida* and *H. aculeifer* body tissues supplied with litter (^{15}N labeled wheat) exposed to Cd contaminated soils. Different letters indicate significant differences ($p < 0.05$) among Cd exposure treatments (LSD test) in *F. candida* or *H. aculeifer*.
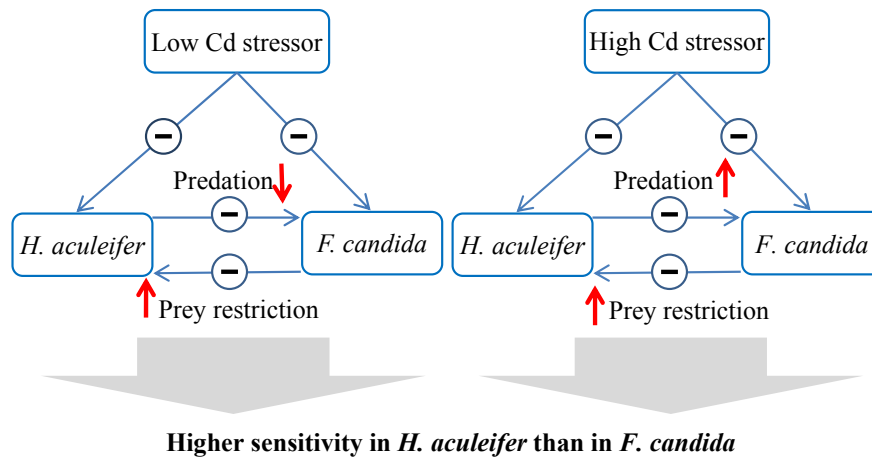
Fig. 6. Conceptual model illustrating the mechanisms by which double-stress (Cd stressor and predator-prey interactions) affects predatory mite and prey collembolan and results of the effect. The direction of the effects (−) shows that the adverse effect on animals increases with increasing Cd exposure concentration. The upward red filled arrows represent the adverse effect of Cd stressor on animals' increases due to predator-prey interactions, and the downward red filled arrows indicate the adverse effect of Cd stressor on animals' declines due to predator-prey interactions. Predator-prey interactions in the soil collembolan-predatory mite food chain test system can influence the toxicity of Cd to animals by predation and food restriction. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

bioavailability is typically estimated using the soil solution or extraction with water or $CaCl_2$ (Hobbelen et al., 2004). Recent studies also show that $CaCl_2$-extractable Pb concentrations in combination with soil properties could best explain the effect on *F. candida* reproduction in a single species test ($R^2 = 0.82$) (Luo et al., 2014a). We also carried out a standard single species test of *F. candida* in the present soil two days after Cd addition (data not published) and the EC50 value (6.12 mg Cd kg^{-1}) of *F. candida* reproduction based on $CaCl_2$ extraction was clearly lower than that (29.4 mg Cd kg^{-1}) in the present study (*t*-test, P < 0.05). This suggests that predator-prey interactions may play an important role in reducing the sensitivity of *F. candida* reproduction to soil Cd pollution. We postulate that in a certain range of Cd concentrations the numbers of collembolans captured by the predatory mites would decline and thus maintain prey population numbers with increasing soil Cd concentrations (Fig. 6). Relatively stable *F. candida* reproduction was observed from 0 to 50 mg Cd kg^{-1} in our study and the fact that the atom% ^{15}N of *H. aculeifer* decreased significantly at a Cd concentration range of 12.5—50 mg Cd kg^{-1} compared to the control, which reflects the decline of predation on collembolans by the mites at the range, supports our hypothesis. Many data in ecological studies have demonstrated that predators can mediate prey population numbers by changing feeding intensity (Yoshida et al., 2003; Lensing and Wise, 2006).

The present study indicates that, compared to the single species test, *H. aculeifer* showed a higher sensitivity to Cd and the sensitivity of *F. candida* would decrease in our model food chain test system and this agrees well with our assumption that the predator-prey relationship would influence species sensitivities to soil Cd pollution (Fig. 6). Similarly, Schnug et al. (2014) also suggested that interspecific competitive interactions might alter the toxicity of biocides in a soil multi-species test system that included collembolans.

Since our test system offers the advantages of smaller numbers of test animals, ease of operation, the availability of more comprehensive information including classic endpoints and predator-prey relationships, and a situation more similar to the natural environment, the improved model food chain test method has great potential for investigating direct and indirect effects of predator-prey relationships under pollution scenarios.

4.2. Cadmium accumulation in the collembolan-predatory mite food chain

The present study shows that Cd can accumulate in a collembolan-predatory mite food chain through soil exposure and can produce biomagnification in the collembolan *F. candida*. The Cd concentration in *F. candida* adults was almost ten times higher than that in *H. aculeifer* adults. In Cd spiked soil exposure, *F. candida* absorbed sufficient Cd from the soil solution by drinking and via the ventral tube (Hopkin, 1997; Ardestani and van Gestel, 2013) and *H. aculeifer* might experience lower exposure to the soil solution due to its continuous exoskeleton with a rigid dorsal shield (Jansch et al., 2005; Pereira et al., 2015). This might be the main explanation for the high Cd accumulation in *F. candida*. The Cd concentration in *F. candida* adults remained stable (500—600 µg Cd g^{-1}) and *F. candida* adults died suddenly between 100 and 400 mg Cd kg^{-1} in soils, suggesting that the lethal body concentration (LBC) of Cd for *F. candida* adults was 500—600 µg Cd g^{-1}. The result is slightly lower than that reported (LBC: 600—700 µg Cd g^{-1}) by van Gestel and Hensbergen (1997) and is about double the value of 200—300 µg Cd g^{-1} reported by Fountain and Hopkin (2001). The LBC of Cd for *F. candida* juveniles in our study ranged from 181 to 267 µg Cd g^{-1}, values clearly higher than the 15—52 µg Cd g^{-1} obtained by van Gestel and van Diepen (1997) and van Gestel and Hensbergen (1997) but in good agreement with the 100—200 µg Cd g^{-1} observed by Crommentuijn et al. (1993). The LBC of *H. aculeifer* juveniles for Cd was 8—10 µg Cd g^{-1} but comparable data on the toxicity of Cd to *H. aculeifer* are not available. The LBC values of *H. aculeifer* juveniles were much lower than those of *F. candida* juveniles, in good agreement with the order of EC50 values of their reproduction. These results also suggest that the internal Cd concentrations of the animals might better explain the toxicity due to soil Cd pollution.

4.3. Changes in N transfer content

The N content of the animal tissues declined sharply from the litter in our study with increasing soil Cd concentrations from 100 to 400 mg Cd kg^{-1} and this strongly supports our hypothesis that Cd pollution restricts N nutrient transfer in the model food chain. *F. candida* obtained N nutrient from litter by feeding on the litter

directly or on fungi decomposing the litter (Chahartaghi et al., 2005; Pfeffer et al., 2010; Semenina and Tiunov, 2011), and the increased N content of *H. aculeifer* tissues was derived from the prey (OECD, 2008). The body length and survival of *F. candida* declined significantly at 100—400 mg Cd kg^{-1} in our study, indicating suppression of its metabolism and suggesting a decrease in the ability of *F. candida* to acquire N from the litter. Alternatively, the amount of litter decomposing fungi consumed by *F. candida* may have declined due to changes in the quantity and quality of the fungi via Cd pollution (Duarte et al., 2008). *F. candida* can discriminate between high and low quality food sources (Pfeffer et al., 2010; Nakamori and Kaneko, 2013) and can thus avoid food highly contaminated with heavy metals (Gillet and Ponge, 2003). *H. aculeifer* survival did not produce any clear change in exposure to Cd pollution in our results, indicating that shortages of prey might be the main explanation for declining N in *H. aculeifer* tissues. At 100 mg Cd kg^{-1} the numbers of *F. candida* adults and juveniles decreased sharply and Cd had exerted substantial toxicity to them, suggesting that more *F. candida* might have been consumed by *H. aculeifer* due to inhibition of *F. candida* by Cd. This may be the main explanation for the increase in N in *H. aculeifer* tissues at 100 mg Cd kg^{-1}. Several comparable studies also show that a high concentration of the insecticide dimethoate dramatically increased the rate of capture of *F. candida* by *H. aculeifer*, likely by suppressing the evasive behavior of *F. candida* (Hamers and Krogh, 1997; Baatrup et al., 2005). Moreover, though the highest atom% (3.35 ± 0.13) of *F. candida* tissues was lower than that (5.5) of its food wheat, the atom% value in *F. candida* clearly showed that N of wheat has been largely absorbed by collembolans and could satisfy our need to reflect N transfer in the model food chain from wheat. These results suggest that the ^{15}N labeled litter addition method can assess soil functional change by detecting the change in N transfer content in animal tissues.

The litter-bag method and bait-lamina method have been conventionally used to assess changes in soil function in previous studies (Römbke, 2014). However, using the litter-bag method it is difficult to identify the contribution of the soil fauna or microbial community to the change in soil function, and the link between the endpoint of the bait-lamina method (feeding rate) and soil function remains controversial (Römbke, 2014). Compared with these two methods, our established ^{15}N labeled litter addition method can quantitatively, easily and directly determine the change in soil function by ^{15}N tracer due to the contribution of soil animals. Moreover, the ^{15}N labeled method can be easily combined with the classic OECD test method.

5. Conclusions

Under conditions of soil Cd exposure, *H. aculeifer* reproduction was a more sensitive parameter than the growth, survival or reproduction of *F. candida* in a soil collembolan-predatory mite food chain test system. The internal Cd concentration of the animals can intuitively reveal the ecological risk of Cd, especially in *H. aculeifer* juvenile tissues. The predator-prey interactions in the food chain will increase the sensitivity of a higher trophic level to pollution by toxicity and food restriction, and the feeding of the predator can maintain the scale of the prey population at low Cd exposure levels and rapidly reduce prey numbers in high Cd conditions by increasing the capture rate. Soil Cd pollution will restrict N nutrient transfer in the model food chain. The improved model food chain test method coupled with our established ^{15}N labeled litter addition method is well suited to increasing our understanding of the secondary effects of pollution driven by predator-prey interactions and soil food chain functional change.

References

Alchami, Z., Eldeen, S.B.Z., AlBitar, L., Atallah, T., 2016. Decomposition of olive-mill waste compost, goat manure and *Medicago sativa* in Lebanese soils as measured using the litterbag technique. Soil Res. 54, 191—199.

André, A., Antunes, S.C., Gonçalves, F., Pereira, R., 2009. Bait-lamina assay as a tool to assess the effects of metal contamination in the feeding activity of soil invertebrates within a uranium mine area. Environ. Pollut. 157, 2368—2377.

Ardestani, M.M., Ortiz, M.D., van Gestel, C.A.M., 2013. Influence of Ca and pH on the uptake and effects of Cd in *Folsomia candida* exposed to simplified soil solutions. Environ. Toxicol. Chem. 32, 1759—1767.

Ardestani, M.M., van Gestel, C.A.M., 2013. Using a toxicokinetics approach to explain the effect of soil pH on cadmium bioavailability to *Folsomia candida*. Environ. Pollut. 180, 122—130.

Baatrup, E., Bayley, M., Axelsen, J.A., 2005. Predation of the mite *Hypoaspis aculeifer* on the springtail *Folsomia fimetaria* and the influence of sex, size, starvation, and poisoning. Entomol. Exp. Appl. 118, 61—70.

Bur, T., Probst, A., Bianco, A., Gandois, L., Crouau, Y., 2010. Determining cadmium critical concentrations in natural soils by assessing Collembola mortality, reproduction and growth. Ecotox. Environ. Safe 73, 415—422.

Burrows, L.A., Edwards, C.A., 2002. The use of integrated soil microcosms to predict effects of pesticides on soil ecosystems. Eur. J. Soil Biol. 38, 245—249.

Chahartaghi, M., Langel, R., Scheu, S., Ruess, L., 2005. Feeding guilds in Collembola based on nitrogen stable isotope ratios. Soil Biol. Biochem. 37, 1718—1725.

Cortet, J., Joffre, R., Elmholt, S., Coeurdassier, M., Scheifler, R., Krogh, P.H., 2006. Interspecific relationships among soil invertebrates influence pollutant effects of phenanthrene. Environ. Toxicol. Chem. 25, 120—127.

Coleman, D.C., Crossley, D.A., Hendrix, P.F., 2004. Soil Food Webs: detritivory and microbivory in soils. In: Coleman, D.C., Crossley, D.A. (Eds.), Fundamentals of Soil Ecology. Elsevier, Amsterdam, pp. 227—246.

Crommentuijn, T., Brils, J., Vanstraalen, N.M., 1993. Influence of cadmium on life-history characteristics of *Folsomia candida* (Willem) in an artificial soil substrate. Ecotoxicol. Environ. Saf. 26, 216—227.

Crommentuijn, T., Doornekamp, A., van Gestel, C.A.M., 1997. Bioavailability and ecological effects of cadmium on *Folsomia candida* (Willem) in an artificial soil substrate as influenced by pH and organic matter. Appl. Soil Ecol. 5, 261—271.

Crossley, D.A., Hoglund, M.P., 1962. A litter-bag method for the study of micro-arthropods inhabiting leaf litter. Ecology 43, 571—573.

Duarte, S., Pascoal, C., Cássio, F., 2008. High diversity of fungi may mitigate the impact of pollution on plant litter decomposition in streams. Microb. Ecol. 56, 688—695.

EK, C., Karlson, A.M., Hansson, S., Garbaras, A., Gorokhova, E., 2015. Stable isotope composition in Daphnia is modulated by growth, temperature, and toxic exposure: implications for trophic magnification factor assessment. Environ. Sci. Technol. 49, 6934—6942.

Fountain, M.T., Hopkin, S.P., 2001. Continuous monitoring of *Folsomia candida* (Insecta: Collembola) in a metal exposure test. Ecotoxicol. Environ. Saf. 48, 275—286.

Fountain, M.T., Hopkin, S.P., 2005. *Folsomia candida* (Collembola): a "standard" soil arthropod. Annu. Rev. Entomol. 50, 201—222.

Geisen, S., Koller, R., Hünninghaus, M., Dumack, K., Urich, T., Bonkowski, M., 2015. The soil food web revisited: diverse and widespread mycophagous soil protists. Soil Biol. Biochem. 94, 10—18.

Gillet, S., Ponge, J.F., 2003. Changes in species assemblages and diets of Collembola along a gradient of metal pollution. Appl. Soil Ecol. 22, 127—138.

Hättenschwiler, S., Field, C.B., 2005. Soil animals alter plant litter diversity effects on decomposition. P. Natl. Acad. Sci. U. S. A. 102, 1519—1524.

Hamers, T., Krogh, P.H., 1997. Predator—prey relationships in a two-species toxicity test system. Ecotoxicol. Environ. Saf. 37, 203—212.

Heemsbergen, D.A., Hal, J.R.V., Faber, J.H., Berg, M.P., Verhoef, H.A., 2004. Environmental heterogeneity in contaminated soils and soil-fauna functioning. Newsl. Stimul. Programme System-Oriented Ecotoxicological Res. 2004, 18—20.

Hentati, O., Abrantes, N., Caetano, A.L., Bouguerra, S., Gonçalves, F., Römbke, J., Pereira, R., 2015. Phosphogypsum as a soil fertilizer: ecotoxicity of amended soil and elutriates to bacteria, invertebrates, algae and plants. J. Hazard. Mater 294, 80—89.

Heckmann, L.-H., Maraldo, K., Krogh, P.H., 2005. Life stage specific impact of dimethoate on the predatory mite *Hypoaspis aculeifer* canestrini (Gamasida: Laelapidae). Environ. Sci. Technol. 39, 7154—7157.

Hobbelen, P.H., Koolhaas, J.E., van Gestel, C.A.M., 2004. Risk assessment of heavy metal pollution for detritivores in floodplain soils in the Biesbosch, The Netherlands, taking bioavailability into account. Environ. Pollut. 129, 409—419.

Hopkin, S.P., 1997. Biology of the Springtails (Insecta: Collembola). Oxford University Press, New York, NY, USA.

Jansch, S., Amorim, M.J., Rombke, J., 2005. Identification of the ecological requirements of important terrestrial ecotoxicological test species. Environ. Rev. 13, 51—83.

Jensen, J., Scott-Fordsmand, J.J., 2012. Ecotoxicity of the veterinary pharmaceutical

ivermectin tested in a soil multi-species (SMS) system. Environ. Pollut. 171, 133—139.

Klimek, B., Niklińska, M., Jaźwa, M., Chodak, M., Tarasek, A., 2015. Application of the bait-lamina method to measure the feeding activity of soil fauna in temperate forests. Pol. J. Ecol. 63, 414—423.

Lavelle, P., Decaëns, T., Aubert, M., Barot, S., Blouin, M., Bureau, F., Margerie, P., Mora, P., Rossi, J.P., 2006. Soil invertebrates and ecosystem services. Eur. J. Soil Biol. 42, S3—S15.

Lensing, J.R., Wise, D.H., 2006. Predicted climate change alters the indirect effect of predators on an ecosystem process. P. Natl. Acad. Sci. U. S. A. 103, 15502—15505.

Luo, W., Verweij, R.A., van Gestel, C.A.M., 2014a. Assessment of the bioavailability and toxicity of lead polluted soils using a combination of chemical approaches and bioassays with the collembolan *Folsomia candida*. J. Hazard. Mater 280, 524—530.

Luo, W., Verweij, R.A., van Gestel, C.A.M., 2014b. Determining the bioavailability and toxicity of lead contamination to earthworms requires using a combination of physicochemical and biological methods. Environ. Pollut. 185, 1—9.

Mackintosh, T.J., Davis, J.A., Thompson, R.M., 2016. Impacts of multiple stressors on ecosystem function: leaf decomposition in constructed urban wetlands. Environ. Pollut. 208, 221—232.

Madani, S., Coors, A., Haddioui, A., Ksibi, M., Pereira, R., Sousa, J.P., Römbke, J., 2015. Effects of contaminated soils from a former iron mine (Ait Amar, Morocco) on enchytraeids (*Enchytraeus bigeminus*) and predatory mites (*Hypoaspis aculeifer*) in standard laboratory tests. Ecotoxicol. Environ. Saf. 119, 90—97.

Nakamori, T., Kaneko, N., 2013. Biomarker responses reveal that food quality affects cadmium exposure in the soil collembolan *Folsomia candida*. Environ. Pollut. 176, 165—170.

Nordberg, G.F., Nogawa, K., Nordberg, M., 2015. Cadmium. In: Nordberg, G.F., Fowler, B.A., Nordberg, M. (Eds.), Handbook on the Toxicology of Metals, fourth ed. Elsevier, Amsterdam, pp. 667—716.

OECD, 2008. Guidelines for the Testing of Chemicals No. 226, Predatory Mite (*Hypoaspis aculeifer*) Reproduction Test in Soil. Organisation for Economic Cooperation and Development, Paris, France.

Owojori, O.J., Waszak, K., Roembke, J., 2014. Avoidance and reproduction tests with the predatory mite *Hypoaspis aculeifer*: effects of different chemical substances. Environ. Toxicol. Chem. 33, 230—237.

Pereira, C.S., Lopes, I., Sousa, J.P., Chelinho, S., 2015. Effects of NaCl and seawater induced salinity on survival and reproduction of three soil invertebrate species. Chemosphere 135, 116—122.

Pfeffer, S.P., Khalili, H., Filser, J., 2010. Food choice and reproductive success of *Folsomia candida* feeding on copper-contaminated mycelium of the soil fungus *Alternaria alternata*. Pedobiologia 54, 19—23.

Römbke, J., 2014. The feeding activity of invertebrates as a functional indicator in soil. Plant Soil 383, 43—46.

Schnug, L., Jensen, J., Scott-Fordsmand, J.J., Leinaas, H.P., 2014. Toxicity of three biocides to springtails and earthworms in a soil multi-species (SMS) test system. Soil Biol. Biochem. 74, 115—126.

Scott-Fordsmand, J.J., Maraldo, K., Brink, P.J.V.D., 2008. The toxicity of copper contaminated soil using a gnotobiotic soil multi-species test system (SMS). Environ. Int. 34, 524—530.

Sechi, V., D'Annibale, A., Maraldo, K., Johansen, A., Bossi, R., Jensen, J., Krogh, P.H., 2014. Species composition of a soil invertebrate multi-species test system determines the level of ecotoxicity. Environ. Pollut. 184, 586—596.

Semenina, E.E., Tiunov, A.V., 2011. Trophic fractionation (Δ^{15}N) in Collembola depends on nutritional status: a laboratory experiment and mini-review. Pedobiologia 54, 101—109.

Smit, C.E., Moser, T., Römbke, J., 2012. A new OECD test guideline for the predatory soil mite *Hypoaspis aculeifer*: results of an international ring test. Ecotoxicol. Environ. Saf. 82, 56—62.

Smith, B.A., Greenberg, B., Stephenson, G.L., 2012. Bioavailability of copper and zinc in mining soils. Arch. Environ. Con. Tox. 62, 1—12.

Soong, J.L., Vandegehuchte, M.L., Horton, A.J., Nielsen, U.N., Denef, K., Shaw, E.A., Tomasel, C.M.D., Parton, W., Wall, D.H., Cotrufo, M.F., 2016. Soil microarthropods support ecosystem productivity and soil C accrual: evidence from a litter decomposition study in the tallgrass prairie. Soil Biol. Biochem. 92, 230—238.

Stankovic, S., Kalaba, P., Stankovic, A.R., 2014. Biota as toxic metal indicators. Environ. Chem. Lett. 12, 63—84.

Törne, E.V., 1990. Assessing feeding activities of soil-living animals. I. Bait-lamina-tests. Pedobiologia 34, 89—101.

van Gestel, C.A.M., van Diepen, A.M.F., 1997. The influence of soil moisture content on the bioavailability and toxicity of cadmium for *Folsomia candida* Willem (Collembola: Isotomidae). Ecotoxicol. Environ. Saf. 36, 123—132.

van Gestel, C.A.M., Hensbergen, P.J., 1997. Interaction of Cd and Zn toxicity for *Folsomia candida* Willem (Collembola: Isotomidae) in relation to bioavailability in soil. Environ. Toxicol. Chem. 16, 1177—1186.

van Gestel, C.A.M., Mol, S., 2003. The influence of soil characteristics on cadmium toxicity for *Folsomia candida* (Collembola: Isotomidae). Pedobiologia 47, 387—395.

van Gestel, C.A.M., 2012. Soil ecotoxicology: state of the art and future directions. ZooKeys 176, 275—296.

van Voris, P., Tolle, D.A., Arthur, M.F., Chesson, J., 1985. Terrestrial microcosms: application, validation, and cost-benefit analysis. In: Cairns, J. (Ed.), Multispecies Toxicity Testing. Pergamon Press, New York, pp. 117—142.

Yoshida, T., Jones, L.E., Ellner, S.P., Fussmann, G.F., Halrston Jr, N.G., 2003. Rapid evolution drives ecological dynamics in a predator-prey system. Nature 424, 303—306.

Zhao, J., Shao, Y., Wang, X., Neher, D.A., Xu, G., Li, Z.A., Fu, S., 2013. Sentinel soil invertebrate taxa as bioindicators for forest management practices. Ecol. Indic. 24, 236—239.

Zhu, D., Ke, X., Wu, L.H., Christie, P., Luo, Y.M., 2016a. Biological transfer of dietary cadmium in relation to nitrogen transfer and ^{15}N fractionation in a soil collembolan-predatory mite food chain. Soil Biol. Biochem. 101, 207—216.

Zhu, D., Ke, X., Wu, L.H., Huang, Y.J., Christie, P., Luo, Y.M., 2016b. Refinement of methodology for cadmium determination in soil microarthropod tissues. Pedosphere (in press).