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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?☆

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ABSTRACT

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)

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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 hemi-edaphic/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