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Species composition of a soil invertebrate multi-species test system determines the level of ecotoxicity☆

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ABSTRACT

A soil multi-species, SMS, experimental test system consisting of the natural microbial community, five collembolan species and a predatory mite along with either *Enchytraeus crypticus* or the earthworm *Eisenia fetida* were exposed to α-cypermethrin. A comparison of the performance of these two types of SMSs is given to aid the development of a standard test system. *E. fetida* had a positive effect on the majority of the species, reducing the negative insecticide effect. *E. fetida* affected the species sensitivity and decreased the degradation of the insecticide due to the organic matter incorporation of earthworm food. After 8 weeks, the EC50 was 0.76 mg kg^{-1} for enchytraeids and ranged between 2.7 and 18.9 mg kg^{-1} for collembolans, more sensitive than previously observed with single species. Changes observed in the community structure and function illustrates the strength of a multi-species test system as an ecotoxicological tool compared to single species tests.

1. Introduction

Soil organisms interact intimately through their contribution to the bio-geochemical cycling of carbon and mineral nutrients (Coleman et al., 2004). General types of ecological interactions are found in soil ecosystems such as predator–prey relationships, competition for resources, mutualism and commensalism. Besides having direct effects on the individual organisms, insecticides also affect these crucial species interactions ultimately having repercussions on the food-web. However, only a few laboratory experimental studies have been done on the effects of pollution on the structure and functioning of food webs in terrestrial ecosystems, especially in soils (Salminen et al., 2002; Cortet et al., 2006).

The decomposition of organic matter is a relevant functional endpoint for toxicity testing of insecticides due to its pivotal role in soil nutrient cycling. It involves a wide range of soil organism and it is employed as the litterbag method (OECD, 2006). However, the litterbag method is relatively laborious and insensitive, and gives no insight into potential impacts on the soil fauna or microbial community structure, as it does not include direct measurements related to diversity. Moreover, the choice of straw of low food quality makes the direct role of soil fauna less important in the initial decomposition phases. Given this, there is a need for new and more sensitive test systems, which incorporate community structural and functional endpoints. Where higher tier methods testing the aquatic toxicity of insecticides are relatively advanced (e.g. Boxall et al., 2002), we advocate for a development of a similar approach for soil environments as the function of individual soil organisms is most meaningful when seen as part of the entire food web (Cortet et al., 2006; Jensen et al., 2009; Knacker et al., 2004).

To evaluate the effect of faunal species interactions under controlled conditions, semi-field systems have been used to simulate field situations (Gyldenkaerne et al., 2000) in various

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degrees, either at indoor or outdoor conditions. Terrestrial Model Ecosystems (TME) are model ecosystems based on intact field soil cores containing an indigenous pool of organisms which are exposed to a toxicant by spiking (Knacker et al., 2004). However, methods closely resembling realistic field situations often have a high variability and a low stability and are difficult to reproduce. Alternatively, the indigenous animals could be extracted from field soil-cores and then added to soil cores containing the contaminant. Still the natural variation inherited from the individual sample cores may result in a relatively high variability between the replicates reducing the statistical power and the repeatability of the test (Scott-Fordsmand et al., 2008) and make it difficult to detect changes at relevant low concentrations of contaminants. The design therefore has to balance gaining realism without losing reproducibility. An attempt to improve the statistical properties is possible by a testing approach with a constructed invertebrate decomposer food web at the expense of some of the natural soil biodiversity (Cortet et al., 2003, 2006; Filser and Krogh, 2002; Pernin et al., 2006; Jensen and Scott-Fordsmand, 2012; Scott-Fordsmand et al., 2008). Compared to the TME approach, such constructed systems represent a specific but reduced faunal food web; nevertheless, they provide the basic characteristics of the soil ecosystem. In this manner, a more homogenous system is obtained with less initial variation and it can be adapted to represent different types of soil habitats. Furthermore, it gives the opportunity to study special ecological topics, such as the importance of functional redundancy and species diversity. Such a more robust higher tier test system still enables quantification of direct and indirect effects of chemicals in terrestrial food webs, and the study of possible links between functional and structural endpoints. Endpoints could include the classical endpoints survival, reproduction, growth and various microbial mediated processes, but also community structure and population dynamics. Indirect effects of pollutants via competition or predation are crucial in most ecosystems and, although community structure is complex and therefore difficult to describe, it should be taken into account (Cortet et al., 2006). Potentially, these indirect effects of a toxicant may play an important role when causality between the observed effects of pollution on the structure and functioning of stressed food webs is evaluated (Salminen et al., 2002).

The aim of the study was to test the performance and validity of a soil multi-species (SMS) test systems, to underpin future adoption for ecotoxicity testing, by measuring the effects of an insecticide, α-cypermethrin, on soil invertebrate populations and microbial community composition. In addition, we wanted to assess the suitability and sensitivity of two alternative worm species in the SMS system and to gain knowledge regarding possible difference in the insecticide effect on different species composition. The performance of two different soil communities was evaluated in soil spiked with a range of α-cypermethrin concentrations. As test organisms we selected, an earthworm or an enchytraeid species, five springtail species, and one predaceous mite species. These organisms are typical inhabitants of temperate agricultural soils (Holmstrup and Krogh, 2001). Collembolans are an appropriately sensitive arthropod group for inclusion in risk assessments to improve risk prediction for soil invertebrate communities (Jänsch et al, 2006). The oligochaete species employed are typically used for testing chemicals according to the OECD procedures (OECD, 2004a, 2004b). While the smaller enchytraeid species is known to co-exist with microarthropods in such a test system, we aimed to establish the feasibility of using this comparably large lumbricid with the much smaller microarthropods, as this may create a risk of microarthropods being too much affected by its presence.

2. Materials and methods

A simplified invertebrate food-web was constructed in the laboratory based on species composition found in a Danish agro-ecosystem (Fjellberg, 2007). Seven culturable species (all cultured at the Dept. of Bioscience. Aarhus University) were selected from different functional groups (Table 1). Mutualism, competition and predation were introduced by addition of five collembolans, one enchytraeid or earthworm, and a predatory mite species. A total of forty-eight soil mesocosms were set-up in a design which included zero (control) and three concentrations (1, 5, 25 mg kg^{-1}) of α–cypermethrin (α-CYP), two different soil fauna communities and two sampling dates.

We chose the pyrethroid insecticide α–cypermethrin (α-CYP) as it has known effects on invertebrates. The recommended application level in Danish agriculture is 100 g ha^{-1}, or approximately 0.07 mg kg^{-1} soil in the upper 10 cm of the ploughing layer. The pyrethroids are a dominant group of plant-protection products widely used as insecticides in agriculture (Hartnik et al., 2008a) and have been used during the past 25 years.

For practical reasons replicates of the experiment were allocated into two blocks with twenty four soil mesocosm per block set up at two dates. The second block was run two weeks later than the first block. The exposure time covered a period of eight weeks. At each sampling date, after four and eight weeks twelve mesocosms were harvested destructively for analyses.

2.1. Communities

Two different faunal communities (abbreviated COM), were constructed, including three trophic levels and different functional groups. All animals employed were obtained from laboratory cultures. Both communities included five species of springtails and the predator mite *Hypoaspis aculeifer* but differed concerning the added oligochaete, which was either juveniles of the earthworm *Eisenia fetida*: COM-F, or adults of the enchytraeid *Enchytraeus crypticus*, COM-C. To avoid pretreatment variability a blocking procedure was used to ensure similar initial weights of earthworms within each treatment (McIndoe et al., 1999). The other organisms were randomly selected from our laboratory culture. The total biomass of oligochaetes introduced per mesocosms was about 14 mg fresh weight (f.w.) for *E. crypticus* and about 300 mg f.w. for *E. fetida*. The enchytraeid number had proved feasible in our previous experiments (e.g. Cortet et al., 2006) and the number of *E. fetida* was comparable to the OECD standard (OECD, 2004b). For the collembolan populations the following species were used representing different ecological niches: *Proisotoma minuta, Protaphorura fimata, Mesaphorura macrochaeta, Heteromurus nitidus* and *Folsomia fimetaria* (see Table 1 for a complete description of mesocosm species composition). The total number of springtails of 23,000 m^{-2} added as the starting populations in the mesocosms is within the normal range of abundance of $5–100 \times 10^3$ individuals m^{-2} found in agricultural soil with annual ploughing (Cortet et al., 2007; Petersen et al., 2003). Both communities were exposed to three different insecticide (α–CYP) concentrations. Due to technical constraints, we settled for four replicates of the lowest and highest concentration (1 and 25 mg kg^{-1}) and eight replicates of control and 5 mg kg^{-1} α-CYP concentration.

2.2. Soil

The soil was collected at Askov, Denmark (N 55° 28.34′, E 9° 6.6′), occasionally selected as a representative for Northern European agricultural soils (Cortet et al., 2006). It is a sandy loam with the following texture: coarse sand (200–2000 mm) 38.0%, fine sand (63–200 μm) 40.6%, silt (20–63 μm) 10.0%, silt (2–20 μm) 12.3%, clay (<2 μm) 9.5.0%, humus 2.1%, furthermore the Water Holding Capacity (WHC) is

Table 1
List of the species used and their relative functional groups and life-forms. The number of individuals introduced at time 0 in each of the 48 experimental units (mesocosms) and the corresponding number of individuals m^{-2}. Twenty-four mesocosms were populated with the enchytraeid *Enchytraeus crypticus* and the other 24 with the earthworm *Eisenia fetida*. (F: Female, J: Juvenile).

Functional group	Species	Life-form	No. indv.	No. indv. m^{-2}
Predator mite	*Hypoaspis aculeifer*	Hemiedaphic	10 F	1270
Collembolan detrivore	*Heteromurus nitidus*	Epedaphic	20	2540
Collembolan detrivore	*Folsomia fimetaria*	Hemi-euedaphic	30	3810
Collembolan detrivore	*Proisotoma minuta*	Hemiedaphic	30	3810
Collembolan detrivore/ herbivore	*Protaphorura fimata*	Euedaphic	50	6350
Collembolan detrivore	*Mesaphorura macrochaeta*	Euedaphic	50	6350
Lumbricid detrivore	*Eisenia fetida*	Epigeic	5 J	635
Enchytraeid detrivore	*Enchytraeus crypticus*	Endogeic	50	6350