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Soil microarthropod community testing: A new approach to increase the ecological relevance of effect data for pesticide risk assessment

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A B S T R A C T

In the present study, a new complementary approach combining the use of the natural soil microarthropod community and conventional test methods was used. The effects of soil contamination with the insecticide carbofuran on two geographically distinct microarthropod communities (Mediterranean and Tropical) were evaluated in their soils of origin under controlled laboratory conditions.

After contamination of two agricultural soils from Portugal and Brazil, a gradient of concentrations was prepared. Soil cores were taken from the respective uncontaminated surrounding areas and the mesofauna of three cores was extracted directly to the test soil. After extracting the microarthropod communities to the test soil, these were incubated under laboratory conditions for 4 weeks, after which the mesofauna was extracted again. The organisms were assorted into higher taxonomic groups and Acari and Collembola were respectively assorted into order/sub-order/cohort and family. Collembolans were still classified according to morphological traits and used as a case-study of trait based risk assessment (TERA; Baird et al., 2008) of pesticides.

The exposure to insecticide contamination caused the impoverishment of the taxonomic diversity in both communities. Significant shifts in the microarthropod community structure in the different carbofuran treatments were found for both soils, although effects were more pronounced in the assay performed with the soil from Brazil. Collembolans were the most affected group with a strong decline in their abundance. A dose–response relationship was observed, showing a consistent decline on the relative abundance of Isotomidae, closely followed by an increase of Entomobryidae. Contrastingly, Acari (especially Oribatida) tended to increase their numbers with higher concentrations.

Trait based analysis of Collembola data suggested that a shift in the functional composition of the communities occurred due to carbofuran soil contamination and that species adapted to deeper soil layers were more vulnerable to insecticide toxicity.

1. Introduction

The toxicity of pesticides to soil fauna is usually evaluated through laboratory assays exposing single standard species to a series of concentrations of the pesticide of concern and measuring their acute and/or chronic effects (Van Straalen, 2002; van Gestel, 2012; Van den Brink, 2008). However, such approach does not take into account the interactions between species within a community, as well as possible differences in the responses of communities from different ecoregions (Van Straalen, 2002; Van den Brink, 2008; Kuperman et al., 2009; Clements and Rohr, 2009).

Higher tier methods include semi-field tests like micro and mesocosms, attempting to combine the controlled laboratory conditions with the complex network of interactions between organisms that naturally occur in the field (Burrows and Edwards, 2002; Scott-Fordsmand et al., 2008; Knacker et al., 2004). Several tools have been developed (for a recent review see Schäffer et al.,

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Table 1
Pedological properties of the tested soils. BR, Brazil; PT, Portugal; OM, organic matter; CEC, cation exchange capacity; WHC, water holding capacity.

	pH (KCl 1 M)	OM (%)	Sand (%)	Silt (%)	Clay (%)	Total N (%)	WHC (%)	Soil type
BR	5.33 ± 0.11	13.5	79.5	18.6	2.17	0.62	67.1 ± 3.48	Loamy sand
PT	3.9 ± 0.03	9.89	40.0	44.5	15.5	0.48	74.7 ± 4.23	Loam

2010) but only Terrestrial Model Ecosystems (TME) have been standardized (ASTM, 1993). Although with higher ecological realism, the semi-field tests are usually associated to increasing variability, higher experimental effort and costs (Van den Brink et al., 2005; Schäffer et al., 2008).

Despite this, the introduction of more ecological information in ecotoxicology, e.g., the use of species abundance and community composition to predict responses and recovery of communities towards anthropogenic disturbances is a current challenge (Filser et al., 2008; Clements and Rohr, 2009; van Gestel, 2012).

A step forward was the proposal of an innovative approach, called Trait-Based Risk Assessment (TERA; Baird et al., 2008). It advocates that morphological/physiological/ecological characteristics of organisms can be used to describe the effects of toxic substances or other stress factors at the community level. Several papers have been published, supplying not only the theoretical background but also proposing frameworks and identifying research needs (e.g. Baird et al., 2008; Van den Brink, 2008; Clements and Rohr, 2009; De Lange et al., 2010; see also the special series on TERA published in IEAM Journal, 2011).

Following the history of ecotoxicology, trait based ecotoxicological studies are being implemented earlier in the aquatic field (e.g. Relyea and Hoverman, 2006; Baird and Van den Brink, 2007; Liess and Beketov, 2011). However, the validation and further consolidation of this approach requires its transposition to the assessment of soil contamination (De Lange et al., 2009).

In the present study, a new complementary approach using the natural soil microarthropod community (that play a key role in the decomposition processes and nutrient cycling; Seastedt, 1984) was tested. The strategy adopted aimed to combine the advantages of both community studies and ecotoxicological conventional tests (performed under a laboratory context during a relatively short period of time, compared, for example, with monitoring and biodiversity studies; Schäffer et al., 2010). In addition, a specific taxonomic group (Collembola) was used as a case-study of TERA in soil.

Carbofuran, a carbamate insecticide, also with nematicidal and acaricidal properties was used as a model pesticide. It works by contact or ingestion and provokes reversible short-term disruption of the nervous system, being highly soluble in water (320 g L^{-1} at 25 °C) and moderately persistent in soil (30–120 days; http://extoxnet.orst.edu/pips/carbofur.htm).

Specifically, the present study had two main objectives: (i) to assess the effects of a carbofuran application on two geographically distinct soil microarthropod communities (warm temperate and tropical); effects were assessed based on traditional taxonomic approaches, namely changes in richness and abundance of different taxonomic entities; and (ii) to describe the potential changes in the composition of soil Collembola communities, the second most abundant group, using an innovative trait-based approach, in which the individuals of – Collembola – were classified according to their life-form traits. Two microarthropod communities from Portugal and Brazil were added to two distinct soils, previously contaminated with carbofuran.

The extraction of soil microarthropods from fresh soil and concomitant inoculation of extracted organisms into contaminated soil was simple, quick, and relatively effortless. Moreover, it allowed the introduction of several species into the test-soil, also minimizing direct handling of animals (and thus, theoretically, diminishing handling related stress).

The exposure to pesticide contamination took place under laboratory controlled conditions, less demanding in terms of space, time and costs, when compared to field and semi-field studies, and with presumably lower associated variability.

2. Materials and methods

2.1. Areas of soil sampling

In Brazil, an experimental area of CRHEA (University of São Paulo, Brazil) with no history of pesticide application, next to sugar cane plantations, and located in São Carlos (SP; −22° 10′ 13.53″, −47° 53′ 58.12″) was chosen. The management practices for the 3 years previous to the experiment consisted in the cut of vegetation two or three times a year, during the rainy season. In Portugal, a parcel of fallow land, not cultivated at least during the last 5 years (only the vegetation layer was cut once a year), located in the surroundings of Coimbra (40° 14′ 46.5066″, −8° 20′ 23.9964″) was selected. The studies took place in the autumn of 2007 (Brazil) and 2009 (Portugal). The soils from Brazil and Portugal were analyzed by CHREA (ESESC, University of São Paulo, Brazil) and Direcção Regional de Agricultura e Pescas do Norte (DRAPN, Porto, Portugal), respectively, as described in Chelinho et al. (2011a, 2012). The pedological properties of the test soils are shown in Table 1.

2.2. Soil contamination

In Brazil, the contamination of soil was performed place under field conditions and was integrated in a broader project (Chelinho et al., 2012). Briefly, the soil was tilled and after three days, two parallel strips of land (3 m × 1 m), separated by a buffer area of 2 m (to avoid cross-contamination) were used to simulate a pesticide spraying over an agricultural field. One of the strips was sprayed with the insecticide Furadan 350 SC (a carbofuran commercial formulation from FMC, SP, Brazil; 350 g a.i. (active ingredient) L^{-1}) at two times the recommended dose (2 × RD) for sugar cane plantations (10l ha^{-1}; ~2.334 mg a.i. kg^{-1} soil oven-dry mass, taking into account an average soil density of 1.5 g cm^{-3} and an incorporation depth of 10 cm). This dose mimicked pesticide overuse, a very common practice among local farmers (Dasgupta et al., 2001). The insecticide was diluted in 5 L of water collected at a nearby reference lagoon. To facilitate the pesticide incorporation, the top 5 cm of soil were mixed and another 10 L of lagoon water were sprayed. The second strip of land, which acted as control, was previously sprayed with the same amount of the lagoon water (5 + 10 L).

In the early morning of next day, soils from both strips were collected (top 10 cm) for ecotoxicological evaluations and chemical analysis.

The contaminated soil samples (as well as the uncontaminated soil which acted as the control) were sieved (5 mm) and defaunated by a freezing–thawing cycle (48 h at −20 °C followed by 8 h at 25 °C and another 24 h at −20 °C). The control soil was mixed with soil sprayed with 2 × RD of Furadan in different proportions to obtain the following dilution series: 0%, 2.5%, 5%, 10%, 25%, 50% and 100% of 2 × RD.

For the assay conducted in Portugal, several samples of soil (top 10 cm) were randomly collected in an area of 40 m^2, mixed, sieved