Applied Soil Ecology xxx (2013) xxx–xxx

Contents lists available at SciVerse ScienceDirect

Applied Soil Ecology

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

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

Sónia Chelinho[a,*], Xavier Domene[b], Pilar Andrés[b], Tiago Natal-da-Luz[a], Cláudia Norte[a], Cristina Rufino[c], Isabel Lopes[d], Anabela Cachada[e], Evaldo Espíndola[f], Rui Ribeiro[a], Armando Costa Duarte[e], José Paulo Sousa[a]

[a] IMAR-CMA, Department of Life Sciences, University of Coimbra, Apartado 3046, P 3001-401 Coimbra, Portugal
[b] CREAF, Universitat Autònoma de Barcelona, Campus de Bellaterra, P08193 Barcelona, Spain
[c] Museu da Ciência, Laboratorio Chimico, Largo Marquês de Pombal, 3000-272 Coimbra, Portugal
[d] CESAM, Departament of Biology, University of Aveiro, P-3810 193 Aveiro, Portugal
[e] CESAM, Departament of Chemistry, University of Aveiro, P-3810 193 Aveiro, Portugal
[f] CRHEA, São Carlos Engineering School, University of São Paulo, P13566590 CP 339, Brazil

ARTICLE INFO

Article history:
Received 5 February 2013
Received in revised form 26 May 2013
Accepted 14 June 2013

Keywords:
Community ecotoxicology
Carbofuran
Diversity
Life-traits
Tropical
Collembola

ABSTRACT

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.,

* Corresponding author at: IMAR-CMA, Marine and Environmental Research Center, Department of Life Sciences, University of Coimbra, Apartado 3046, 3001-401 Coimbra, Portugal. Tel.: +351 239 855760; fax: +351 239 855789.
E-mail addresses: sonia.chelinho@iav.uc.pt, soniachelinho@sapo.pt (S. Chelinho).

0929-1393/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.apsoil.2013.06.009

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

(5 mm) and defaunated through one freezing (F)–thawing (T) cycle (48 h F–8 h T–24 h F). Afterwards, the soil was spiked in the laboratory with different proportions of a stock solution of Furadan 350SC (the same commercial formulation of carbofuran referred above) and diluted in deionised water to create the same range of concentrations as in the Brazilian soil.

In both assays, the amount of solutions added per treatment was adjusted to achieve initial moisture content of 50% of the WHC. Afterwards, the contaminated soil was distributed in plastic boxes (9.5 cm height × 11.5 cm diameter) with perforated lids (~300 g dry weight, ~80 cm^3 × 7 replicates per treatment).

2.3. Sampling, extraction and incubation of soil microarthropods

For the assay performed in Brazil, in the uncontaminated surroundings of the area where the test soil was collected, the upper vegetation layer was removed and soil cores (7 cm Ø × 10 cm) were taken along chosen transects randomly outlined and placed in plastic bags. A similar procedure was undertaken in the fallow land used as study area in Portugal.

In both assays, the content of 3 soil cores (randomly selected; ~450 g fresh weight) was mixed and used in each test replicate as a source of microarthropods. The microarthropod communities were extracted using Berlese funnels (Brazil) or a Macfadyen high-gradient extractor (Portugal). The replicates of the different treatments were randomly assorted. Organisms were extracted directly to the treated (contaminated with carbofuran), control soils and 8 smaller vessels (per assay) containing 80% ethanol, during 14 or 7 d (respectively in the Brazilian and Portuguese assay). The vessels with ethanol were used to further characterize the initial communities (ICs) of both countries, in terms of abundance and richness of microarthropods.

After this extraction period, the vessels containing the treated soil and the microarthropods were incubated under laboratory conditions (23 ± 1 °C or 20 ± 1 °C, respectively for Brazilian and Portuguese assays; 16:8 – light:dark photoperiod) for 4 weeks. The vessels used to characterize the ICs were stored at room temperature for further processing (see Section 2.4). Following the incubation period, microarthropods were extracted again (during the same period reported above for the first extraction) and preserved in 80% ethanol.

2.4. Microarthropod sorting and identification

The extracted organisms, preserved in 80% ethanol, were initially counted and sorted into higher taxonomic entities under a stereomicroscope (40× magnification) according to Barrientos (1988) and Minor and Robertson (2006).

In a second phase, mites were sorted into four main groups: (suborder) Oribatida, (order) Mesostigmata, (suborder) Prostigmata, and (cohort) Astigmata, according to Lindquist et al. (2009).

For the identification of collembolans, taxonomic and trait-based approaches were followed. These organisms were identified and assorted into five families (Entomobryidae, Isotomidae, Onychiuridae, Poduridae and Sminthuridae) according to Gisin (1960). In addition, the individuals were classified according to five morphological traits, namely the ocelli, furca, antenna, pigmentation and the presence of hairs and scales, that define Collembola life-form (Gisin, 1943; see Table 2).

Within each Collembola family, the organisms exhibiting a different combination of trait scores were considered as representing different morphospecies (see Table A.1). Thus, for the Portuguese and Brazilian assays, 19 and 18 morphospecies were considered, respectively.

Table 2
Collembola species traits and corresponding scores used to define the morphospecies and to calculate the mean trait per community index (mT).

Trait	Trait modality	Score
Ocelli	Absent	1
	Present	5
Antenna length	$0 < X < 0.5$ body length	1
	0.5 body length $< X < 1$ body length	3
	>1 body length	5
Furca	Absent	1
	Reduced/short	3
	Long	5
Hairs/scales	Absent	1
	Presence of hairs only	3
	Presence of hairs and scales	5
Pigmentation	White	1
	Coloured, no patterns	3
	Coloured, patterns	5

2.5. Chemical analysis

Samples (~400 g, w/w) from each dilution/concentration were stored at −20 °C for analysis of carbofuran concentrations. The analyses of the Brazilian and Portuguese samples were performed by IQSC, University of São Paulo (Brazil) and CESAM – Department of Chemistry, University of Aveiro (Portugal), respectively, as described before (Chelinho et al., 2011b, 2012).

2.6. Statistical analysis

The data were previously analyzed for normality (Shapiro–Wilk test) and for variance homogeneity (Levene test). If violations of normality and/or homogeneity occurred, a $\log(x+1)$ (for total abundance data) or a arcsin (for relative abundance data) transformation were applied. To investigate the effects of carbofuran on the total or relative abundance of Acarina and Collembola (the two most abundant groups; see Section 3.2), a One-Way ANOVA followed by post hoc comparisons with the control (Dunnets' test) or a Kruskall–Wallys test followed by multiple comparisons with the control were used. The latter tests were used whenever normality and variance homogeneity assumptions were not fulfilled even after data transformation. The same analyses were performed for the Collembola families and the four groups of mites (see Section 2.5).

All analyses were performed in Statistica 7.0 (available at http://www.statsoft.com/). The relative abundance of the four groups of Acari and five families of Collembola was calculated as a function of to the total numbers of each group (Acari or Collembola) found per treatment.

Potential effects of carbofuran on overall microarthropod community composition and on the Collembola and mite groups separately (for the last two endpoints, only relative abundance data was used) were evaluated by analysis of similarity (ANOSIM), comparing the community composition of the carbofuran contaminated samples with those of the control. Whenever significant differences were found, the similarity of percentages (SIMPER) analysis was used to identify the families or groups responsible for the observed change and their contribution (in terms of percentage) for the overall shift. Both ANOSIM and SIMPER, analysis were performed in Primer 5.2.6 (Clarke and Gorley, 2001) using $\log(x+1)$ transformed data (for microarthropod community composition).

Regarding the morphological traits of Collembola, data were pooled per treatment and used to calculate two functional trait indices per treatment: the mean trait per community (mT) and the Functional Diversity (FD), following a similar approach to that

Table 3
Group composition of soil microarthropod communities from Portugal (PT) and Brazil (BR) (expressed as total abundance of major taxonomic groups per treatment) exposed to soil contaminated with Furadan (see Section 2.2 for details). RD, recommended dose; IC, initial community; Aca, Acari; Coll, Collembola; Prt, Protura; Symph, Symphyla; Paurop, Pauropoda; Chil, Chilopoda; Aran, Araneae; Pseudosc, Pseudoscorpionidae; Staph, Staphylinidae; Coleo, other Coleoptera; Aphid, Aphididae; Form, Formicidae; Larv, Larvae; Others, Isopoda + Diplopoda + Isoptera + Psocoptera + Thysanoptera.

Doses (% 2 × RD)	Aca	Coll	Prt	Symph	Paurop	Chil	Aran	Pseudosc	Staph	Coleo	Aphid	Form	Larv	Others
PT														
IC	2317	568	4	24	0	5	0	1	0	3	12	170	27	0
0	709	199	0	1	0	1	1	0	0	0	1	0	2	0
2.5	845	97	0	1	0	1	1	0	0	0	1	2	3	0
5	1032	108	0	1	0	1	1	0	0	0	0	1	1	0
12.5	828	133	0	1	0	1	0	2	1	0	0	0	3	1
25	862	79	0	2	0	2	0	0	0	0	0	0	3	0
50	550	12	1	0	0	2	0	0	0	0	0	3	6	0
100	622	8	0	0	0	0	0	0	0	0	0	1	3	0
BR														
IC	4301	487	460	13	8	2	1	0	4	40	0	175	5	14
0	1371	1214	24	1	1	1	1	0	5	7	0	15	3	0
2.5	1514	651	89	0	2	0	3	0	6	21	0	42	4	0
5	1657	658	217	1	0	0	3	0	4	16	0	19	2	1
10	1028	382	0	1	0	1	2	0	1	8	0	67	1	0
25	1528	279	3	0	0	1	1	0	1	10	0	60	0	0
50	2049	133	0	0	0	1	6	0	0	9	0	25	0	0
100	2294	27	0	0	0	0	0	0	0	7	0	0	1	2

carried out by Vanderwalle et al. (2010). For each morphospecies, the scores of individual traits were summed up to determine the "Life-form" trait (LFT) that was used for the calculation of indices indicated above (see Vanderwalle et al., 2010). It ranged between 5 (minimum, indicative of euedaphic species) and 25 (maximum, indicative of epigeic species).

The mT index consisted of the average of the trait values, which were calculated for each morphospecies of each treatment (the latter considered as a different community). The trait value was calculated from the multiplication of the LFT (described above) by the relative abundance of each morphospecies. The mT values calculated for each carbofuran treatment were compared with the respective control using a *t*-test.

The FD index reflected the range of trait values within each treatment (or community; Díaz et al., 2007) and was calculated according to Lepš et al. (2006).

Simpson (1949) and Shannon diversity indexes (Shannon and Weaver, 1949) were also calculated.

3. Results

3.1. Composition of the microarthropod community

The initial communities (ICs) of Portugal and Brazil were composed by 12 and 16 groups of microarthropods, respectively, with clear dominance of mites (74% and 78% of total individuals, respectively; Table 3).

Prostigmatid and oribatid mites dominated the initial Portuguese microarthropod community (46% and 16%, respectively; data not shown). While in the Brazilian assay, Oribatid and mesostigmatid mites were the most abundant (51% and 20%, respectively; data not shown) Collembolans were the second most abundant group and, together with mites, represented approximately 92% and 87% of the total of individuals found, respectively for the Portuguese and Brazilian assays (Table 3).

Also, the dominance of collembolan families differed between the two countries. In Portugal, the majority of collembolans belonged to the Onychiuridae and Isotomidade (66% and 30% of the total Collembola, respectively; data not shown) while in Brazil, Entomobryidae and Isotomidae represented approximately 3% of all microarthropods (39% and 47% of total Collembola, respectively; data not shown).

Among the less abundant microarthropod groups, some exclusively occurred within one of the communities. For example, Aphididae and Pseudoscorpionidae were only found within the Portuguese (IC, control and/or treated) samples while Coleoptera (other than Staphylinidae) Pauropoda, Thysanoptera, Isoptera and Diplopoda were specific of Brazilian communities (IC, control and/or treated) samples (Table 3).

3.2. Effects of carbofuran on community composition and abundance

For the two assays, the total number of microarthropods recovered in the controls, when compared with the IC, decreased strongly (to 70% and 52%, respectively for the Portuguese and Brazilian experiments; Table 3). Also, in general, a high variability was found between replicates in the total number of individuals and in the relative abundance of the microarthropod groups.

Exposing both soil communities to a gradient of carbofuran concentrations (measured values are available in Table 4) caused a decrease in the community richness since the number of microarthropod groups progressively decreased (Fig. 1 and Table 3).

Significant differences in the microarthropod community composition were also detected by ANOSIM. In the Portuguese experiment, the community at the two highest doses (50% and 100% of 2 × RD) was significantly different from the control (ANOSIM, $p < 0.05$); Collembola and Acarina (both with decreased abundance at the two highest doses if compared to the control) were the groups that most contributed to this dissimilarity (SIMPER analysis; see Table A.2).

Stronger effects of carbofuran were observed within the communities extracted from the Brazilian soil since, with one exception (5% of 2 × RD), all doses caused significant changes in the community structure when compared with the control (ANOSIM, $p < 0.05$); the SIMPER analysis highlighted Protura, Collembolla (in both cases, their abundance was negatively affected by the treatments) and Formicidae (more abundant in the treated doses than in the control) as the groups that mostly contributed to the dissimilarity detected (SIMPER analysis; see Table A.2).

Focusing on the two most abundant groups, a common pattern of response was found for the collembolans: the average number of individuals decreased along the contamination gradient, with significant negative effects found for the highest doses (50% and

Table 4
Carbofuran concentrations (expressed as milligrams per kilogram of soil dry mass) in the laboratory spiked soil (Portuguese experiment) or in the field contaminated soil (Brazililian experiment) with 2 times the recommended dose (2 × RD) of Furadan. n.a., not applicable.

Doses (% of 2 × RD)	Predicted carbofuran concentrations[a] (mg/kg)	Carbofuran concentrations (mg/kg)	
		Portugal	Brazil
0	n.a.	<0.020	<0.010
2.5	0.058	0.054	0.039
5	0.117	0.113	0.079
10	0.233	n.a.	0.460
12.5	0.292	0.509	n.a.
25	0.583	0.964	0.400
50 (RD)	1.167	2.025	1.520
100	2.333	3.438	2.460

[a] Assuming a soil density of 1.5 g/dm^3 and a pesticide incorporation up to 5 cm depth.

100% of 2 × RD or 25%, 50% and 100% of 2 × RD, respectively for the Portuguese and Brazilian experiments; One-Way ANOVA, Dunnett test; $p < 0.05$; Fig. 1).

With respect to mites, in the Portuguese experiment, the variability among replicates impaired the establishment of statistically significant effects (One-Way ANOVA, Dunnett test; $p > 0.05$; Fig. 1A).

The increase in carbofuran concentrations was generally linked with a higher number of mites in the Brazilian experiment with a significant effect found for the highest dose (One-Way ANOVA, Dunnett test, $p < 0.05$; Fig. 1B).

3.3. *Effects of carbofuran in the community composition of mites and collembolans*

The shifts in the overall community structure of Collembola in the different carbofuran treatments were somehow similar for the two geographical communities.

In the Portuguese experiment, the relative abundance of Entomobryidae tended to increase, while the opposite happened for the Isotomidae (Fig. 2A). Onychiuridae slightly increased with concentrations, excepting at the two highest doses, where a sharp decline in the relative abundance was observed (Fig. 2A). Despite

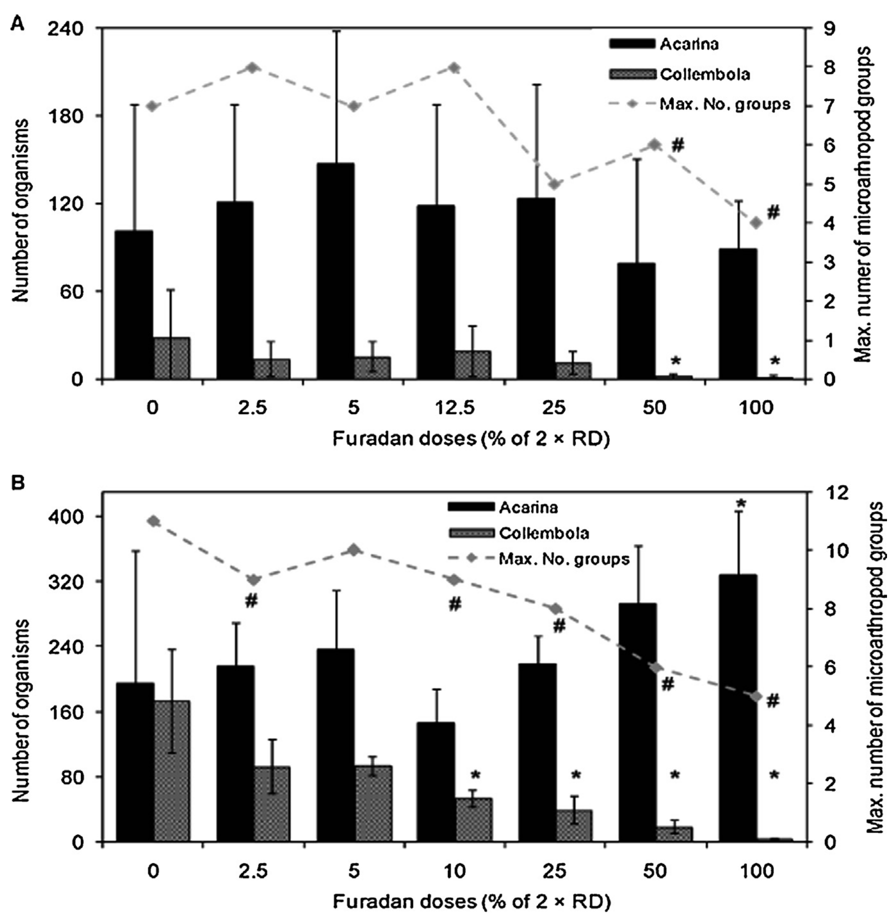

Fig. 1. Effects of Furadan (a.i. carbofuran) contaminated soil on the total abundance of Acarina (black bars), Collembola (grey bars, left axis) and maximum number of soil microarthropod groups (dotted line, right axis) found in two soil communities from Portugal (A) and Brazil (B). Values express average (±standard deviation – SD) values. RD, recommended dose. *Statistically different from Acarina or Collembola found in the control (One-Way ANOVA, Dunnett test, $p < 0.05$). #Microarthropod community (diversity and abundance of taxonomic groups) statistically different from the control (ANOSIM, $p < 0.05$).

Please cite this article in press as: Chelinho, S., et al., Soil microarthropod community testing: A new approach to increase the ecological relevance of effect data for pesticide risk assessment. Appl. Soil Ecol. (2013), http://dx.doi.org/10.1016/j.apsoil.2013.06.009

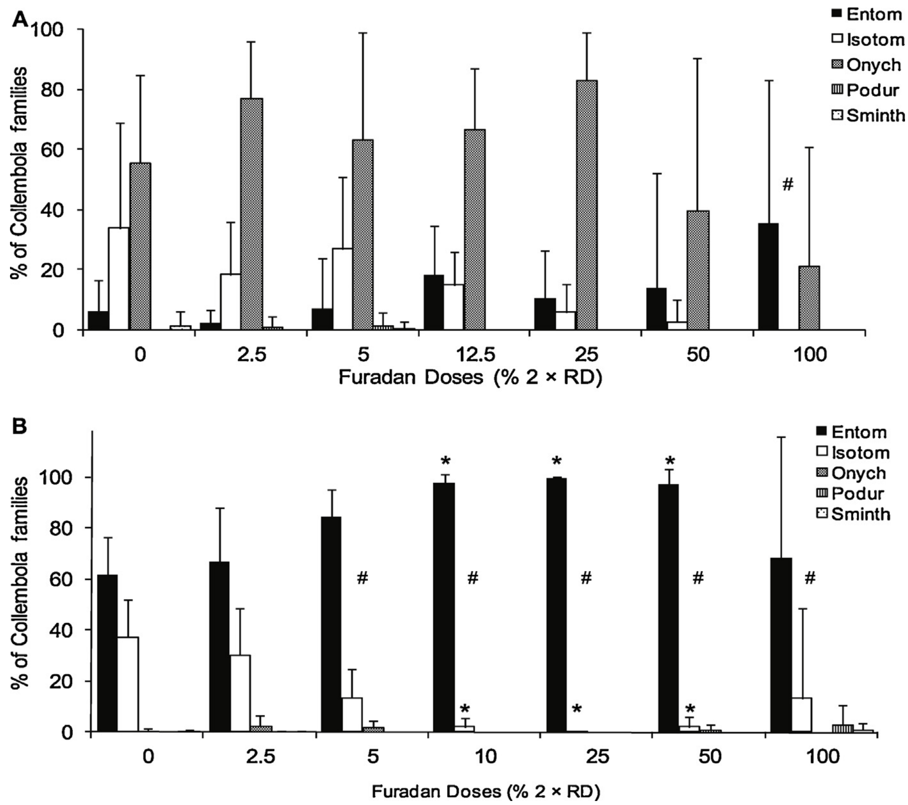

Fig. 2. Effects of Furadan (a.i. carbofuran) contaminated soil on the relative abundance of the families of Collembola found in two soil microarthropod communities from Portugal (A) and Brazil (B). Values express average (±standard deviation – SD) values. RD, recommended dose. *Statistically different from the respective Collembola family of the control (Kuskall–Wallys test and multiple comparisons with the control; $p < 0.05$). #Collembolan community (diversity and abundance of families) statistically different from the control (ANOSIM, $p < 0.05$). Entom, Entomobryidae; Isotom, Isotomidae; Onych, Onychiuridae; Podur, Poduridae; Sminth, Sminthuridae.

this, no significant differences were found for any family (One-Way ANOVA, Dunnett test, $p > 0.05$; Fig. 2A).

A significant increase in the relative abundance of Entomobryidae was also observed in the Brazilian experiment, which contrasted with the significant decline of Isotomidae, in all Furadan doses, except at the highest dose, where a high variability was found (significant differences found for the doses 10%, 25% and 50% of 2 × RD, for both families; Kruskall–Wallys test and multiple comparisons with the control, $p < 0.05$; Fig. 2B).

In terms of the overall community composition of Collembola, in the Portuguese experiment, significant differences were found only at the highest dose (100% of 2 × RD) when compared to control (ANOSIM, $p < 0.05$; Fig. 2A). Entomobryidae, Isotomidae and Onychiuridae explained 95% of the observed dissimilarity (SIMPER analysis; see Table A.2 and Fig. 2A). The effects of the insecticide were more clear within the Brazilian experiment, since ANOSIM detected significant differences at all the doses, except the lowest (2.5% of 2 × RD), relatively to the control (ANOSIM, $p < 0.05$). Again, the most abundant families, Isotomidae, Entomobryidae and Onychiuridae contributed most to these differences (SIMPER analysis, see Table A.2 and Fig. 2B).

The mite community in the Portuguese soil appeared to be more resistant to carbofuran contamination, compared to Collembola communities, since no significant differences were found between the relative abundance of any of the four groups individually and that of the control (One-Way ANOVA, $p > 0.05$; Fig. 3A) nor for the overall community structure (ANOSIM, $p > 0.05$).

Contrastingly, in the Brazilian experiment, a significant drop in the relative abundance of Mesostigmata and Prostigmata (doses 10%, 25%, 50% and 100% of 2 × RD lower than the control; One-Way ANOVA, Dunnett test, $p < 0.05$; Fig. 3B) was observed. In parallel, the relative abundance of oribatid mites consistently increased (all doses, excepting 2.5% of 2 × RD, higher than the control; One-Way ANOVA, Dunnett test, $p < 0.05$; Fig. 3B).

The community of mites was different from the control at all doses, except for the lowest (ANOSIM, $p < 0.05$); these differences were mainly influenced by the decrease in relative abundance of Mesostigmata and Prostigmatid mites (SIMPER, see Table A.2).

The effects of carbofuran on the total abundance of Collembola and Acarina groups were quite similar to the ones described above for the relative abundance data, and are available in Figs. A and B of supplementary material, respectively.

3.4. Effects of carbofuran on functional traits of collembolans

In both assays, the soil contamination generally decreased the diversity of morphospecies, as evidenced by the decreases of both Simpson and Shannon diversity indices, excepting for the dose "50% of 2 × RD" in the Brazilian experiment (Table 5).

Among the two indexes used to describe changes in functional traits of collembolans along the gradient of insecticide treatments (mT and FD), the patterns were not always similar. The mT values tended to increase in both assays, reflecting the higher representation of morphospecies adapted to surface soil layers (Table 5). Relatively to the controls, this variation was statistically significant at the two highest Furadan doses for the Portuguese experiment (t-test, $p < 0.05$; Table 5) and at the highest dose for the Brazilian experiment (t-test, $p < 0.05$, Table 5).

On the other hand, for the Portuguese experiment, there was a tendency for FD values to increase along the contamination gradient (Table 5), which were not correlated with the decrease in the diversity indices reported above. An opposite scenario was observed for the Brazilian experiment, since lower FD values were obtained for the high Furadan treatments and this trend was

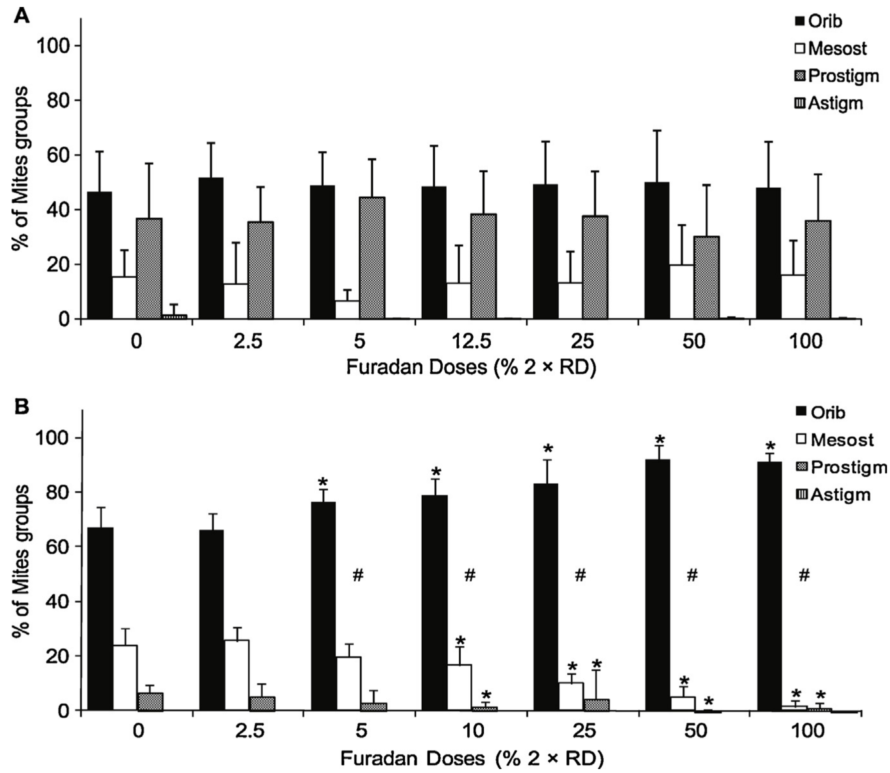

Fig. 3. Effects of Furadan (a.i. carbofuran) contaminated soil on the relative abundance of the four groups of Acarina found in two soil microarhopod communities from Portugal (A) and Brazil (B). Values express average (±standard deviation – SD) values. RD, recommended dose. *Statistically different from the respective Acarina groups of the control (One-Way ANOVA, Dunnett test, $p < 0.05$). #Mites community (diversity and abundance of mite groups) statistically different from the control (ANOSIM, $p < 0.05$). Orib, Oribatida; Mesost, Mesostigmata; Prostigm, Prostigmata; Astigm, Astigmata.

strongly correlated with the variation of both Simpson and Shannon indices ($r = 0.90$ and 0.82 and $p < 0.001$ and <0.005, respectively for FD vs Simpson and FD vs Shannon).

4. Discussion

4.1. Testing strategy

From a methodological point of view, one of the major limitations of the approach adopted in the present study was the low recovery rate of the microarthropods in the controls and treated samples, compared with the initial community. This might be related to the fact that organisms were confined to a small area from which it was not possible to escape, either from potential predators (e.g. Staphylinidae and/or Araneae; Bohac, 1999; Marc et al., 1999) or from unfavourable environmental conditions (e.g. temperature, humidity and/or light). In fact, under laboratory conditions, it was not possible to simulate the environment of a real soil system. Also, the possibility that the extraction procedures might have worked as a stress factor to some of the organisms cannot be discharged. Improvements can be further adopted by using larger test-boxes, extending the exposure period for at least four more

Table 5
Summary of the responses of two soil communities of Collembola, from Portugal (PT) and Brazil (BR) to increasing Furadan treatments. Results are expressed in terms of the mean trait per community (mT), functional diversity (FD) and diversity of morphospecies (Simpson and Shannon indices). RD, recommended dose; IC, initial community; *n*, number of morphospecies (organisms exhibiting a different combination of trait scores; see Section 2.4).

	Furadan doses (% of 2 × RD)							
PT								
Indices	IC	0	2.5	5	12.5	25	50	100
mT	0.637	0.805	1.374	0.975	1.164	1.166	3.944[a]	7.250[a]
FD	0.129	0.142	0.120	0.150	0.244	0.135	0.375	0.256
Simpson	0.616	0.669	0.622	0.711	0.590	0.468	0.542	0.375
Shannon	1.251	1.387	1.165	1.434	0.995	0.985	0.888	0.562
n	13	11	6	9	8	7	3	2
BR								
Indices	IC	Ct	2.5	5	10	25	50	100
mT	1.097	1.111	1.508	1.457	1.706	2.830	1.665	5.198[a]
FD	0.321	0.270	0.262	0.240	0.125	0.099	0.203	0.108
Simpson	0.735	0.704	0.710	0.743	0.622	0.538	0.710	0.524
Shannon	1.680	1.435	1.399	1.584	1.184	0.967	1.578	0.814
n	12	13	10	11	10	6	10	3

[a] Statistically different from Ct (*t*-test; $p < 0.05$).

Please cite this article in press as: Chelinho, S., et al., Soil microarthropod community testing: A new approach to increase the ecological relevance of effect data for pesticide risk assessment. Appl. Soil Ecol. (2013), http://dx.doi.org/10.1016/j.apsoil.2013.06.009

weeks (possibly allowing the reproduction of some species and thus, the assessment of sub-lethal effects) and by considering the supply of food during the test.

Despite the methodological constrains, results showed that this testing approach was valid and sensitive enough to detect the effects of a pesticide on two soil microarthropod communities. Moreover, its further use as a tool to introduce more ecological realism in the data gathered from ecotoxicology-related studies seems promising.

4.2. Effect of carbofuran contamination on the microarthropod communities

Both soil microarthropod communities from Portugal and Brazil were negatively affected by the insecticide contamination. Notwithstanding, stronger effects were registered within the Brazilian assay, where even low dosages of carbofuran induced significant shifts when compared to the control.

These observations are not in agreement with the chemical analysis of the test soils that showed carbofuran concentrations generally higher in the laboratory spiked Portuguese soil than in the field contaminated Brazilian soil (Table 4). The different levels of contamination may be explained by the high dispersion usually occurring for soil sprayings under field conditions (Schulz, 2004). On the other hand, the pedological properties of the two soils might have determinated a lower bioavailability of the pesticide for the organisms in the Portuguese soil. Indeed, higher adsorption of carbofuran seems to occur in silt loam and loam soils (such as the Portuguese) than in sandy loam soils (such as the Brazilian) (Singh and Srivastava, 2009). In addition, carbofuran seems to degrade faster (and thus, causing lower toxicity) under moist conditions (Shelton and Parkin, 1991), which were higher for the Portuguese soil. Moreover, in a more moist soil, carbofuran will be more diluted in the soil pore water, an important exposure route for soil microarthropods. An intrinsically higher sensitivity of the organisms from communities of Brazil to pesticides (not investigated) is also a possible explanation.

Direct comparisons with the available literature should be made with caution, due to the differences in experimental design and exposure conditions. Despite this, the impoverishment of taxonomic diversity as well as the general decrease in the overall abundance of microarthropods (more evident in the Brazilian assay; Table 3) observed in the present study is in agreement with data previously reported for carbofuran (Broadbent and Tomlin, 1982; Bambaradeniya and Edirisinghe, 2008) and other insecticides (Joy and Chakravorty, 1991; Frampton, 1999; Joy et al., 2005; Endlweber et al., 2006; Scholz-Starke et al., 2011). However, other authors found low or no toxicity of insecticides (endosulfan, deltamethrin and diflubenzuron) for the same groups of organisms (Osler et al., 2001; Griffiths et al., 2006; Adamski et al., 2009).

As expected, Collembola, known to be particularly sensitive towards carbofuran (Frampton, 1994; Bambaradeniya and Edirisinghe, 2008) showed a dose–response pattern, with a lower abundance relatively to the control. This was most pronounced at the highest dose, with 5% or less of the abundance of the control.

The opposite response occurred for mites, which generally increased along the contamination gradient. However, in the Portuguese experiment, at the doses 5%, 50% and 100% of $2 \times$ RD, a slight decline (maximum 22%, compared with the control) was registered. Especially in the case of the Brazilian assay, the increase in the number of mites might be related to the concurrent decline of predators. Data from literature, where other acetylcholinesterase inhibitors (e.g. dimethoate and chlopyriphos) were tested, also reported toxic effects for collembolans (Joy and Chakravorty, 1991; Martikainen et al., 1998; Frampton, 1999; Endlweber et al., 2006; Frampton and Van den Brink, 2007) but not for mites (Joy and Chakravorty, 1991). However, the absence of toxic effects of chlorpyrifos in tropical arthropod assemblages (including collembolans and mites) was also reported by Michereff-Filho et al. (2004). Also, Frampton (1999) and Frampton and Van den Brink (2007) did not find toxic effects of another carbamate insecticide, pirimicarb, on the collembolan community.

4.3. Effects of carbofuran on the taxonomic groups and community structure of Acari

The response of mites over the increasing Furadan dosages was again stronger within the Brazilian assay. The continuous increase in the relative abundance of Oribatids, which are particle feeding saprophages and mycophages (Krantz, 2009), may be related to a reduction in the number of competitors (namely, collembolans) for the available organic matter (Filser, 2002). Moreover, their typically high body sclerotization (Norton and Behan-Pelletier, 2009), may work as a biological barrier to pesticide penetration, conferring them higher resistance against carbofuran (Martin, 2007). Their long-life cycles (with slow metabolic rates) and conservative ontogeny, if compared to other mite groups (Norton and Behan-Pelletier, 2009), might have also conferred increased resistance or delayed effects of carbofuran. In parallel, the decline observed for predatory mites (Mesostigmata), was probably a consequence of the strong decrease of collembolans, their potential preys (Koehler, 1997). Despite this, a direct toxic effect of the insecticide cannot be excluded (Koehler, 1997).

In the Portuguese assay, the taxonomic profile of the mite community was different, with a shared dominance of Oribatida and Prostigmata. The observed decrease of collembolans was also expected to cause the reduction of mesostigmatid mites. However, the relative abundance of the four taxonomic groups of mites remained more or less constant at all Furadan doses, causing no significant effects at the community level. The maintenance of the community structure might have been facilitated by prostigmatid mites, which are extremely diverse in their feeding habits (Walter et al., 2009). Thus, these mites might have competed with oribatids for organic detritus and with mesostigmatids for collembolans or could also be used as food for predatory mites (Koehler, 1997). However, the lower level of body sclerotization of prostigmatid mites (Walter et al., 2009) would anticipate a higher sensitivity to carbofuran (Martin, 2007).

4.4. Taxonomic and morphological trait changes of communities of collembola induced by carbofuran contamination

The most significant shifts in the relative abundance of collembolan families and, consequently, in the overall community structure due to carbofuran contamination were observed for the Brazilian experiment. The two major trends observed were the significant increase of the relative abundance of Entomobryidae and the decline in Isotomidae, observed at the four highest Furadan doses.

For the Portuguese experiment, the same tendencies were observed, although the low number of organisms found per treatment (compared with the Brazilian assay) might have contributed to a higher variability and impaired the establishment of more reliable trends. Despite this, significant shifts in the overall community structure (in relation to that of the control) were detected at the highest Furadan dose.

Similarly, in an 8 week study, the total abundance of Entomobryidae collected in pitfall traps of Brazilian cornfields subjected to chlorpyrifos spraying, increased by 18%, while for Isotomidade a decline of 77% was registered (Michereff-Filho et al., 2004). However, under temperate conditions, the 44 d exposure of collembolans to the same insecticide caused a significant decline in the abundance of both Isotomidae and Entomobryidae (Frampton and Van den Brink, 2007).

Trait analysis also revealed significant changes in the community of collembolans induced by carbofuran. The diversity of combinations of trait scores, considered in the present study as morphospecies richness, decreased along the contamination gradient, as revealed by the two diversity indices (Table 5).

In both assays, the increasing mT values calculated for the communities of collembolans along the contamination gradient (Table 5) suggest that species adapted to deeper soil layers are more vulnerable to toxic effects of this insecticide. Moreover, these results also indicate a shift in the functional composition of the communities, namely that epigeic species increased their representativeness with increasing carbofuran concentrations.

A consistent match is noticeable when linking these results with the life form traits assigned to the organisms of the most abundant families, and with the variation in their relative abundances. Indeed, individuals from the family Entomobryidae, which are mostly epigeic (Hopkin, 1997), presented the highest values of LFT and increased their relative abundance. The opposite pattern (i.e. low values of LFT and decreased relative abundance) was registered for organisms assigned to the family Isotomidae, which, in the present study, possessed mostly life form traits related to euedaphic life.

Members of the family Onichiuridae, which are euedaphic (Hopkin, 1997) and dominated the Portuguese communities, theoretically, could have also decreased in numbers, being substituted by epigeic species. However, this pattern was only observed for the two highest Furadan doses and it was not statistically significant. Despite this, hemiedaphic species may have also contributed to the observed increase in the mT.

A possible explanation to these community shifts might be indirectly related to one of the Collembola adaptations against drought. Epigeic collembolans developed a low cuticular permeability, which provides them high resistance towards desiccation, contrasting with the high cuticular permeability of euedaphic species (Kærsgaard et al., 2004). The cuticle also constitutes a biological barrier against the penetration of water-soluble pesticides (Gillott, 1995; Martin, 2007) such as carbofuran. Therefore, epedaphic collembolans, which in addition are less in contact with soil pore water (Hopkin, 1997) would be less exposed to this insecticide. The higher mobility and lower contact with the soil pore water of epedaphic Collembola when compared with euedaphic ones was the explanation pointed by Fountain and Hopkin (2004) for the lower toxicity observed for epigeic springtails along a gradient of metal contamination.

FD index decreased with taxonomic diversity only in the Brazilian assay, indicating that carbofuran contamination decreased the diversity of morphospecies traits within each community (Díaz et al., 2007).

The opposite pattern found for the Portuguese assay is difficult to explain as the diversity of morphospecies drastically declined at the highest carbofuran concentrations (e.g. from 11 in the control to 2 at the highest dose, Table 5). However, considering that FD index is a sum of the trait dissimilarity of all pairs of species, weighted by the their relative abundance (Vanderwalle et al., 2010) and that along the contamination gradient the relative abundance of morphospecies more dissimilar in their LFT increased, the final FD value would also be higher. This was observed for the Portuguese assay.

Despite it is not mandatory to find a correlation between species diversity indices and FD (Vanderwalle et al., 2010), the inclusion of more trait data (e.g. association to disturbed systems and stress tolerance) as well as the extension of the exposure period might clarify these responses.

In the present study, the more sensitive descriptor of the community responses to the insecticide disturbance was the mT index rather than the overall functional diversity as reported in other case studies presented before (Vanderwalle et al., 2010).

Also, the application of a trait based assessment of effects on Collembola allowed to identify which morphological characteristics make the organisms more vulnerable to carbofuran contamination. Ecological relevance was improved by using the original community of organisms as test-groups instead of laboratory introduced species. Further research, especially with other groups of soil organisms is needed to gain clearer insights into the sensitivity of communities to pesticides and other toxic substances.

5. Conclusions

The present study showed the feasibility of assessing effects of pesticide applications at community level under laboratory conditions. Also, the response patterns of both, Portuguese and Brazilian microarthropod communities to carbofuran contamination were similar, although a higher toxicity was observed for the latter assay. Significant shifts in the overall community structure of both microarthropod communities, reflected by a decrease in abundance and the impoverishment of taxonomic diversity were detected. Direct and strong negative effects were observed for Collembola while the abundance of Acari tended to increase with higher carbofuran concentrations.

Lowering the taxonomic level of assessment in the two most abundant groups, Acari and Collembola, the patterns of response were clarified. Thus, for mites, significant community shifts were only detected for the Brazilian organisms and were reflected by the increase of Oribatids and the reduction of mesostigmatids.

Among collembolans, data revealed that Entomobryidae seemed to have replaced Isotomidae along the contamination gradient.

Trait-based effects assessment was sensitive in revealing the response of Collembola to carbofuran. Main trends comprised the decrease in taxon diversity (expressed in the present study as different combinations of trait scores) in treated soils. A major functional shift was also observed, namely the favouring of epedaphic species and loss of euedaphic species. This shift was consistent with the changes observed at the family level and may be a consequence of their ecophysiological characteristics, namely the cuticular permeability. This is usually reduced in epigeic species and higher in euedaphic ones. Further methodological refinements are necessary to improve the information taken from this type of approach.

Funding sources

This study was sponsored by Fundação para a Ciência e Tecnologia – Portugal, trough PhD grants to Sónia Chelinho (SFRH/BD/27719/2006), Tiago Natal-da-Luz (SFRH/BD/29437/2006) and Anabela Cachada (SFRH/BD/38418/2007) and Post-doctoral grant to I. Lopes (SFRH/BPD/7192/2001) and FSE and POPH funds (Programa Ciência 2007).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.apsoil.2013.06.009.

References

Adamski, Z., Bloszyk, J., Piosik, K., Tomczak, K., 2009. Effects of diflubenzuron and mancozeb on soil microarthropods: a long-term study. Biol. Lett. 46 (1), 3–13.

ASTM, 1993. Standard guide for conducting a terrestrial soil-core microcosm test. Annual Book of ASTM 1197. American Society for Testing and Materials, Philadelphia, USA.

Baird, D.J., Rubach, M.N., Van den Brink, P.J., 2008. Trait-based ecological risk assessment (TERA): the new frontier. Integrated Environ. Assess. Manag. 4 (1), 2–3.

Baird, D.J., Van den Brink, P.J., 2007. Using biological traits to predict species sensitivity to toxic substances. Ecotoxicol. Environ. Saf. 67, 296–301.
Bambaradeniya, C.N.B., Edirisinghe, J.P., 2008. Composition, structure and dynamics of arthropod communities in a rice agro-ecosystem. Cey. J. Sci. (Bio. Sci.) 37 (1), 23–48.
Barrientos, J.A., 1988. Bases para un curso práctico de Entomologia. Associación Española de Entomología, Salamanca.
Bohac, J., 1999. Staphylinid beetles as bioindicators. Agric. Ecosyst. Environ. 74, 357–372.
Broadbent, A.B., Tomlin, A.D., 1982. Comparison of two methods for assessing the effects of carbofuran on soil animal decomposers in corn fields. Environ. Entomol. 11 (5), 1036–1042.
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.
Chelinho, S., Lopes, I., Natal-da-Luz, T., Domene, X., Nunes, M.E.T., Espíndola, E.L.G., Ribeiro, R., Sousa, J.P., 2012. Integrated ecological risk assessment of pesticides in tropical ecosystems: a case study with carbofuran in Brazil. Environ. Toxicol. Chem. 31, 437–445.
Chelinho, S., Domene, X., Campana, P., Natal-da-Luz, T., Scheffczyk, A., Römbke, J., Andrés, P., Sousa, J.P., 2011a. Improving ecological risk assessment in the Mediterranean area: selection of reference soils and evaluating the influence of soil properties on avoidance and reproduction of the oligochaetes *Eisenia andrei* and *Enchytraeus crypticus*. Environ. Toxicol. Chem. 30, 1050–1058.
Chelinho, S., Sautter, K.D., Cachada, A., Abrantes, I., Brown, G., Duarte, A.C., Sousa, J.P., 2011b. Carbofuran effects in soil nematode communities: using trait and taxonomic based approaches. Ecotoxicol. Environ. Saf. 74, 2002–2012.
Clarke, K.R., Gorley, R.N., 2001. Software PRIMER v5. PRIMER-E, Plymouth, UK.
Clements, W.H., Rohr, J.R., 2009. Community responses to contaminants: using basic ecological principles to predict ecotoxicological effects. Environ. Toxicol. Chem. 28, 1789–1800.
Dasgupta, S., Mamingi, N., Meisner, C., 2001. Pesticide use in Brazil in the era of agroindustrialization and globalization. Environ. Dev. Econ. 6, 459–482.
De Lange, H.J., Lahr, J., Van der Pol, J.J.C., Wessels, Y., Faber, J.H., 2009. Ecological vulnerability in wildlife: an expert judgment and multicriteria analysis tool using ecological traits to assess relative impact of pollutants. Environ. Toxicol. Chem. 28 (10), 2233–2240.
De Lange, H.J., Sala, S., Vighi, M., Faber, J.H., 2010. Ecological vulnerability in risk assessment – a review and perspectives. Sci. Total Environ. 408, 3871–3879.
Díaz, S., Lavorel, S., de Bello, F., Quetier, F., Grigulis, K., Robson, M., 2007. Incorporating plant functional diversity effects in ecosystem service assessments. Proc. Natl. Acad. Sci. U. S. A. 104, 20684–20689.
Endlweber, K., Schädler, M., Scheu, S., 2006. Effects of foliar and soil insecticide applications on the collembolan community of an early set-aside arable field. Appl. Soil Ecol. 31, 136–146.
Filser, J., 2002. The role of Collembola in carbon and nitrogen cycling in soil. Proceedings of the Xth international Colloquium on Apterygota, České Budějovice 2000: Apterygota at the beginning of the third millennium. Pedobiologia 46, 234–324.
Filser, J., Koehler, H., Ruf, A., Römbke, J., Prinzing, A., Schaefer, M., 2008. Ecological theory meets soil ecotoxicology: challenge and chance. Basic Appl. Ecol. 9, 346–355.
Fountain, M.T., Hopkin, S.P., 2004. A comparative study of the effects of metal contamination on Collembola in the field and in the laboratory. Ecotoxicology 13, 573–587.
Frampton, G.K., 1994. Sampling to detect effects of pesticides on epigeal Collembola. Aspects Appl. Biol. 37, 121–130.
Frampton, G.K., 1999. Spatial variation in non-target effects of the insecticides chlorpyrifos, cypermethrin and pirimicarb on Collembola in winter wheat. Pesticide Sci. 55, 875–886.
Frampton, G.K., Van den Brink, P.J., 2007. Collembola and macroarthropod community responses to carbamate, organophosphate and synthetic pyrethroid insecticides: direct and indirect effects. Environ. Pollut. 147, 14–25.
Gillott, C., 1995. Entomology, second ed. Plenum Press, New York.
Gisin, H., 1960. Collembolenfauna Europas. Muséum d'Histoire Naturelle, Geneva.
Gisin, H., 1943. Ökologie und Lebensgemeinschaften der Collembolen im Schweizer Exkursionsgebiet Basels. Rev. Suisse Zool. 50, 131–224.
Griffiths, B.S., Caul, S., Thompson, J., Birch, A.N.E., Scrimgeour, C., Cortet, J., Foggo, A., Hackett, C.A., Krogh, P.H., 2006. Soil microbial and faunal community responses to Bt maize and insecticide in two soils. J. Environ. Qual. 35 (3), 734–741.
Hopkin, S.P., 1997. Biology of the Springtails (Insecta: Collembola). Oxford University Press, Oxford.
Joy, V.C., Chakravorty, P.P., 1991. Impact of insecticides on nontarget microarthropod fauna in agricultural soil. Ecotoxicol. Environ. Saf. 22 (1), 8–16.
Kærsgaard, C.W., Holmstrup, M., Malte, H., Bayley, M., 2004. The importance of cuticular permeability, osmolyte production and body size for the desiccation resistance of nine species of Collembola. J. Insect Physiol. 50, 5–15.
Knacker, T., Van Gestel, C.A.M., Jones, S.E., Soares, A.M.V.M., Schallnaß, H-J., Förster, B., Edwards, C.A., 2004. Ring-testing and field validation of a terrestrial model ecosystem (TME) – an instrument for testing potentially harmful substances: conceptual approach and study design. Ecotoxicology 13, 5–23.
Koehler, H.H., 1997. Mesostigmata (Gamasina Uropodina), efficient predators in agroecosystems. Agric. Ecosyst. Environ. 62, 105–117.
Krantz, G.W., 2009. Habits and habitats. In: Krantz, G.W., Walter, D.E. (Eds.), A Manual of Acarology. , third ed. Texas Tech University Press, Lubbock, TX, pp. 64–82.
Kuperman, R.K., Checkai, R.T., Garcia, M.V., Römbke, J., Stephenson, G., Sousa, J.P., 2009. Ecotoxicological assessment of contaminated land: the state-of-practice and the way forward. Pesqui. Agropecu. Bras. 44, 811–824.
Lepš, J., de Bello, F., Lavorel, S., Berman, S., 2006. Quantifying and interpreting functional diversity of natural communities: practical considerations matter. Preslia 78, 481–501.
Liess, M., Beketov, M., 2011. Traits and stress: keys to identify community effects of low levels of toxicants in test systems. Ecotoxicology 20, 1328–1340.
Lindquist, E.E., Krantz, G.W., Walter, D.E., 2009. Classification. In: Krantz, G.W., Walter, D.E. (Eds.), A Manual of Acarology. , third ed. Texas Tech University Press, Lubbock, TX, pp. 97–103.
Marc, P., Canard, A., Ysnel, F., 1999. Spiders (Araneae) useful for pest limitation and bioindication. Agric. Ecosyst. Environ. 74, 229–273.
Martikainen, E., Haimi, J., Ahtiainen, J., 1998. Effects of dimethoate and benomyl on soil organisms and soil processes – a microcosm study. Appl. Soil Ecol. 9, 381–387.
Martin, J.M., 2007. Concise Encyclopedia of the Structure of Materials. Elsevier Science Publishers, Amsterdam.
Michereff-Filho, M., Guedes, R.N.C., Della-Lucia, T.M.C., Michereff, M.F.F., Cruz, I., 2004. Non-target impact of chlorpyrifos on soil arthropods associated with no tillage cornfields in Brazil. Int. J. Pest Manage. 50 (2), 91–99.
Minor, M.A., Robertson A.W., 2006. Soil Bugs – An Illustrated Guide to New Zealand Soil Invertebrates (updated 16-Nov-2010), Available at: http://soilbugs.massey.ac.nz/key.php. Assessed 20th of November 2012.
Norton, R.A., Behan-Pelletier, V.M., 2009. Suborder Oribatida. In: Krantz, G.W., Walter, D.E. (Eds.), A Manual of Acarology. , third ed. Texas Tech University Press, Lubbock, TX, pp. 430–546.
Relyea, R., Hoverman, J., 2006. Assessing the ecology in ecotoxicology: a review and synthesis in freshwater systems. Ecol. Lett. 9, 1157–1171.
Scott-Fordsmand, J.J., Maraldo, K., van den Brink, P.J., 2008. The toxicity of copper contaminated soil using a gnotobiotic Soil Multi-species Test System (SMS). Environ. Int. 34, 524–530.
Schäffer, A., Van den Brink, P., Heimbach, F., Hoy, S., de Jong, F., Römbke, J., Roß-Nickoll, M., Sousa, P., 2010. Semi-Field Methods for the Environmental Risk Assessment of Pesticides in Soil. SETAC (CRC) Press, Boca Raton, FL.
Schäffer, A., van den Brink, P.J., Heimbach, F., Hoy, S., Jong, F., de, Römbke, J., Sousa, J.P., Roß-Nickoll, M., 2008. Semi-field methods are a useful tool for the environmental risk assessment of pesticides in soil. Environ. Sci. Pollut. Res. 15, 176–177.
Scholz-Starke, B., Nikolakis, A., Leicher, T., Lechelt-Kunze, C., Heimbach, F., Theissen, B., Toschki Ratte, H.T., Schäffer, A., Roß-Nickoll, B., 2011. Outdoor terrestrial model ecosystems are suitable to detect pesticide effects on soil fauna: design and method development. Ecotoxicology 20 (8), 1932–1948.
Schulz, R., 2004. Field studies on exposure, effects, and risk mitigation of aquatic nonpoint-source insecticide pollution: a review. J. Environ. Qual., 419–448.
Seastedt, T.R., 1984. The role of microarthropods in decomposition and mineralization processes. Annu. Rev. Entomol. 29, 25–46.
Shannon, C.E., Weaver, W., 1949. The Mathematical Theory of Communication. University of Illinois Press, Urbana, IL.
Shelton, D.R., Parkin, T.B., 1991. Effect of moisture on sorption and biodegradation of carbofuran in soil. J. Agric. Food Chem. 39, 2063–2068.
Simpson, E., 1949. Measurement of diversity. Nature 163, 688.
Singh, R.P., Srivastava, G., 2009. Adsorption and movement of carbofuran in four different soils varying in physical and chemical properties. Adsorpt. Sci. Technol. 27 (2), 193–203.
Vanderwalle, M., de Bello, F., Berg, M.P., Bolger, T., Doledec, S., Dubs, F., Feld, C.K., Harrington, R., Harrison, P.A., Lavorel, S., da Silva, P.M., Moretti, M., Niemela, J., Santos, P., Sattler, T., Sousa, J.P., Sykes, M.T., Vanbergen, A.J., Woodcock, B.A., 2010. Functional traits as indicators of biodiversity response to land use changes across ecosystems and organisms. Biodivers. Conserv. 19 (10), 2921–2947.
Van den Brink, P.J., 2008. Ecological risk assessment: from book-keeping to chemical stress ecology. Environ. Sci. Technol. 42, 8999–9004.
Van den Brink, P.J., Tarazona, J.V., Solomon, K.R., Knacker, T., Van den Brink, N.W., Brock, T.C.M., Hoogland, J.P., 2005. The use of terrestrial and aquatic microcosms and mesocosms for the ecological risk assessment of veterinary medicinal products. Environ. Toxicol. Chem. 24 (4), 820–829.
van Gestel, C.A.M., 2012. Soil ecotoxicology: state of the art and future directions. ZooKeys 296 (176), 275–296.
Van Straalen, N.M., 2002. Assessment of soil contamination – a functional perspective. Biodegradation 13, 41–52.
Walter, D.E., Lindquist, E.E., Smith, I.M., Cook, D.R., Krantz, G.W., 2009. Order Trombidiformes. In: Krantz, G.W., Walter, D.E. (Eds.), A Manual of Acarology. , third ed. Texas Tech University Press, Lubbock, TX, pp. 233–420.