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Collembola in ecotoxicology—Any news or just boring routine?

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ABSTRACT

Despite the uncontested significance of soils for human nutrition and drinking water quality, the majority of ecotoxicological testing is confined to aquatic test systems. Among the standardised tests for soils, the reproduction test with the springtail *Folsomia candida* is among the most widely used ones. First steps towards its standardisation were undertaken in the late 1980s. Here we review major advances that have been made since then, with respect to mechanistic, pragmatic and ecological aspects. Specifically we address the ecological relevance of any modifications of the standardised tests. We introduce a miniaturised version of the reproduction test which allows reducing the amount of soil per test unit to one third and the number of synchronised individuals to 40% as compared to the standard test. In addition, we developed an assay using Collembola eggs instead of synchronised adults. First results of a three-species test indicate that the presence of other species may affect choice behaviour. We point out a potential biased view of existing ecotoxicological data with Collembola due to the fact that most results refer to metal contamination. Finally, recommendations for future research are given, with special reference to avoidance and microcosm tests involving Collembola.

1. Introduction

Along with water and oxygen, soils are a crucial resource of human life: 95% of our food is directly or indirectly based on soils. In its report on degradation of land and water resources FAO recently stated "The greatest threat is the loss of soil quality" (FAO, 2011). Global problems such as desertification, salinisation, erosion, biodiversity loss and pollution have increasingly raised public awareness and called many political initiatives into action. However, only recently considerably more emphasis was put on international action plans and initiatives (e.g. Beck et al., 2005; EC, 2012).

Despite enormous anthropogenic input and technical advancements in agriculture (e.g. soil tillage, plant breeding, mineral fertilisers, pesticides) the essential services associated with crop production are mainly provided by the extremely diverse organism communities inhabiting soils (Nielsen et al., 2011). Studying their species composition and overall performance delivers information on the ecological state and quality of a soil (Beck et al., 2005). Potential hazards of new and existing chemicals to soils are being assessed by standardised test protocols with simplified models, usually involving single species of soil invertebrates or activity measures of the microorganism community. Van Gestel (2012) just reviewed ecotoxicological testing in soils in general, with special respect to invertebrates and isopods. Invertebrate tests cover mortality, reproduction and behaviour, and the two best studied groups with standard tests are earthworms and Collembola.

In this mini-review we focus on Collembola as a group which is highly abundant in almost any environment (Hopkin, 1997) and usually dominates the individual numbers of arthropods in most arable soils worldwide (Filser et al., 2002). We sketch the development towards standardised soil assays involving Collembola since the late 1980s and throw some spotlights on pragmatic, mechanistic and biological aspects related to these. Furthermore, we introduce a miniaturised version of the Collembola reproduction test and a test with Collembola eggs. We also present a new aspect in testing choice behaviour and make suggestions for future research in ecotoxicological testing with Collembola.

2. Recent developments in Collembola ecotoxicology

2.1. Steps towards standardisation

Until 1999, no standardised test guideline involving Collembola existed. However, considerably earlier much was known about the impact of toxic substances, especially heavy metals and pesticides, on Collembola (e.g. Hüther, 1961; Fox, 1967; Tomlin, 1975; Joosse and Buker, 1979). This included the interesting fact that our present standard species, *Folsomia candida* (Fountain and Hopkin, 2005), is resistant towards the most famous pesticide of all, DDT (Manley, 1971).

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From the end of the 1980s, intensive discussions towards a standardised test system with Collembola – involving a large selection of species – took place (e.g. Wiles and Frampton, 1996; Wiles and Krogh, 1998), and finally the ISO guideline using reproduction of the parthenogenetic species *F. candida* as an endpoint came into force (ISO, 1999). Ten years later, also the OECD included this assay into its guidelines for the testing of chemicals (OECD, 2009), with some modifications such as the possibility to perform the test with another – sexually reproducing – test species, *Folsomia fimetaria*.

Due to their long duration and the multitude of animals that have to be counted in the end, reproduction tests are very time-consuming and expensive. This drawback can be overcome by behavioural observations, which may indicate negative effects in much shorter time. Following promising results of choice experiments with contaminated food or soils (Filser and Hölscher, 1997; Filser et al., 2000), Natal-da-Luz et al. (2004) suggested an avoidance test which finally resulted in a standardised guideline (ISO 17512-2, 2011).

2.2. The setting: test conditions

All test guidelines involving Collembola are performed in natural or artificial soil. Since the early days of soil ecotoxicology, a huge body of literature has been published on how soil properties such as pH, salinity, water, clay or organic matter content affect bioavailability and thus toxicity of chemicals (e.g. Sandifer and Hopkin, 1996; Smit and van Gestel, 1998; Van Gestel, 2012). For example, ageing of zinc in soil reduced its toxicity by a factor of 5–8 (Smit and van Gestel, 1998). However, toxicity of spiked soils may also considerably increase over time, which we demonstrated with sewage sludge containing silver nanoparticles (Filser et al., unpublished data).

It should be expected that the problems associated with the large diversity and heterogeneity of natural soils can be overcome by using a standardised, artificial substrate such as the OECD soil (consisting of sand, clay, peat, and $CaCO_3$). All the more striking is the outcome of a study on OECD soil prepared in 25 different laboratories: Bielská et al. (2012) reported an enormous variety of those standards, with pH ranging from 4 to 7 and organic carbon content from 1.4% to 6.1%.

The result of a test is also affected by spatial heterogeneity and food availability: both inhomogeneous distribution of the substance and the presence of separate food (yeast) patches reduced toxic effects of dimethoate (Krogh, 1995). Contrarily, nonylphenol (NP) caused stronger effects when applied together with sewage sludge than without (EC_{10} = 6 and 23 mg NP/kg, respectively; Scott-Fordsmand and Krogh, 2004). Thus, the standard assay is not always conservative, and the large variety and heterogeneity of both natural and artificial soils often complicate the interpretation of the results and even question standardisation procedures (Bielská et al., 2012).

2.3. The mechanistic perspective

Given all these soil-related problems, it is evident that a simplified procedure should additionally be available, in particular when it comes to mechanistic questions with respect to bioavailability, toxicokinetics and toxicodynamics. Especially for the latter, stress biomarkers such as heat shock proteins were already tested decades ago (e.g. Tranvik et al., 1994; Köhler et al., 1999), culminating in a seminal paper by van Straalen (2003). Since then the focus has shifted towards gene expression profiling, proteomics and microarrays, delivering a plethora of information with a single assay (e.g. Nota et al., 2013). However, with respect to ecological risk assessment these methods still suffer from "some formidable problems" (van Straalen and Feder, 2012).

With respect to simplified exposure of soil animals, there is an OECD test for earthworms using spiked filter paper (see Van Gestel, 2012), yet nothing equivalent for Collembola. It is somewhat surprising that one promising method appears to be forgotten: Houx et al. (1996) developed an acute toxicity assay in which they exposed *F. candida* to toxicants in a simple aqueous medium. We have recently tested this assay with silver nanoparticles and can only recommend it whenever the confounding effects of soil properties are to be controlled for.

2.4. Biology of the test species

Thus far we have briefly covered the regulatory, physicochemical and biochemical aspects of Collembola ecotoxicology. Turning to the biological part of the issue, we start with the test organisms. Despite some sensitivity differences compared to other collembolan species (e.g. Wiles and Krogh, 1998; Holmstrup and Krogh, 2001), *F. candida* was considered representative for the group and selected as standard species. One main reason was its preference for warm temperatures, humus-rich soils and parthenogenetic reproduction, which makes culturing easy. The main differences of the related *F. fimetaria* are smaller size, more common occurrence in European agricultural soils and sexual reproduction (OECD, 2009). The biology of *F. candida* and *F. fimetaria* has been extensively described by Fountain and Hopkin (2005) and Krogh (2009).

The variability of the substrate does not only influence substance bioavailability (see Section 2.2) but also the performance of the test species. *F. candida* avoided OECD soil and reproduced worst in this substrate whereas they performed well in many natural soils (Domene et al., 2011). Adjustment of soil moisture, pH and organic matter content are crucial to avoid biased results of both reproduction and avoidance tests (Crouau et al., 1999; Domene et al., 2011).

Variation in other conditions may also affect the outcome of a test. Crouau and Cazes (2003) found that small temperature differences (1 °C) did not affect the variability of reproduction, yet one day difference in age did: The coefficient of variation in offspring from 11 day old *F. candida* was almost 50% higher than in offspring of 10 day old animals. In the same study, prolonging the test from 35 to 49 days reduced the variation as well, along with a 50% lower LOEC level and a slightly lower EC_{50} value (114 vs. 105 μg Cd/g soil).

Besides differences with respect to life stage and nutritional status, the main reason for variability in reproduction is genetic variability. Although this should be low in a parthenogenetic species such as *F. candida*, clones from separate laboratories may differ considerably: The EC_{50} of cadmium for four clones of *F. candida* ranged between 800 and more than 2000 μg Cd/g soil (Crommentuijn et al., 1995). A more recent study (Chenon et al., 2000) revealed no sensitivity differences of nine clones towards Cd and minor differences towards phenantrene.

Extending the selection of species, differences between their sensitivity towards chemicals become apparent (e.g. Holmstrup and Krogh, 2001), which is logical in view of their varying morphology, physiology, microhabitat or feeding preferences. Generalisations based on the animals' biology are however difficult. For instance, *Folsomia quadrioculata* is highly sensitive to copper but thrives very well in lead-contaminated soils (Filser et al., 2000).

Unfortunately, theoretical concepts seem somewhat underrepresented in Collembola ecotoxicology, although there are some powerful and promising options. In recent years, trait-based approaches have been advocated for analysing the vulnerability of wildlife to pollutants (de Lange et al., 2009) and for linking biodiversity and associated ecosystem services (de Bello et al., 2010). This concept was applied to effects of land use change on biodiversity and extended, with special attention to animal groups including

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Collembola (Vandewalle et al., 2010). One critical point for all theoretical approaches is that the necessary biological information for most Collembola species is lacking (Vandewalle et al., 2010).

The Dynamic Energy Budget (DEBTox theory), released in 1996 (Kooijman and Bedaux, 1996), combines external stress, energy budget and population biology of species. Applying it to *F. candida*, Jager et al. (2004) found that exposure to Cd decreased energy assimilation from the food whereas the opposite was true for triphenyltin. This underpins the importance of resource availability when it comes to extrapolating the results from laboratory tests to outdoor conditions.

2.5. Ecological interactions

Single-species tests have always been criticised since they do not reflect realistic conditions. Test batteries comprising a range of endpoints, trophic groups and habitats are available (e.g. Matzke et al., 2007) but cannot overcome these shortcomings. Holmstrup and Krogh (2001) studied the same substance with six different soil animal species, including a test in which *F. candida* was combined with its predator, the gamasid *Hypoaspis aculeifer*. We have recently developed a microcosm test with a food chain of three standard species, consisting of the soil bacteria *Arthrobacter globiformis*, *F. candida* and *H. aculeifer*. Upon exposure with silver nanoparticles, reproduction of the Collembola was slightly increased in microcosms with *F. candida* and *A. globiformis* only, but in one soil type a strong decrease was found when *H. aculeifer* was included (Hackmann et al., unpublished data). This sheds light on the importance of ecological interactions for assessing the effects of toxicants, which was already shown in a more complex test setup for phenantrene (Cortet et al., 2006).

The most realistic risk assessment tools for Collembola thus far are terrestrial model ecosystems (TMEs), which are larger intact soil cores exposed to chemicals under controlled conditions in the laboratory or in the field (Scholz-Starke et al., 2011). The authors found clear dose-response curves and long-lasting effects on microarthropod communities following excessive application of lindane under field conditions. Other test endpoints were not affected during one year in this system, rendering microarthropods more sensitive than plant biomass, earthworms or the bait-lamina feeding test. However, much effort both in terms of equipment and personnel is needed for performing tests with TMEs.

On the XVI International Colloquium on Soil Zoology in 2012 in Coimbra, Chelinho et al. (2013) presented an interesting alternative which is both simple and ecologically relevant: exposure of extracted native communities of nematodes and microarthropods in the same soils spiked with different concentrations of pollutants. This is clearly an advantage over single-species tests, rendering a much more reliable hazard assessment for the soil from which the native community was extracted. Although the setup complicates test comparisons between different regions or countries, exactly this can be regarded as a potential advantage: comparable effects in a range of soils provide much more reliable information than only one standard substrate.

2.6. Adaptation of test guidelines

Van Gestel (2012) argued that existing test guidelines might need to be adjusted in order to make them applicable to new chemicals such as nanoparticles. Part of this need is also the miniaturisation of test systems, which is not only desirable for ethical and practical reasons, but also necessary because new materials can often be produced in only very small amounts since synthesis is expensive and time-consuming. For instance, OECD tests on plant growth or earthworm reproduction require 500–600 g soil per test unit and are simply not feasible if only a few milligrams of a new substance are available.

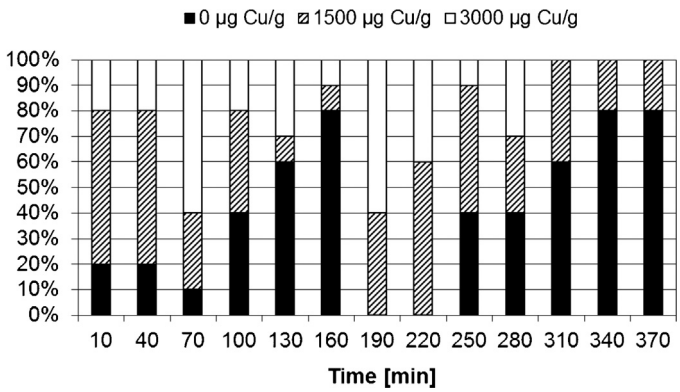

Fig. 1. Percentage of 10 introduced *Folsomia candida* found in 3 × 4 filter paper discs soaked with solutions corresponding to either 0, 1500 or 3000 mg Cu/kg dw. The Petri dishes with the animals and paper discs were incubated and observed for 370 min. See Filser and Hölscher (1997) for more details.

This was our initiative to design an experiment in order to explore if a miniaturisation of the Collembola reproduction test is feasible or not. A test system with eggs instead of young adults – the second part of this experiment – would not only explicitly expose the earliest development stages from the very beginning and reduce damage of the tiny animals during handling but also save time for synchronisation.

2.7. Avoidance behaviour

Substrate avoidance by Collembola has proven to be a good and sensitive early-warning tool in ecological hazard assessment (Natal-da-Luz et al., 2004; ISO, 2011). One disadvantage of this assay is the often very high variation, which may obscure significant results. There are at least two reasons for this variation, namely aggregation behaviour and temporal variation. The former can be easily controlled for by using single specimens in small test chambers (Filser et al., 2000), although their tests were conducted without soil. In an earlier experiment (Filser and Hölscher, 1997) we did not present all data of our choice tests (using 10 individuals on Petri dishes without soil), in which we counted individual numbers every 30 min over 6–10 h. We observed clear and apparently coordinated oscillations of preference behaviour (Fig. 1). Despite overall avoidance of copper patches, sometimes most animals were counted in copper patches. Although the conditions in that study were rather artificial, such behaviour is very likely in soils as well. The associated problems can be overcome by sufficient replication and by counting the animals at several time intervals.

Evidently, not only intraspecific but also interspecific interactions affect avoidance and food choice behaviour (Pfeffer and Filser, 2010, and references therein). Independent of Chelinho et al. (2013) we developed the idea of an avoidance test in which a community of different species is introduced. Here we present first experiments into this, based on food choice tests with single species and a mixture of these.

3. Materials and methods

The test design for the miniaturisation of the reproduction assay with *F. candida* followed ISO 11267 (1999). *F. candida*, originally obtained from DMU-NERI, Silkeborg, DK, has been cultured in our laboratory since 1998. All tests were conducted in a temperature-controlled cabinet (ET 618-4, Aqualytic, Dortmund, Germany) at 20 °C with a light–dark cycle of 16:8 h. We used Lufa 2.2 soil (Landwirtschaftliche Untersuchungs- und Forschungs-Anstalt, Speyer,

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Germany) and systematically varied soil mass (30, 20 or 10 g dry weight) and individual numbers of Collembola at start of the test (10, 7 or 4 animals). Tests were performed in glass vials with PE lids (Omnilab, Bremen, Germany) varying in volume (50, 30 and 20 mL) but not in shape to keep the surface-to-volume ratio approximately constant.

The test system with eggs followed the same design, with the only exception that at start of the test instead of adults 12, 9 or 6 eggs were added with a fine brush to 30, 20 or 10 g soil. A second experiment followed a regression design where egg numbers were continuously increased from 2 to 15 for each of the three different soil masses. The eggs had been collected from several cultures with 30 adult individuals each, which had been incubated in darkness at 20 °C for 3 days. The test vessels were aerated twice a week and the water content was adjusted once per week. After hatching of the first individuals, they were fed with dried baker's yeast. After 28 days the experiment was terminated. Individuals were counted as in the reproduction test. In addition the length of the individuals was measured: animals floating on the water surface were transferred to Petri dishes (5 cm diameter) containing a moistened plaster of Paris/charcoal mixture (9:1, w/w). The Petri dishes were placed on ice for 15 min to slow down the movement of the animals and then photographed under a stereomicroscope (Olympus SZX 12) at 51-fold magnification with a video camera (Sony Color CCD-IRIS). Body length was measured by means of Optimas 6.5 (Media Cybernetics, L.P., 1999). Every treatment combination of both tests was replicated 10 times.

The choice test was performed with the collembolan species *Protaphorura fimata*, *F. candida* and *Heteromurus nitidus* with baker's yeast, *Saccharomyces cerevisiae*, and monocellular green algae, *Pseudokirchneriella subcapitata*, as food choices. *P. fimata* and *H. nitidus* were obtained from L. Ruess, Humboldt University Berlin; *F.candida* came from our laboratory culture.

The three-chamber choice system consisted of Petri dishes (5 cm diameter) containing a moistened plaster of Paris/charcoal mixture (9:1, w/w). The starting chamber had two holes in its sides (diameter 5 mm) about 5 mm from bottom; the choice chambers had one hole. The chambers were connected by a 25 mm piece of silicone tubing (inner diameter 4 mm). A strip of moistened filter paper (2 mm × 45 mm) was laid through the tube to ease transition for the Collembola. A piece of agar (1 cm^2) with algae/yeast was placed in each choice chamber. A total of 12 adult Collembola was introduced into each starting chamber, for single species tests 12 of one species, for multi-species tests 4 of each species. All chambers were closed with transparent lids to enable observation without opening the chambers. The chambers were kept in the dark at 22 °C, covered with black cloth. In the pre-test, after 1, 2, 4, 6, 8, 10 and 24 h the individuals per chamber were counted in red light, if necessary using a magnifying glass. In the following test, counting took place only after 24 h. Every treatment was replicated six times.

Statistical analyses of the miniaturisation experiments were performed with SPSS 16.0. For the choice tests we used R 2.14.1, with paired *T*-tests for comparisons between algae and yeast patches and a Wilcoxon rank sum test with continuity correction for not normally distributed data. For comparisons between single and multispecies choice test a Welch Two-Sample *t*-test was used, and the Wilcoxon signed rank test with continuity correction for not normally distributed data.

4. Results

4.1. Miniaturisation of the reproduction test

In our miniaturisation experiment recovery rate of adults was generally low. Reducing the soil mass had no consistent effect on the number of retrieved individuals, nor had the number of introduced animals (Fig. 2). Only in the vessels with 30 g soil and four animals the recovery rate was distinctly lower than in all other combinations. With 10 individuals, reproduction was slightly lower when soil mass was reduced to 10 g (Fig. 3). Reducing the number of animals resulted in considerably higher offspring numbers, particularly with simultaneously reducing the soil mass (GLM; individual numbers: $p = 0.000$, soil mass: $p = 0$ 0.009, interaction: $p = 0.000$). In the system with four individuals and 10 g soil the number of juveniles per adult was almost doubled compared to the standard assay (30 g/10 individuals).

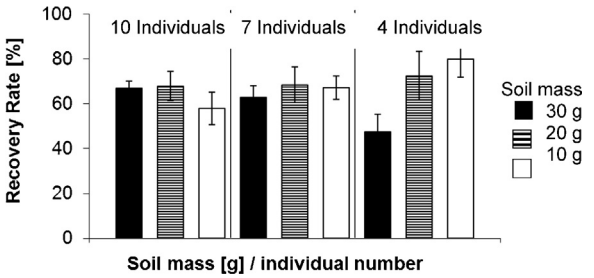

Fig. 2. Recovery rate of *Folsomia candida* in the miniaturisation experiment with varied soil mass and individual numbers. Each test had been incubated for 28 days. Shown are mean values ± SE, $n = 10$.

4.2. A test system with eggs

In the first egg test the average hatching rate was generally low, ranging between 35% and 60%, and individuals on average grew to a size between 0.75 and 0.85 mm (data not shown). In the regression design an overall better hatching rate was obtained. With 30 g soil it tended to slightly decrease with increasing egg number (Fig. 4). This trend was not found with 20 g soil, where the hatching rate was again very low since in some replicates no hatching took place. When only 10 g soil and 2–5 eggs were used, the hatching rate was by far highest and the variation lowest (Fig. 4).

4.3. Community choice behaviour

The preference of either algae or yeast was studied in choice experiments with three Collembola species, added either singly or as a mixture of all species. In both tests, within 24 h only few of the 12 introduced specimens had moved to one of the chambers with food patches, and all species preferred yeast to algae (Table 1), which partly could be statistically supported in the multispecies choice test: In the pre-test (data not shown), a trend was found for *F. candida* ($p = 0.0533$). In the following test (Table 1), the preference for yeast was significant ($p = 0.03501$) for *P. fimata*.

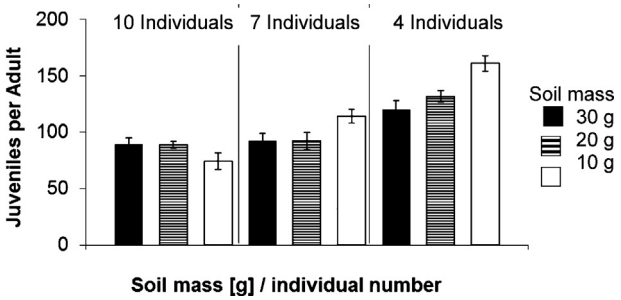

Fig. 3. Reproduction rate, shown as number of juveniles per introduced adult *Folsomia candida*, in the miniaturisation experiment with varied soil mass and individual numbers. Incubation time: 28 days; shown are mean values ± SE, $n = 10$.

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Table 1
Individual numbers (mean ± SE, $n = 6$) of three Collembolan species in test chambers with unicellular green algae (*Pseudokirchneriella subcapitata*) and bakers' yeast (*Saccharomyces cerevisiae*) in food choice experiments after 24 h. Single: starting chamber with 12 individuals of one species per replicate (calculated to 4 in table to enable comparison with multispecies test); Multi: multispecies choice test with 4 individuals of each of the three species.

Choice	*Protaphorura fimata*		*Folsomia candida*		*Heteromurus nitidus*	
	Single	Multi	Single	Multi	Single	Multi
Algae	0.9 ± 0.5	0.3 ± 0.2	0.9 ± 0.3	1.0 ± 0.4	0.9 ± 0.5	0.7 ± 0.3
Yeast	1.6 ± 0.5	3.2 ± 0.5	1.5 ± 0.3	2.0 ± 0.5	2.3 ± 0.5	2.7 ± 0.2

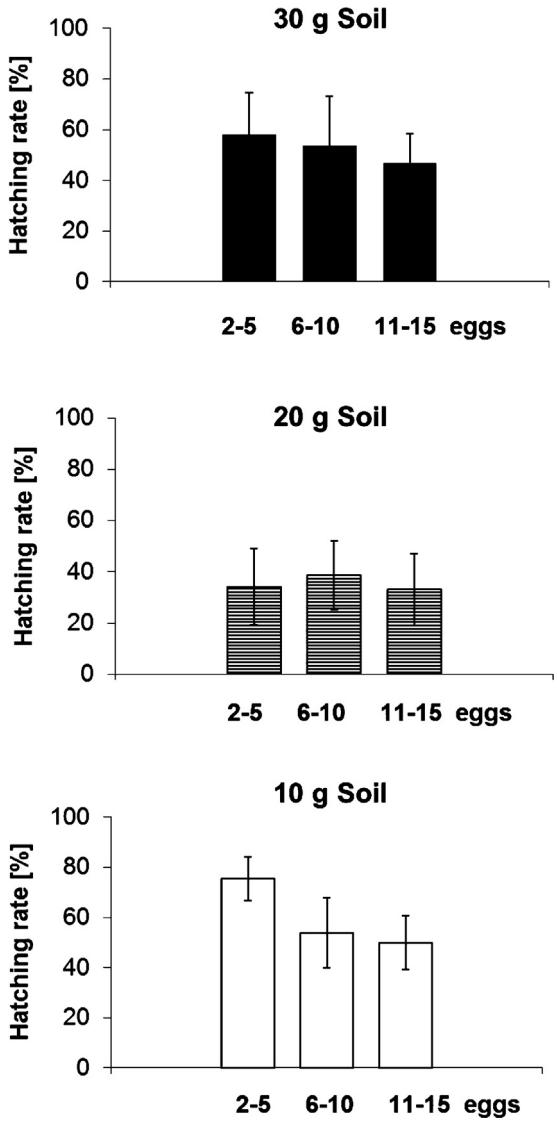

Fig. 4. Hatching rate of *Folsomia candida* (percentage of hatched eggs) in the egg test with varied soil mass and egg numbers. Data from a regression design where egg numbers increased constantly. Incubation time: 28 days; shown are mean values ± SE of egg number classes ($n = 4$ for 2–5 and $n = 5$ for 6–10 and 11–15 eggs).

Comparing single-species with multi-species tests, no differences were found in the pre-test except a significantly higher preference ($p = 0.04186$) of yeast in the single species choice test for *H. nitidus*. In the following test, a significantly higher preference ($p = 0.0407$) of *P. fimata* for yeast in the multispecies choice test was found (Table 1).

5. Discussion

The miniaturisation experiment revealed the best validity and highest reproduction rates in the treatment with the smallest amount of soil and the lowest number of Collembola. Therefore we recommend 10 g soil with four adults as miniaturised Collembola reproduction test. We have meanwhile performed several comparisons of this miniaturised and the standard reproduction test (data not shown) and never found any notable difference in the outcome of both, with or without toxic substances.

According to our data, an egg test seems feasible, yet still requires experiments with toxic substances and a careful look at the overall variability. The latter was agreeable in one constellation (2–5 eggs in 10 g soil) but very high in all others. If the variability can be kept in a reasonable range, which should be possible by increasing the number of replicates, the egg test provides several advantages. First, it is an "early life stage" test, exposing only eggs and juveniles to the substance, second, the typical problem that some adults lay many, others few eggs would be overcome, and third, the number of juveniles to be counted per test unit – five at most in the suggested design – is much smaller than in the reproduction test. Finally, much less time would be required for the synchronisation which would be a substantial improvement with respect to flexibility in the planning of a test.

The preliminary results of our last experiments indicate that the choice behaviour of Collembola may indeed be affected by intraspecific interactions. One has to bear in mind that a three-chamber system like the one used here (to avoid mixing of the olfactory cues as much as possible) is rather insensitive. The analysis of the pre-test showed best results after 24 h, when the numbers of individuals that had chosen a food source was highest, yet even then the majority of the 12 introduced animals were still in the starting chamber. The number of replicates ($n = 6$) was too low for detecting a significant difference in choice between food sources in all species. Further development of the test is in preparation.

6. Perspectives and suggestions for the future

Collembola ecotoxicology has definitely advanced a lot during the past decades and meanwhile offers a broad array of standard tests and alternatives. The first phase was dominated by mostly descriptive research, studying which species were affected by what chemicals, and how chemicals differed in their toxicity. A second phase focused on the chemical–biochemical perspective, in particular bioavailability, bioaccumulation, molecular biomarkers and mixture toxicity. The fast developing field of ecotoxicogenomics, together with the sequencing of *F. candida*, already has brought a lot of insight in toxic modes of action and will likely become more important in risk assessment. Recently, the biological perspective has gained increasing attention, with tests and experiments covering behaviour and biotic interactions. Biotic interactions definitely need more attention in the future, and so do long-term effects involving several generations: A diet of fungi that had been avoided by *F. candida* in choice experiments resulted in substantially decreased reproduction, whereas preferred fungi had the opposite effect. These effects were even more pronounced in the F1 than in the parent generation. In ecotoxicology (Klironomos et al., 1999), multi-generation tests would allow for much more realistic predictions on long-term population development, but have been

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performed only rarely thus far (Campiche et al., 2007; Leon Paumen et al., 2008).

Interestingly, after the "pesticide rush" in the 1960s and 1970s, it seems that the majority of studies, especially mechanistic ones, have focused on metals. Although this impression has not been exhaustively covered here, a potential bias must be kept in mind whenever it comes to generalisations. On the other hand, such a large body of evidence also provides an advantage for studying pollutant mixtures and new substances. Most common nanomaterials are metal-based, and the experience with metals and their modes of action helps in distinguishing effects of the metal (ions) themselves from those caused by the special properties of the nanomaterials.

The facts summarised here should make clear that the future of collembolan ecotoxicology is definitely not a one-way road. Chemical and biological expertise is required to judge, complement and improve existing standardised test systems, we need simplified assays, molecular markers and high-throughput methods for fast screening of new substances, and we need more basic knowledge on the biology of the organisms in order to improve (or only apply) existing theoretical approaches. Most of all, we need interdisciplinary cooperation in order to understand and regulate the challenges posed to soils by human actions (Filser et al., 2008).

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