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Exploring the impacts of plastics in soil – The effects of polyester textile fibers on soil invertebrates

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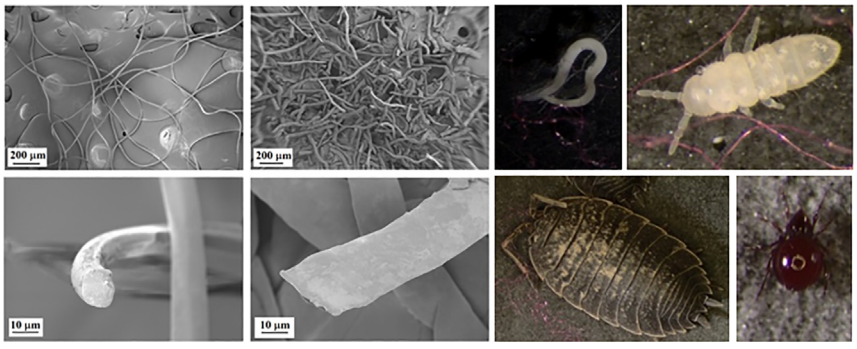
HIGHLIGHTS

- The effects of polyester fibers on soil animals were studied for the first time.
- Enchytraeid reproduction decreased up to 30% but only by long fibers in soil.
- Isopod energy reserves and feeding activity were affected by fibers in soil.
- Polyester fibers were not very harmful to soil invertebrates in 21–28-days exposure.
- Polyester fibers can enter terrestrial food webs by ingestion by soil invertebrates.

GRAPHICAL ABSTRACT



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ABSTRACT

Polyester fiber is one of the most abundant types of microplastics in the environment. A major proportion of the fibers entering wastewater treatment plants end up in sewage sludge, which is used as a soil fertilizer in many countries. As their impacts in the terrestrial environment are still poorly understood, we studied the effects of polyester fibers on enchytraeids (*Enchytraeus crypticus*), springtails (*Folsomia candida*), isopods (*Porcellio scaber*) and oribatid mites (*Oppia nitens*), all playing an important role in soil decomposer food webs. We exposed these invertebrates in the laboratory to short (12 µm–2.87 mm) and long (4–24 mm) polyester fibers, spiked in soil or in food at five concentrations ranging from 0.02% to 1.5% (w/w) and using five replicates. Overall the effects of polyester fibers on the soil invertebrates were slight. Energy reserves of the isopods were slightly affected by both fiber types, and enchytraeid reproduction decreased up to 30% with increasing fiber concentration, but only for long fibers in soil. The low ingestion of long fibers by the enchytraeids suggests that this negative impact arose from a physical harm outside the organism, or from indirect effects resulting from changes in environmental conditions. The short fibers were clearly ingested by enchytraeids and isopods, with the rate of ingestion positively related to fiber concentration in the soil. This study shows that polyester fibers are not very harmful to soil invertebrates upon short-term exposure. However, longer lasting, multigeneration studies

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with functional endpoints are needed to reveal the possible long-term effects on soil invertebrates and their role in the decomposition process. This study also shows that polyester fibers can enter terrestrial food web via ingestion of fibers by soil invertebrates.

1. Introduction

The presence of microplastics in the ocean environment has raised concerns since the early 2000s (Thompson et al., 2004). Lately the contamination of soils by microplastics has also gained increasing attention (Rillig, 2012, Zhu et al., 2019). Polyester fibers have been identified as one of the most abundant types of microplastics in these environments (Dris et al., 2016; Burton, 2017; Carr, 2017; De Falco et al., 2018; Henry et al., 2019). In addition to atmospheric deposition (Dris et al., 2016; Henry et al., 2019), polyester fibers can enter the environment via municipal wastewater treatment plants (WWTPs). Up to several million synthetic fibers can be released to wastewater from a typical 5 kg wash load of polyester fabrics in a household washing machine (Sillanpää and Sainio, 2017; De Falco et al., 2018). Thus, it is not surprising that the dominant form of microplastics in wastewater entering the WWTP is synthetic fiber, with the major type being polyester (Talvitie et al., 2017; Kang et al., 2018). Some 80–99% of all microplastics present in wastewater are retained in WWTPs, ending up in the sewage sludge, resulting in concentrations ranging from 1 000 up to 56 400 particles kg^{-1} dry sewage sludge (Zubris and Richards, 2005; Lusher et al., 2017; Mahon et al., 2017; Mintening et al., 2017; Talvitie et al., 2017; Li et al., 2018a). As sewage sludge and sewage sludge-based biosolids are commonly used as soil fertilizers, polyester fibers eventually enter the terrestrial environment (Nizzetto et al., 2016). In agricultural soils amended with sewage sludge or biosolids, synthetic fibers have been found to comprise 92% (Zhang and Liu, 2018) and even 97% (Corradini et al., 2019) of the number of microplastic particles present.

Even though fibers have been identified as one major type of plastics in soil, their effects on soil invertebrates and possible entry into terrestrial food webs are still unknown. Current reports on the effects of microplastics on soil invertebrates mostly studied polyethylene (PE), polystyrene (PS) and polyvinylchloride (PVC) non-fibrous particles (microbeads, films, pellets, fragments). Such studies included for instance exposures of the earthworm *Lumbricus terrestris* to PE particles in litter (Huerta Lwanga et al., 2016; 2017a), of the earthworm *Eisenia andrei* to PE pellets in soil (Rodriguez-Seijo et al., 2017, 2018), of the springtail *Folsomia candida* to PVC particles (Zhu et al., 2018a) and PE microbeads (Ju et al., 2019) in soil, and of the enchytraeid worm *Enchytraeus crypticus* to PS nanobeads in food or soil (Zhu et al., 2018b). Effects on isopods *Porcellio scaber,* exposed to plastic bag fragments and cosmetic microbeads in food pellets, have also been reported (Jemec Kokalj et al., 2018). However, for assessing their risks in soil, knowledge on the effects of polyester fibers on soil animals is urgently needed, since exposure of soil invertebrates to polyester fibers is more probable than exposure to most of the other types of microplastics.

The adverse effects and gut retention of microplastics may depend on their size and shape (Lei et al., 2018). For example, synthetic fibers induced stronger adverse effects on some aquatic invertebrates (Watts et al., 2015; Jemec et al., 2016; Ziajahromi et al., 2017) than PE microbeads (Au et al., 2015; Ziajahromi et al., 2017), and their egestion took longer than the egestion of food (Au et al., 2015) or sediment particles (Hurley et al., 2017). Characteristics of fibers, such as their chemical composition and size, have been suggested to be important determinants of toxicity (Blake et al., 1998). It is, therefore, of high interest to investigate the potential adverse effects of differently sized fibers on soil organisms.

The present study aims to shed light on the effects of synthetic fibers on soil invertebrates. We investigated the influence of polyester fibers of two lengths: short (220 ± 200 μm; range 12 μm – 2.9 mm) and long (11.9 ± 3.7 mm; range 4–24 mm) fibers. The effects of fibers were tested on isopods *P. scaber* (Arthropoda: Crustacea), springtails *F. candida* (Arthropoda: Entognatha), enchytraeids *E. crypticus* (Annelida: Oligochaeta), and oribatid mites *Oppia nitens* (Arthropoda: Arachnida). All these species play important roles in decomposition and nutrient cycling processes in the soil. Isopods participate in the decomposition of leaf litter with fragmentation of organic materials into smaller pieces (Špaldoňová and Frouz, 2014), springtails and oribatid mites are predominantly microbial feeders, releasing nutrients from microbial biomass by feeding, and earthworms and enchytraeids ingest detritus and soil, maintaining soil fertility (Briones, 2018) but also allowing the possible ingestion of microplastics (Huerta Lwanga et al., 2017a; Rillig et al., 2017). Soil invertebrates have also been shown to play an important role in the transport of microplastics in the soil (Huerta Lwanga et al., 2017a; Maaß et al., 2017; Rillig et al., 2017, Zhu et al., 2018c). All these test animals are established models in soil ecotoxicity testing (OECD, 2004; OECD, 2009; Princz et al., 2010; van Gestel et al., 2018).

2. Materials and methods

2.1. Fibers

Two fiber materials with a different size range were prepared for the tests, defined here as “long fibers” and “short fibers”. A pink polyester fleece blanket (Skogsklocka, IKEA) was used as material for both fibers, since pink fibers are easy to visually detect from soil. Long fibers were obtained by brushing the blanket with a dog brush with bent wires. To prepare the short fibers, the fleece blanket was cut to pieces (average size 0.5 cm^2) which were placed in a closed steel chamber that was soaked in liquid nitrogen for 5 min. Subsequently, the material was milled for 2.5 min using a ball mill (MillMix 20, Domel, Slovenia) at the highest frequency (Jemec et al., 2016). After preparation, the fibers were stored in a sealed paper container.

The length of 319 short fibers and 213 long fibers was measured using a stereo microscope Leica MZ FLIII (Leica, Germany), ImageJ® Image Analysis Software and AxioVision 4.8.2. The dimensions of the cross-section of the fibers were determined using SEM micrographs, after inspection with field emission scanning electron microscope (FE-SEM, Zeiss ULTRA plus, Carl Zeiss, Germany), at an accelerating voltage of 2 kV and 30 μm aperture size. Fibers were sputtered with a thin layer of platinum-palladium and fixed on an aluminium holder using double sided adhesive carbon tape.

The longer fibers were cylindrical with length 11 880 ± 3 710 μm (mean ± standard deviation; n = 232), ranging from 4 000 to 24 000 μm, an average diameter of 14.71 μm and round cross-section. The shorter textile fibers were shaped as narrow strips with an approximate length 220 ± 200 μm (n = 319), ranging from 12 to 2 870 μm. As a result of the use of grinding to prepare short

fibers, it was found that the cross-section was also deformed, in addition to a reduction in fiber length (Fig. A.1).

According to the most recent suggestion for classification of plastic debris (Hartmann et al., 2019) the short fibers can be considered as microfibers (i.e. microplastics) and long fibers as mesofibers (i.e. mesoplastics). However, as there still is no consensus for the size classification of plastic particles, we refer to short and long fibers here.

2.2. Test organisms

E. crypticus, *F. candida* and *O. nitens* were obtained from a laboratory culture at the Department of Ecological Science, Vrije Universiteit, Amsterdam. *P. scaber* were collected from a compost heap in a non-polluted garden in Ljubljana, Slovenia.

E. crypticus were cultured on agar media prepared with aqueous soil extracts at 16 °C, 75% relative humidity, complete darkness, and fed with a mixture of oatmeal, dried yeast, fish oil, and egg yolk powder. Mature individuals of a similar size were used in the experiments.

F. candida were cultured on moist plaster of Paris amended with charcoal (10:1, w/w) at 16 °C, 75% relative humidity, and illumination (12:12 h light:dark), and fed with baker's yeast. The tests were performed with age-synchronized springtails.

O. nitens were cultured on moist plaster of Paris amended with charcoal (10:1, w/w) at 20 °C, 75% relative humidity, and illumination (12:12 h light:dark), and fed with baker's yeast. Young adult mites were used for the tests.

P. scaber were synchronized under constant temperature (20 ± 2 °C) and illumination (16:8h light:dark) regimes in a climate-controlled chamber at the University of Ljubljana. Isopods were caged in glass containers with a mixture of loamy sand and peat (moistened at 40% of the water holding capacity; WHC) at the bottom, fed on dry leaves from common hazel (*Corylus avellana*), common alder (*Alnus glutinosa*) and carrots, as described by Jemec Kokalj et al. (2018). For the experiment we used only healthy, adult animals (30–60 mg fresh body mass) of both sexes. Molting individuals and females with marsupia were excluded.

2.3. Exposures

Standard agricultural soil Lufa 2.2 (Lufa Speyer, Germany) was used in all experiments. For *E. crypticus*, *F. candida* and *P. scaber* tests, nominal concentrations of 0.02, 0.06, 0.17, 0.5 and 1.5% w/w of fibers in the soil and control soil without fibers were prepared one day before starting the exposures. These concentrations correspond with nominal 200, 600, 1 700, 5 000 and 15 000 mg kg^{-1} dry soil and fall in the concentration range of 0.062 – 28 % w/w of microplastics that has previously been tested for soil invertebrates and ranges reported in the literature; see discussion (Huerta Lwanga et al., 2016; Huerta Lwanga et al., 2017a; Rodriguez-Seijo et al., 2018; Zhu et al., 2018b).

The fibers were first mixed in with dry soil and prior to exposure the moisture content was adjusted to 40% (*P. scaber*) or 50% (*E. crypticus, F. candida*) of the WHC by adding deionized water and mixing again. After preparation, the test soils were transferred into glass test jars, 20–30 g moist soil in each 100 mL jar. Six replicates were prepared for each concentration and control in all tests, with one jar being used to determine soil pH at the beginning and at the end of the tests.

In addition to exposure via spiked soil, the enchytraeids, springtails and oribatid mites were also exposed to fibers via spiked food. For exposing *E. crypticus* and *F. candida* to short fibers, concentrations of 0.02, 0.06, 0.17, 0.5 and 1.5% w/w were spiked in with food, whilst the long fibers were only tested at a concentration of 0.5% in the food. For *F. candida* the fibers were spiked in a mixture of baker's yeast and water (1:2; w:w), for *E. crypticus* in a mixture of finely ground oats and water (1:4; w:w). Small amounts of food were placed on the soil surface in test jars containing clean soil moistened at 50% of the WHC.

O. nitens was exposed only to 0.5% long fibers spiked in soil, 0.5% long fibers spiked in food and controls without fibers, all in five replicates. For the food exposure, the fibers were spiked in a mixture of baker's yeast and water (1:2; w:w). The moisture content of the soil was adjusted to 50% of its WHC.

All exposures were performed at 20 ± 2 °C in 16:8h light:dark, except the exposure of isopods which was in the dark.

2.3.1. Enchytraeid reproduction test

Enchytraeids were exposed following OECD guideline 220 (OECD, 2004) with modifications following Castro-Ferreira et al. (2012). Ten mature individuals were randomly added to each test jar. The enchytraeids were fed once a week with 10 mg of finely ground rolled oats, mixed with water at a ratio of 1:4.

After 3 weeks of exposure, surviving adults were removed by hand sorting the soil and four individuals from each replicate were depurated for 6 h in isotonic solution. After depuration, the solution containing the feces was collected. Depurated individuals were weighed, frozen at −20 °C, digested with 10% KOH at 60 °C for 30 min and filtered. Ingestion and egestion as well as the retention of the fibers in the organisms were assessed by measuring the number of the fibers present in the feces and in the digested tissue of the organisms after depuration using a stereo microscope. The length of fibers in the feces and enchytraeids exposed to fibers spiked in soil was measured using Olympus cellSens imaging software.

To assess reproduction, after removing the adults from the soil the juveniles were fixated and dyed by adding 10 mL of 96% ethanol and 200 μL of Bengal rose (Sigma Aldrich, 1% in ethanol) to the soil of each replicate. After 24 h staining, the soils were washed through a sieve (0.25 mm) and the animals remaining on the sieve were spread out in a white tray to take pictures (Nikon D5200). The juveniles were counted from the pictures using Adobe Photoshop CC 2018.

2.3.2. Springtail reproduction test

Springtails were exposed following OECD guideline 232 (OECD, 2009). Ten 10–12 day old *F. candida* were added to each jar, and fed once a week with 5 mg of baker's yeast, mixed with water at ratio of 1:2. After 4 weeks of exposure, the soil was transferred to a 250 mL beaker using 100 mL of water, after which a picture of the collembolans floating on the water surface was taken (Nikon COOLPIX P510). The numbers of adults and juveniles were counted using ImageJ®.

2.3.3. Oribatid mite test

Oribatid mites were tested following a method developed by Princz et al. (2010). Fifteen young adults, being similar light brown in color, were introduced into test jars and exposed for four weeks. Once a week the soil moisture content was adjusted and the oribatid mites were fed with 5 mg baker's yeast mixed with water at a ratio of 1:2. At the end of the test, the oribatid mites were extracted from the soil using a Tullgren apparatus and collected in jars containing moistened plaster of Paris. After three days of extraction, water and a drop of ink was added to the collecting jars and adults and juveniles were counted under a stereo microscope.

2.3.4. Isopod test

Five isopods were placed into each replicate 200 mL glass jar containing soil spiked with long or short fibers and some dry leaves of common hazel (*C. avellana*). Soil moisture content was checked once every three days and the common hazel leaves were replaced

every week. In all jars the dry mass of common hazel leaves was measured before and after 7, 14, 21 and 28 days and also upon replacing the leaves. The mass of the isopods was recorded before and after exposure. Isopod feeding activity was calculated as the quotient of common hazel dry leaf weight mass loss (initial dry leaf weight (mg) – final dry leaf weight (mg)) and number of animals in the test jar (mg per animal). Mortality was assessed once a week and at the end of the experiment.

After the experiment, 15 animals per test group of the second and third test were dissected, the gut was discharged, and the rest of the body stored at −20 °C to measure energy reserves. Isopod energy reserves were determined according to Ferreira et al. (2015) and Jemec Kokalj et al. (2018). Dissected animals were weighed and homogenized in 1000 μL of 100 mM potassium phosphate buffer (pH = 7.0) using a T10 IKA Ultra-turrax homogenizer for 1 min. All three energy rich molecules – proteins, lipids and carbohydrates – were analyzed from the homogenate. Measurements were done in triplicate using a microplate reader Multiskan Spectrum 2005 (Thermo Scientific, ZDA). The total triglyceride (lipids), carbohydrate and protein contents were calculated from the standard curve of corresponding standards (tripalmitin, glucose and BSA protein, respectively), and expressed as μg triglycerides/protein/carbohydrate per mg fresh weight animal. Available energy (E_a) was calculated as described by Verslycke et al. (2004).

2.4. Quantification of fibers in soil

As mixing the fibers in soil may have changed the size distribution of the fibers, the size range of the fibers in soil as well as the spiked concentrations were measured after spiking the soils.

Long fibers were extracted from soil by density separation using saturated NaI solution (1.8 g cm^{-3}) after reducing the organic matter with Fenton's reagent according to Hurley et al. (2018). Soil samples were handled in a laminar flow cabinet to minimise inputs from airborne contamination whilst they were exposed for subsampling. Following this, samples were kept covered using glass or aluminium foil lids to protect the sample during organic matter digestion, density separation, and filtering. All equipment was prewashed with filtered (0.22 μm) RO water, and all reagents were filtered (1.2 μm) prior to use. All fibers extracted on What GF/A filters were counted, and the length of 232 fibers was measured under a stereo microscope. The fibers were also weighed, when the mass exceeded the measurement limit of the balance. The recovery rate of long fibers was calculated using the amount of fibers extracted versus that added for each test concentration.

As this extraction protocol was not efficient for short fibers, they were extracted from the test soil using tweezers. As short fibers could not be weighed, their mass was estimated from the data on the volume of individual fiber and density of polyester (1.39 g/cm^{-3}). Due to the varying shapes of the cross-sections of short fibers (Fig. A.1), the cross-section of long fibers (Fig. A.1) was used to calculate the volume of the short fibers, assuming that the cross-section area of the fibers does not change during the grinding process. The fiber lengths were measured from light microscopy images of fibers collected on filter papers using AxioVision 4.8.2. Detailed description regarding the methodology is found in Supplementary material (Methodology A.1).

2.5. Data analysis

Before data analysis, apparent outliers deriving from consequences in the experiment or analysis phase were removed. The response data were plotted against nominal fiber concentrations in soil or food. Due to the low expression rate in the responses, no EC50 or LC50 values could be determined. Instead, no observed effect concentrations (NOEC) for each parameter were determined using one-way-ANOVA followed by Dunnett's posthoc test for comparisons between the control and the fiber treatments. When the data were not normally distributed, the data was analyzed using Kruskal-Wallis nonparametric test, followed by pairwise comparison with Mann-Whitney *U* test.

As in the tests with long fibers the animals were exposed via spiked food using only one concentration, the differences between the soil exposure and food exposure were tested with one-way ANOVA, using 0.5% fiber treatment in soil, 0.5% fiber treatment in food, and the control as fixed factors. The pairwise-comparisons between the treatments were analyzed with Tukey's test. When the data were not normally distributed, the data were analyzed with Kruskal-Wallis nonparametric test, followed by pairwise comparison with Mann-Whitney *U* test.

Statistical analyses were run with IBM SPSS Statistics 23.

3. Results

3.1. Characterisation of fibers in soil, animals and feces

The recovery of the long fibers extracted from the soil after spiking and measured by weighing increased considerably with increasing nominal concentration (Table 1). The mass of long fibers at the lowest concentration was below the measurement limit and thus the recovery rate was 0%. The number concentrations of short fibers in soil ranged from 1 548 g^{-1} (0.02%) to 112 170 g^{-1} (1.5%). The average recovery rate of short fibers, evaluated by the size and number of fibers, ranged from 52 to 81% (Table 1, Table A.1).

In the case of long fibers, six out of 120 analyzed enchytraeid worms (four individuals in the same composite sample) contained one fiber. These six fibers were found in tissue extract, whilst no fibers were found in the feces. The average length of the fibers was 1 288 ± 454 μm (mean ± SE), the shortest fiber being 375 μm and the longest one 3 254 μm. The length of the long fibers extracted from the spiked soil varied between 650 μm and 17 400 μm and was on average 5 760 μm ± 200 μm.

Table 1
The measured concentrations (w%, mean ± SE), recovery rate (%) and the corresponding concentrations in number of short and long fibers for each nominal concentration in Lufa 2.2 soil used for toxicity testing with soil invertebrates.

Nominal mass concentration (%)	Long fibers		Short fibers		
	Measured mass concentration (%)	Recovery rate (%)[1]	Measured mass concentration (%)	Recovery rate (%)[2]	Measured number concentration (g^{-1})
0.02	0	0	0.005 ± 0.001	64	1 550 ± 84
0.06	0.006 ± 0.006	9	0.013 ± 0.002	52	3 890 ± 310
0.17	0.13 ± 0.053	75	0.063 ± 0.020	81	17 800 ± 1 510
0.5	0.39 ± 0.16	78	0.15 ± 0.033	69	48 900 ± 4 020
1.5	1.6 ± 0.58	105	0.35 ± 0.011	55	112 200 ± 7 860

[1] Measured by weighing.
[2] Evaluated from the number and size of fibers.

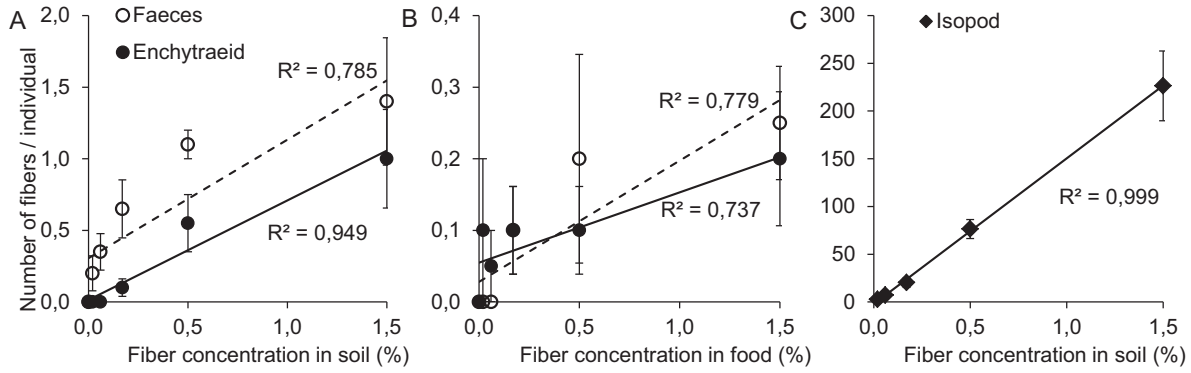

Fig. 1. The number of fibers ingested by the enchytraeid *Enchytraeus crypticus* (A, B), when exposed to short fibers spiked in Lufa 2.2 soil (A) and in food (B), and the number of fibers ingested by the isopod *Porcellio scaber* (C), when exposed to short fibers spiked in Lufa 2.2 soil. The open circles show the number of fibers egested by the enchytraeids via feces and the black dots the number of fibers found in the enchytraeids after 6 h of depuration (mean ± SE). The black diamonds show the number of fibers found in the isopod gut after exposure without any depuration. Coefficients of determinations (R^2) have been determined using average data.

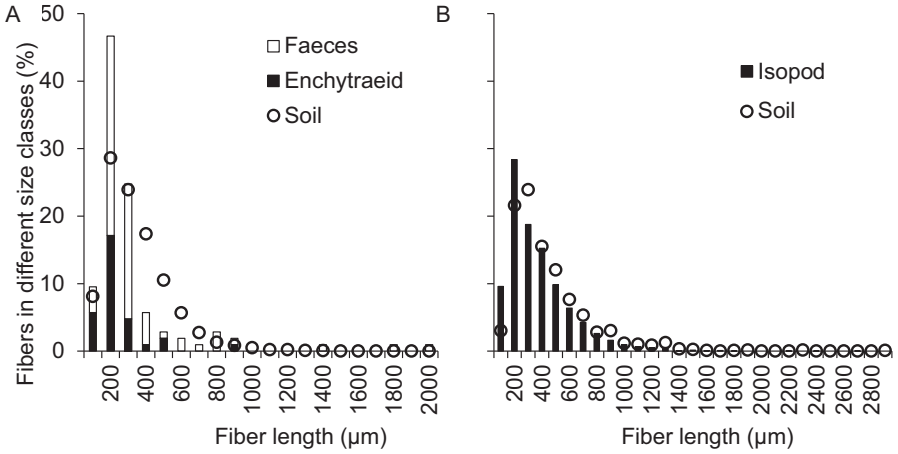

Fig. 2. The size range of the fibers in test soil and the fibers ingested by the enchytraeid *Enchytraeus crypticus* (A) and isopod *Porcellio scaber* (B) exposed to different concentrations of short fibers in Lufa 2.2 soil. The bars show the proportion of ingested fibers in different size classes, the circles the proportion of fibers in different size classes in soil. In panel A, the white bars show the portion of ingested fibers that were egested by enchytraeids and the black bars the proportion of fibers found in the enchytraeids after 6 h of depuration. The range for each size class is 100 µm, and the values on the × axis show the upper limit of each class.

In the experiment with short fibers spiked in soil, a total of 74 fibers were found in enchytraeid feces and 33 in depurated, digested enchytraeid out of the 120 enchytraeids analyzed. Between 0 and 12 fibers were found in the feces of the four enchytraeids per sample. All feces samples from animals exposed to soil concentrations of 0.17% and higher contained fibers. Between 0 and 9 fibers were found after depuration of the four individuals per replicate, and all enchytraeid samples from the two highest soil concentrations contained fibers. The number of ingested and egested fibers was positively related with the fiber concentration in soil (Fig. 1). The length of the fibers varied between 45 and 871 µm in the digested enchytraeid samples and between 85 and 1 900 µm in the feces samples. The average length of the fibers in the worms and in the feces were 191 ± 27 µm (mean ± SE; n = 72) and 304 ± 39 µm (n = 33), respectively (Fig. 2). The fibers in the spiked soil measured average 291 ± 4 µm (n = 2 017), varying between 49 and 1 314 µm (Fig. 2). The size distribution of the ingested fibers was more skewed towards the shorter size classes than that of the fibers in soil (Fig. 2).

When the enchytraeids were exposed to the short fibers spiked in food, 11 fibers were found in digested enchytraeid worm samples and 11 in the feces from the 120 enchytraeids analyzed. The number of fibers was 0–3 in the feces of four individuals and 0–2 per four enchytraeids after depuration. The number of ingested and egested fibers were positively related with the fiber concentration in food (Fig. 1).

Every analyzed isopod gut from exposure concentrations higher than 0.02% contained short fibers, and the number of ingested fibers was strongly correlated with the concentration in the soil (Fig. 1). The length of the fibers ingested by the isopods ranged between 50 and 2 653 µm, and the average length was 326 ± 5 µm (mean ± SE, n = 2 731; Fig. 2). The fibers extracted from the test soil were on average 389 ± 8 µm long (n = 1 298), varying between 62 and 2 807 µm (Fig. 2). The size distribution of ingested fibers resembled that of the fibers in soil, except in the smallest size classes which seemed to be ingested in a greater proportion (Fig. 2).

3.2. The effects of fibers on soil invertebrates

Upon exposure to long fibers, the survival of *E. crypticus* was slightly decreased only at moderate fiber concentrations of 0.17 and 0.5% in the soil. Long fibers in soil negatively affected the reproduction of *E. crypticus* at all concentrations except for 0.06% (Fig. 3). Thus, 0.06% was the NOEC for the effects of long fibers on *E. crypticus* reproduction. Enchytraeid survival and reproduction

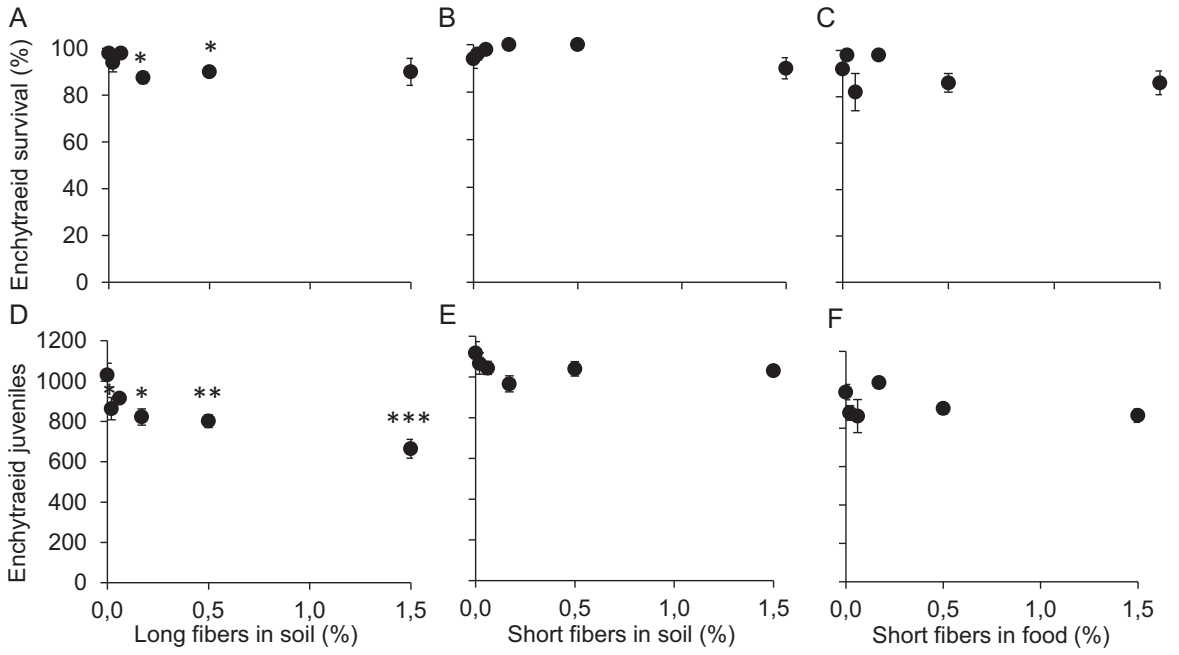

Fig. 3. Survival (A, B, C) and reproduction (D, E, F) (mean ± SE) of the enchytraeid *Enchytraeus crypticus* exposed for 3 weeks to long polyester fibers spiked in Lufa 2.2 soil (A, D), short polyester fibers spiked in Lufa 2.2 soil (B, E) and short polyester fibers spiked in food (C, F). The asterisks represent the level of significance in the comparison with control (*< 0.05; **< 0.01; ***< 0.001).

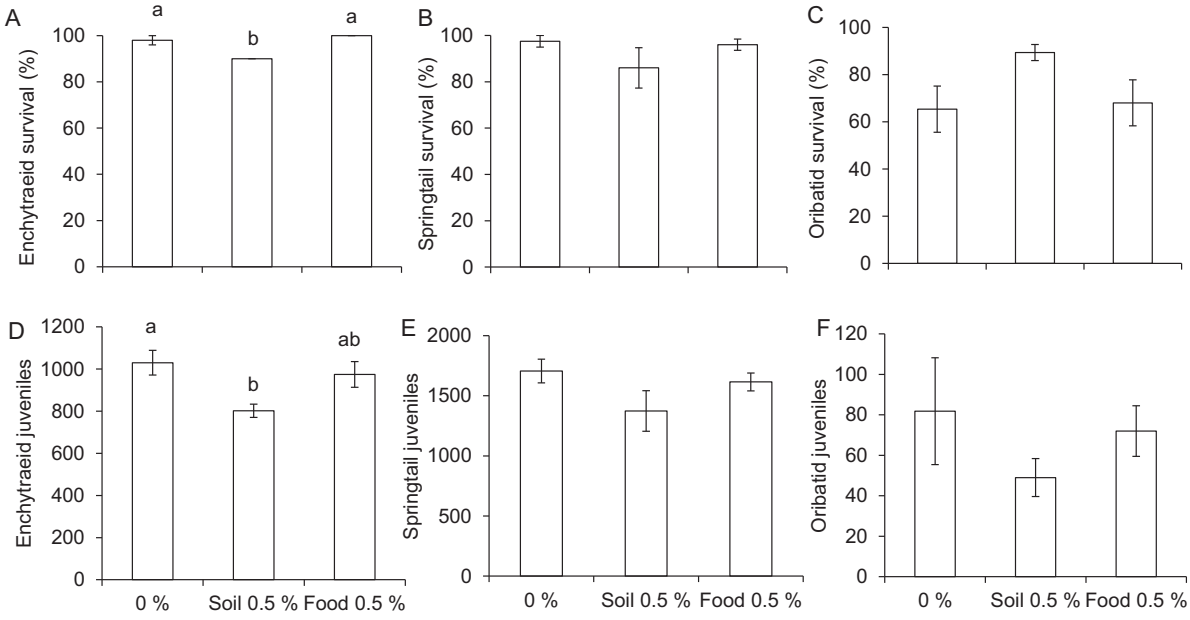

Fig. 4. Survival (A, B, C) and reproduction (D, E, F) (mean ± SE) of the enchytraeid *Enchytraeus crypticus* (A, D), the springtail *Folsoma candida* (B, E) and the oribatid mite *Oppia nitens* (C, F) exposed to long polyester fibers spiked in Lufa 2.2 soil and food at 0.5% (w/w). Treatments marked with different letters differ significantly ($p < 0.05$) from each other.

were not affected upon exposure to long fibers in food (0.5%; Fig. 4, Table A.2), or to short fibers in soil or in food (Fig. 3, Table A.3).

There were no differences in springtail survival or reproduction between the soil or food fiber treatments and the control for any of the fiber exposures (Figs. 4 and A.2, Tables A.2 and A.3). Survival and reproduction of the oribatid mite *O. nitens* were also not affected by the polyester fibers (Fig. 4, Table A.2).

The presence of polyester fibers in the soil had no significant effect on the survival, feeding activity or biomass change of isopods compared to the control. However, even though the differences were not statistically significant ($F_{5,24} = 2.52$, $p = 0.057$), a dose-related decrease in feeding activity with increasing concentration of short fibers was observed at concentrations higher than 0.06% w/w. The most noticeable difference in feeding activity was

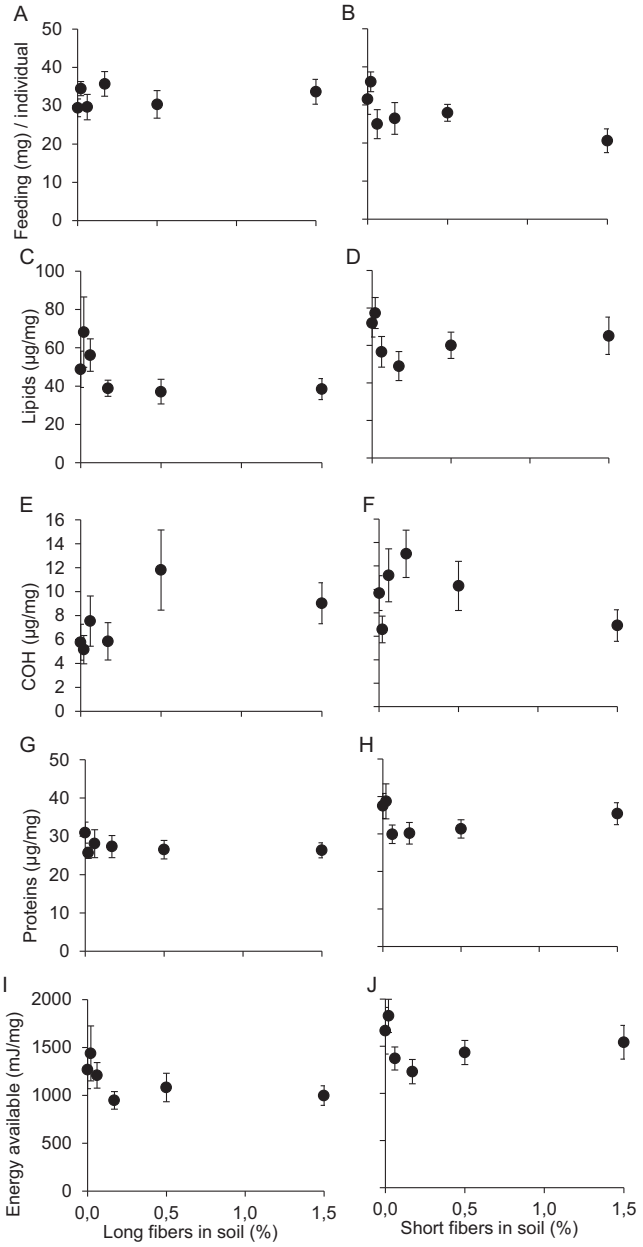

Fig. 5. Feeding activity (A, B) expressed as mass of food consumed per individual isopod *Porcellio scaber*, and the total contents of energy rich molecules: lipids (C, D), carbohydrates (COH; E, F) and proteins (G, H) as well as the total energy content (I, J) in *P. scaber* exposed for 4 weeks to long (A, C, E, G, I) and short (B, D, F, H, J) polyester fibers in Lufa 2.2 soil (mean ± SE).

observed at the highest concentration of short fibers (1.5% w/w), where the feeding activity was 34% lower than in the control (Fig. 5).

The total contents of energy-rich molecules (lipids, carbohydrates and proteins) in *P. scaber* exposed to textile fibers in the soil were not significantly different from the control group (Fig. 5). However, there was a regular pattern in isopod energy reserves and feeding activity. When isopods were exposed to short fibers, protein and lipid concentrations as well as total energy reserves decreased at soil concentrations of 0.06 and 0.17% and increased to the control level at the highest concentration. Carbohydrates showed the opposite trend, increasing at 0.06 and 0.17% and decreasing to the control level at higher concentrations (Fig. 5). In the exposure to long fibers, lipid concentration and total energy reserves decreased at soil concentrations of 0.17% and higher, whilst carbohydrate concentration increased in the two highest concentrations (Fig. 5).

4. Discussion

This is the first study to investigate the effects of textile fibers on soil invertebrates with different ecological niches and feeding strategies. For both short and long sized polyester fibers, no statistically significant effects on *P. scaber, O. nitens* and *F. candida* were found at concentrations in soil or food up to 1.5% w/w (on *O. nitens* 0.5% w/w).

The only statistically significant effects were found for *E. crypticus* exposed for 21 days to long fibers in soil. The survival was reduced only by about 10%, but reproduction of *E. crypticus* was clearly decreased by 20%, 20% and 30% at long fiber concentrations of 0.17, 0.5 and 1.5%, respectively. However, no effects were detected in the exposure of enchytraeids to short fibers. Surprisingly, the ingestion of long fibers was very limited, while the short fibers that did not induce effects on survival or reproduction were clearly ingested by the enchytraeids. This suggests that for *E. crypticus* the effect of long fibers arose from the physical harm outside the organism, or from indirect effects resulting from changes in the environmental conditions. These effects seem to depend on the length and amount of fibers in the surrounding environment.

These findings on the enchytraeid *E. crypticus* are in line with observations on the negative effects of fibers on the survival, reproduction and growth of the water flea *Ceriodaphnia dubia*, without evidence of fiber ingestion (Ziajahromi et al., 2017). Instead, malformations in the external morphological traits of the daphnids were observed. This shows that negative effects of micro- or mesoplastics on invertebrates are not always caused by ingestion. Synthetic fibers can damage or otherwise hamper the fitness of the organism externally and result in responses at the population level due to reduced reproduction rate.

It is also possible that changes in the properties of the surrounding habitat affected the reproductive success of the enchytraeids. Microplastics can affect the microbial activity and physicochemical properties of the soil (de Souza Machado et al., 2018; Zhang et al., 2019). Such biological, physical and chemical changes in the environment, in turn, can be reflected in the different compartments of the soil food web (Tsiafouli et al., 2015). De Souza Machado et al. (2018) showed that the intensity and even the direction of the changes in soil properties are dependent on the type of microplastic. In addition, the effects of microplastics on organisms may not only depend on the microplastic type but also on the size (Gray and Weinstein, 2017, Ziajahromi et al., 2017), which makes the risk assessment of microplastics challenging. The effects of microplastics cannot be extrapolated among different types or even different sizes, as shown in the present study.

In addition to applying traditional endpoints such as survival and reproduction, we also investigated the potential effects of polyester fibers on the feeding activity, growth and energy reserves of isopods. It has previously been suggested that microplastics may affect the energy reserves of organisms. Wright et al. (2013) reported that 28-day exposure of the lugworm *Arenicola marina* to polyvinyl chloride particles (1–5% w/w, 130 µm mean diameter) resulted in reduced feeding activity and significantly depleted energy reserves. Although we did not record such an evident effect on terrestrial isopods after 28 days (this study) or 14 days exposure to 0.4% of plastic fragments (Jemec Kokalj et al., 2018), a change in energy reserves was detected, and feeding activity slightly decreased with increasing concentrations of short fibers. A decreasing trend in feeding activity and growth of *P. scaber* was also found in a preliminary test (Fig. A.3). Our findings suggest a

potential depletion in energy reserves and allocation of energy resources from proteins and lipids to carbohydrates at short fiber concentrations in soil of 0.06 and 0.17% and at concentrations 0.17% and higher of long fibers.

Our findings support the previous conclusions that population level responses (survival and reproduction) during exposure of one generation of soil invertebrates on microplastics generally occur at relatively high concentrations, while biochemical level responses, like energy reserve reductions, and also functional endpoints, like feeding activity, may be more sensitive to microplastics. Our earlier study with the isopod *P. scaber* showed no effects on survival after 14-day exposure to 0.4% w/w microplastics in food, which were obtained from a plastic bag (183 ± 93 μm) and facial cleaner (137 ± 51 μm) (Jemec Kokalj et al., 2018). Similarly, Rodriguez-Seijo et al. (2017) found no effects on reproduction, survival, and growth of *Eisenia andrei* after 28 days exposure to 0.1% w/w polyethylene pellets (250–1000 μm) in soil. However, damage in the intestinal epithelium, in the immune response of coelomocytes and signs for oxidative stress were found already at lower concentrations (Rodriguez-Seijo et al., 2017, 2018). Nevertheless, some studies reported a variety of effects not only on biochemical but also on population level endpoints at higher concentrations, and in some cases even at lower concentrations of microplastics. There also seems to be considerable variability in the responses according to the type of the particles tested. Although we did not observe effects of polyester fibers on *F. candida* reproduction, in the study of Ju et al. (2019) with polyethylene beads (<500 μm) reproduction *F. candida* was inhibited at concentrations ≥ 0.1%, reaching up to 70% reduction at 1% in soil. When earthworms *Lumbricus terrestris* were exposed to polyethylene particles (200–300 μm) in litter on the soil surface, increased mortality and decreased growth were seen at ≥ 28% (Huerta Lwanga et al., 2016) and higher formation of burrows was detected at 7% w/w (Huerta Lwanga et al., 2017a). Reduced weight and altered gut microbial composition of *E. crypticus* were recorded after 7 days exposure to 10% w/w polystyrene nanobeads (0.05–0.1 μm) spiked in oatmeal (Zhu et al., 2018b). These concentrations sound extremely high, especially when considering the low density of most plastic types compared to mineral soil. However, concentrations up to 6.7% (w/w) in soil have been reported, although these soils were sampled from an industrial area with a history of plastic production in Australia (Fuller and Gautam, 2016). In any case, it is still unknown whether synthetic fibers and other microplastics commonly found in the environment can induce population level effects upon long-term exposure to lower concentration levels. Multigeneration studies are needed to reveal the possible long-term effects of microplastics on soil invertebrates.

From the existing literature on the effects of microplastics on terrestrial invertebrates we conclude that (i) various exposure conditions are used (spiked litter, spiked food or soil), (ii) exposure concentrations are commonly very high, and (iii) expressed only on a mass basis. Since particles have different shapes and densities, the number concentrations may differ substantially among treatments. Also, none of the studies thus far have provided measured size and number distributions of particles after spiking in soil. All these facts hamper a proper comparison of different studies using terrestrial invertebrates and comparison with field concentrations that usually are expressed as number of particles and not as mass concentrations. In the present study, we measured the concentrations and length distributions of fibers also after spiking the soil. We also measured the number concentrations of short fibers, allowing better comparison with field studies.

Besides the data from the Australian industrial site, with concentrations up to 6.7% (Fuller and Gautam, 2016), microplastic mass-concentrations have also been reported for Swiss floodplain soils (Scheurer and Bigalke, 2018), Swedish agricultural soils (Ljung et al., 2018) and Chinese agricultural soils. In Swiss floodplain soils the average microplastic concentration was 0.0005% and the maximum concentration 0.0056% (Scheurer and Bigalke, 2018). In Swedish agricultural soil receiving mineral fertilizers or sewage sludge, the microplastic concentrations were 0.000032% and 0.00034%, respectively. In Chinese agricultural soils the average microplastic concentration varied between 0.0008% and 0.054% (Zhang et al., 2018). However, even in these studies the measured size ranges were different. The Swiss study focused on particles over 125 μm and the Swedish study on the particle size range 10–500 μm, which makes it hard to compare these studies. When considering organic soil fertilizers, Ljung et al. (2018) reported a microplastic mass concentration of 0.042% in sewage sludge, and Bläsing and Amelung (2018) referred to unpublished data on compost samples with highest concentrations of 0.12% and 0.018%. The concentrations used in our study (0.02–1.5%) correspond to the microplastic levels found in industrial soils, some Chinese agricultural soils, sewage sludge or compost. It should, however, be noted that these data concern all microplastic types, not only synthetic fibers as in the present study. In any case, since data on microplastics in soils is so scarce and measured plastic size ranges vary greatly between the studies, no definite conclusion on realistic exposure concentrations in soils can be drawn.

More data is available on the number-concentrations of microplastics in soil. On average 40–42 960 microplastics kg^{-1} soil have been reported in Chinese farmlands (Liu et al., 2018; Zhang and Liu, 2018; Zhang et al., 2018), up to 12 800 items kg^{-1} in Chinese sandy beaches and mangrove wetlands (Li et al., 2018b) and up to 14 700 items kg^{-1} soil in Chinese coastal soils (Zhou et al., 2018), but only on average 0.34 items kg^{-1} soil in German farmlands that never received agricultural plastic applications (Piehl et al., 2018). Zubris and Richards (2005) found synthetic fibers to indicate sewage sludge application already in the early 21st century, with 580–1 210 fibers kg^{-1} sludge-amended soil compared to 0–80 fibers kg^{-1} in control soils. Compared to these concentrations, the levels of exposure in our experiment were high, ranging from 1 548 short fibers g^{-1} soil at the lowest concentration (0.02%) to 112 170 g^{-1} soil at the highest concentration (1.5%). However, these number concentrations are for short fibers with an average length of approximately 300 μm, the corresponding number concentrations of longer fibers would be lower. It is also possible that fiber concentrations measured in the field are underestimated. In the present study we found that density separation after reduction of organic matter, a method commonly used to quantify microplastics in soil samples, was not efficient for short fibers. We also found that the shortest fibers extracted from soil were about four times longer than the smallest pieces of the original material, indicating that the smallest microplastic particles are very hard to extract and detect from a solid matrix such as soil. These findings suggest that the concentrations of small microplastics in soil are easily underestimated. Finally, we conclude that mass quantification of fibers in soil is not a trivial task, and multiple approaches are required and still need to be developed to estimate microplastic concentrations in soil.

The distribution of fibers in soil is also heterogeneous, as indicated by the poor recovery rate of long fibers at low concentrations. This is likely to be the case also in the field, meaning that some soil invertebrate individuals may be exposed to considerably higher concentrations of microplastics than others, especially if the animal does not avoid microplastic contamination. In another study we conducted, isopods did not show any avoidance behavior towards polyester fibers (unpublished data). However, for some types of microplastics or some invertebrate species avoidance of microplastics may occur, as *F. candida* was shown to avoid PE microbeads (<500 μm; Ju et al., 2019) and PVC particles (80–250 μm; Zhu et al., 2018c).

In addition to determining the effects, we also assessed the ingestion of fibers by *E. crypticus* and *P. scaber*. For both the enchytraeids and isopods, the ingestion of short fibers increased linearly with increasing concentration in soil. The number of short fibers ingested by the enchytraeids was also higher when they were spiked in soil compared to food, suggesting that enchytraeid worms are either more selective when consuming food compared to ingesting soil, or that the rate of ingestion of test soil is overall higher than the ingestion of the ground oats that served as food. In any case, it seems that the more fibers are present in the surrounding environment, the more they are ingested by soil invertebrates.

Whilst the enchytraeids clearly ingested short fibers, the ingestion of long fibers was very limited. The ingested fibers were on average shorter than the fibers extracted from the spiked soil. When exposed to short fibers, there was a greater proportion of shorter fibers in the animals compared to the size distribution of the fibers in spiked soil, with more than half of the ingested fibers being shorter than 200 μm. In the isopods, the size of ingested fibers was more similar to that of the fibers in soil, except that the proportion of fibers shorter than 100 μm was higher in the isopod gut than in the surrounding soil. These findings suggest that especially in the small sized enchytraeid worms the ingestion of fibers depends on the length of the fiber. Our results suggest that the ingestion of fibers by soil invertebrates depends not only on the concentration, but also on their size in relation to the size of the exposed species.

It is also possible that the greater portions of shorter fibers found in the feces and in depurated enchytraeids and in the isopod gut than in spiked soil indicate that the fibers may be fragmented upon passing the digestive tract of animals. This possibility is supported by the finding that the shortest fiber found in the digested enchytraeid after exposure to long fibers was shorter than the ones extracted from the spiked soil, even though the limited number of the ingested fibers hampers drawing definite conclusions. We also found that the length of the long fibers extracted from spiked soil was considerably lower than in the original material, indicating that physical forces, present also in field conditions, can break larger fibers and result in the formation of microplastic sized particles. As proven from the evidently higher ingestion rate of shorter fibers in the present study, the possible degradation of synthetic fibers by the activity of soil invertebrates and physical stresses in the environment can make the fibers more easily ingestible by soil organisms.

Synthetic fibers have been shown to be retained in the digestive tract of aquatic organisms longer than food or sediment particles (Au et al., 2015; Hurley et al., 2017). As such information on soil organisms is still lacking, we studied whether fibers retain in the gut of *E. crypticus* by assessing the number and size distribution in the organisms after a 6 h depuration period. Although most of the short fibers were egested during 6 h of depuration, on average 30–50% of the fibers remained in the organism after depuration. To confirm whether some fibers are retained in the organism after complete depuration of the digestive tract, the elimination needs to be investigated more closely. The finding that no long fibers were egested during the depuration period suggests that long fibers could be depurated less efficiently than short ones. However, in the experiment with short fibers in spiked soil the egested fibers were on average longer than the ones retained in the organism. In addition, the number of long fibers ingested was very low, not allowing for a conclusion on the egestion rate. More research is needed to investigate the importance of the size of fibers for their retention in the digestive tract of soil organisms. Although the ingested fibers are not assimilated in the tissues of the organism, the fibers retained in the digestive tract will be transferred to organisms at the higher trophic level possibly inflicting negative impacts. Evidence of trophic transfer of microplastics already is available, not only in the aquatic environment (Setälä et al., 2014, Au et al., 2017), but also in the terrestrial food web (Huerta Lwanga et al., 2017b). More information on factors affecting the retention of microplastics in digestive tract and more evidence on the trophic transfer in terrestrial ecosystem are still needed for assessing the risks of microplastics in soils.

5. Conclusions

This study shows that synthetic polyester fibers, which are among the most important fractions of microplastics entering the soil through sludge application, are not very harmful to soil invertebrates at the exposure concentrations used in this study. Nevertheless, effects were seen on enchytraeid reproduction and also on the energy reserves and feeding activity of isopods. However, enchytraeid reproduction was decreased only by long fibers that were hardly ingested, suggesting that the impact of microplastics on soil animals is not always related to the ingestion of the particles but may be due to physical harm outside the organism or to changes in the surrounding environment. Although only slight effects were seen in this one-generation study, we found clear evidence for fiber uptake in enchytraeids and isopods, indicating the entry of polyester fibers into terrestrial food webs and potential long-term risks for these organisms and their predators. Our results ask for more research on the uptake and effects of different types of microplastics in soil invertebrates.

Declaration of Competing Interest

There is no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2019.134451.

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