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Invited paper

Transport of microplastics by two collembolan species☆

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ABSTRACT

Plastics, despite their great benefits, have become a ubiquitous environmental pollutant, with microplastic particles having come into focus most recently. Microplastic effects have been intensely studied in aquatic, especially marine systems; however, there is lack of studies focusing on effects on soil and its biota. A basic question is if and how surface-deposited microplastic particles are transported into the soil. We here wished to test if soil microarthropods, using Collembola, can transport these particles over distances of centimeters within days in a highly controlled experimental set-up. We conducted a fully factorial experiment with two collembolan species of differing body size, *Folsomia candida* and *Proisotoma minuta*, in combination with urea-formaldehyde particles of two different particle sizes. We observed significant differences between the species concerning the distance the particles were transported. *F. candida* was able to transport larger particles further and faster than *P. minuta*. Using video, we observed *F. candida* interacting with urea-formaldehyde particles and polyethylene terephthalate fibers, showing translocation of both material types. Our data clearly show that microplastic particles can be moved and distributed by soil microarthropods. Although we did not observe feeding, it is possible that microarthropods contribute to the accumulation of microplastics in the soil food web.

1. Introduction

Plastic as a cheap but long-lived material has had an enormous and beneficial effect on our everyday life (Andrady and Neal, 2009; Cole et al., 2011; Thompson et al., 2009) and makes up about 10% of the solid waste, depending on the country (Barnes et al., 2009). It has also become a serious threat to our environment (Cole et al., 2011; Lechner et al., 2014). First evidence for pollution by plastic came from aquatic systems (Buchanan, 1971; Colton et al., 1974; Kenyon and Kridler, 1969). Especially the various sizes of plastic can cause a wide range of threats, i.e. plastic bottles and fishing nets (Ivar do Sul and Costa, 2014) vs. fibers or abrasive materials. The latter fraction, microplastics, are particles smaller than 5 mm in size (Cole et al., 2011) which can be of primary or secondary origin, being directly manufactured as such particles or derived from the fragmentation of larger plastic items, respectively (Wright et al., 2013), and there is increasing evidence that these particles can be accumulated in the aquatic food chain (Wright et al., 2013). Additionally, they provide large surface areas which can absorb a range of other pollutants in aquatic systems (e.g. Bakir et al., 2012; Mato et al., 2001). Especially in soils, these properties have not yet been examined in detail (Browne et al., 2011; Rillig, 2012) although it is assumed that any soil with anthropogenic influence may show a certain degree of pollution by (micro-)plastics over years if not decades (e.g. Fuller and Gautam, 2016; Nizzetto et al., 2016; Zubris and Richards, 2005).

In the last years, the potential negative effects of plastics on soil biota have been investigated with a special focus on earthworms (Huerta Lwanga et al., 2016). Earthworms have large dispersal capabilities and hence potentially a huge influence on the distribution of also larger plastic particles from the soil surface to deeper layers, which has been demonstrated (Huerta Lwanga et al., 2016; Rillig et al., unpublished). However, soil harbors a multitude of organisms of different size classes. We here focus on a highly abundant group of microarthropods, Collembola, which can occur in high numbers in soils, i.e. 10,000–100,000 individuals per square meter

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(Hopkin, 2007), and which hence should be considered as potential agents of microplastic movement. Microarthropods reach highest densities within the first 10 cm of the soil profile with a presumably small home range (Widenfalk et al., 2015), however, especially soil surface dwelling species might contribute strongly to the incorporation of plastic particles into the soil.

In this study we wanted to test (1) if collembolans could act as agents for the transport of microplastic particles; and, if so, (2) at which temporal and spatial scales such transport could potentially occur. As direct observation in soil is nearly impossible we here used a highly controlled arena experiment and video-filming approaches. We hypothesized that the distribution of particles can occur via (a) feeding and defecation, (b) attachment of particles to the cuticle of Collembola (although this process might only play a minor role in soil), (c) animal movement like crawling over particles and jumping, respectively. Such processes would be expected to be highly dependent on the size and type of the particles and the organism size. For this reason we here studied two different microplastic types and sizes as well as two collembolan species varying in body size. We expected to see that the smaller collembolan *P. minuta* would in general transport particles to a lesser degree and not as far as *F. candida* within a given time, with this difference in transport being most pronounced for the larger size (100–200 μm) microplastic particles.

2. Material & methods

For the arena experiment, we used urea-formaldehyde microplastic (WIWOX ST KS 002, particle size 200–400 μm, WIWOX GmbH Surface Systems, Erkrath, Germany) which was washed with VE water and dried at 40 °C for 24 h to remove any toxic substances from the particle surface. After freeze-drying with liquid nitrogen, the material was ground by hand with a mortar and sieved to produce two particles fractions (<100 μm and 100–200 μm). We used specimen cups which we filled with a 5 mm thick layer of a mixture of plaster of Paris and activated charcoal (3:1) and let it dry. As treatments we used 5 mg of the 100–200 μm fraction and 2.5 mg of the <100 μm fraction, which corresponds to the amount of particles needed to evenly cover a circle of 0.5 cm in diameter ('feeding station') in the middle of the specimen cups. No additional food source was provided. In order to avoid the distribution of the particles by airflow, we carefully placed lids on the specimen cups.

As target organisms we used the two collembolan species, *Folsomia candida* (up to 3 mm body size) and *Proisotoma minuta* (up to 1.1 mm in body size). We set up 7 replicates for each combination of collembolan species (n of ind = 25) and particle fraction, the controls did not contain any collembolan species, resulting in a total of 42 samples. The cups were incubated at room temperature (20 ± 2 °C). For seven days, each sample was photographed once a day with a Canon 70D at a distance of 30 cm. For image analysis, four concentric circles of 1, 2, 3 and 4 cm diameter were placed around the feeding station and the amount of particles was counted in each ring (Fig. 1).

We analyzed the data with R version 3.3.1 (R Development Core Team, 2016). We used generalized least square models of the 'nlme' package (Pinheiro et al., 2016) to account for heterogeneity in our data; for this we used the function 'varIdent' (Zuur et al., 2009). Model residuals were checked for normal distribution and variance homogeneity. Pairwise comparisons of least square means of factors were performed with the eponymic package 'lsmeans' (Lenth, 2016) and for figures we used 'ggplot2' (Wickham, 2009).

In order to capture representative animal behavior we recorded videos, for which we used rectangular breeding boxes (polystyrene, 180 × 135 × 60 mm, W&V Becker and Hauger, Leichlingen, Germany) filled with a 3 mm layer of plaster of Paris and activated charcoal (3:1) and 10 individuals of *Folsomia candida*. We offered two different microplastic types in the box: (1) particles of organic plastic abrasive (urea-formaldehyde WIWOX ST KS 002, particle size 200–400 μm) and (2) scraped-off parts of a polyethylene terephthalate (PET) bottle. Videos were taken with the help of a NIKON EL-Nikkor with a Novoflex and bellows attachment.

Fig. 1. Examples of image analysis with four concentric circles of 1, 2, 3 and 4 cm diameter placed around the feeding station (left: initial photo, right: day 5). The amount of particles was counted in each ring and used for analysis. (photos: D. Daphi).

Supplementary video related to this article can be found at http://dx.doi.org/10.1016/j.envpol.2017.03.009.

3. Results

3.1. Arena experiment

In general, we found significant differences between the species ($F_1 = 27.1$, $p < 0.001$) and the rings, i.e. the distance the particles were transported ($F_1 = 4.3$, $p = 0.001$). Additionally, there was a significant interaction term for species and ring ($F_1 = 5.6$, $p = 0.001$). Interestingly, we did not observe significant differences between the two particle sizes ($F_1 = 0.7$, $p = 0.41$) (see Table 1).

The biggest differences can be seen between the species in regard to the distribution into ring 1, i.e. 1 cm diameter around the feeding station, with *F. candida* distributing far more particles than the smaller species *P. minuta* (Fig. 2). A smaller but still significant difference can be observed at the 2 cm distance. Distances of more than 3 cm diameter around the feeding station are regularly reached by *F. candida* but only rarely by *P. minuta*.

The smaller collembolan *P. minuta* was able to move particles to a lesser extent than the larger bodied *F. candida,* and this was the case for both offered microplastic particle sizes (Fig. S1). After one week, particles transported by *P. minuta* were about 1 cm in diameter around the feeding station, whereas *F. candida* moved particles up to 4 cm already after day 4 (<100 μm) or day 5 (100–200 μm), respectively (Fig. S1). We observed most particle movement for the size class <100 μm when acted upon by *F. candida*.

Table 1
Results of three factors (collembolan, horizontal distance and particle size) ANOVA. Significant p-values < 0.05 (shown in bold).

	df	F	p
(Intercept)	1	529.180	<**0.0001**
species	1	27.050	<**0.0001**
ring	3	3.428	**0.02**
particle	1	0.681	0.41
species:ring	3	5.563	**0.001**