Environmental Pollution 225 (2017) 456–459

Contents lists available at ScienceDirect

Environmental Pollution

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

Invited paper

Transport of microplastics by two collembolan species☆

Stefanie Maaß [a, c, *], Daniel Daphi [b], Anika Lehmann [b], Matthias C. Rillig [b, c]

[a] *Universität Potsdam, Institut für Biochemie und Biologie, Plant Ecology and Nature Conservation, Am Mühlenberg 3, D- 14476 Potsdam, Germany*
[b] *Freie Universität Berlin, Institut für Biologie, Plant Ecology, Altensteinstr. 6, D- 14195, Berlin, Germany*
[c] *Berlin-Brandenburg Institute of Advanced Biodiversity Research (BBIB), D- 14195, Berlin, Germany*

ARTICLE INFO

Article history:
Received 17 November 2016
Received in revised form
28 February 2017
Accepted 3 March 2017
Available online 17 March 2017

Keywords:
Microplastics
Soil
Collembolans
Transport
Pollution

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

☆ This paper has been recommended for acceptance by Maria Cristina Fossi.

* Corresponding author. Universität Potsdam, Institut für Biochemie und Biologie, Plant Ecology and Nature Conservation, Am Mühlenberg 3, D - 14476 Potsdam, Germany.

E-mail addresses: stmaass@uni-potsdam.de (S. Maaß), da.da@fu-berlin.de (D. Daphi), lehmannanika@zedat.fu-berlin.de (A. Lehmann), rillig@zedat.fu-berlin.de (M.C. Rillig).

http://dx.doi.org/10.1016/j.envpol.2017.03.009
0269-7491/

(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**

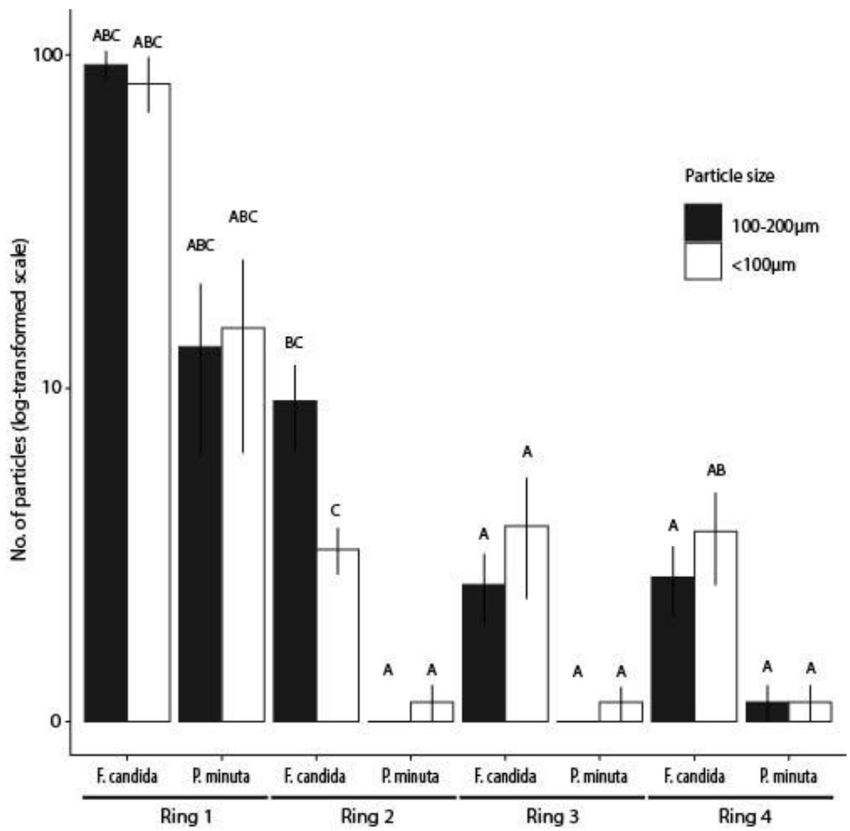

Fig. 2. Number of particles moved horizontally over the four defined rings (with ring 1 = 1 cm, ring 2 = 2 cm, ring 3 = 3 cm and ring 4 = cm diameter) around feeding station by different collembolan species. The color of bars represents the different particles sizes used. Mean ± SE, n = 7. Bars with same letters are not significantly different according to pairwise comparisons of least square means at alpha level 0.05. Y-axis was log-transformed for better visualization.

3.2. Video

In the video (see supplementary material) we directly see that *F. candida* is indeed capable of moving microplastic particles of both offered types. As expected, the lighter, scraped-PET material could easily attach to the collembolans' body and thus be transported. Also in the case of the heavier urea-formaldehyde particles, there is potential to at least move them within a certain range by crawling over the material or by pushing particles aside with the legs or the head. We did not observe feeding on the particles. Interestingly, we observed that the microplastic clusters were used as oviposition site (Fig. S2). However, in this setup we did not test for toxicity and fecundity effects.

4. Discussion

In terms of studying the transport of particles of various kinds, including organic matter, soil or microplastics, the focus has so far been clearly on macrofauna, i.e. earthworms, ants and termites (e.g. Anderson, 1988; Benckiser and Schnell, 2007; Bunnenberg and Taeschner, 2000), neglecting the highly abundant group of microarthropods which is usually present in the soil. We here provide first evidence that soil inhabiting collembolan species are indeed able to move and distribute microplastic particles, an effect that was strongly dependent on the particle type and its size class as well as the size of the organism. Compared to earthworms, the distribution range is far smaller; however, we have to assume that microplastics can be translocated at the same speed and distance as organic matter. Although we have evidence from our experiment that particles can become attached to the collembolan's cuticle and thus be transported, we assume that in soil this process might only play a role for very small microplastic particles. Thus collembolans might be able to introduce microplastic particles even into microaggregate pore spaces and introduce potential hazardous chemical loads to previously isolated and protected soil microbial communities with unforeseeable consequences for their performance and hence ripple-on effects on soil structure and health.

Moreover, collembolans might be involved in the production of microplastics from bigger pieces of plastic by scraping or chewing activities (Rillig, 2012), however, we did not find evidence for this in our experiment. At the same time, passive ingestion of microplastic particles and the defecation at another location in the soil especially by collembolans (Hopkin, 2007) should also be considered in further studies. Due to their small body size, microarthropods might just play an important role for microplastics of sizes smaller than 100 µm; however, their typically high abundance might increase the effect we have shown here when occurring in soil.

As presented in the video, we also saw that clusters of microplastic were used as oviposition sites by *F. candida*. In the western Atlantic, viable eggs of *Halobates* species were found on 24% of the microplastic pellets (Ivar do Sul and Costa, 2014). It has also been shown for two aquatic bug species, *Halobates micans* and *H. sericeus,* that the choice of these oviposition sites potentially have an effect on their abundance and dispersion (Goldstein et al., 2012; Majer et al., 2012). In soils, there are no data available on this issue, especially not in regard to potential toxicity of the particles due to adsorbed substances which might affect the fitness of the offspring. It is possible that the ingestion of microplastics might cause internal abrasion and blockage with lethal outcome which might have impacts on the whole population (Wright et al., 2013).

Our study sheds light on the role of microarthropods in the transport of microplastics horizontally; however, we have to assume that this transport might also occur from the surface into the soil (as has been shown for biochar summarized in Ralebitso-Senior and Orr, 2016 and for macrofaunal transport of soil in Bunnenberg and Taeschner, 2000) which should be tested in more detail. Due to this translocation, there might also be strong impacts on the physical structure of soil, e.g. the incorporation of microplastics into soil aggregates (Rillig, 2012). Additionally, there is a lack of data concerning the amount and release of contaminants as well as the general degradability of microplastics in soil (Rillig, 2012). However, it is likely that small microplastic particles and pollutants on their surfaces can enter the soil food web via microarthropods and persist and accumulate over long periods with so far unknown consequences for the soil and its fauna.

5. Conclusion

Although the pollution of the environment by (micro-)plastic has gained increasing attention during the last decades, interactions with soil and its fauna has so far not been investigated in detail. We here provide first evidence that collembolans can translocate microplastic particles; studies aimed at testing for potential toxic effects on collembolans, also following long-term exposure, should be a future priority. The latter issue is especially important as it will be impossible to remove huge quantities of especially small plastic fragments from soils in the near future.

Acknowledgements

We thank Freie Universität Berlin for financial and Christian Reinhart and Christopher Maaβ for technical support.

Appendix A. Supplementary data

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

References

Anderson, J.M., 1988. Invertebrate-mediated transport processes in soils. Agriculture, Ecosyst. Environ. 24, 5–19. http://dx.doi.org/10.1016/0167-8809(88)90052-7.

Andrady, A., Neal, M., 2009. Applications and societal benefits of plastic. Philos. Trans. R. Soc. 364, 1977–1984. http://dx.doi.org/10.1098/rstb.2008.0304.

Bakir, A., Rowland, S.J., Thompson, R.C., 2012. Competitive sorption of persistent organic pollutants onto microplastics in the marine environment. Mar. Pollut. Bull. 64, 2782–2789. http://dx.doi.org/10.1016/j.marpolbul.2012.09.010.

Barnes, D.K.A., Galgani, F., Thompson, R.C., Barlaz, M., 2009. Accumulation and fragmentation of plastic debris in global environments. Philos. Trans. R. Soc. B 364, 1985–1998. http://dx.doi.org/10.1098/rstb.2008.0205.

Benckiser, G., Schnell, S. (Eds.), 2007. Biodiversity in Agricultural Production Systems. CRC Press Inc, Boca Raton, Fl, USA, 429 pp.

Browne, M.A., Crump, P., Niven, S.J., Teuten, E., Tonkin, A., Galloway, T., Thompson, R., 2011. Accumulation of microplastic on shorelines worldwide: sources and sinks. Environ. Sci. Technol. 45, 9175–9179. http://dx.doi.org/10.1021/es201811s.

Buchanan, J.B., 1971. Pollution by synthetic fibres. Mar. Pollut. Bull. 2, 23. http://dx.doi.org/10.1016/0025-326X(71)90136-6.

Bunnenberg, C., Taeschner, M., 2000. Soil fauna transport versus radionuclide migration. Radiat. Prot. Dosim. 92, 35–38. http://dx.doi.org/10.1093/oxfordjournals.rpd.a033280.

Cole, M., Lindeque, P., Halsband, C., Galloway, T.S., 2011. Microplastics as contaminants in the marine environment: a review. Mar. Pollut. Bull. 62, 2588–2597. http://dx.doi.org/10.1016/j.marpolbul.2011.09.025.

Colton, J.B., Knapp, F.D., Burns, B.R., 1974. Plastic particles in surface waters of the Northwestern Atlantic. Science 185, 491–497. http://dx.doi.org/10.1126/science.185.4150.491.

Fuller, S., Gautam, A., 2016. A procedure for measuring microplastics using pressurized fluid extraction. Environ. Sci. Technol. 50, 5774–5780. http://dx.doi.org/10.1021/acs.est.6b00816.

Goldstein, M.C., Rosenberg, M., Cheng, L., 2012. Increased oceanic microplastic debris enhances oviposition in an endemic pelagic insect. Biol. Lett. 8, 817–820. http://dx.doi.org/10.1098/rsbl.2012.0298.

Hopkin, S.P., 2007. A Key to the Collembola (Springtails) of Britain and Ireland. Aidgap, Field Studies Council, Shrewsbury.

Huerta Lwanga, E., Gertsen, H., Gooren, H., Peters, P., Salánki, T., van der Ploeg, M., Besseling, E., Koelmans, A.A., Geissen, V., 2016. Microplastics in the terrestrial ecosystem: implications for *Lumbricus terrestris* (Oligochaeta, lumbricidae). Environ. Sci. Technol. 50, 2685–2691. http://dx.doi.org/10.1021/acs.est.5b05478.

Ivar do Sul, J.A., Costa, M.F., 2014. The present and future of microplastic pollution in the marine environment. Environ. Pollut. 185, 352–364. http://dx.doi.org/10.1016/j.envpol.2013.10.036.

Kenyon, K.W., Kridler, E., 1969. Laysan Albatross swallow indigestible matter. Auk 86, 339–343. http://dx.doi.org/10.2307/4083505.

Lechner, A., Keckeis, H., Lumesberger-Loisl, F., Zens, B., Krusch, R., Tritthart, M., Glas, M., Schludermann, E., 2014. The Danube so colourful: a potpourri of plastic litter outnumbers fish larvae in Europe's second largest river. Environ. Pollut. 188, 177–181. http://dx.doi.org/10.1016/j.envpol.2014.02.006.

Lenth, R.V., 2016. Least-squares means: the R package lsmeans. J. Stat. Softw. 69, 1–33. http://dx.doi.org/10.18637/jss.v069.i01.

Majer, A.P., Vedolin, M.C., Turra, A., 2012. Plastic pellets as oviposition site and means of dispersal for the ocean-skater insect Halobates. Mar. Pollut. Bull. 64, 1143–1147. http://dx.doi.org/10.1016/j.marpolbul.2012.03.029.

Mato, Y., Isobe, T., Takada, H., Kanehiro, H., Ohtake, C., Kaminuma, T., 2001. Plastic resin pellets for toxic chemicals in the marine environment. Environ. Sci. Technol. 35, 318–324. http://dx.doi.org/10.1021/es0010498.

Nizzetto, L., Langass, S., Futter, M., 2016. Pollution: do microplastics spill on to farm soils. Nature 537, 488. http://dx.doi.org/10.1038/537488b.

Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D., R Core Team, 2016. nlme: Linear and Nonlinear Mixed Effects Models. R package version 3.1-128. http://CRAN.R-project.org/package=nlme.

Ralebitso-Senior, T.K., Orr, C.H. (Eds.), 2016. Biochar Application: Essential Soil Microbial Ecology, first ed. Elsevier. 344 pp.

R Development Core Team, 2016. R: a Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/.http://www.rstudio.com/.

Rillig, M.C., 2012. Microplastic in terrestrial ecosystems and the soil? Environ. Sci. Technol. 46, 6453–6454. http://dx.doi.org/10.1021/es302011r.

Rillig, Matthias, Ziersch, Lisa, Hempel, Stefan, Microplastic transport in soil by earthworms. submitted.

Thompson, R.C., Swan, S.H., Moore, C.J., vom Saal, F.S., 2009. Plastics, the environment and human health: current consensus and future trends. Philos. Trans. R. Soc. B 364, 1973–1976. http://dx.doi.org/10.1098/rstb.2009.0053.

Wickham, H., 2009. ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag, New York.

Widenfalk, L.A., Bengtsson, J., Berggren, A., Zwiggelaar, K., Spijkman, E., Huyer-Brugman, F., Berg, M.P., 2015. Spatially structured environmental filtering of collembolan traits in late successional salt marsh vegetation. Oecologia 179, 537–549. http://dx.doi.org/10.1007/s00442-015-3345-z.

Wright, S.L., Thompson, R.C., Galloway, T.S., 2013. The physical impacts of microplastics on marine organisms: a review. Environ. Pollut. 178, 483–492. http://dx.doi.org/10.1016/j.envpol.2013.02.031.

Zubris, K.A.V., Richards, B.K., 2005. Synthetic fibers as indicator of land application of sludge. Environ. Pollut. 138, 201–211. http://dx.doi.org/10.1016/j.envpol.2005.04.013.

Zuur, A.F., Ieno, E.N., Walker, N.J., Saveliev, A.A., Smith, G., 2009. Mixed Effects Models and Extensions in Ecology with R. Springer, New York.