Environmental Pollution 220 (2017) 523–531

Contents lists available at ScienceDirect

Environmental Pollution

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

Incorporation of microplastics from litter into burrows of *Lumbricus terrestris*☆

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ARTICLE INFO

Article history:
Received 9 June 2016
Received in revised form
29 September 2016
Accepted 30 September 2016
Available online 8 October 2016

Keywords:
Microplastics
Lumbricus terrestris burrows
Risk assessment

ABSTRACT

Pollution caused by plastic debris is an urgent environmental problem. Here, we assessed the effects of microplastics in the soil surface litter on the formation and characterization of burrows built by the anecic earthworm *Lumbricus terrestris* in soil and quantified the amount of microplastics that was transported and deposited in *L. terrestris* burrows.

Worms were exposed to soil surface litter treatments containing microplastics (Low Density Polyethylene) for 2 weeks at concentrations of 0%, 7%, 28%, 45% and 60%. The latter representing environmentally realistic concentrations found in hot spot soil locations. There were significantly more burrows found when soil was exposed to the surface treatment composed of 7% microplastics than in all other treatments. The highest amount of organic matter in the walls of the burrows was observed after using the treatments containing 28 and 45% microplastics. The highest microplastic bioturbation efficiency ratio (total microplastics (mg) in burrow walls/initial total surface litter microplastics (mg)) was found using the concentration of 7% microplastics, where *L. terrestris* introduced 73.5% of the surface microplastics into the burrow walls. The highest burrow wall microplastic content per unit weight of soil (11.8 ± 4.8 g kg^{-1}) was found using a concentration of 60% microplastics. *L. terrestris* was responsible for size-selective downward transport when exposed to concentrations of 7, 28 and 45% microplastics in the surface litter, as the fraction $\leq$50 μm microplastics in burrow walls increased by 65% compared to this fraction in the original surface litter plastic. We conclude that the high biogenic incorporation rate of the small-fraction microplastics from surface litter into burrow walls causes a risk of leaching through preferential flow into groundwater bodies. Furthermore, this leaching may have implications for the subsequent availability of microplastics to terrestrial organisms or for the transport of plastic-associated organic contaminants in soil.

1. Introduction

Microplastics are defined as plastics that have a size lower than 5 mm (GESAMP, 2015) and originate from primary as well as from secondary sources such as plastic fragmentation at sea and on land (Barnes et al., 2009; Claessens et al., 2011). Microplastics have ecologically relevant effects on aquatic organisms. For instance, ingesting microplastics led to a decrease in weight and feeding activity in *Arenicola marina* (Besseling et al., 2013; Browne et al., 2013; Wright et al., 2013) with subsequent mortality (Besseling et al., 2013). The transfer of microplastics between food web components has been observed, such as those occurring between mesozooplankton copepods and pelagic macrozooplankton mysid

☆ This paper has been recommended for acceptance by Eddy Y. Zeng.

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http://dx.doi.org/10.1016/j.envpol.2016.09.096
0269-7491/

shrimps ie. *Mysis mixta*, *Mysis relicta* (Setälä et al., 2014), or between *Mytilus edulis* and *Carcinus maenas* (Farrell and Nelson, 2012), or between microalgae (ie. *Rhodomonas baltica, Tetraselmis chuii*) and estuarine fish (Oliveira et al., 2012; Ivar do Sul and Costa, 2014). Microplastics can enter terrestrial ecosystems via sewage sludge applications on agricultural land (Hohenblum et al., 2015; Zubris and Richards, 2005), aerial transport, degradation of plastic mulch on agricultural land in semiarid regions (GESAMP, 2015; Steinmetz et al., 2016), and mismanagement of plastic waste, which occurs in developing or transitional economies where plastic waste is often incorporated into the soil of home gardens (van der Wal et al., 2011) or agricultural land (Duis and Coors, 2016). Hot spots of (micro) plastics in soil are mainly found on roadsides (Hohenblum et al., 2015) as well as home gardens (van der Wal et al., 2011) and agricultural lands treated with plastic mulch (Briassoulis et al., 2010). In these hotspots, high amounts of (micro) plastics are found on the soil surface or in the topsoil. For example, plastic mulch used for treatment on Chinese agricultural land with 60–100% coverage is often not removed after harvesting and is left on the field, leading to plastic waste hotspots on the fields (Fig. 1). Another example is the incorporation of plastic waste into the topsoil of home gardens in Tabasco, Mexico, where we found plastic covering up to 40% of the soil surface (personal observation). After fragmentation and downward transport due to bioturbation, 0.2–1.2% of the plastic could be found in the soil (Huerta Lwanga et al., 2016). Furthermore, surface plastic litter concentrations are expected to increase even further in the near future because the production, emission and leakage of plastics into the environment is expected to increase by an order of magnitude (Jambeck et al., 2015).

Hardly any information about the effects of microplastics on soil organisms in terrestrial ecosystems is available (Rillig, 2012). A recent study demonstrated that an earthworm's fitness can be affected by microplastics present on the soil surface at high yet realistic concentrations (Huerta Lwanga et al., 2016) which may consequently impact the ecosystem processes in which they are involved.

As ecosystem engineers, earthworms participate in important ecosystem processes like organic matter decomposition and water infiltration (Lavelle et al., 2006). *Lumbricus terrestris* is an anecic earthworm, widely distributed across Europe and North America (Hendrix and Bohlen, 2002). *L. terrestris* ingests soil surface litter and then moves inside the soil, forming burrows. Through this bioturbation, the earthworm modifies the soil structure (Meysman et al., 2006). *L. terrestris* is known to produce long vertical burrows (Lee, 1985) through which water and pollutants are transported (Worrall et al., 1997). *L. terrestris* plays an important role in the incorporation of organic matter from the surface into the soil (Jégou et al., 2000, 2001) and modifies the soil porosity (Görres et al., 2001). *L. terrestris* burrows are richer in carbon, fungi, bacteria, NO_3–N, and enzymes than the non-ingested soil (Devliegher and Verstraete, 1997; Jégou et al., 2001; Tiunov and Scheu, 2000). When adverse compounds like pesticides are present in the soil, *L. terrestris* burrows can decrease in volume (Dittbrenner et al., 2011), affecting the ecosystem service infiltration. Stress factors such as microplastics are scarcely documented in the terrestrial ecosystem and the way and extent to which *L. terrestris* moves microplastics through burrows is not known. There were two objectives in the present study. Our first objective was to study the effect of different concentrations of Low Density Polyethylene (LDPE) microplastics in soil surface litter (0, 7, 28, 45, 60% w/w) on *L. terrestris* biomass, burrow formation, and burrow characteristics. Our second objective was to examine biogenic transport and the incorporation of the different concentrations of microplastics into the burrows. Furthermore, we assessed the differences that appeared between the particle sizes of the microplastics found in the litter compared to those deposited in the burrows. It is important to note that the doses of up to 60% microplastics on the soil surface correspond to realistic surface litter content. Concomitantly, the actual concentrations of microplastics in the soil after bioturbation are lower. The lower end of the selected range of doses represents realistic surface litter concentrations. The higher end represents concentrations already encountered presently at hot spot locations.

2. Material and methods

2.1. Set up and procedures

The mesocosm experiments used sandy soil (50% sand, 50% loamy silt, with 0.2% organic matter). Previous testing showed that *L. terrestris* would ingest the surface litter when the soil was low in nutrients (Huerta Lwanga et al., 2016). A mesocosm size of 40 × 30 × 3 cm (Fig. 2) was used which included five microplastic concentration treatments: 0 (C), 7 (T7), 28 (T28), 45 (T45) and 60 (T60) % w/w, where 'C' stands for control, and 'T' stands for treatment. There were 3 mesocosm systems per treatment and the experiment lasted for 14 days.

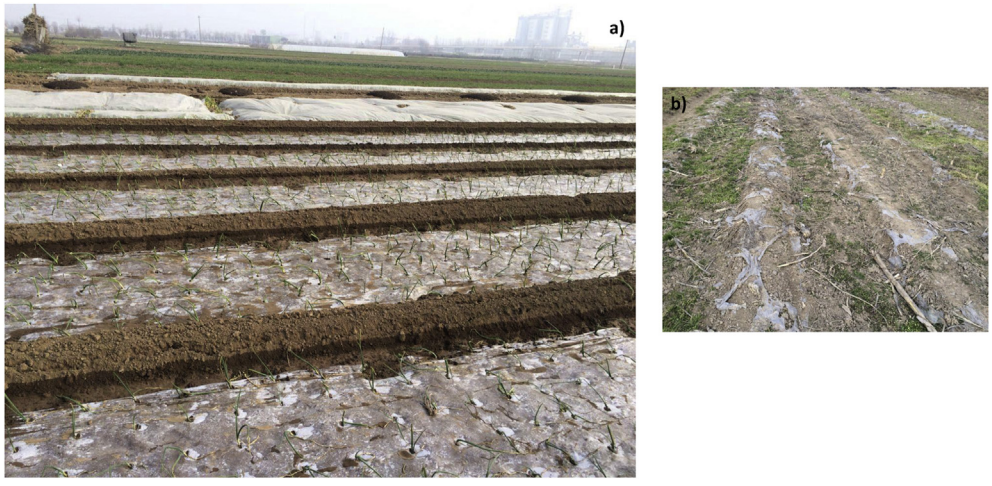

Fig. 1. Plastic mulch in China; a) covered agricultural land, b) fragmented plastic. After Huerta Lwanga et al. (2016).

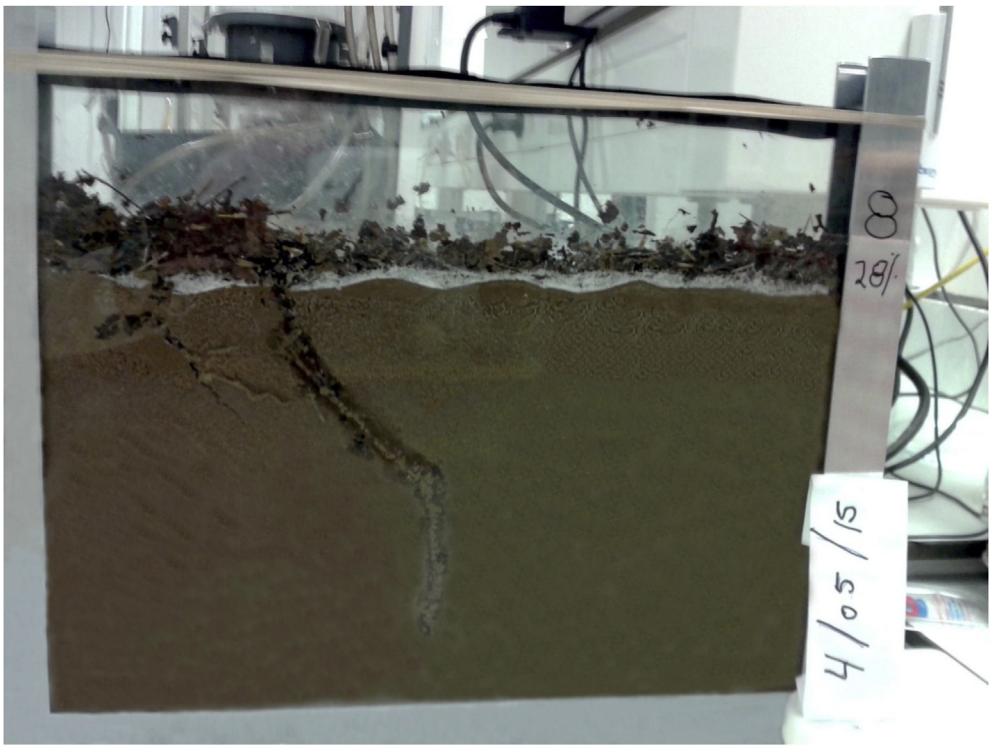

Fig. 2. Mesocosms used during the experiment.

Low Density Polyethylene (LDPE, Riblon, Ter Hell Plastic GMBH) was used as a representative microplastic since it is a very commonly produced polymer and happens to be the most commonly encountered polymer in the environment (Hohenblum et al., 2015). The plastic particles were ground up by ter Hell Plastic GMBH (Herne, Germany) to <400 μm, with the bulk of particles ranging between 200 and 300 μm. This microplastic powder was then sieved at IMARES (Institute for Marine Resources & Ecosystem Studies), The Netherlands to produce particles with a size distribution of ≤50 μm (40%) and 63–150 μm (60%). This size range was chosen based on the results of previous aquatic studies. These sizes represent the smaller microplastics that can be expected in soils. Such particles are formed due to embrittlement, photo-oxidation, abrasion and fragmentation under the influence of UV light and mechanical wear. The microplastics were washed with octane and pentane to extract any solvent-soluble plastic monomers. The microplastics were then rinsed with demineralized water to remove (any) remaining solvent. The microplastics were air dried at 60 °C and vacuum sealed.

The microplastics were mixed with *Populus nigra* dry litter to obtain the treatment concentrations mentioned above and then placed on the surface of the mesocosms. Four adults of *L. terrestris* were washed with demineralized water, dried with paper towels and weighted. After 2 days of starvation, they were placed in the mesocosms. The initial earthworm biomass was similar among individuals (20.3 ± 2.3 g). During exposure, conditions included a light: dark cycle of 8:16, a temperature of 16.5 ± 1 °C (average room temperature of 17.2 °C) and soil moisture content of 21%. The soil moisture was kept constant using a mobile soil-moisture sensor (TRIME PICO 64, IMKO). Due to the fact that earthworms are photo sensible animals and the mesocosms were made out of transparent glass, all mesocosms were covered with dark textile and kept in a cardboard box. To ensure equal test conditions for all systems, mesocosms were randomly distributed in the box and frequently rotated.

After 14 days of exposure, the complete mesocosms including earthworms, burrows, litter and plastic were quickly frozen, which secured the burrow structure. The freezing procedure consisted of placing all of the mesocosms inside a −17 °C freezer room. As far as we know, this is the first experiment applying this sudden freezing technique to preserve the state of the earthworms as well as the burrows. To assess the size and form of *L. terrestris* burrows, previous studies made a profile in the field (Pérès et al., 2010) or they used an X-ray-computed tomography scan procedure (Jégou et al., 2002). In this study, we froze *L. terrestris* burrows in order to collect the microplastics from undisturbed burrows. Frozen earthworms were carefully collected, washed, dried with paper towels and weighted. Their weight loss was expressed as a growth rate (k_{gr}) according to the following equation:

$$k_{gr} = \frac{\frac{M_{org,2} - M_{org,1}}{M_{org,1}}}{t} \quad (1)$$

where $M_{org,1}$ and $M_{org,2}$ are the initial and final body weights of *L. terrestris* (mg) respectively, and t is exposure time (days).

The number of iced burrows from each mesocosm was noted and the length and the diameter of the burrows (including tunnel and wall) were measured (Fig. 3a, b, c). The material (litter transported mechanically, casts and microplastics) forming the burrow walls (Fig. 3d) was carefully collected. With the use of a clean metal knife, each burrow was excised, after which each burrow, complete or in pieces, was placed individually in a metal container. Subsequently, they were dried at 40 °C, and sieved to obtain the size fractions >250, 250–150, 150–100, 100–63, 63–50, and <50 μm. The burrow formation was recorded as a function of time (3, 6, 8 and 14 days) by taking pictures and the burrows were counted. The number of burrows was normalized by the initial weight of the earthworms (burrow formation by mass) according to the

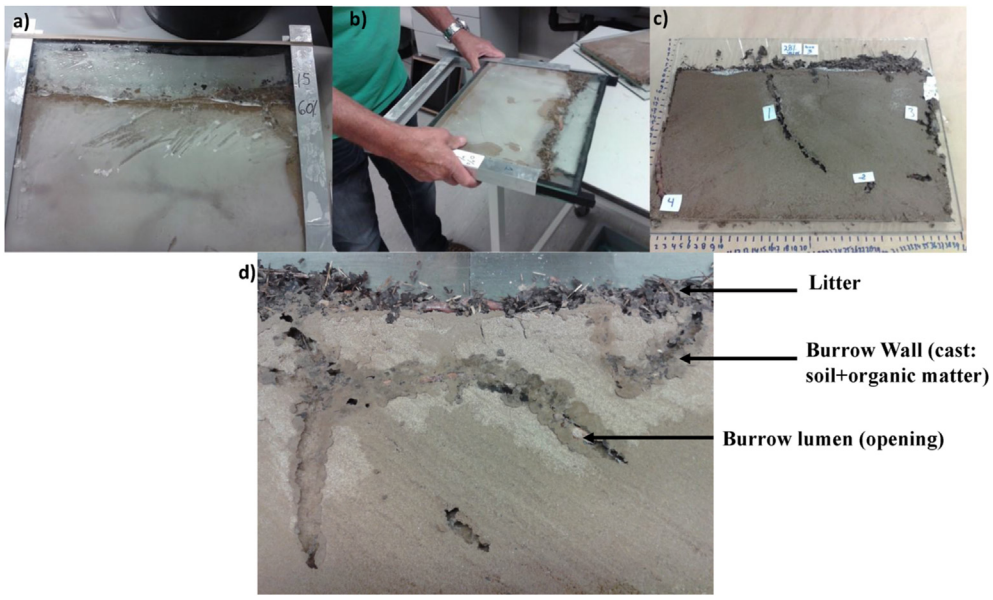

Fig. 3. Burrow measurement procedure (after 14 days of experimentation), a) frozen mesocosms, b) defreazing mesocosmos, c) counted and measured burrows, d) burrow walls.

equation:

$$BFW = \frac{NB}{W_{EW}} \quad (2)$$

in which BFW (no. burrows/g) is the number of burrows per earthworm's biomass, NB is the number of burrows per mesocosm, and W_{EW} (g) is the total initial earthworm biomass per mesocosm.

The burrow volume was calculated by using the length of the burrows and the diameter of the lumen (Fig. 3d), according to the followed equation:

$$Bv = l\pi r^2 \quad (3)$$

where *Bv* (cm^3) is the volume per burrow, *r* (cm) the radius per burrow and *l* (cm) the length of the burrow. Dried burrow wall weight was recorded using a digital scale (Denver Instrument, XL-410, 0.001 g readability).

The number of burrows and galleries was determined for each mesocosm. A gallery (G [cm^3]) was defined and calculated as the sum of the burrow volumes per mesocosm:

$$G = \sum_{1}^{n} Bv \text{ per mesocosms} \quad (4)$$

Microplastics were extracted from the burrow walls by flotation per size fraction as described in Huerta Lwanga et al. (2016). Subsequently, burrow wall organic matter content per fraction was determined by loss on ignition after 3 h at 550 °C. Microplastics were removed prior to the treatment at 550 °C and did not interfere with the measurement of the organic matter content. The microplastic incorporation rate per worm was determined by the amount of microplastics found in the burrows divided by the mass of the earthworms in the mesocosm (4 earthworms per mesocosm), following the equation:

$$MPi = \frac{W_{MP}}{W_{EW} N_{EW} t} \quad (5)$$

where *MPi* (mg microplastics g^{-1} worm d^{-1}) is the microplastics incorporation rate, W_{MP} is the amount of microplastics per gallery (mg), W_{EW} is the total earthworm biomass present in the mesocosms (g/mesocosm), N_{EW} is the number of earthworms per mesocosm, and t is the duration of the experiment (days).

The relative bioturbation efficiency ratio (*BE)* was determined according to the equation:

$$BE = \frac{W_{MP}}{W_{MPs}} \quad (6)$$

where W_{MPs} (mg) is the initial total amount of microplastics in the surface litter, and W_{MP} (previously described) is the amount of microplastics per gallery (mg).

Microplastic density (*MPd*, mg/cm^3) was determined by dividing W_{MP} by the volume of the gallery (V_G [cm^3]), according to the equation:

$$MPd = \frac{W_{MP}}{V_G} \quad (7)$$

Finally, the concentration of microplastics (g kg^{-1}) in the soil was calculated.

2.2. Statistical data analysis

The statistical significance of the differences among treatments, normality of the data and equality of variance were tested using KS and Levene's test and a one-way analysis of variance (ANOVA), followed by a post hoc (Tukey's or Duncan's) test using STATISTICA version 12. The Mann-Whitney *U* test was used when the data were not normally distributed. An effect was considered significant at $p \leq 0.05$. When looking at the variation within a burrow, the ANOVA analysis per treatment was performed at the burrow level.

3. Results

3.1. Effects of microplastics on earthworm biomass and burrow characteristics

3.1.1. Biomass of L. terrestris

In all treatments, *L. terrestris* showed weight loss. The earthworms lost the most weight when exposed to treatment T7

(10.12 ± 0.24 mg day^{-1}), followed by T60, 28, 45 and C (6.4 ± 4.7, 3.4 ± 0.4, 2.7 ± 1.7, 1.7 ± 1.6 mg day^{-1} respectively, ANOVA, $p < 0.05$).

3.1.2. Burrow formation and characteristics

On the 3rd day, the number of burrows in each treatment was equal, whereas after 6 days, the number of burrows in T7 and T60 was significantly higher than in C, T28 and T45 (Fig. 4) (ANOVA, $p < 0.05$). On day 8, there were no significant differences between the treatments and on day 14, T7 showed a significantly higher number of burrows than the other treatments (Fig. 4, ANOVA, $p < 0.05$). Burrow formation per earthworm biomass was not significantly different among the treatments (Table 1).

Burrow length and volume varied from 13.2 to 18.3 cm and 10.7 ± 6 cm^3 to 14 ± 7.4 cm^3 respectively, T60 > T28 > T7>C > T45, but no significant differences were observed among the treatments (Table 1). Burrow weight, however, was significantly higher for treatments T7 and T45 than for treatment C (ANOVA, $p < 0.05$), i.e. 69.7 ± 37.5 and 67.6 ± 35.2 g respectively; control 46.1 ± 25.2 g (Table 1). The same was observed with the burrow wall density (Table 1, Mann-Whitney *U* test $p \leq 0.05$).

In all microplastic treatments, the material forming the burrow walls was significantly enriched with particles with size fractions <50, 50–100, 100–150 μm, compared to the control burrows (Fig. 5a). Also, the amount of organic matter per fraction was higher in the burrow walls exposed to the microplastic treatments, compared to the burrow walls in the control (Fig. 4b, ANOVA, $p < 0.05$). T28 and T45 showed significantly higher organic matter in the burrow walls compared to T7 and T60 (Fig. 5b). Furthermore, the *P. nigra* litter content in all of the microplastic treatment burrows was significantly higher than in the control burrows (Table 1).

3.2. Microplastics in the burrow walls

The incorporation of microplastics per worm biomass was significantly higher for worms exposed to the treatments T28, T45 and T60 (1.34 ± 0.1, 1.95 ± 0.72, 3.82 ± 0.21 mg g^{-1} day^{-1}, respectively) than those exposed to T7 0.55 ± 0.13 mg g^{-1} day^{-1} (ANOVA, $p < 0.05$ Table 1). In T7, the microplastic density (*MPd*) and the concentration (g.kg-^{1}) of microplastics in the galleries were also significantly lower (7.4 ± 1.3 mg/cm^3 and 1.6 ± 0.7 g kg^{-1}, respectively: Table 1, Fig. 6a) than in T28, T45 and T60.

The vertical distribution of the microplastics in the gallery walls showed a peak in the center of the gallery, as 48% of the microplastics were found in the middle of the gallery for treatments T28 and T60 (Fig. 7).

The size of microplastics found in the litter were significantly higher than those found inside the galleries in treatments T7, T28, T45 and T60 (ANOVA, $p < 0.05$, Fig. 8). The fraction of microplastics ranging from 63 to 150 μm was significantly lower inside the gallery walls than in the litter, whereas the fraction of ≤50 μm was significantly higher inside the galleries. The latter size fraction (<50 μm) made up 65% of all the plastic inside the burrow walls (7, 28 and 45%, Fig. 8), whereas this fraction made up only 40% of the original microplastics in the surface litter (Fig. 8).

The relative bioturbation efficiency (*BE*) (microplastic inside the gallery (mg)/microplastics in the initial surface litter (mg)) was significantly higher in T7 (0.73 ± 0.15), compared to T28, T45 and

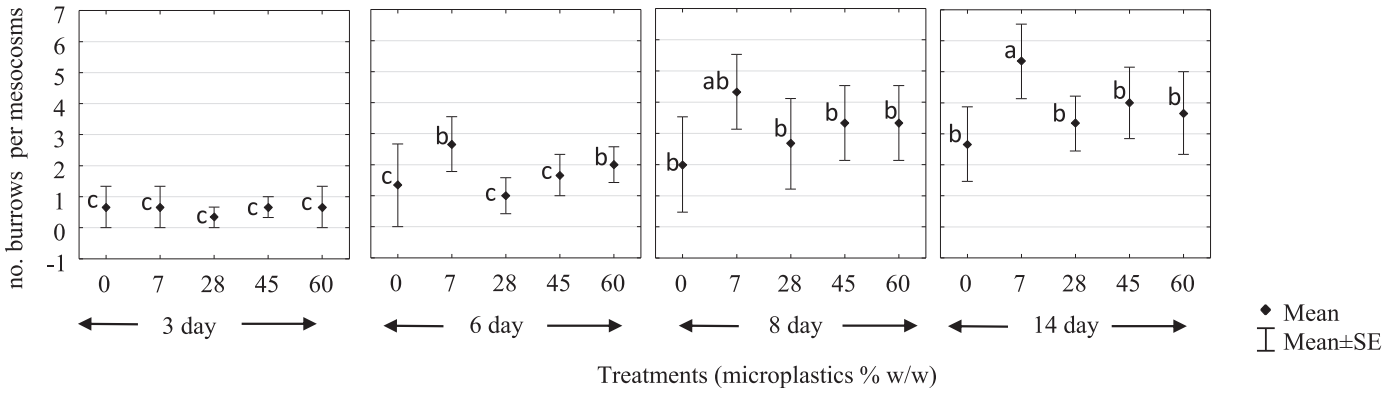

Fig. 4. Burrows formation in 14 days, under the exposure of surface microplastics. Different letters indicate significant differences amongst treatments. a>b > c (ANOVA, $p \leq 0.05$).

Table 1
Characteristics of microplastics (MP) in *L. terrestris* mesocosms.

Microplastics (MP) added to litter	Treatments (microplastics)				
MP weight (mg)	0	910	3640	5850	7800
Resulting % w/w litter	0	7	28	45	60
earthworm weight loss (mg. worm.day-^{1})	−1.7 ± 1.6 **b**	−10.12 ± 0.24 **a**	−3.4 ± 0.4 **ab**	−2.7 ± 1.7**b**	−6.4 ± 4.7 **ab**
Burrow formation per earthworms mass (no. burrows/g)	0.25 ± 0.08	0.28 ± 0.08	0.24 ± 0.03	0.31 ± 0.02	0.29 ± 0.07
burrow length (cm)	14.9 ± 8.4	15.5 ± 8	17.5 ± 7.3	13.2 ± 5.8	18.3 ± 8.4
burrow volume (cm^3)	10.7 ± 6	11.7 ± 2.5	14.1 ± 6.6	9.4 ± 6.4	14 ± 7.4
burrow walls weight (g)	46.1 ± 25.2**b**	67.6 ± 35.2**a**	64.3 ± 30.8 **ab**	69.7 ± 37.5**a**	63.3 ± 33.8 **ab**
burrow walls density (g. cm^3)	4.6 ± 2.9 **b**	7.9 ± 7.1 **a**	5.1 ± 2 **b**	16 ± 20 **a**	5.8 ± 3.1 **b**
gallery volume (cm^3)	53.7 ± 25.9	70.3 ± 12.8	75.5 ± 33.4	56.6 ± 16.5	84.1 ± 21.1
MP density (*MPd*) per gallery (mg. cm^3)	–	9.6 ± 1.8 **c**	22.7 ± 5.8 **b**	43.5 ± 34.8 **ab**	55.1 ± 14.11**a**
litter inside the burrows (g)	1 ± 0.53 **b**	1.6 ± 0.7 **a**	1.6 ± 0.7 **a**	1.7 ± 0.9 **a**	1.7 ± 0.7 **a**
total MP inside the gallery walls (mg)	–	669.1 ± 143.7 **c**	1603.5 ± 350.2 **bc**	2093.7 ± 958.7 **b**	4439.9 ± 421.4 **a**
% of MP from total found in the gallery walls	–	73.5 ± 15.7 **a**	37.1 ± 2.6 **b**	35.7 ± 16.3 **b**	56.9 ± 5.4 **a**
MP incorporation rate (*MPi*) by earthworm (mg MP g worm-1 day-1)	–	0.55 ± 0.13 **c**	1.34 ± 0.11 **b**	1.95 ± 0.72 **b**	3.82 ± 0.21 **a**

Different letters indicate significant differences amongst treatments. a>b>c (ANOVA, p<0.05).

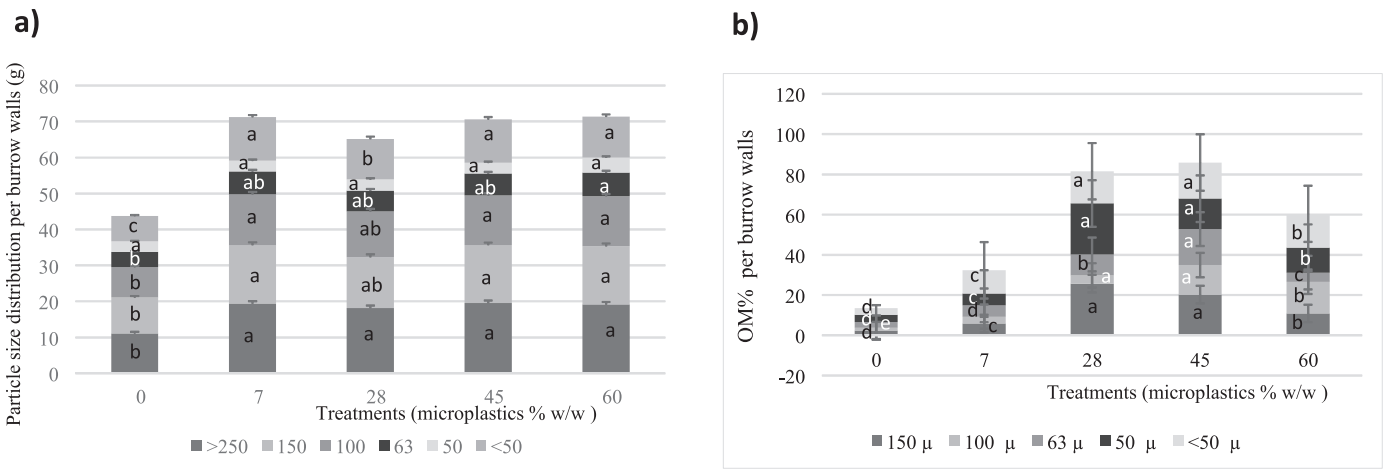

Fig. 5. Burrow wall characteristics, a) particle size distribution, b) organic matter, per fraction per burrow. Different letters indicate significant differences among treatments (ANOVA, $p \leq 0.05$).

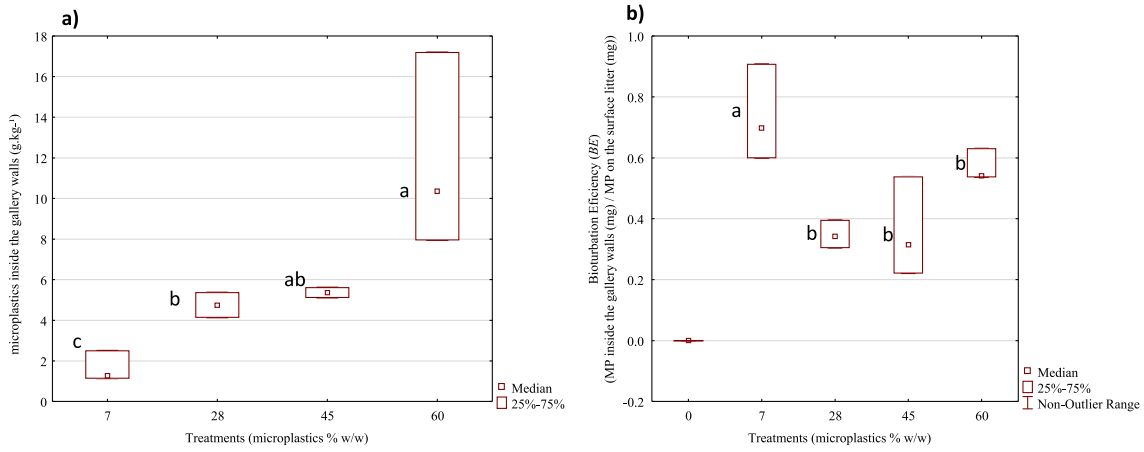

Fig. 6. a) Microplastic (MP) concentration (g.kg^{-1}) inside the *L. terrestris* gallery walls, b). Bioturbation Efficiency (*BE*, Microplastics (MP) inside the *L. terrestris* gallery walls)/ Microplastics (MP) in the surface litter). Different letters indicate significant differences among treatments (a) Mann-Whitney *U* test, $p \leq 0.05$, b) ANOVA, $p \leq 0.05$.

T60 (range 0.34 ± 0.04–0.56 ± 0.05, Fig. 6b).

4. Discussion

Whilst the impact of microplastics on aquatic ecosystems has gathered increasing attention over the years, so far only our study (Huerta Lwanga et al., 2016) has shown that earthworms are negatively impacted by the presence of microplastics due to their decreased growth rates and increased mortality. The present paper provides the first experimental evidence that earthworms enhance the transport of microplastics from the surface down into deeper soil layers and may increase preferential leaching to deeper soil through burrow formation. The present study, testing a range of microplastic concentrations in litter, shows several distinct impacts of low-concentration microplastics in *L. terrestris* burrow walls. Of the four surface litter microplastic concentrations used in these experiments, the T7 treatment had the highest worm weight loss, a significantly greater number of burrows at day 14, and a significantly higher microplastic mass inside the gallery relative to the surface litter. The T7 treatment also had a higher burrow weight and density compared to the control. Across all of the microplastics treatments, there was a 25% increase in the presence of <50 µm particles inside galleries relative to surface litter. In the sections that follow, we present the findings in the context of the literature and expand on the implications of our findings with regards to the earthworms and their habitat.

4.1. Effects of microplastics on earthworm biomass

Earthworms lost weight in all of the treatments, including the control treatment. However, *L. terrestris* lost significantly more weight when exposed to treatment T7 than in all other treatments. We hypothesized that in T7, earthworms probably used more energy for larger burrow formation, and therefore lost weight while burrowing during the first days of the experiment. In our previous study (Huerta Lwanga et al., 2016), we observed that the concentrations of >28% caused the highest weight loss in *L. terrestris*. In the present experiment, this was not the case. No dose-dependent relationship was observed. We can explain this by looking at the differences in the methods that we used at the end of the experiment. In order to collect the burrows, we froze the experimental systems from all of the treatments. This means that the earthworm weight was measured after death, whereas in the previous experiments, the weight of the worms was measured while they were still alive. We assume that freezing has caused extra stress on the worms, which is likely to enhance the secretion of coelomic fluids

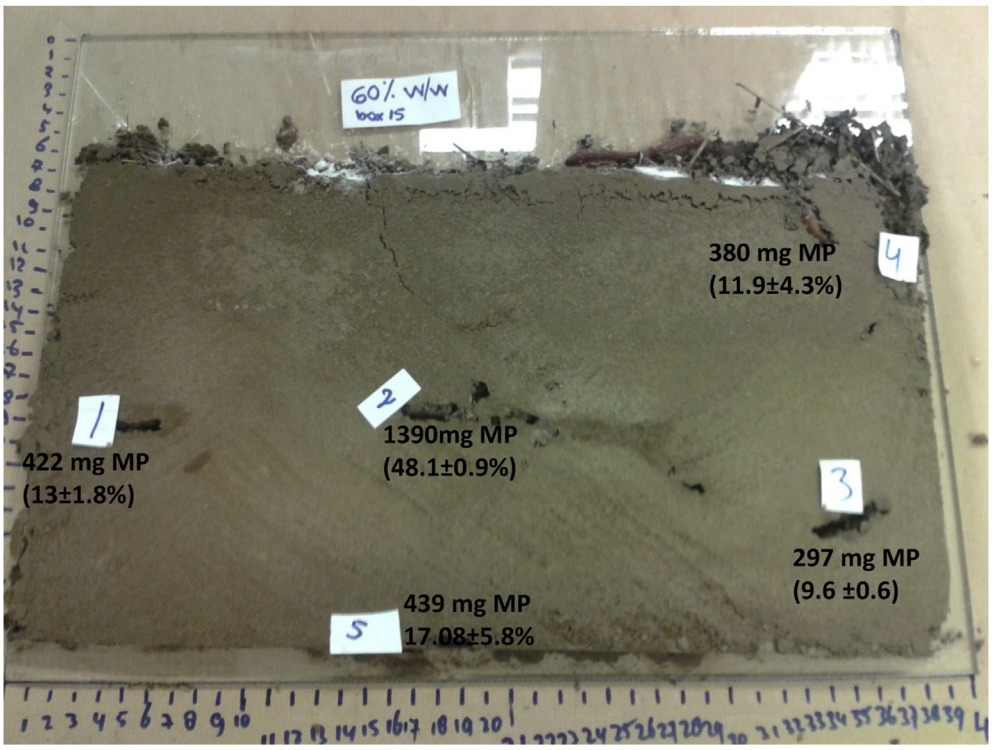

Fig. 7. Microplastic (MP) vertical distribution along the gallery walls (mg). 60% w/w microplastics on the surface, media of the percentage is done between 60% and 28% w/w, box 15 and 8).

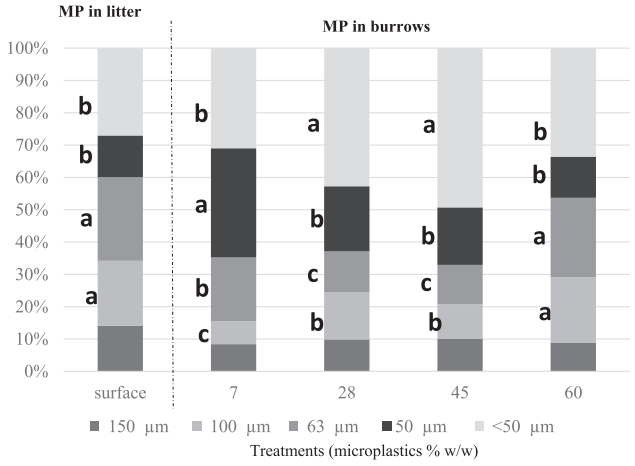

Fig. 8. Microplastic (MP) size distribution among the treatments, different letters indicate significant differences a>b > c (Maan-Whitney *U* Test, $p \leq 0.05$).

from their bodies (Vail, 1972). This, combined with exposure to the treatments, resulted in the observed weight loss.

4.2. Effect of microplastics on burrow characteristics

At the beginning of the present study, all earthworms weighed about the same and the formation of burrows seemed like it was not affected by the earthworm mass in this study.

Burrowing earthworms bioturbate the soil according to the soil conditions and the earthworms evolutionary characteristics. If a perturbation is present, earthworms will react/adapt to the stress factor or they perish (Meysman et al., 2006). *L. terrestris* burrows can reach a length of 70 cm (Nuutinen and Butt, 2003) but when an adverse factor is present, pesticides for example, the volume of the burrow tends to decrease (Dittbrenner et al., 2011; Pelosi et al., 2014). Burrow walls are normally compacted and denser than the soil matrix, due to organic residues present in the casts (Babel and Kretzsmar, 1994). However, in the present study, the *L. terrestris* burrow walls were significantly heavier and more dense at T7 and T45 than in the control, although the volume was not significantly affected. More studies are required in order to better understand what causes *L. terrestris* to create heavier and denser burrow walls in the presence of surface microplastics. The heavier and denser burrow walls had a higher concentration of organic matter (in litter and casts). This causes high concentrations of organic matter locally, where microbial organisms can be more active than elsewhere in the soil. A change in the burrow structure and an increase in the organic matter content along the burrow walls can have implications such as a higher affinity of contaminants for sorption, as has been shown by Edwards et al. (1992).

L. terrestris burrows under surface microplastics are richer in organic matter, which may cause a higher gas exchange than burrows under natural conditions. Microplastics as pollutants have a negative effect on the soil environment (ie. 8–25% mortality of *L. terrestris* at ≥28% microplastics in 60 days of exposure, Huerta Lwanga et al. (2016), therefore it is important to avoid them on and in the soil. Yet, there is a trade-off between positive and negative effects. For example in degraded soils, a restorative measure may use compost with a low amount of microplastics on top of the soil thus enhancing the integration of organic matter and microplastics into the soil. However, such a measure may come at a possibly high environmental cost. Microplastics will be available to other soil organisms, possibly accumulate in the soil trophic chain and burrows being preferential pathways for vertical water movement and will increase groundwater pollution risks. Therefore, further studies are needed to assess the use of microplastics in soil organic matter restoration projects in order to prevent

pollution of the soil and water resources.

In the present study, burrow formation significantly increased with treatment T7 between days 6 and 14 compared to all of the other treatments. We observed that a surface consisting of 7% microplastics actually enhanced the activity of *L. terrestris*. This contrasts with the findings of Besseling et al. (2013) which showed that marine worms (*Arenicola marina*) delayed burrow formation after microplastics exposure. However, we would like to emphasise that the *Arenicola marina* and the *Lumbricus terrestris* have different physiological responses to environmental factors (i.e. different response to cations exposure, Ochiai and Weber, 2002). *L. terrestris* tolerates microplastics better due to its symbiotic relationship with soil microflora (Jolly et al., 1993), a characteristic that is not present in *Arenicola marina* due to its gut bacteriolytic property (Plante and Mayer, 1994). *A. marina* reduced its weight and activity in 28 days when exposed to 7.4% v/v microplastics (Besseling et al., 2013), while *L. terrestris* increased its activity in 14 days under 7% w/w microplastic but reduced its weight significantly when exposed to under >28% w/w microplastics for 60 days (Huerta Lwanga et al., 2016).

Under natural circumstances, the number of burrows depends on the quality of food available on the soil surface as mentioned above. *L. terrestris* forms more burrows when organic matter residues are limited or of a poor quality (Pérès et al., 2010). Apparently, this phenomenon occurs for treatment T7 but not for the treatments with higher microplastic concentrations in the litter. More research is needed in order to understand this behaviour.

4.3. Biogenic transport of microplastics

The transport of microplastics by *L. terrestris* occurs in two ways: the microplastics are pushed together with the litter inside the burrows and they are bioconcentrated and expelled in the casts that are forming the burrow walls.

4.4. Microplastics in the burrow walls

We observed the highest bioturbation efficiency (*BE*) in treatment T7. In T7, the microplastics were concentrated in the burrow walls just like they are in their casts. This is in line with the findings from our previous study on the effects of microplastics on earthworms (Huerta Lwanga et al., 2016). The bioturbation efficiency refers to the ratio of the amount of microplastics (mg) present on the soil surface and the amount of microplastics (mg) present inside the burrows. Bioturbation by L. *terrestris* in the soil was significantly higher with the treatment consisting of 7% surface microplastics compared to those with 28, 45 and 60% microplastics on the surface. In T7, there was a higher proportion of the surface microplastics introduced into the burrows by bioconcentration in the cast. According to Huerta Lwanga et al. (2016) this can be explained by the fact that at this dose, the percentage of organic matter in the ingested litter was highest at 93%, leading to a large increase in the concentration of the microplastics in the cast due to partial digestion of this organic matter. More studies are required in order to better understand the physiology of this mechanism.

4.5. Microplastic size and transport

Under the influence of microplastics, *L. terrestris* appeared to select microplastics in a particle size-dependent manner. After all, the concentration of the fraction $\leq$50 μm increased from the initial 40% at the surface to 65% inside the burrow walls. This is relevant because the smaller plastic particles remain available for re-uptake by terrestrial organisms and at the same time may be more mobile, bioavailable and efficient in taking up toxic chemicals and transferring them to the food web, as also occurs in aquatic systems (Ivar do Sul and Costa, 2014). According to Judas (1992), *Lumbricus terrestris* mainly bioturbates particles of 0.5–1 mm however the main particle size inside its gut are smaller than 0.25 mm. Mariani et al. (2001) have shown that a large number of ingested particles are found to measure between 0.008 and 0.057 mm in *Martiodrilus carimaguensis*, an anecic tropical earthworm. This agrees with our finding that smaller particles are concentrated in the gut.

4.6. Implications for environmental risk

The presence of microplastics on the surface of the soil affected the burrowing activity of *L. terrestris* which lead to burrows that were more enriched in organic matter and microplastics compared to the control burrows without microplastics. Under the presence of litter microplastics, *L. terrestris* not only transported more organic matter into the burrows but also more microplastics. This resulted in highly dense burrow walls which can be spots in the below-ground microtopography that absorb more pollutants than the bulk of the soil because both organic matter and microplastics strongly absorb other contaminants (Koelmans et al., 2016; Endo and Koelmans, 2016). In these areas of high concentrations of organic matter and/or microplastics, microplastics may be more readily available to plants and other organisms. Microplastics also affect local soil porosity as *L. terrestris* built more dense burrow walls when exposed to microplastic treatments. Preferential flow through the burrow lumen is a common way of transport for metalloids (Sizmur et al., 2011) and pesticides (Pelosi et al., 2014), resulting in them leaching into deeper soil layers or into the groundwater (Tomlin et al., 1993). In the burrow walls, microplastics are more abundant than in the bulk soil such that the risk for leaching is higher. Once microplastics are present in the soil pore water or in the groundwater, they may be transferred to other organisms including plants. We are not aware of any studies addressing the risks of microplastics due to the uptake by plants from the soil or uptake in terrestrial food webs. However, given the present findings, such studies are highly recommended.

5. Conclusions

L. terrestris moves microplastics from the soil surface into their burrows in a size-selective way. The concentration of organic matter within the burrows was higher given higher concentrations of microplastics which may in fact be directly related to the worm's response to stress. The uptake of microplastics by worms and the resulting biogenic transport into the soil may lead to the pollution of groundwater and consequent uptake by terrestrial plants as well as terrestrial food webs. We argue that our understanding of the implications of microplastics in terrestrial food webs is too limited, which urges for specific research in this area.

Acknowledgments

We would like to acknowledge Michiel Kotterman and thank him for providing the microplastics for this study and for his advice. We would like to thank the WIMEK Institute of Wageningen University for providing a fellowship for E.H. as a visiting scientist in 2015.

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