Leaching of microplastics by preferential flow in earthworm (Lumbricus terrestris) burrows

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Title: Leaching of microplastics by preferential flow in earthworm (*Lumbricus terrestris*) burrows

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Environmental context

Recent research results show that microplastics on the soil surface can be ingested by earthworms (*Lumbricus terrestris*) and transported into soil. There are several potential pollution risks posed by MP found in soil. One example being the fact that MPs may enter into the food chain and groundwater systems, especially in cases where the water table is shallow or there is preferential flow.

Abstract

In the current study, we examined how the activities of earthworms (*Lumbricus terrestris*) affect MP distribution and concentration in soil, focusing on Low Density Polyethylene (LDPE). We also wanted to see if MP could be flushed out with water. We used a laboratory sandy soil column (PVC) experimental set-up and tested five different treatments: (1) treatment with just soil (control) and check if saturated conductivity Ks could be impacted by MP, (2) treatment with MP, (3) treatment with MP and litter, (4) treatment with earthworms and litter as a second control for treatment five, (5) treatment with MP, earthworms and litter. Each treatment consisted of eight replicates. For treatments with MP, the concentration added at the start of the experiment was 7% weight percent (3.97 g, Polyethylene, 50% 1 mm-250 μm, 30% 250 μm-150 μm and 20% <150 μm) based on 52.78g of dry litter from *Populusnigra* (52.78 g). In treatments using earthworms, two adult earthworms, with an initial average weight of (7.14±0.26) g, were placed in each column. Results showed that the LDPE particles could be introduced into the soil by earthworms. MP particles could be detected in each soil sample and in different soil layers in the earthworm treatments. Earthworms showed a tendency of transporting smaller MP particles and that MP size class <250 μm weight percentage increased in soil samples with increasing soil depth. After leaching, MPs were only detected in leachate from the treatments with earthworms and the MP had similar size distributions as the soil samples in 40-50 cm layer of treatment with MP, earthworms and litter. The results of this study clearly show that biogenic activities can mobilize MP transport from the surface into the soil and even be leached into drainage. It is highly likely that biogenic activities constitute a potential pathway for MPs to be transported into soil and groundwater.

Keywords: Microplastics, earthworms, soil column, litter, floating method, leaching, ground water

1. Introduction

Plastics are manufactured and consumed ubiquitously in many areas as it is a cheap, lightweight, waterproof and stable material (Blasing and Amelung, 2018; Crawford and Quinn, 2017a). As a consequence, plastic wastes, and particularly microplastics (Thompson et al.), have caused environmental problems, both in water and terrestrial systems (Duis and Coors, 2016; Hurley and Nizzetto, 2018; Van Cauwenberghe et al., 2015; Wang et al., 2016).

MPs as a plastic pollutant in aquatic and terrestrial environments are currently a very hot topic but the potential risk is still unclear for terrestrial environments (Huerta Lwanga et al., 2017b; Ivar do Sul and Costa, 2014; Rillig, 2012). For the purpose of this article, we consider microplastics (Thompson et al., 2009) to be plastic particles having a size between 1 μm and 5 mm. Sources of MPs can be mainly divided into primary and secondary sources (Lots et al., 2017). Primary sources of MPs are purposely produced and used directly in domestic and industrial products such as artificial fibre, detergent, personal care products and cleaning agents (Andrady, 2011; Salvador Cesa et al., 2017). Secondary sources of MPs mainly originate from photo degradation and mechanical abrasion of larger plastic pieces (Gewert et al., 2015; Siegfried et al., 2017; Van Cauwenberghe et al., 2015). Primary MPs can be detected in rivers, sewage sludge, and waste water irrigated systems (Nizzetto et al., 2016a). Secondary MPs can be found in landfills, mulch film covered farmland, marine surface water, sediment, seabed sand shorelines (Horton et al., 2017). Both primary and secondary MPs can enter rivers and lakes through sewer overflows, especially in urban areas (Besseling et al., 2017; Crawford and Quinn, 2017b). MPs ending up in river systems connected to oceans will be eventually transported into marine ecosystems (Andrady, 2017; Chubarenko et al., 2016; Crawford and Quinn, 2017c; Duis and Coors, 2016; Horton and Dixon, 2018). Over 180 marine species were found to have ingested MP debris. Ingested MPs might bio accumulate in predators and eventually end up in the top of food chain (Wang et al., 2016).

A significant body of research on MP in aqueous systems documents MP sourcing, retention and reactions in rivers, lakes, and sediments as well as the mechanism of MP particle transport within the marine food chain (Besseling et al., 2017; Nizzetto et al., 2016a; Siegfried et al., 2017). Research on MP pollution in terrestrial ecosystems has gained momentum as well. It is estimated that land is storing almost 21% to 42% of the world's plastic waste (Nizzetto et al., 2016b) which has contributed to secondary MP pollution. Moreover, chemical additives are widely used in plastic manufacturing to adjust the characteristics of individual plastics in order to meet different needs. These additives include substances like bisphenol A, phthalates and metals all of which have been identified as either toxic or endocrine disruptors (Horton et al., 2017). These added chemicals can easily leach from the plastic compounds over time when exposed to high temperatures or simply normal degradation processes. (Andrady, 2011). Taking into consideration the combined physical (e.g. thermoplastic, absorb) and chemical (e.g. additives) properties of plastic (Yang et al., 2011), not only do the degrading plastics release chemicals into the soil but the degraded plastic and its by-products also aggregate with soil particles. These products in turn affect soil physical properties (Horton and Dixon, 2018) where MP accumulate (Hurley and Nizzetto, 2018). For instance, plastic mulch films are widely used in agriculture worldwide. The use of these films helps to reduce surface evaporation, increase soil water content and improve land production per unit water consumption. Most of these films are thin (less than 0.008 mm) and light (0.90 kg/m^3, LDPE, low density Polyethylene) thus making it difficult to recycle and reuse the plastic in an efficient way, especially considering the small pieces involved (Liu et al., 2014). The residues of mulch film (LDPE) significantly affect soil physical properties: initial gravimetric water content, bulk density, total porosity and saturated hydraulic conductivity in the top soil layer (0-20 cm) and also influence water distribution in the maize root zone (Jiang et al., 2017). These kinds of film residues in soil can degrade and contribute to secondary MP sources (Horton et al., 2017). Another direct source of MP may come from sewage sludge when it is applied as a fertilizer in agriculture (Carr et al., 2016; Mahon et al., 2017). Once MPs are present in the soil, they may be transported by runoff or wind (Horton and Dixon, 2018). This could cause the MPs to wind up in rivers but most MP likely remain on site (Wang et al., 2018). A recent study showed that MPs incorporated into litter on the soil surface could be ingested by earthworms (*Lumbricus terrestris)*and reduce their growth (Huerta Lwanga et al., 2016, 2017a). Furthermore, MP ingestion increased earthworm mortality at MP concentrations of 28%, 45%, and 60 % w/w with litter compared to 7% w/w and the control (Huerta Lwanga et al., 2016). At the same time, as the earthworm burrows system of *Lumbricus terrestris* can be deeper than 30 cm (Capowiez et al., 2014), burrows may serve as a potential pathway for solute and contaminant to be leached into deeper soil layer directly with preferential flow (Sander and Gerke, 2009; Zhang et al., 2016). Although soil was thought to be a good filter for groundwater (Keesstra et al., 2012), preferential flow in macropores can transport contaminants into groundwater within a shorter time and higher concentration, especially where groundwater is shallow (Bogner et al., 2012; Jarvis et al., 2016). Thus, the risk of MPs leaching into groundwater through burrows with preferential flow is thought to be high (de Souza Machado et al., 2018; Huerta Lwanga et al., 2016, 2017a). Rillig (2017) pointed out that earthworms can transport MP (Polyethylene) debris from the soil surface into the bottom layer, between 7-10.5 cm, in a plant pot, while most of smaller particles (710-850 μm) had been introduced into the deepest layer (10.5 cm). Meanwhile, MP remained on the soil surface when no earthworm activity was present. Lwanga (2017a) indicated that the earthworm (*Lumbricus terrestris*) exhibited particle size selection when transporting MP when MP concentrations were 7%, 25% and 45% w/w litter on the surface. MPs smaller than 50 μm were found to be up to 65% more abundant in soil as compared to the soil surface. These studies clearly showed that biogenic activities could be potential pathways for MP transport through the soil towards groundwater, especially for MPs smaller than 50 μm and Nano-sized material. MP accumulation, distribution and transportation through the soil, and especially MP transportation into the soil under preferential flow has so far not been studied.

Preferential flow in most types of soil is a common physical phenomenon where water or solute often infiltrates the soil matrix through cracks, fissures and biopores such as earthworm burrows and root channels (Li et al., 2018). Preferential flow also occurs in non-macroscopically uniform soils when water infiltration fronts are not stable anymore, resulting in so-called fingered flow. Preferential flow is a combination of high speed flow and large volumes of water. This phenomenon is considered an important factor affecting soil hydrological properties, soil water cycling, environmental pollution, and agricultural soil management (Guo and Lin, 2018; Li et al., 2018; Nimmo, 2012). Water or solute can be transported from the surface to groundwater more rapidly than expected based on soil texture and related soil hydraulic properties (Bogner et al., 2012; Jarvis et al., 2016; Zhang et al., 2016). Highly absorbing pollutants like pesticides or nutrients like phosphorus maybe transported by preferential flow without penetrating the soil matrix, presenting a hazard to surface and ground water quality. In order to investigate preferential flow paths and make them visible dye tracers are used to stain the channels (Allaire et al., 2009; Sander and Gerke, 2009; Wang and Zhang, 2011). A breakthrough curve experiment is another method which is widely used to examine water flow, especially in laboratory soil column experiments (Guo and Lin, 2018; Jarvis et al., 2012). With technical innovation progressing, the geophysical method and the soil moisture network provide relative more accurate ways to study the mechanisms and dynamics of preferential flow in the field than before(Triantafilis et al., 2013).

This study is based on results reported by Huerta Lwanga (2017a) and Rillig (2017) and focuses on the possibility of MP leaching through the soil with or without the influence of biogenic activities (such as earthworm movement) under laboratory soil column set-up. The aim of this study was to investigate whether biogenic activities increase MP particle distribution and concentration in soil and leachate.

2. Materials and Methods

2.1 Experiment set-up

A laboratory soil column experiment was designed to investigate whether MP could be transported into and leached out of soil with or without the influence of earthworm activity. A low density microplastic (Polyethylene, Riblon, Ter Hell Plastic GMBH) was applied in this experiment based on recent results (Huerta Lwanga et al., 2017a; Rillig et al., 2017) at a concentration of 7% (3.97 g) w/w with *Populusnigra* dry litter (52.78 g). Particle distribution of MP was 50% 1 mm-250 µm, 30% 250 µm-150 µm and 20% <150 µm. The MP was washed in demineralized water and then dried in the oven at 60°C to remove any toxic solvent (Huerta Lwanga et al., 2017).

Sandy soil (94.40% sand, 3.20% silt and 2.40% clay) with average organic matter content of 3.37% was used since this material had a high saturated hydraulic conductivity of the soil matrix compared to other soil types. The soil columns were made of clean PVC tubes. Four rings (10 cm in height and 12 cm in diameter) and one taller ring (20 cm in height and 12 cm in diameter) were placed on top of each other, with the taller ring on the top of the column (Fig. 1). Duct tape was then used to keep the separated rings together and to avoid water leaking out the sides of the column. At the bottom of each column, a perforated metal plate covered by a mesh cloth was used to keep soil from flowing out of the column, while facilitating water and possible MP transport through the mesh.

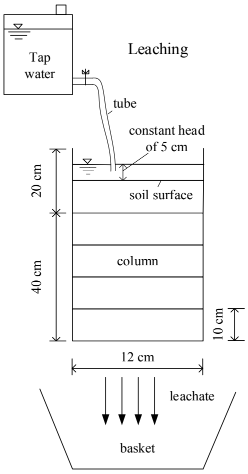


Fig. 1 Soil column set up

Fig. 2 Picture of experiment equipment. Eight soil columns with Mariotte bottles on top of the shelf to supply tap water.

2.1.1 Incubation exposure

Four treatments of eight replicates were carried out in this laboratory experiment (Table 1). Each column was filled with 7.00 kg of air-dried sandy soil mixed with 1.00 litre of tap water. All of the columns were flushed with 5 litres of tap water to saturate and compact the soil. The height of soil column was 50 cm after compaction by tap water. 52.78 g of litter mixed with 3.97g of MP was placed on the top of each column. Two adults earthworms (*Lumbricus terrestris*) were added to each column for treatment EW(earthworm)-L(litter) and MP-EW-L after being washed in demi water, dried with paper towel and scaled. The weight of the two adults earthworms added to each column was (7.14±0.26) g. 25 ml of tap water was sprayed on top of litter and MP mixture, which was placed on the surface of soil column. The columns were covered with PVC lids which had ten holes (2 mm in diameter) on top to prevent earthworms escaping from soil columns. All of the columns in treatments MP-b, MP-L, EW-L and MP-EW-L were kept in the laboratory for 14 days with a controlled temperature of (16±1)°C and a humidity of (40±5)% (Huerta Lwanga et al., 2017). Treatment MP-a (no incubation) was started with a direct exposure to leaching. MP-b (similar incubation as all other treatments) and MP-L were designed as controls to help find out which factors in this study affected MP distribution and leaching properties the most.

Table 1 The various treatments of the experiment, each experiment contained 8 replicated soil columns.

	Control	MP-a	MP-b	MP-L	EW-L*	MP-EW-L
Air dry sandy soil (7.00 kg)	✓	✓	✓	✓	✓	✓
Tap water (1.00 litre)	✓	✓	✓	✓	✓	✓
Microplastics (3.97 g)	×	✓	✓	✓	×	✓
Dried litter (52.98 g)	×	×	×	✓	✓	✓
Earthworms (2 adults)	×	×	×	×	✓	✓

*EW-L served as control for MP-EW-L.

2.1.2 Leaching exposure

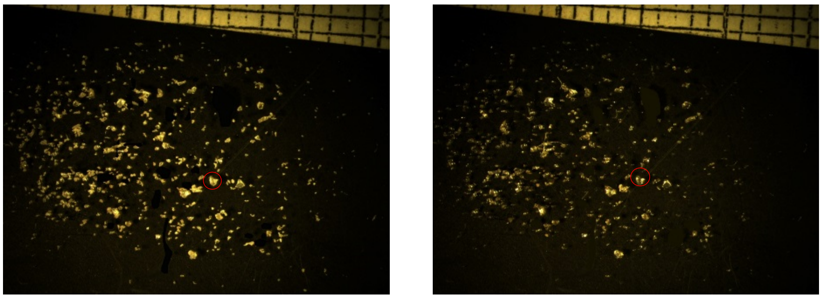
100 ml of Potassium Bromide (KBr) solution with a concentration of 0.0167 M was added to each column as a tracer before leaching was started to ensure the complete replacement of all of the water present in the soil column and to study the breakthrough curve of each soil column. Eight 10 litre Mariotte bottles were placed on a wooden shelf above the columns to supply tap water (Fig .2). Leaching was carried out under steady state conditions with a water layer of 5 cm at the top and free drainage at the bottom. In treatment MP-a, leaching was started directly with no incubating exposure in order to investigate whether MPs bypassed the soil matrix without burrows and litter. In treatments MP-b, MP-L, EW-L and MP-EW-L, the leaching experiments began after 14 days of incubation. During the whole leaching procedure, electrical conductivity, measured with an EC meter, and the volume of drain water were monitored. When electrical conductivity value dropped to the starting value or even smaller than that one, all of the water within the column had been totally replaced by new tap water and the supply of tap water was stopped at that moment. When leaching was finished, all drain water was directly filtered and the filter papers were subsequently dried in an oven at 40 °C for 24 hours.

2.1.3 Collecting soil samples

After finishing the leaching parts of the experiments, the duct tape was removed and all columns were cut horizontally following the 10 cm rings to collect soil samples. All of the collected 10 cm soil samples and earthworm burrow samples were then cut vertically into five slices. In the treatments EW-L and MP-EW-L, earthworm burrows were separated carefully from the opened soil column layer by layer (horizontally), slice by slice (vertically). Pictures were taken after the PVC column rings were removed to check burrow formation by noting burrow positions, which could be present at both the outside (as a result of the earthworm moving along the inside cylinder wall and the soil present in the cylinder) and within soil), diameter and length in the columns. The basic parameters of earthworm burrows were measured including diameter, length, cast dry weight (60°C for 24 hours) and organic matter content of cast (Huerta Lwanga et al., 2017a; Jégou et al., 2000; Jégou et al., 2001). A muffle furnace was used to heat soil samples to 550 °C for 4 hours to calculate soil organic matter content. In treatments MP-a, MP-b and MP-L, soil samples were extracted from soil slices by each 10 cm and dried in the oven at 60 °C for 24 hours.

2.2 Microplastics extraction

The floating method (Zhang et al., 2017) was applied in this study to extract MPs from soil. It is an easy and fast method used to collect low density MP in soils by using the buoyancy of specific solutions to isolate MP. 5.00 g of soil from soil samples collected in each treatments were weighed. 30 ml of distilled water was added into soil samples and then stirred three times. The soil solution was kept overnight and filtered on next morning. This procedure was repeated three times or more till all of the floating particles had been removed. Glass cups with soil solutions were put into an ultrasonic machine for two hours to facilitate the breakdown of soil aggregates and extract small particles of MP which might be aggregated with soil by organic matter. Soil samples were subsequently removed from the machine and kept in the laboratory overnight. The floating materials were filtered the next morning. Filter paper was dried in the oven at a temperature of 60°C for 3-4 hours. All materials on the filter paper were transferred to glass slides and a microscope (Leica wild M3C, Type S, simple light, 6.4×) was used to take pictures of these samples. Firstly, a picture of the MP including impurities (not all floated material was MP)was taken and then the glass slide was heated at a temperature of 130 °C for 5 seconds. The plastics used in this study were thermoplastics and easily changed shape and colour into a round and shiny spot when heated. After being heated for 5 seconds, MP debris melted and changed shape which helped us to distinguish MP from dust or sand. Comparing particle shape and colour in these two pictures, the round shiny spots are melted MP while the other particles are not (Fig. 3).



(a) MP before being heated (b) MP after being heated

Fig. 3 MP in soil samples of column 1 at 0-10 cm soil layer in MP-EW-L. The particle circled in red is a MP which became round and shiny after being heated for 5 seconds at 130 °C.

Photo editor and ImageJ were used to measure MP particle diameter, area and vertical angle. Pictures which were taken under microscope before and after heating were compared in photo editor and Image J. Quantity of MP particles were counted in picture taken after heating. At the same time, Image J could also be used to measure spot area and vertical angles in pictures in order to help calculate MP particle diameter (before heating) and volume (after heating). With these data on MP particles, equation (1) (Zhang et al., 2017) was applied to calculate the weight of MP extracted from soil samples. MP weight concentration in soil could then be calculated based on the MP weight results.

$$m = \frac{4}{27}\rho \sum_{i=1}^{n} \sqrt{\frac{S_i^3}{\pi}} \tag{1}$$

In equation 1, *m* is the weight of the plastic particles (g),ρ is the density of the plastic (0.90 g cm^{-3}),*n* is the quantity counted, and S_i is the vertical angle of the viewing area occupied by plastics *i* after melting at 130°C for 3-5s (in pixels, 1 pixel=0.585/60 mm). S_i was calculated in Image J.

2.3 Data analysis

IBM SPSS statistics version 23 was used to perform statistical significance analysis among treatments. All of the data was checked using K-S and Levene's test for normality and equality. Kruskal-Wallis test (nonparametric test) was used if the data was not normally distributed even after log transferring.

3. Results

3.1 MP particle distribution and concentration along soil column

The MP particle concentration and size distribution results in the soil samples from the burrows and layers are displayed in figures 4 and 5. MP weight concentration in soil samples decreased with increased soil depth (Fig.

4). Maximum MP weight concentration in soil samples was detected in treatment MP-b in the surface layer, measuring 0.33% w/w, and it was significantly higher than that in MP-L and MP-EW-L (Kruskal-Wallis, $p \leq 0.05$; Fig. 4). The minimum value was measured in treatments MP-a, MP-b, and MP-Lin the soil layer between 10 and 50 cm ,which was zero. In treatment MP-EW-L, the weight concentration in the lowest soil layer was 0.01×10^{-2}%. MP weight concentration in treatment MP-L was lower than that found in treatments MP-a and MP-b. In MP-L, litter absorbed most of the particles and stopped MPs from being flushed directly onto the soil surface and consequently, from entering the soil matrix.

When evaluating MP concentrations in treatments with MPs but without earthworms, MP remained in the first soil layer 0-10 cm in high concentrations while no particles were found in the deeper soil layers (10-50 cm). In other words, MP particles were detected in each soil layer for treatment MP-EW-L only, in which the MP concentration decreased as the soil depth increased. In treatment MP-EW-L, 99.67% of MP particles found at a depth of 0-10 cm were bigger than 250 µm (Fig. 5). The value of MP particles (size class 250µm-1mm) weight percentage dropped when the soil depth increased, which means the largest size class 250µm-1mm contributed less to the sum of all particle size classes. The percentage value of MP size class <250µm increased from 0.33% at a depth between 0 and 10 cm to 41.89% at a depth between 40 and 50 cm. Within the soil layer between 40 and 50 cm, MP particles <50 µm weight percentage was 0.17%, which was significantly more than that in the other layers but less than the original distribution (Kruskal-Wallis, $p \leq 0.05$). However, in treatments MP-a, MP-b and MP-L, even the smallest MP size class,<50 µm, was not detected in the soil layer 40-50 cm. According to the accuracy of the MP floating method, approximately 90% of MP could be extracted from the soil when the MP concentration in the soil was higher than 0.05% w/w with 50% of the particle sizes<50 µm(Zhang et al., 2017).

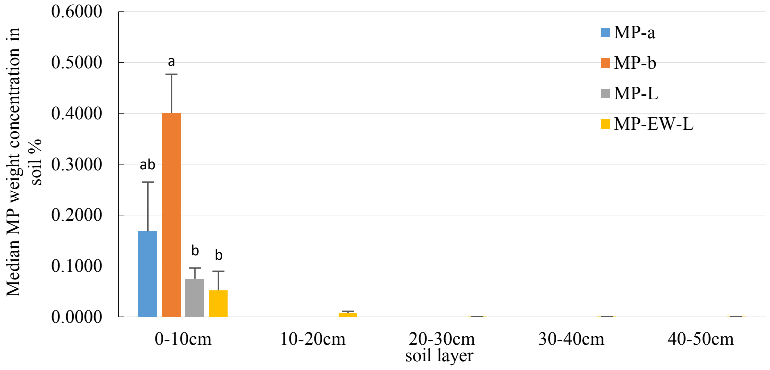


Fig. 4. Median of MP weight concentration in soil samples (n=8) in treatments with MP. For explanations of abbreviations MP-a, MP-b, MP-L and MP-EW-L see Table 1. Different letters (a, b) on each column top indicate significant differences among each treatment in soil layer 0-10 cm (Kruskal-Wallis, $p \leq 0.05$). Bars indicate median absolute deviation.

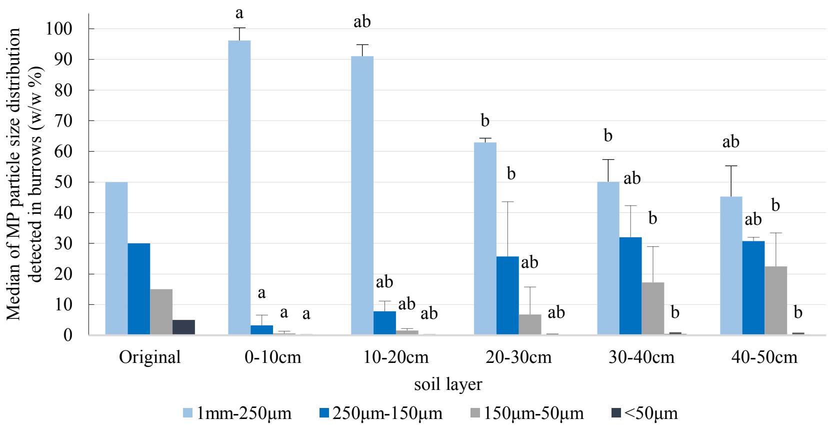


Fig. 5. Median of MP particles size distribution in different soil layers (n=8 per soil layer) in treatment MP-EW-L. Different letters(a, b) on each column top indicate significant difference of same size classification among each layers (Kruskal-Wallis, p≤0.05). Bars indicate median absolute deviation.

3.2 Earthworm Burrows

The boxplot for burrow number, volume and organic content per soil layer in treatment EW-L and MP-EW-L are displayed in fig. 6. The data of burrow quantity, volume and organic matter did not have a normal distribution which can be seen in fig .6. Comparing quantity of burrows of these two treatments, the median was different. Median of burrow quantity in each soil layers was close to the 25% quartile and skewed to the left. In treatment EW-L, one outlier was found with value of 5.. However, burrow volume in each soil layer of treatment EW-L was skewed to left and had a longer tail than that of treatment MP-EW-L with one outlier observed. The median burrow volume of treatment MP-EW-L was smaller than that of treatment EW-L. But shorter box length (Inter Quartile Range IQR) of treatment MP-EW-L showed that values were more concentrated near median. Organic matter content in the casts of these two treatments are different as that in treatments EW-L had two outliers with one was extremely higher than the others. The median of these two treatments are similar but treatment MP-EW-L had a longer whisker and quartile.. In treatment EW-L, box lengths and whiskers were shorter than that in MP-EW-L. In conclusion, data of earthworm activities implied random properties in both of these two treatments EW-L and MP-EW-L.

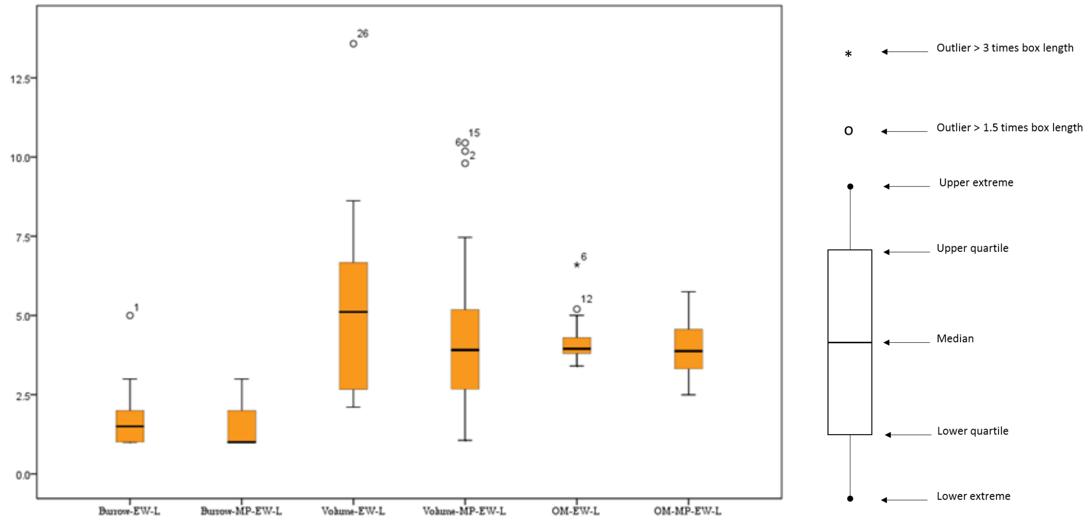


Fig. 6 Left: Box and whisker plot of earthworm burrow quantity, volume and organic matter content in cast in treatment EW-L and MP-EW-L(median, n=8 replicate *5 layers). No significant difference was found between treatments (Kruckal-Wallis, P≤0.05). ° indicates outlier bigger than 1.5 times box length. * indicate outliers bigger than 3 times box length. Length of boxes and horizontal lines within boxes indicate inter quantile range (IQR) and median respectively. Right: explanation of associated statistical information derived from box and whisker plot.

3.3 MP in Leachate

No MP particles, even those smaller than 50µm, were detected in the filtered drainage water for treatments MP-a, MP-b and MP-L. Treatment MP-EW-L was the only treatment in which MP was detected both in the soil samples collected from each layer and also in the leachate. The MP particle weight distribution in the leachate is shown below in Fig. 7. The weight percentage of MP particles between 250 µm and 1 mm was 58.53%, and it was 28.08% for particles between 150 µm to 250 µm. Compared with the initial distribution of MP applied on the soil column surface, MP debris bigger than 250 µm increased by 8.53%. MP size between 150 µm to 250 µm decreased by 1.92%. MP particles measuring between 50 µm and 150 µm decreased by 2% in the leachate. Moreover, there were only 0.36% particles < 50 µm, which was 0.33% in soil samples from the soil layer 40-50 cm.

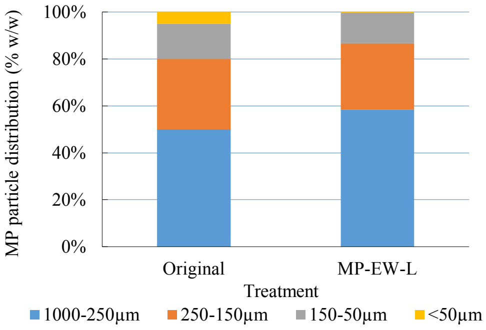


Fig. 7 Comparison of MP particles size distribution in leachate of treatment MP-EW-L and litter

To determine if burrow parameters had a statistical correlation with particles distribution in leachate in treatment MP-EW-L, correlation analysis(Spearman, p<0.05, two -tailed) was performed(Table 2). No significant correlations were found between burrow parameters and the amount of MP particles of different sizes used within this experimental set-up. However, there was a significant correlation between the amount of particle of different sizes which was 0.738 (p<0.05, two-tailed).

Table 2 Correlation between parameters of burrows and MP particle distribution in treatment MP-EW-L

Correlation	Burrows quantity	Burrow Diameter	Burrow Length	Total volume of burrow	Organic content (%) of burrow cast	MP in cast size class 150μm-50μm	MP in cast size class <50μm
Burrows quantity	1						
Burrow diameter (cm)	0.381	1					
Burrow length (cm)	0.135	0.095	1				
Total volume of burrow	0.737*	0.786*		1			
organic content (%) of burrow cast	-0.123	0.405	-0.500	-0.024	1		
MP in cast size class 150μm-50μm	-0.233	-0.262	0.381	-0.143	-0.595	1	
MP in cast size class <50μm	-0.184	0.310	0.333	0.238	-0.405	0.738*	1

*. Correlation is significant at the 0.05 level (2-tailed).

4. Discussion

4.1 MP transport and distribution in soil

This study presents the first evidence showing that MP can be leached out of soil in the presence of earthworm activities in a soil column experimental set-up. MP applied on soil surface mixed with dried litter could be vertically transported by earthworms and be further leached out by preferential flow. For treatments without earthworms, no MP were detected in the leaching water or the deeper soil layers(10-50 cm). The results of biogenic activities affected transportation of MPs within the soil columns. This result confirms previous studies of MP incorporation into burrows. For instance, Rillig (2017) demonstrated that earthworms could transport MPs vertically and that smaller size classes were preferred. Maaß (2017) implied that under a well-controlled experimental set-up, soil micro arthropods could transport and distribute MP horizontally (<100μm and 100μm-

200μm, urea-formaldehyde and polyethylene terephthalate). The MP type and size class significantly influenced translocation distance. In our study, earthworms also showed the size selective trend when transporting MP particles. MP size class <250 μm percentage of weight distribution in soil samples kept increasing with increasing soil depth. In leachate, MP size distribution implied a similar percentage of class <50 μm with the distribution found in the bottom soil layer (40-50 cm depth). For all treatments, except MP-EW-L, MP concentration in soil samples decreased when soil depth increased. For treatment MP-EW-L, MP concentration in soil layer 30-40 cm was higher than those in soil layer 20-30 cm and 40-50 cm. A possible reason is the relatively short incubation time of 14 days, leading to fewer MP particles being transported into the deeper soil layers. Another reason can be the MP size selective ingestion by earthworms when they were feed with mixture of MP and litter (Huerta Lwanga et al., 2017a; Huerta Lwanga et al., 2018). Furthermore, the applied floating method for extracting MPs in soil and water samples has its own limitations, such as nanoparticles passing through the filter paper.

Pictures of soil layers taken before soil sample collection for the treatment with earthworms revealed that many burrows were formed around the soil cylinder surface, which followed the columns' interior walls. When earthworms were moved to a new place, the first thing they did was to dig a tunnel in the easiest way possible to hide themselves in the soil and only later were more rooms built(Perreault and Whalen, 2006; Rastetter and Gerhardt, 2017). The tiny gaps between the soil and PVC columns provided the earthworms with a better opening to start their new life in soil columns. Treatment MP-EW-L had visibly bigger and deeper burrows than those in treatment EW-L. When considering the positions of the burrows in these two treatments, no significant differences were found. Capowiez (2014) pointed out that with longer incubation time, more and deeper burrows would be formed by earthworms (*Allolobophora Chlorotica)* in soil cores 20 cm in length and 11.8 cm in diameter. Huerta Lwanga (2016) obtained similar results for earthworms *Lumbricus terrestris*: the number of burrows increased with longer incubation times of 60 days at the MP concentration of 7% w/w litter. Consequently, increased incubation time resulted in deeper and more abundant burrows which could lead to more MPs being transported into deeper soil layers and more MPs may have been leached out with water.

4.2 Potential risk of microplastic translocation in soil and groundwater

According to the results in this study, where MP particles residing on the soil surface together with litter were transported by earthworms into deep soil layers and were found in leachate, there may be a potential risk of MPs leaching into ground water. Biogenic activities affected the MP particle distribution and concentration over soil depth, with MP < 250 µm more likely to be transported and leached. Although MP >250 µm were detected in each layer in MP-EW-L, its concentration decreased with soil depth.

Comparing MP treatments with and without earthworm presence, there seems to be a low risk of leaching MPs through soil matrix itself, which confirmed the results of previous studies (Blasing and Amelung, 2017; de Souza Machado et al., 2018). The difference between MP treatments with or without earthworm presence shows the different processes that play a role in MP transport through the soil. It seems likely that transport of MP to deeper soil layers can be attributed to bioturbation by earthworms. MP in leachate may be transported with water through preferential flow paths which the earthworm burrows provide. These MP may therefore mainly originate from the soil surface or burrow walls and not from the surrounding soil matrix. The sandy soil used in this study had large pores compared to other soil types, and was expected to be the most vulnerable to MP pollution by soil water infiltration. For the MPs>50 µm ,this did not seem to happen, whereas for MP <50 µm, the detection method used in this study seemed to be insufficient and therefore the risk of leaching by soil water infiltration for MP <50 µm cannot be excluded. Moreover, biogenic activities may give MP a pathway to be transported within and through soil and even leaching to groundwater in places with shallow groundwater levels.

This study sheds light on the necessity to pay attention to terrestrial pathways for MP transport and its potential toxic effects within the terrestrial system. Earthworms are not the only species of macrofauna being used to test MP transportation and biodegradation. Yang (2018) suggested that yellow mealworms (larvae of *Tenebrio molitor* Linnaeus)could degrade polystyrene (PS) during their life cycle and the second generation showed favourable PS degradation. That means that high PS concentrations being fed into the environment can gradually change the yellow mealworms' diet. While the MP diet effecting earthworm feeding behaviour in the second generation has rarely been reported, MPs can significantly influence mortality and reproduction rates of earthworms(Huerta Lwanga et al., 2016). Moreover, earthworms have been observed digesting and biodegrading MPs (Huerta Lwanga et al., 2018). These phenomenon confirmed the results of our study that MP can be detected in deeper soil layer because earthworms may ingest MP and aggregate it with soil to build their tunnel systems. Earthworms' burrow system can help to form preferential flow when leaching, which transporting MP within burrows out even reaching into groundwater. Without earthworms activities, MP on soil surface cannot be transport directly through soil matrix even soil porous is high.

5. Conclusion

Although MP pollution in the terrestrial environment is rapidly gaining attention, its influence on soil biota and its presence in deep soil layers and groundwater is unknown. This study showed that terrestrial biogenic activities (earthworm movement) provided pathways (burrows) for MP transport from the soil surface through the soil allowing the MP to end up in leaching water in an experimental soil column set-up. MP particle<250 µm were more easily transported by earthworms. MPs>50 µm were not found at 20-50 cm soil depth or in leachate from columns without earthworms. The detection of MPs<50 µm was not possible with the current methodology used. The risk of MPs leaching into terrestrial systems with well-developed biogenic activities and shallow groundwater seems to be high. The results of this study point out the urgent need to screen for the presence of MPs in soil and groundwater systems.

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Conflicts of interest

The authors declare no conflicts of interest.

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