Lahive, Elma; Cross, Richard; Saarloos, Aagje I.; Horton, Alice A; Svendsen, Claus; Hufenus, Rudolf; Mitrano, Denise M. 2022. **Earthworms ingest microplastic fibres and nanoplastics with effects on egestion rate and long-term retention.**

The definitive version was published in *Science of the Total Environment*, 807 (3), 151022. https://doi.org/10.1016/j.scitotenv.2021.151022

Earthworms ingest microplastic fibres and nanoplastics with effects on egestion rate and long-term retention

Elma Lahive[1*], Richard Cross[1], Aafke. I. Saarloos[1,2], Alice A Horton[1,3], Claus Svendsen[1], Rudolf Hufenus[4], Denise M Mitrano[5]

[1] UK Centre for Ecology and Hydrology, Maclean Building, Benson Lane, Crowmarsh Gifford, Oxfordshire, OX10 8BB, UK

[2] Department of Toxicology, Wageningen University, Wageningen, The Netherlands

[3] National Oceanography Centre, European Way, SO14 3ZH, Southampton, UK

[4] Laboratory of Advanced Fibers, Empa, 9014 St. Gallen, Switzerland

[5] Department of Environmental Systems Science, ETH Zurich, 8092, Zürich, Switzerland

*corresponding author, elmhiv@ceh.ac.uk

Abstract

Microplastic fibres (MPFs) and nanoplastics (NPs) have the potential to be hazardous to soil organisms. Understanding uptake into organisms is key in assessing these effects, but this is often limited by the analytical challenges to quantify smaller-sized plastics in complex matrices. This study used MPFs and NPs containing inorganic tracers (In, Pd) to quantify uptake in the earthworm *Lumbricus terrestris*. Following seven days exposure, tracer concentrations were measured in earthworms and faeces. Earthworms exposed to 500 µg MPFs/g soil retained an estimated 32 MPFs in their tissues, while at 5000 µg MPFs/g earthworms retained between 2 and 593 MPFs. High variation in body burdens of MPFs was linked to soil retention in earthworms and reduced faeces production, suggesting egestion was being affected by MPFs. NPs uptake and elimination was also assessed over a more extended time-period of 42 days. After 1 day, NPs were no longer detectable in faeces during the elimination phase. However, some retention of NPs in the earthworm was estimated, not linked to retained soil, indicating not all NPs were eliminated. MPFs and NPs uptake can be quantified in earthworms and both particle types can be retained beyond the depuration period, suggesting the potential for longer-term accumulation.

Keywords: Plastic, soil, terrestrial, bioaccumulation*, Lumbricus terrestris*

1. Introduction

Terrestrial environments are subject to extensive pollution by plastics, prompting concern about their potential negative consequences for soil biodiversity and function, and the overall health of soils.[1] Although macroplastic pollution is more easily visualized in the environment, smaller-sized plastic particles such as nanoplastics (NPs) and microplastics (MPs) are more numerous and of more biological relevance as they can be taken up by organisms. [2, 3] NPs and MPs can enter the terrestrial environment directly as primary plastic materials, for example, from polymer-coated fertilisers.[4] However, it is anticipated that secondary NPs and MPs, generated from the breakdown of larger macroplastic items, are likely to dominate emissions to soils. For example, in agricultural systems, sources include the degradation of plastic mulch films and the application of soil conditioners (sludge and composts) which contain NPs and MPs. [5, 6 7 8] More generally, terrestrial systems will also receive inputs from littering and atmospheric deposition. [9, 10] However, large disparities between plastic inputs are expected between residential, industrial, natural and agricultural areas for different types of plastic pollution, since specific uses of plastics will determine the magnitude of the corresponding emissions.[11]

While early research on MP and NP effects on soil dwelling organisms showed limited or no effects on life history traits such as survival, growth, or reproduction,[12, 13] there is emerging evidence that ingestion of plastic particles by some soil organisms has the potential to cause detrimental effects, albeit at high concentrations.[14-16] One reason for these seemingly contradictory findings is that many of the effects of NPs and MPs on soil organisms appear to be mediated by physical parameters, such as particle shape and size, rather than by overt chemically-mediated toxicity. The feeding traits and size of the organism, as well as the characteristics of the particles to which they are exposed, can determine the likelihood of ingestion. For example, larger MPs (fragments), similar in size to the mouthparts of *E. crypticus,* were ingested less compared to MPs much smaller than their mouthparts, which in turn was linked with greater effects on reproduction associated with these smaller MPs.[15] Longer-term studies, or those that investigated biochemical markers of toxicity (e.g. altered gene

50 expression, signs of oxidative stress, changes in energy metabolism), more consistently demonstrated
51 negative impacts.[14, 16] In soil invertebrates, effects on food intake, cast production and invertebrate
52 biomass have been shown.[13, 17 16] Particle morphology has also proven important in changing soil
53 aggregates, water holding capacity, and microbial diversity and functioning.[18] Therefore, particles of
54 different sizes and/or morphology may impact organisms directly, by affecting life history traits or
55 inducing biochemical stress responses, or indirectly, by changing the soil properties in which the
56 organisms reside.
57 Microplastic fibres (MPFs) have the potential to cause physical harm while outside of the organism,
58 for example through abrasion[19], or once ingested can cause damage to the intestine and stomach.[16]
59 They may also become trapped in the gut of organisms resulting in lower assimilation of food or
60 reducing egestion of faeces.[13] In many studies, however, only toxicological endpoints were measured
61 and the actual body burden of MPs or MPFs were less frequently assessed, since the latter metric still
62 remains analytically challenging. Analysis of MPFs in soil, organic residues and soil dwelling organisms
63 is an involved process requiring specific sampling, extraction/separation and concentration analysis
64 steps, which collectively makes for a demanding and time-consuming task. For particles below 10
65 µm, there are few documented protocols to measure these materials.[20] These analytical challenges of
66 plastic detection and quantification are exacerbated when considering particles of even smaller sizes
67 (e.g. NPs), and thus the impacts of NPs have focused on effects on organisms and to date, few have
68 considered the extent of retention of the particles within soil organisms. [21, 22] However, the study of
69 nanoparticulate matter in terrestrial systems and their impacts is not entirely new, as NPs have been
70 studied in the context of engineered nanomaterials as a representative non-dissolving nanoparticle.
71 It is only recently that their inherent toxicity or potential for adverse effects has been considered from
72 the perspective of plastic pollution. [23] Organisms can easily ingest nano-sized plastics; with particles
73 of this size having the potential to cross biological barriers and penetrate tissues, and consequently
74 bioaccumulate in tissues, and thus this area remains active in current research investigations.
75 The aim of this study was to quantify the uptake of NPs and MPFs in the soil invertebrate *Lumbricus*

terrestris. Earthworms are ecosystem engineers important to soil functioning, and thus their fitness is essential for a healthy soil ecosystem. Measurements of the uptake and retention of plastics in organisms are key to identifying mechanisms of effect and potential for hazard. We have circumvented some of the analytical limitations and challenges posed by these materials, by synthesizing NPs and MPFs containing an inorganic tracer.[24, 25] Metal-doped plastics greatly benefit the assessment of uptake in a laboratory setting, increasing the speed and precision of analysis using standardized techniques for trace metals analysis, allowing measurement of smaller sized particles at lower concentrations than with most currently available plastic detection methods.[26] In this current study, it was possible to accurately assess the mass of NPs and MPFs retained in the body of an earthworm and importantly to determine whether NPs and MPFs were retained in the gut as part of soil aggregates or not. In addition, we assessed the uptake and elimination kinetics of NPs, by measuring body concentrations over a 21-day uptake phase in NP-spiked soil followed by a 21-day elimination phase in clean soil. This approach allowed us to 1) assess the homogeneity of NPs and MPFs in the test soil and quantify true exposure concentrations to the earthworms, 2) quantify uptake and elucidate differences between soils contaminated with NPs or MPFs, and 3) determine the mass and number of plastics that were retained by earthworms after depuration.

2. Materials and methods

2.1 Metal-doped plastic materials

The production steps used in creating the microplastic fibres (MPFs) are described in more detail in Frehland et al 2020.[8] Briefly, a polyethylene terephthalate (PET) compound containing indium oxide was prepared with a twin-screw extruder (Dr. Collin GmbH, Germany) in two steps. First, 5 wt% of In2O3 was melt mixed with 95% PET, followed by a second compounding with 95% PET, resulting in the final In nominal concentration of approximately 0.25 wt%. Fibre melt-spinning was carried out on

100 a customized pilot melt-spinning plant described elsewhere. [27] The fully drawn PET filaments were
101 subsequently passed through our fibre-cutting device. The MPFs were cut to an intended length of
102 approximately 500 µm, corresponding to the length of the MPFs released by textiles when
103 laundering.[28] The MPFs underwent several washing and clean up steps to remove oil residues and
104 metal filings from the cutting process. The MPFs were washed six times in water and detergent to
105 remove the oil residue before being rinsed five times with water to ensure all detergent was removed.
106 Following the washing steps, the MPFs were placed in water with a magnetic flea and placed on a
107 magnetic stirrer. This was repeated until no more filings were found to collect on the flea. The cleaned
108 MPFs were then dried in preparation for being used in the experiments. A subsample of cut MPFs was
109 observed and measured under a stereomicroscope (Figure S1, S2). Average MPFs length was 633.7 ±
110 282.8 µm (n=140) and 30 µm in diameter (see SI and Figure S1). The indium content of randomly
111 selected fibres from each spool averaged 0.213 ± 0.005 wt %.

112 Emulsion polymerization of nanoplastic spheres (NPs) containing entrapped Pd were made in-house
113 and characterized following the protocol described in SI and Mitrano et. al. 2019.[24] Briefly, the
114 procedure consisted of a two-step emulsion polymerization in which first the particle core was
115 synthesized (which contained the metal). After this, a further shell of polystyrene (PS) was grown
116 through feeding a second monomer-containing solution over time to augment the surface chemistry
117 and morphology of the original particle (scanning electron microscopy, Figure S1). The solids content
118 of the stock dispersion content was approximately 11.5% dry weight (d.w.). The total metal content
119 was 253.6 mg Pd/L and the particle size and electrophoretic mobility was measured with the Malvern
120 Zetasizer (z-average: 187 nm, polydispersity index: 0.04, zeta-potential (derived from the
121 electrophoretic mobility): -43 mV). Pd was homogeneously incorporated into the center of the particle
122 with an even mass of Pd across the particle population, and the surface of the particle was fully plastic.
123 Previous studies found negligible Pd-leaching different experimental systems, warranting Pd as a
124 conservative tracer for the plastic. [29, 30]

Since both the nanoplastics and microplastic fibers were homogeniously doped with a known concentration of metal (Pd and In, respectively), by measuring the concentration of metal in the target sample, we could then back calculate the plastic concentration by using this known metal:plastic ratio. Both the raw measurements of the metal concentrations and plastic equivalent concentrations are reported throughout the text and figures.

2.2 Organisms and soil

The test organism used in this study was the anecic earthworm, *Lumbricus terrestris*. Earthworms were sourced from a commercial supplier (Worms Direct, UK). Adult earthworms (5.5 ± 1.3 g fresh weight) were used in the experiments.

The selected soil was a topsoil from a former agricultural site at Sprowston, Norfolk, UK (52°39'18.8"N 1°20'23.7"E). The soil was classified as sandy loam (60% sand, 28% silt, 12% clay), with pH 7.2-7.6 and 5% organic matter content. The measured water holding capacity (WHC) was 44.3 ml per 100g soil.

2.3 Short-term MPFs and NPs accumulation assays

Soils were spiked with three different nominal concentrations of MPFs: 50, 500 and 5000 µg MPFs/g d.w. soil; equivalent to 0.11, 1.1 and 11 µg In/g d.w. soil. NPs concentrations were 22, 221 and 2206 µg NPs/g d.w., 0.12, 1.2 and 12 µg Pd/g d.w. Two separate exposure assays were established, one containing MPFs and a second containing NPs. These highest concentrations represent the upper limit of the plastic content permitted in compost added to soils as soil conditioner (0.25% w/w).[31] Soil without any added plastics were also included as a control. There were four replicates for each treatment and the controls. The dried MPFs were added to the dry soil and mixed to create a

148 homogenous distribution (Figure S3). The NPs were added as a dispersion to the dry soil before being
149 mixed thoroughly to ensure homogeneity. The soils were then wet to 40% of their WHC and mixed.
150 Soils were distributed to small containers (12 cm diameter, 7 cm height) with 400 g d.w. equivalent in
151 each and held for three days in a temperature-controlled chamber (13 ± 1 ^{o}C) before the earthworms
152 were introduced.

153 To increase the earthworm's appetite, and encourage burrowing into the soil, each individual was
154 placed on a moist filter paper for 24 hours to void its gut before being introduced to the soil. The fresh
155 weight of each earthworm was recorded and one earthworm was added to each container. No food
156 was added to increase ensure soil consumption was the only source for the earthworms. The
157 containers were covered with perforated lids, weighed and kept in a temperature controlled room (13
158 ± 1 ^{o}C with a 12:12 hr light:dark cycle) for the duration of the experiment. After seven days incubation
159 in the soil, the earthworms were gently removed from the soil. They were rinsed, weighed and then
160 placed individually on moist filter paper for 48 hours to allow them void their gut contents. After 24
161 hours, the filter paper was changed. The faeces produced by the earthworms were collected at the
162 end of the 24 and 48-hour periods and these were pooled for each individual. Following depuration,
163 the earthworms were snap-frozen in liquid nitrogen and freeze-dried in preparation for analysis of In
164 (MPFs exposure) or Pd (NPs exposure).

165

166 *2.4. NPs uptake and elimination experiment*

167 Following the short-term assays, a longer-term assay was set up to assess the uptake and elimination
168 of NPs over an extended period (21 days uptake and 21 days elimination) following the OECD test
169 guideline 317. Based on the outcomes of the above-mentioned short-term NPs assay, a single
170 concentration above the limit of quantification (LOQ) for quantification of Pd in the earthworms was
171 chosen: 464 µg NPs/dry soil (= 2.32 µg Pd/g dry soil). This concentration is equivalent to the permitted
172 plastic content in compost added in a 1:5 ratio to soil. Soils were spiked in the same manner as before.

A total of 32 containers were spiked with NPs and individual earthworms added to each as before. No food was added as for the short-term experiments. Four replicate containers were sampled at each sampling point during the 21-day uptake phase, after 3, 9, 15 and 21 days of incubation. At the end of the 21-day uptake phase, earthworms in the remaining containers were removed from spiked soil, rinsed and transferred to containers with uncontaminated control soil, one earthworm per container, to start the 21-day elimination phase of the experiment. Earthworms were sampled during the elimination phase after 1, 3, 10 and 21 days incubation in the uncontaminated soil, with four replicates sampled per time point. Earthworms sampled during the uptake and elimination phases were allowed to void their gut as in the short-term assay and were preserved in the same manner. Faeces samples were also collected at each uptake and elimination sampling time. Soil samples were collected from the freshly spiked soils (top, middle and bottom of container) and from replicate containers sampled on day 21 of the uptake phase and on day 21 of the elimination phase.

2.5 MPFs and NPs detection in organisms, faeces and soil

For sample digestions, hydrogen peroxide (H_2O_2, >30%, Sigma Aldrich, stored in the refrigerator at 5 ^{o}C wrapped in aluminium foil) and nitric acid (HNO_3, 65%, Merck) were used. Sample digestion was performed in a microwave system (ultra-CLAVE 4, MLS GmbH), operated at a pressure of 120 bar and temperature of 250 ^{o}C for 10 min. Digestion tubes and caps were made of Teflon, with sample volumes of 10 ml. The digestion protocol was derived from a standardized protocol for plastic digestion (Berghof Instruments, 2004) and was modified to additionally mineralize the organic matter in the samples. First, 0.4 ml H_2O_2 was added to the sample directly in the digestion tube and allowed to stand for 30 min. Second, 4 ml HNO_3 was added and again the sample was left to rest for 30 min. After digestion, the samples were quantitatively transferred to polypropylene Falcon tubes with DI H_2O to a final volume of 50 ml. In some samples, particularly those which contained large amounts of silicates (soil, worm faeces); there was a precipitate remaining after digestion. In these cases, the precipitate

198 was allowed to fully settle, the liquid was decanted into a fresh tube and then was subsequently
199 analysed. Spike recovery tests of Pd into soil and following this digestion and analysis protocol showed
200 good recovery (99 +/- 3%), indicating that Pd did not bind to undigested residual materials. Elemental
201 analysis was performed by inductively coupled plasma mass spectrometry (ICP-MS) (Agilent
202 Technologies, QQQ 8900) featuring an integrated sample introduction system (ISIS), microMIST spray
203 chamber and nickel cones. A standard calibration was performed on each day of ICP-MS analysis (see
204 SI for details).

205

206 *2.6 Data analysis*

207 The earthworm body concentrations and faeces concentrations were checked for normality using the
208 Anderson-Darling test. Non-normal data was log-transformed where appropriate in order to carry out
209 ANOVA analysis. Significant differences between body burdens at different exposure concentrations
210 were tested using a one-way ANOVA with post-hoc Tukey (Minitab 18).
211 To establish the likelihood of soil retention in the earthworm to explain measured body burdens in
212 the short-term assays, the total Pd or In in the earthworms and the soil concentration were used to
213 calculate the mass of soil that would need to be retained in the earthworm to result in the measured
214 body burdens:

215 $$Sr = \frac{Et}{Cexp} \qquad (1)$$

216 Where *S*r = mass of soil that would need to be retained (g d.w.), *E*t = total mass of Pd or In in the
217 earthworm minus background Pd or In (µg) and *C*exp = measured concentration of Pd or In in the soil
218 minus background Pd or In (µg/g).
219 Two kinetic models were tested to describe the uptake and elimination of Pd (NPs) in the earthworms'
220 uptake and elimination experiment. These were run using GenStat 19. Model A was a first order one-
221 compartment model, which considers the organism to be one compartment to which the NPs are
222 taken up at a given rate and eliminated at a given rate. Model B was also a first-order one-

compartment model but alongside uptake and elimination, it includes an inert fraction. This allows for a proportion of NPs to be stored and not eliminated during the elimination phase.[32] In both cases, the uptake and elimination were fitted simultaneously.

For the uptake phase, the following equation was used in both models:

$$C_{\text{int}} = C_0 + \left(\frac{k_1}{k_2}\right) * C_{\text{exp}} * (1 - e^{-k_2 t}) \qquad 0 \le t \le tn \qquad (2)$$

Where C_{int} = concentration earthworm tissues at time t (µg Pd/g), k_1 = uptake rate constant (g dry soil/g earthworm dry tissue/ day, k_2 = elimination rate constant (d^{-1}), C_0 = Pd concentration in the earthworms at the start of the experiment (µg Pd/g), C_{exp} = exposure concentration (soil, mg Pd/kg dry soil), t = time (days), tn = time where the earthworms were transferred to clean soil (days),

For the elimination phases, two different equations were used in the model, Eq3 in Model A and Eq4 in Model B.[33]

$$C_{\text{int}} = C_0 + \left(\frac{k_1}{k_2}\right) * C_{\text{exp}} * (e^{-k_2 * (t - tn)} - e^{-k_2 t}) \qquad 0 \le t \le tn \qquad (3)$$

$$C_{\text{int}} = C_0 + \left(\frac{k_1}{k_2}\right) * C_{\text{exp}} * (F\text{i} + (1 - F\text{i}) - e^{-k_2 * (t - tn)} \qquad t > tn \ (4)$$

Fi = the fraction (ranging from 0 to 1) that cannot be eliminated and is stored in the body.

Using the metal content in the plastic particles, it was possible to covert mass of metal measured in a matrix (soil, faeces, earthworm) to mass of plastics in that matrix. Using the density and volume of the particle types used, it was possible also to estimate the number of particles in the matrix with this plastic mass calculations, based on the measurement of Pd (NPs) or In (MPFs).

3. Results and Discussion

Considerations for using doped plastics in biota tracer studies

An advantage in using plastics doped with scarce metal tracers is the ability to overcome the background interferences faced when using alternative tracing methods, such as fluorescence. In addition, they avoid the need for complex and extensive extraction procedures that are required for

247 microscopy or spectroscopy-based analyses. Using metal-doped plastics, in particular for smaller
248 microplastics and nanoplastics, makes them traceable in complex matrices and at low concentrations,
249 with effective digestion procedures and standard methods for trace metal analysis being readily
250 available. The background In (MPFs tracer) concentrations in the test soil used in this study was 0.018
251 ± 0.001 μg In/g which is within the range of measured background In concentrations in unpolluted
252 soils.[34] The background earthworm In concentrations were also low, 0.015 ± 0.002 μg In/g d.w. (Figure
253 1). In comparison, the background Pd (NPs tracer) soil concentration were comparatively more
254 elevated, 0.094 ± 0.0026 μg Pd/g d.w (Table 1). Natural background Pd concentrations have been
255 reported to be as low as 0.015 μg Pd/g, but can be as high as 0.1 μg Pd/g, particularly in soils from
256 urban settings where Pd sources include inputs from vehicle catalytic converters.[35, 36] This contrasts
257 with surface waters which usually have concentrations that are <0.022 μg Pd/l. [37] Background Pd
258 concentrations in the earthworms were also elevated, 0.032 ± 0.01 μg Pd/g d.w., even when measured
259 directly from culture, which utilised a different soil matrix (Figure 2). This highlights that although Pd
260 is often considered a scarce metal, its increasing use, in manufactured items such as cars, over the
261 past 20 years has led to elevated levels in the terrestrial environment. Despite this, our accumulation
262 studies demonstrated that uptake of NPs could still be assessed in the earthworms and importantly,
263 NPs could be reliably detected in earthworms using the Pd tracer at environmentally relevant
264 concentrations (> 0.02 % w/w) (Figure 2).

265

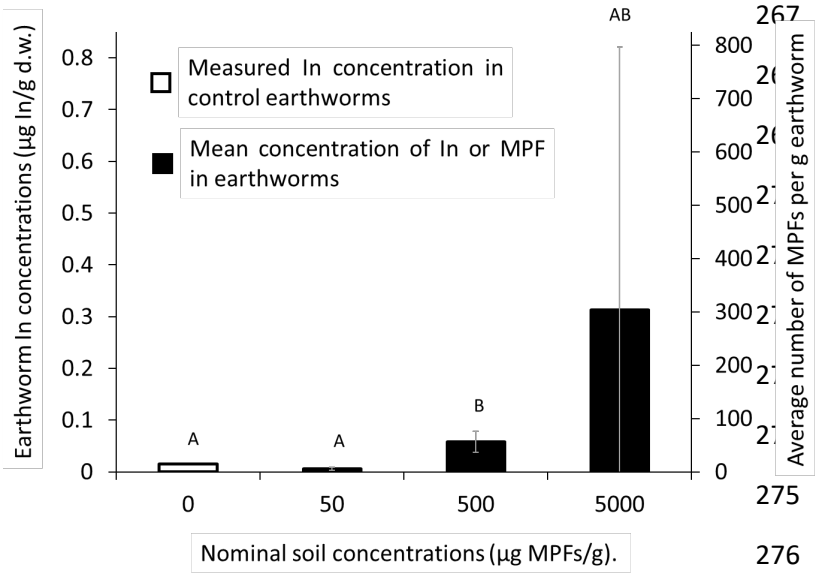

Figure 1: *The concentration of In measured in earthworm tissues (and the corresponding number of MPFs per gram earthworm, secondary y-axis) following 7 days exposure to three concentrations of In-doped microplastic fibres (MPFs) 50, 500 and 5000 µg MPFs/g (0.11, 1.1 and 11 µg In/g). Earthworms were also exposed in soil not spiked with MPFs (control = 0 µg/g). The columns show the mean value and the error bars the standard deviation (n=4). Different letters indicate treatments that are significantly different from one another.*

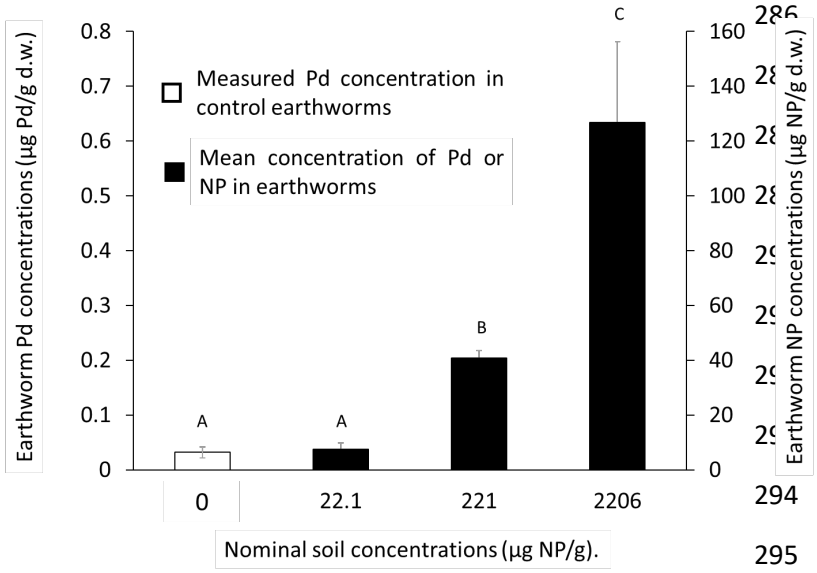

Figure 2: *The concentration of Pd and the corresponding NPs concentration in earthworm tissues following 6 days exposure to three concentrations Pd-doped polystyrene NPs 22.1, 221 and 2206 µg NPs/g (nominal = 0.12, 1.2 and 12 µg Pd/g). Earthworms were also exposed in soil not spiked with plastic (control = 0 µg/g). The columns show the mean value and the error bars the standard deviation (n=4). Different letters indicate treatments which are significantly different from the other treatments.*

In laboratory studies using soil organisms, plastics have often been spiked in food or liquid media, to guarantee ingestion or to reduce the experimental effort. [16, 21, 38, 39] Although this can give more controlled exposures, earthworms live in intimate contact with, and ingest, soil which means using spiked soil provides a more realistic route of exposure. Where plastics have been dosed to a soil matrix, large variation in exposure concentrations have sometimes been observed, particularly in the case of MPFs, where validation of the dosing has been challenging or heterogeneous distributions have been observed visually in spiked soil. [13, 19] High variation in spiking can preclude confident interpretation of bioaccumulation data. For example, when assessing the retention of plastics in biota, it is necessary for the concentration in the exposure media to be as homogenous as possible so that

accumulated plastic in the organism can be distinguished from plastic associated with any soil residues retained in the gut. In this study, it was possible to confirm the homogeneity of our spiking by evaluating the variation in the recovery of In and Pd from the soil, when samples were collected randomly from the spiked batches of soil (Table 1, Figure S3). The coefficient of variance in the spiked soil concentrations in the MPFs exposure was between 2 and 20 times lower when compared with other MPFs soil bioaccumulation studies. [13, 19] Similarly, the coefficient of variance in NPs concentrations in spiked soils was below 10%. This confirmed that the spiking procedure was reliable, achieving reproducible spiking with consistent exposure across replicates. The recovery rate of MPFs from the soil was 102-115% of the nominal concentrations (Table 1). In the short-term accumulation assay, the spiked concentrations of NPs in the soils were mostly lower compared to the nominal concentrations, with exposure concentrations measuring between 47.6% and 70.1% of the nominal concentrations (Table 1). . The resultant NPs concentrations were then calculated as 29.2, 137 and 1566 µg NPs/g d.w., respectively (Table 1).

Table 1: *The nominal microplastic fibre (MPFs) and nanoplastics (NPs) mass concentration in soil, the corresponding nominal In and Pd concentration, the measured In and Pd concentrations in the soils and corresponding actual MPFs and NPs mass concentrations in soils spiked at three different concentrations of microplastic fibres or nanoplastics. The % recovery rate is the measured soil concentration as a percentage of the nominal soil concentration (% recovery = (measured concentration/nominal concentration)*100). The concentration of In and Pd measured in earthworm faeces. All data show mean ± standard deviation. Faeces concentrations marked with ¥ indicate where faeces concentrations were significantly lower compared to the soil concentrations in that treatment.*

Microplastic fibre exposures (MPFs)					
Nominal MPFs concentration (µg MPFs /g d.w. soil)	**Nominal In concentration** (µg In/g d.w. soil)	**Measured In concentration** (µg In/g d.w. soil)	**Actual MPFs concentration*** (µg MPF/g d.w. soil)	**% recovery rate**	**Measured In concentration in faeces** (µg In/g d.w. faeces)
0	0	0.018 ± 0.002	0	NA	0.016 ± 0.001
50	0.11	0.141 ± 0.033	65.9	115	0.110 ± 0.034
500	1.1	1.13 ± 0.024	528.8	104	0.881 ± 0.031¥
5000	11	10.9 ± 0.671	5107	102	9.821 ± 0.497¥
Nanoplastic particle exposures (NPs)					
Nominal NPs concentration (µg NPs/g d.w. soil)	**Nominal Pd concentration** (µg Pd/g d.w. soil)	**Measured Pd concentration** (µg Pd/g d.w. soil)	**Actual NPs concentration*** (µg NPs/g d.w. soil)	**% recovery rate**	**Measured Pd concentration in faeces** (µg Pd/g d.w. faeces)
0	0	0.094 ± 0.006	0	NA	0.149 ± 0.027
22.1	0.12	0.146 ± 0.016	29.2	47.6	0.182 ± 0.039
221	1.2	0.686 ± 0.027	137	53.7	0.645 ± 0.052
2206	12	7.83 ± 0.586	1566	70	5.908± 1.135¥

*Based on measured In/Pd concentration in the soil

Earthworms ingest and retain MPFs and NPs but variation in the body burden is greater at higher MPFs concentrations in soil

Based on the variation in background In concentration of the earthworms, and the In content in the MPFs, the LOQ for measuring In (and therefore MPFs) in the earthworms was calculated as 0.039 µg In/g d.w., equivalent to 23 MPFs in an average-sized earthworm (based on average mass of all earthworms used in the study). For earthworms exposed to 500 and 5000 µg MPFs/g d.w. soil, this limit was exceeded, with earthworms retaining an estimated average of 32 ± 9 MPFs and 180 ± 280 MPFs per earthworm, respectively (Figure 1). Earthworms exposed in the highest MPFs treatment displayed large variations (155% variance) in body burdens compared to earthworms from the lower MPFs treatments (16-28% variance). Excluding the highest MPFs treatment, and associated large variation, from the dataset showed there were significantly higher body burdens in earthworms exposed at 500 µg MPFs/g d.w. compared to the control and the lowest MPFs exposure ($F=58.1$, $P<0.05$).

For the short-term NPs bioassay, the LOQ for measuring Pd above background in the earthworms was comparatively higher, 0.103 µg Pd/g d.w., equivalent to 16.5 µg NPs /g d.w. This concentration was exceeded in earthworms exposed in the two highest NPs treatments and there was a significant increase in Pd body burdens with increasing soil concentration compared to the control (Figure 2). Earthworms exposed to the highest treatment reached tissue Pd concentrations that were equivalent to 121 ± 29 µg NPs/g d.w compared to 34 µg NPs/g d.w in the lower treatment. This corresponds to an average number of NPs retained in the earthworms being 2.04×10^{10} NPs and 7.54×10^{9} NPs, respectively. In contrast with the MPFs exposure, variation in body burdens was less for the NPs exposed worms (between 6 and 23% variance across treatments).

Concentrations of MPFs in the earthworm faeces and soil help us interpret the MPFs concentrations in the earthworms

371 Assessment of ingestion by earthworms can be problematic due to their immersion in soil, as well as
372 the soil itself acting as their food source in the exposure. Earthworm depuration, even for extended
373 periods (> 48 hours), does not always successfully result in full clearance of soil from the gut. [40] Thus,
374 it is possible that soil being retained in the gut is accounting for the high variation in body burdens,
375 particularly in the MPFs treatments. If it is assumed the earthworm In concentrations were the result
376 of soil still residing in the gut following depuration, using the soil and earthworm In concentrations,
377 the amount of soil that would need to be retained in the gut was estimated, *Sr* (Equation 1). It was
378 estimated that 17.1 ± 26.5 mg d.w. soil was remaining in the earthworm gut in the highest MPFs
379 treatment and 30 ± 8.1 mg d.w. soil in their gut (= 30 MPFs), in the lower treatment (500 µg/g) (Table
380 S1). These soil masses are between 3% and 5% of the earthworm whole body weight. There was also
381 a trend showing a decrease in the amount of faeces produced (normalised to the weight of the
382 earthworm) with increasing MPFs concentration in the soil, further suggesting some soil retention in
383 the gut ($F=7.17$, $P<0.05$) (Figure 3). In the highest treatment, there was high variation (88% variance)
384 in the amount of faeces produced between replicates, although the mean was consistent with the
385 downward trend. This is in line with the large variation in body burdens for exposed earthworms
386 (Figure 1). A similar study assessing MPFs ingestion and egestion in *L. terrestris*, found a comparable
387 trend for the lowered production of faeces, although with higher MPFs concentrations in the soil (1%
388 MPFs w/w compared to 0.1% MPFs w/w). [13]

389

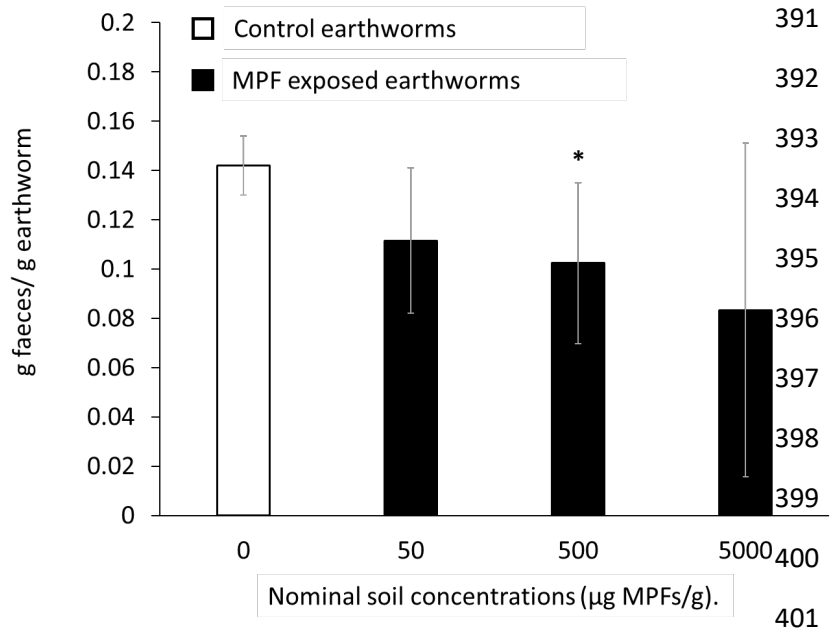

Figure 3: *The biomass of faeces produced per gram earthworm (all dry weight) during 48 hours depuration following 7 day exposures to three concentrations of In-doped microplastic fibres (MPFs) 50, 500 and 5000 µg MPFs/g (0.11, 1.1 and 11 µg In/g). Earthworms were also exposed in soil not spiked with MPFs (control = 0 µg/g). The columns show the mean value and the error bars the standard deviation (n=4). * indicate treatments which are significantly different from the control.*

Avoidance of MPFs-spiked soil was not observed in this study or in other similar soil studies, [13 41] but it is possible that reduced or irregular consumption of soil could also explain some of the variation in body burdens in the highest MPFs treatment. Reduced ingestion or filtration of food has also been observed in other organisms when spiked with MPFs due to plastic particles creating a feeling of satiation or aversion of the food, which could be responsible for lower egestion. [16, 42-44] There was no significant change in earthworm weight over the 7-day exposure; regardless of MPFs loading treatments (P>0.05) (Table S2), although indeed this would not be expected due to the short test duration. The presence of large numbers of MPFs in the earthworms would seem to be more consistent with ingestion and retention by the earthworms. The trend for reduced faeces production

417 suggests that egestion is being impacted by the presence of the large numbers of MPFs in the soil,
418 with clearance of soil from the gut being impeded in some way. Finally, the concentration of In in the
419 faeces of the earthworms was compared with the soil concentrations for each treatment. This
420 revealed significantly lower MPFs concentrations in the faeces compared to the soil for the two
421 highest MPFs treatments, indicating retention of some fibres from the soil within the worms that is
422 not egested with the rest of the soil material (Table 1). The doped MPFs made it possible to look in
423 detail at the ingestion and egestion of MPFs by the earthworms and provide support for the conclusion
424 that MPFs are being retained in the earthworm guts at higher MPFs soil concentrations, regardless of
425 the extent of soil retention in the gut.

426

427 *NPs uptake in the earthworms*

428 Studies assessing uptake of NPs in organisms are less common compared to micron-sized plastics,
429 particularly those studies quantifying uptake from complex matrices such as soil, largely due to the
430 analytical challenges associated with detecting NPs in tissues. The majority of studies have used
431 fluorescently-labelled NPs which can be prone to artefacts of the dissociation of the fluorescent tag
432 leading to sometimes erroneous conclusions about NP absorption. [42] This study is the first to our
433 knowledge which has been able to use realistic exposures (i.e. in soil at relatively low concentrations)
434 to assess uptake of NPs to soil organisms and understand their potential to be retained in tissues.

435 The size and shape of the NPs compared to the MPFs means they are less likely to interfere with
436 egestion. They are, however, more likely to be incorporated into tissues due to their small size. The
437 mass of faeces produced by earthworms exposed to NPs in the short-term assay did not vary
438 significantly with increasing soil NPs concentrations (Figure S4). The estimated mass of soil that would
439 need to be retained in the earthworm to explain the tissue Pd concentrations were > 40 mg d.w. (> 7-
440 8% of their body weight). This seems unlikely given smaller soil masses that were estimated for the
441 MPFs. Instead, it is likely that there are some NPs being retained within the gut, independent of soil

retention, or even in the tissues. In the highest NPs treatment, the faeces concentrations of NPs were significantly lower compared to the soil concentrations ($P<0.05$) supporting the retention of NPs in the earthworms.

Longer-term uptake and elimination of NPs in earthworms

To assess NPs uptake in more detail, and over a longer timescale than 7 days, the longer-term NPs assay allowed the uptake and elimination kinetics of NPs in earthworms to be determined at a relatively low exposure concentration (464.2 μg NP/g d.w. = 0.046% w/w). The Pd concentration in the earthworm tissues increased as a result of exposure but tissue and faeces concentrations were also highly variable, with an average 50% variance among replicates (Figure 4a). The faeces collected from the exposed earthworms had Pd concentrations that were above background soil concentrations, and slightly lower compared to the spiked Pd concentration in the soil during the uptake phase (Figure 4b). When the earthworms were transferred to clean soil, after 24 hours the concentration of Pd in the faeces was comparable to background soil concentrations, which indicated that earthworms did not egest the NPs over an extended period of time (Figure 4b).

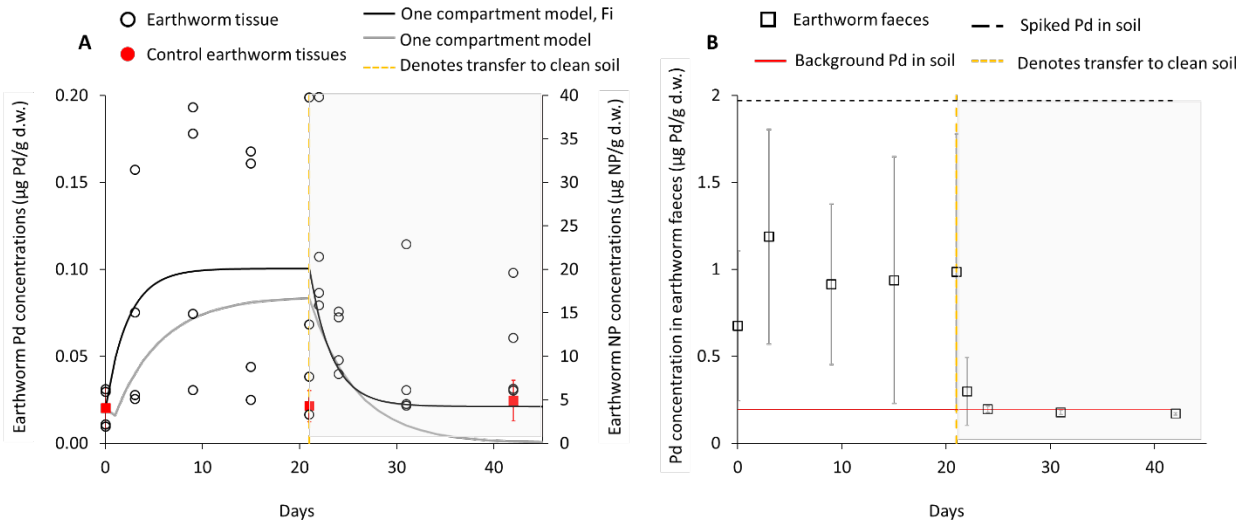

Figure 4: *The concentration of Pd in earthworm tissues (A) and earthworm faeces (B) exposed for 21 days to a single concentration of Pd-doped NPs, 464 mg NP/kg (1.97 mg Pd/kg). The earthworms were also exposed in soil not spiked with plastic (control earthworms). Following 21 days exposure, earthworms were transferred to clean soil and the tissue and faeces concentrations measured during the elimination period. In (A) the one-compartment model fit (Model A = grey solid line) and the one compartment model with the inert fraction (Fi) (Model B = black solid line) are shown along with the concentration in the control earthworms (mean ± standard deviation). In (B) the mean faeces concentrations ± standard deviation are shown along with the Pd concentration in the soil during the uptake phase and the background concentration of Pd in the soil. The vertical yellow line indicates where the earthworms were transferred to clean soil.*

The kinetic parameters obtained by fitting Model A (one-compartment model) and Model B (one-compartment model with an inert fraction) are in Table S3. Including an inert fraction as a parameter in the model (Model B) increased the uptake rate (k_1) and in particular the elimination rate (k_2) (0.432 ± 0.312 d^{-1}), which reflects that NPs were eliminated from the earthworms quickly. Although the inert fraction was small (*F*i=0.015), it still suggests that not all of the ingested NPs were completely egested by the earthworms, or egestion was too low to be detectable after more than one day in clean soil.

These measurements are limited by detection limits for analysing Pd in the earthworms which means that if NPs were present in the earthworms in a concentration < 5 µg NP/g earthworm d.w., they would not be detected. The variability in the uptake amongst replicates also resulted in larger uncertainty in the kinetic parameters so higher exposure concentrations or lower background concentrations would be needed to improve the certainty of these uptake and elimination parameters. Regardless, based on the elimination parameters, the half-life the NPs in the earthworms was estimated to be 1.6 days. This timescale of elimination (1 - 2 days) has also been observed for small microplastics (< 10 µm) in other organisms such as fish and mussels previously. [46, 47]

What does this mean for assessing plastic accumulation in organisms in the environment?

Accumulation of particulate plastics in organisms in the environment has been assessed more often for aquatic organisms than terrestrial organisms. [48, 49] Typically, analysis is carried out using individuals preserved *in-situ* (i.e. they are preserved as captured and not allowed to void their gut). This could be considered representative of true exposure for organisms in the environment. However, it is also recognised that there can be great heterogeneity in the presence of particulate plastics in the environment and so it is possible that organisms will ingest particulates more randomly compared to other non-particulate chemical pollutants. The distribution of MPFs and NPs in the individual replicate containers of soil were not assessed at the end of the exposure, but it is possible that the distribution was not as homogenous as it was in the beginning due to earthworms turning over the soil, particularly for the MPFs due to their size. This is likely more reflective of a real world scenario where MPFs are found incorporated into soil aggregates to a larger degree as opposed to being freely dispersed. [50] Thus, the likelihood for uptake of MPFs may be more random or stochastic in the environment compared with a carefully controlled exposure, such as the one conducted here. Considering the high variability already observed in body burdens of earthworms exposed to NPs and MPFs under these very controlled exposures, it is likely that predicting MPFs or NPs accumulation and trophic transfer in

real environments will be challenging. Better understanding of particulate plastic behaviour in soils and the role and influence of patchiness and heterogeneity in exposure on bioaccumulation kinetics over the longer term could help to provide some insights. [51, 52] However, mechanistic studies allowing for the assessment in controlled conditions gives some power towards making this prediction of uptake of particles and their likelihood to remain in organisms for longer times than either food or soil, which could then be validated in the field.

Another consideration is the size and shape of the particles that are detectable in environmental samples using contemporary analytical techniques for plastics analysis. While there have been valuable advances in the use of spectroscopic methods (e.g. μFTIR) for plastics identification, a significant amount of work has relied on visual identification and staining of microplastics. This means that detection is constrained by the approach (e.g. visual identification means they must be visible *via* microscope) or limitations of the instrument (e.g. size detection limit). For example, MPFs can be difficult to observe and identify using μFTIR because their width can be close to the limit for detection for the instrument. [53] Consequently, it is very challenging for environmental surveys of biota to detect MPFs, and certainly NPs, which might be present and thus assessing bioaccumulation will be difficult. Alongside this, the potential for an organism to ingest particles will also relate to the interplay between the organism's size, feeding traits and the size and shape of the (plastic) particle. [15, 54] For example, in soil exposures at the same concentrations as in this study (0.5% w/w), MPFs with an average size of 220 μm, found 1-2 MPFs per individual for the small (< 1 cm) earthworm *E. crypticus* (following depuration) and 100-150 MPFs in the relatively larger (~ 2 cm) isopod *P. scaber.* [19] *L. terrestris,* used in this study, are larger again (~ 10-20 cm), with a demonstrated greater capacity to retain more MPFs. This underlines the importance of understanding the role of organism physiology in uptake and retention as well as their functional grouping in the environment, as this can help determine their potential susceptibility to ingest MPs or NPs. The relationship between particle characteristics and characteristics of key species in these functional groups must be understood when aiming to predict the potential for accumulation, trophic transfer and ultimately the impact of plastic

pollution on ecosystems. In this study, we were able to determine the number of particles that were retained in the earthworms and link this with responses in earthworm egestion, which could result in altered assimilation longer term.

Acknowledgements

We would like to thank colleagues at Empa, Swiss Federal Laboratories for Materials Science and Technology, including Mathias Lienhard, Benno Wüst (for extrusion and melt spinning trials of fibres), and Markus Hilber (design and operation of the fibre-cutting device).

Funding

D.M.M. was funded through the Swiss National Science Foundation (grant number PCEFP2_186856). Additional support was provided by the Zürcher Stiftung für Textilforschung, Switzerland, for fibre development and processing. E.L., R.C., A.I.S., A.A.H. and C.S. were all supported by funding from the EU, H2020 grant agreement numbers 686239 (caLIBRAte) and 720952 (AceNano).

References

1. Rillig, M. C.; Lehmann, A., Microplastic in terrestrial ecosystems. *Science* **2020,** *368*, (6498), 1430-1431.

2. Enders, K.; Lenz, R.; Stedmon, C. A.; Nielsen, T. G., Abundance, size and polymer composition of marine microplastics ≥10μm in the Atlantic Ocean and their modelled vertical distribution. *Mar Pollut Bull* **2015,** *100*, (1), 70-81.

549 3. Erni-Cassola, G.; Gibson, M. I.; Thompson, R. C.; Christie-Oleza, J. A., Lost, but Found with
550 Nile Red: A Novel Method for Detecting and Quantifying Small Microplastics (1 mm to 20 µm) in
551 Environmental Samples. *Environmental Science & Technology* **2017,** *51*, (23), 13641-13648.
552 4. Kah, M.; Tufenkji, N.; White, J. C., Nano-enabled strategies to enhance crop nutrition and
553 protection. *Nature Nanotechnology* **2019,** *14*, (6), 532-540.
554 5. Liu, M. T.; Lu, S. B.; Song, Y.; Lei, L. L.; Hu, J. N.; Lv, W. W.; Zhou, W. Z.; Cao, C. J.; Shi, H. H.;
555 Yang, X. F.; He, D. F., Microplastic and mesoplastic pollution in farmland soils in suburbs of Shanghai,
556 China. *Environmental Pollution* **2018,** *242*, 855-862.
557 6. Yang, X. M.; Bento, C. P. M.; Chen, H.; Zhang, H. M.; Xue, S.; Lwanga, E. H.; Zomer, P.;
558 Ritsema, C. J.; Geissen, V., Influence of microplastic addition on glyphosate decay and soil microbial
559 activities in Chinese loess soil. *Environmental Pollution* **2018,** *242*, 338-347.
560 7. Li, Q.; Wu, J.; Zhao, X.; Gu, X.; Ji, R., Separation and identification of microplastics from soil
561 and sewage sludge. *Environmental Pollution* **2019,** *254*, 113076.
562 8. Frehland, S.; Kaegi, R.; Hufenus, R.; Mitrano, D. M., Long-term assessment of nanoplastic
563 particle and microplastic fiber flux through a pilot wastewater treatment plant using metal-doped
564 plastics. *Water Research* **2020,** *182*, 115860.
565 9. Dris, R.; Gasperi, J.; Saad, M.; Mirande, C.; Tassin, B., Synthetic fibers in atmospheric fallout:
566 A source of microplastics in the environment? *Marine Pollution Bulletin* **2016,** *104*, (1), 290-293.
567 10. Allen, S.; Allen, D.; Phoenix, V. R.; Le Roux, G.; Durántez Jiménez, P.; Simonneau, A.; Binet, S.;
568 Galop, D., Atmospheric transport and deposition of microplastics in a remote mountain catchment.
569 *Nature Geoscience* **2019,** *12*, (5), 339-344.
570 11. Kawecki, D.; Nowack, B., Polymer-Specific Modeling of the Environmental Emissions of Seven
571 Commodity Plastics As Macro- and Microplastics. *Environmental Science & Technology* **2019**.
572 12. Kokalj, A. J.; Horvat, P.; Skalar, T.; Krzan, A., Plastic bag and facial cleanser derived
573 microplastic do not affect feeding behaviour and energy reserves of terrestrial isopods. *Science of*
574 *the Total Environment* **2018,** *615*, 761-766.

13. Prendergast-Miller, M. T.; Katsiamides, A.; Abbass, M.; Sturzenbaum, S. R.; Thorpe, K. L.; Hodson, M. E., Polyester-derived microfibre impacts on the soil-dwelling earthworm Lumbricus terrestris. *Environmental Pollution* **2019,** *251*, 453-459.

14. Lwanga, E. H.; Gertsen, H.; Gooren, H.; Peters, P.; Salanki, T.; van der Ploeg, M.; Besseling, E.; Koelmans, A. A.; Geissen, V., Microplastics in the Terrestrial Ecosystem: Implications for Lumbricus terrestris (Oligochaeta, Lumbricidae). *Environmental Science & Technology* **2016,** *50*, (5), 2685-2691.

15. Lahive, E.; Walton, A.; Horton, A. A.; Spurgeon, D. J.; Svendsen, C., Microplastic particles reduce reproduction in the terrestrial worm Enchytraeus crypticus in a soil exposure. *Environmental Pollution* **2019,** *255*, 113174.

16. Song, Y.; Cao, C.; Qiu, R.; Hu, J.; Liu, M.; Lu, S.; Shi, H.; Raley-Susman, K. M.; He, D., Uptake and adverse effects of polyethylene terephthalate microplastics fibers on terrestrial snails (Achatina fulica) after soil exposure. *Environmental Pollution* **2019,** *250*, 447-455.

17. Boots, B.; Russell, C. W.; Green, D. S., Effects of Microplastics in Soil Ecosystems: Above and Below Ground. *Environmental Science & Technology* **2019,** *53*, (19), 11496-11506.

18. de Souza Machado, A. A.; Kloas, W.; Zarfl, C.; Hempel, S.; Rillig, M. C., Microplastics as an emerging threat to terrestrial ecosystems. *Global Change Biology* **2018,** *24*, (4), 1405-1416.

19. Selonen, S.; Dolar, A.; Jemec Kokalj, A.; Skalar, T.; Parramon Dolcet, L.; Hurley, R.; van Gestel, C. A. M., Exploring the impacts of plastics in soil – The effects of polyester textile fibers on soil invertebrates. *Science of The Total Environment* **2020,** *700*, 134451.

20. Koelmans, A. A.; Besseling, E.; Shim, W. J., Nanoplastics in the Aquatic Environment. Critical Review. In *Marine Anthropogenic Litter*, Bergmann, M.; Gutow, L.; Klages, M., Eds. Springer International Publishing: Cham, 2015; pp 325-340.

21. Zhu, B.-K.; Fang, Y.-M.; Zhu, D.; Christie, P.; Ke, X.; Zhu, Y.-G., Exposure to nanoplastics disturbs the gut microbiome in the soil oligochaete Enchytraeus crypticus. *Environmental Pollution* **2018,** *239*, 408-415.

600 22. Kwak, J. I.; An, Y.-J., Microplastic digestion generates fragmented nanoplastics in soils and
601 damages earthworm spermatogenesis and coelomocyte viability. *Journal of Hazardous Materials*
602 **2021,** *402*, 124034.
603 23. Mitrano, D. M.; Wick, P.; Nowak, B., Placing nanoplastics in the context of global plastic
604 pollution. *Nature Nanotechnology* **2021,** *accepted*.
605 24. Mitrano, D. M.; Beltzung, A.; Frehland, S.; Schmiedgruber, M.; Cingolani, A.; Schmidt, F.,
606 Synthesis of metal-doped nanoplastics and their utility to investigate fate and behaviour in complex
607 environmental systems. *Nature Nanotechnology* **2019**.
608 25. Schmiedgruber, M.; Hufenus, R.; Mitrano, D. M., Mechanistic understanding of microplastic
609 fiber fate and sampling strategies: Synthesis and utility of metal doped polyester fibers. *Water*
610 *Research* **2019,** *155*, 423-430.
611 26. Koelmans, A. A., Proxies for nanoplastic. *Nature Nanotechnology* **2019,** *14*, (4), 307-308.
612 27. Hufenus, R.; Reifler, F. A.; Maniura-Weber, K.; Spierings, A.; Zinn, M., Biodegradable
613 Bicomponent Fibers from Renewable Sources: Melt-Spinning of Poly(lactic acid) and Poly[(3-
614 hydroxybutyrate)-co-(3-hydroxyvalerate)]. *Macromolecular Materials and Engineering* **2012,** *297*,
615 (1), 75-84.
616 28. Hernandez, E.; Nowack, B.; Mitrano, D. M., Polyester Textiles as a Source of Microplastics
617 from Households: A Mechanistic Study to Understand Microfiber Release During Washing.
618 *Environmental Science & Technology* **2017,** *51*, (12), 7036-7046.
619 29. Keller, A. S.; Jimenez-Martinez, J.; Mitrano, D. M., Transport of Nano- and Microplastic
620 through Unsaturated Porous Media from Sewage Sludge Application. Environmental Science &
621 Technology 2020, 54, (2), 911-920.
622 30. Redondo-Hasselerharm, P. E.; Vink, G.; Mitrano, D. M.; Koelmans, A. A., Metal-doping of
623 nanoplastics enables accurate assessment of uptake and effects on Gammarus pulex. Environmental
624 Science: Nano 2021, 8, (6), 1761-1770.
625 31. BSI, Specification for composted materials. In *PAS 100*, British Standards Institute: 2018.

626 32. Vijver, M. G.; Vink, J. P. M.; Jager, T.; van Straalen, N. M.; Wolterbeek, H. T.; van Gestel, C. A.
627 M., Kinetics of Zn and Cd accumulation in the isopod Porcellio scaber exposed to contaminated soil
628 and/or food. *Soil Biology and Biochemistry* **2006,** *38*, (7), 1554-1563.
629 33. Tourinho, P. S.; van Gestel, C. A. M.; Morgan, A. J.; Kille, P.; Svendsen, C.; Jurkschat, K.;
630 Mosselmans, J. F. W.; Soares, A. M. V. M.; Loureiro, S., Toxicokinetics of Ag in the terrestrial isopod
631 Porcellionides pruinosus exposed to Ag NPs and AgNO3 via soil and food. *Ecotoxicology* **2016,** *25*, (2),
632 267-278.
633 34. Asami, T.; Yoshino, A.; Kubota, M.; Gotoh, S., Background level of indium and gallium in soil
634 with special reference to the pollution of the soils from zinc and lead smelters. *Zeitschrift für*
635 *Pflanzenernährung und Bodenkunde* **1990,** *153*, (4), 257-259.
636 35. Jackson, M. T.; Sampson, J.; Prichard, H. M., Platinum and palladium variations through the
637 urban environment: Evidence from 11 sample types from Sheffield, UK. *Science of The Total*
638 *Environment* **2007,** *385*, (1), 117-131.
639 36. Clément, N.; Muresan, B.; Hedde, M.; François, D., Assessment of palladium footprint from
640 road traffic in two highway environments. *Environmental Science and Pollution Research* **2015,** *22*,
641 (24), 20019-20031.
642 37. Melber, C.; Keller, D.; Mangelsdorf, I.; International Programme on Chemical, S., Palladium.
643 In World Health Organization: Geneva, 2002.
644 38. Kim, S. W.; An, Y.-J., Soil microplastics inhibit the movement of springtail species.
645 *Environment International* **2019,** *126*, 699-706.
646 39. Kim, S. W.; Kim, D.; Jeong, S.-W.; An, Y.-J., Size-dependent effects of polystyrene plastic
647 particles on the nematode Caenorhabditis elegans as related to soil physicochemical properties.
648 *Environmental Pollution* **2020,** *258*, 113740.
649 40. Arnold, R. E.; Hodson, M. E., Effect of time and mode of depuration on tissue copper
650 concentrations of the earthworms Eisenia andrei, Lumbricus rubellus and Lumbricus terrestris.
651 *Environmental Pollution* **2007,** *148*, (1), 21-30.

652 41. Baeza, C.; Cifuentes, C.; González, P.; Araneda, A.; Barra, R., Experimental Exposure of
653 Lumbricus terrestris to Microplastics. *Water, Air, & Soil Pollution* **2020,** *231*, (6), 308.
654 42. Woods, M. N.; Stack, M. E.; Fields, D. M.; Shaw, S. D.; Matrai, P. A., Microplastic fiber uptake,
655 ingestion, and egestion rates in the blue mussel (Mytilus edulis). *Marine Pollution Bulletin* **2018,** *137*,
656 638-645.
657 43. Cole, M.; Coppock, R.; Lindeque, P. K.; Altin, D.; Reed, S.; Pond, D. W.; Sørensen, L.;
658 Galloway, T. S.; Booth, A. M., Effects of Nylon Microplastic on Feeding, Lipid Accumulation, and
659 Moulting in a Coldwater Copepod. *Environmental Science & Technology* **2019,** *53*, (12), 7075-7082.
660 44. Coppock, R. L.; Galloway, T. S.; Cole, M.; Fileman, E. S.; Queirós, A. M.; Lindeque, P. K.,
661 Microplastics alter feeding selectivity and faecal density in the copepod, Calanus helgolandicus.
662 *Science of The Total Environment* **2019,** *687*, 780-789.
663 45. Catarino, A. I.; Frutos, A.; Henry, T. B., Use of fluorescent-labelled nanoplastics (NPs) to
664 demonstrate NP absorption is inconclusive without adequate controls. *Science of The Total*
665 *Environment* **2019,** *670*, 915-920.
666 46. Hu, L.; Su, L.; Xue, Y.; Mu, J.; Zhu, J.; Xu, J.; Shi, H., Uptake, accumulation and elimination of
667 polystyrene microspheres in tadpoles of Xenopus tropicalis. *Chemosphere* **2016,** *164*, 611-617.
668 47. Kinjo, A.; Mizukawa, K.; Takada, H.; Inoue, K., Size-dependent elimination of ingested
669 microplastics in the Mediterranean mussel Mytilus galloprovincialis. *Marine Pollution Bulletin* **2019,**
670 *149*, 110512.
671 48. Bour, A.; Avio, C. G.; Gorbi, S.; Regoli, F.; Hylland, K., Presence of microplastics in benthic and
672 epibenthic organisms: Influence of habitat, feeding mode and trophic level. *Environmental Pollution*
673 **2018,** *243*, 1217-1225.
674 49. Windsor, F. M.; Tilley, R. M.; Tyler, C. R.; Ormerod, S. J., Microplastic ingestion by riverine
675 macroinvertebrates. *Science of the Total Environment* **2019,** *646*, 68-74.
676 50. Zhang, G. S.; Liu, Y. F., The distribution of microplastics in soil aggregate fractions in
677 southwestern China. *Science of the Total Environment* **2018,** *642*, 12-20.

678 51. de Souza Machado, A. A.; Lau, C. W.; Till, J.; Kloas, W.; Lehmann, A.; Becker, R.; Rillig, M. C.,
679 Impacts of Microplastics on the Soil Biophysical Environment. *Environmental Science & Technology*
680 **2018,** *52*, (17), 9656-9665.
681 52. Zhang, G. S.; Zhang, F. X.; Li, X. T., Effects of polyester microfibers on soil physical properties:
682 Perception from a field and a pot experiment. *Science of The Total Environment* **2019,** *670*, 1-7.
683 53. Horton, A. A.; Cross, R. K.; Read, D. S.; Jürgens, M. D.; Ball, H. L.; Svendsen, C.; Vollertsen, J.;
684 Johnson, A. C., Semi-automated analysis of microplastics in complex wastewater samples.
685 *Environmental Pollution* **2021,** *268*, 115841.
686 54. Scherer, C.; Brennholt, N.; Reifferscheid, G.; Wagner, M., Feeding type and development
687 drive the ingestion of microplastics by freshwater invertebrates. *Scientific Reports* **2017,** *7*.

688

689