Journal of Insect Science, (2022) 22(1): 21; 1–8
https://doi.org/10.1093/jisesa/ieac005
Research

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Soil Invertebrates Generate Microplastics From Polystyrene Foam Debris

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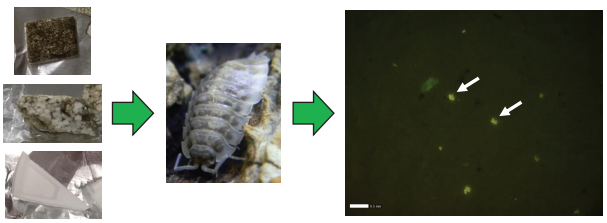
Subject Editor: Phyllis Weintraub

Received 28 November 2021; Editorial decision 12 January 2022

Abstract

To fully understand microplastics' impact on soil ecosystems, one must recognize soil organisms as not just passively enduring their negative effects, but potentially contributing to microplastics' formation, distribution, and dynamics in soil. We investigated the ability of four soil invertebrates, the cricket *Acheta domesticus* L. (Orthoptera: Gryllidae), the isopod *Oniscus asellus* L. (Isopoda: Oniscidae), larvae of the beetle *Zophobas morio* Fabricius (Coleoptera: Tenebrionidae), and the snail *Cornu aspersum* Müller (Stylommatophora: Helicidae) to fragment macroscopic pieces of weathered or pristine polystyrene (PS) foam. We placed invertebrates into arenas with single PS foam pieces for 24 h, then collected and assessed the microplastic content of each invertebrate's fecal material, its cadaver, and the sand substrate of its arena via hydrogen peroxide digestion, filtration, and fluorescent staining. All taxa excreted PS particles, though snails only to a tiny extent. Beetle larvae produced significantly more microplastics than snails, and crickets and isopods fragmented the weathered PS foam pieces more than the pristine pieces, which they left untouched. A follow-up experiment with pristine PS foam assessed the effect of different treatments mimicking exposure to the elements on fragmentation by isopods. PS foam pieces soaked in a soil suspension were significantly more fragmented than untreated pieces or pieces exposed to UV light alone. These findings indicate that soil invertebrates may represent a source of microplastics to the environment in places polluted with PS foam trash, and that the condition of macroplastic debris likely affects its palatability to these organisms.

Graphical Abstract



Key words: soil, isopod, snail, cricket, beetle

Though primarily studied in aquatic systems, microplastics (plastic particles <5 mm in diameter) are now known to contaminate soils across the globe (Bläsing and Amelung 2018, He et al. 2018, Jacques and Prosser 2021, Zhang et al. 2021). Significant effort has been devoted to unearthing microplastics' effects on soil organisms and broader ecology (Accinelli et al. 2020, Barreto et al. 2020, Lozano et al. 2021, Mueller et al. 2020, Yan et al. 2021) as well as the overall risk they pose to soil biota (Jacques and Prosser 2021). The reverse, effects of soil organisms on microplastics, are also receiving increased attention (Ng et al. 2018, Helmberger et al. 2020b, Song et al. 2020, Kwak and An 2021). Earthworms and springtails have been shown to disperse microplastics (Maaß et al. 2017, Rillig et al.

2017, Zhu et al. 2018) and may thus influence their distribution in soil. However, the potential of soil animals to create microplastics by fragmenting larger pieces of plastic debris, as has been observed in aquatic systems (Hodgson et al. 2018, Mateos-Cárdenas et al. 2020), has so far received less attention.

Microplastics were found in the guts of *Cryptopygus antarcticus* Willem (Collembola: Entomobryidae) springtails collected on a piece of polystyrene (PS) foam flotsam on King George Island in the Antarctic (Bergami et al. 2020), suggesting fragmentation by even these minute soil animals. The land snail *Achatina fulica* Férussac (Stylommatophora: Achatinidae) was also found to fragment PS foam (Song et al. 2020). Also, terrestrial invertebrates' inclination and ability to feed on plastic has been studied in waste management contexts. Some insects, alone or in combination with their gut microbiota, have proven capable of ingesting and possibly biodegrading plastics (Brandon et al. 2018, Yang et al. 2018b, Kundungal et al., 2019, Peng et al., 2019), including polyethylene and PS foam, mostly via mineralization into carbon dioxide. However, fragments of undegraded or partially-degraded plastic can persist in the insects' fecal material (Yang et al., 2015, Kundungal et al., 2019). Thus, microplastics are formed even if digestion reduces the total mass of plastic. To be sure, not all of these studied taxa live in soil. Two of the pyralid moth larvae shown to fragment plastics (Yang et al., 2014, Kundungal et al. 2019, Zhang et al. 2020), are parasites of bee nests and the third is a graminivorous stored products pest. Tenebrionid beetle larvae, the other major group of known plastic-degrading insects, live in soil, leaf litter, and rotting wood (Lawrence, 1991), though they are not especially abundant members of the soil fauna. Nevertheless, insects possess great species diversity (Stork et al. 2015) and many insects spend at least part of their active life in soil, so the ability likely exists in other soil insects and invertebrates more broadly. For example, bacteria from earthworm guts have been found to biodegrade polyethylene (Huerta Lwanga et al. 2018), though to our knowledge this ability has not been tested in vivo. Also, even if biodegradation in the strict sense is rare, physical fragmentation by the mouthparts and/or gut may still occur, even if the plastic is only chewed, not ingested.

In this study, we tested the ability of four soil-dwelling invertebrates, the cricket *Acheta domesticus* (L.) (Orthoptera: Gryllidae), the isopod *Oniscus asellus* (L.) (Isopoda: Oniscidae), larvae of the beetle *Zophobas morio* (F.) (Coleoptera: Tenebrionidae), and the snail *Cornu aspersum* Müller (Stylommatophora: Helicidae) to contribute microplastics to terrestrial food chains by fragmenting larger debris, in our case, macroscopic PS foam sourced from a commercial supplier or collected from natural environments. We also conducted a more targeted experiment on *O. asellus* to evaluate how exposure to ultraviolet (UV) light and/or soil solutions might sensitize macroscopic PS foam to faunal fragmentation.

Materials and Methods

Both experiments reported in this study used a common set of procedures. Thus, we will first detail this common protocol, then describe where the two experiments differ.

Experimental Arenas

Experimental arenas consisted of cylindrical glass jars with an inner diameter of 7 cm and height of 5 cm. We filled the bottom of each arena with mixed and hardened Plaster of Paris to a depth of 10 mm to retain moisture, followed by an additional 5 mm of sand. The sand had been heated in a muffle furnace at 500°C for 24 h to burn away organic matter and any contaminating plastic (Liu et al. 2019). To provide additional sustenance for the invertebrates, we placed a single oat flake into each arena (Quaker Oats Company, Chicago, IL). Providing non-plastic food does not necessarily prevent organisms from consuming plastic, and may even increase plastic consumption (Yang et al. 2018a, 2021). The plaster bottoms of the arenas were then moistened to saturation.

Animal Exposure to Plastics

We placed each invertebrate into a 60 ml glass jar for 24 h to starve them and allow them to defecate before placing each into its experimental arena with a piece of PS foam (see experiment-specific sections for the treatments present in each). After 24 h at room temperature in the arenas, we placed the invertebrates into clean jars for 48 h to let them defecate again before removing and freezing them.

Sample Processing – Feces, Sand, and Cadavers

We assessed the microplastic content of the invertebrates' fecal material, the sand in the arenas, and the frozen invertebrate cadavers themselves, to obtain as complete as possible metric of the microplastic generated by the animal.

To assess fecal material, we added 10 ml of filtered 30% hydrogen peroxide solution to the defecation jars to digest the contents at room temperature for 48 h. Following the peroxide digestion, jar contents were vacuum filtered onto 1.5 µm glass fiber filters (Whatman) for staining and counting.

For the sand, we washed the contents of each arena into 100 ml glass beakers using 25 ml of hydrogen peroxide, then washed the PS foam piece into the beaker as well to ensure any fecal or microplastic material on it would be dislodged into the sand. After 48 h of peroxide digestion, we then filled the beakers to a depth of 50 ml with filtered DI water (water and hydrogen peroxide being sufficient to separate PS foam and sand by density) and agitated their contents by stirring with a glass rod. We let the beakers stand for 2 min and carefully filtered the supernatant as described above, though we refilled and reagitated each beaker with DI water two additional times to ensure as much floating material as possible was transferred onto the filters.

Cadavers were dried at 65°C for 72 h and finely ground with a glass rod within a glass jar, digested for 48 h in 10 ml of 30% hydrogen peroxide solution, and filtered like the other samples.

Anti-contamination Protocols

Because contamination is a significant problem in microplastic research (Prata et al. 2021), we used the following protocols to mitigate it. Forceps, funnels, and other equipment were triple-rinsed with filtered DI water in between each sample. To further assess any contamination due to particles persisting on the equipment, we placed procedural blanks between every five to seven samples, filtering 10 ml of clean 30% hydrogen peroxide from a glass jar as if it contained digested material. Finally, we placed four moistened glass fiber filters throughout the work area during the vacuum filtration process to assess deposition of airborne microplastics (air blanks).

Microplastic Staining, Visualization, and Counting

We stained all filters (including controls and blanks) with a combination of Calcofluor White/Evans Blue (Sigma-Aldrich) and Nile Red (Santa Cruz Biological) fluorescent dyes following Helmberger et al. (2020a). We stained each filter with 10–15 drops of Calcofluor White/Evans Blue blend, washed with a similar quantity of filtered DI water, stained again with Nile Red, and washed again with filtered *n*-hexane. Once dry, we observed the filters under a Leica S8

APO stereomicroscope fitted with a Nightsea fluorescence adapter (440–460 nm excitation light, 500 nm long-pass emission filter, and an additional AmScope LED UV excitation light, 395 nm) to locate and count PS foam fragments. By turning the 440–460 nm excitation light on and off, we could determine which particles fluoresced under Nile Red *only*, as opposed to Calcofluor White only or under both dyes' excitation wavelengths. Any particles fluorescing under Nile Red but not Calcofluor White/Evans Blue were considered potential microplastics, although fibers, films, and any other obviously non-PS-foam particles were ignored. Particles resembling PS foam were prodded with the finely filed tip of a soldering iron (Chicago Electric, operating temperature 390°C) to confirm their identity as plastic if they melted deformed in response to the heat. Visual detection methods, however, are limited in their ability to detect very small plastic particles (Lv et al. 2021), and though fluorescent staining can facilitate identification of microplastics with diameters in the tens of microns (Sfriso et al. 2020), particles smaller than that could escape observation and go uncounted.

To account for sample contamination, we then subtracted the highest number of suspected PS foam particles found on any of that experiment's negative control, procedural blank, or airborne blank filters from each sample's count. Thus, for each replicate, since we counted microplastics in fecal material, arena sand, and cadaver biomass separately, we applied this correction to each count and summed the corrected counts to produce a total microplastic value.

Experiment 1: Four-Species

This experiment compared fragmentation of PS foam into microplastics by four invertebrate species; the cricket *A. domesticus* L. (Orthoptera: Gryllidae), the isopod *O. asellus* L. (Isopoda: Oniscidae), larvae of the beetle *Z. morio* Fabricius (Coleoptera: Tenebrionidae), and the snail *C. aspersum* Müller (Stylommatophora: Helicidae). All four are to some extent decomposers, feeding on decaying plant and/or animal material. We collected the isopods from a wooded area in East Lansing, MI (42°44′16″ N, 84°27′4″ W), obtained the crickets and beetle larvae from a pet supply store (Preuss Pets, Lansing, MI), and obtained the snails from a private hobbyist. Taxonomy for *Z. morio*, *A. domesticus*, and *C. aspersum* were confirmed by the suppliers and the isopods were identified as *O. asellus* following Shultz (2018).

We prepared three treatments of PS foam out of pieces cut from larger items, two 'weathered' and one 'pristine.' For the two weathered treatments, we collected two items of PS foam trash, a shard of a drink cup, and an irregular lump of indeterminate origin, from the wooded bank of the Red Cedar River in East Lansing, MI, USA (42°44′01″ N, 84°29′28″ W). We specifically sought pieces showing wear, discoloration, or other visible signs of age and prolonged exposure to the elements. The pristine treatment used freshly-purchased PS foam cup lids (Dart Container Corporation, Mason, MI) stored indoors. We cut all smaller pieces in such a way that original outer surface area was equally distributed between pieces, since the surfaces exposed by cutting may have had different properties than the outer surfaces exposed directly to the elements. We triple-rinsed each piece in filtered DI water to remove any clinging microplastic fragments potentially created via cutting, then wrapped the pieces in aluminum foil and stored them at room temperature until needed.

Prior to use, we confirmed our field collected pieces as PS via attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR), conducted on a FTIR spectrometer (Vertex 70, Bruker) equipped with an A225/Q Platinum ATR Diamond accessory operating in the 4500–400 cm^{-} mid-IR region, using a 4 cm^{-1} resolution with 60 scans/sample and medium Norton-Beer apodization. We took background spectra of an empty and cleaned system before each sample, as recommended by Andrade et al. (2020). We then identified the background-subtracted spectra with the online Open Specy tool (Cowger et al. 2021), without processing. Our plastics, cut pieces, and identification spectra are shown in Fig.1.

This experiment used 5 replicates of each invertebrate × plastic combination. We also included three types of negative control; arenas containing a piece of PS foam and an oat flake but no invertebrates (n = 3 for each PS foam type), arenas containing invertebrates and an oat but no PS foam (n = 3 for each fauna type), and arenas containing only an oat atop the sand (n = 3). These allowed us to account for; any shedding of microplastics from the PS foam via physical, chemical, or microbial processes; any microplastic present on or in the invertebrates before being placed in the arenas; and any microplastic present in the arena substrate itself, respectively.

Positive control samples consisted of fecal material from each of the four invertebrates (n = 2 for *Z. morio*, n = 3 for all others) mixed with a known 50 PS foam fragments counted out for each sample (fragments were grated from the cup lids used for the pristine treatment, in a room separate from the one in which most other protocols took place), and subject to the same processing as fecal material from the arenas (see next section). This method, however, did not simulate any potential loss of particles due to biodegradation or apparent addition of particles via further physical fragmentation in the gut, and was restricted to larger-sized particles that could be reliably counted and transferred. We also created positive controls for the sand samples, in which the same numbers of PS foam fragments were added to 10 g of sand, along with fecal material, as some would be present in the arena sand after invertebrates were removed.

Experiment 2: Isopod-only

This experiment compared fragmentation by the isopod *O. asellus* between initially identical PS pieces subject to different treatments. All of the PS foam pieces placed into the arenas were cut from the cup lids used for the previous experiment's pristine treatment and were subjected to different treatments mimicking exposure to the elements. These treatments were; 1) immersion in sterile DI water for 48 h, 2) exposure to 405 nm UV light (Comgrow, Shenzhen, China) for 24 h per side, then immersion in sterile DI water, 3) immersion in an aqueous suspension of agricultural field soil collected from East Lansing, MI, and 4) exposure to UV light, then immersion in the soil suspension, as well as 5) an untreated control. We then air-dried all plastics under aluminum foil for 24 h at room temperature.

For the experiment, we placed lone, starved isopods into arenas with either no plastic or a piece of untreated plastic, water-treated plastic, UV-and-water-treated plastic, soil-suspension-treated plastic, or UV-and-soil-suspension-treated plastic (n = 10 for all treatments). Negative controls consisted of arenas with an isopod but no plastic (n = 10), with plastic but no isopod (n = 5), and neither plastic nor isopod (n = 5).

Statistical Analysis

For the four-species experiment, we tested the effect of invertebrate species and plastic treatment on the number of total PS foam particles with Kruskal-Wallis tests (Zar 1999), then used a post-hoc Kruskal Nemenyi test with a Chi-square distribution to correct ties (Zar 1999), via the R package PMCMR (Pohlert, 2014), to determine which species produced more fragments across all three plastic types. We used the same procedure in the isopod-only experiment to

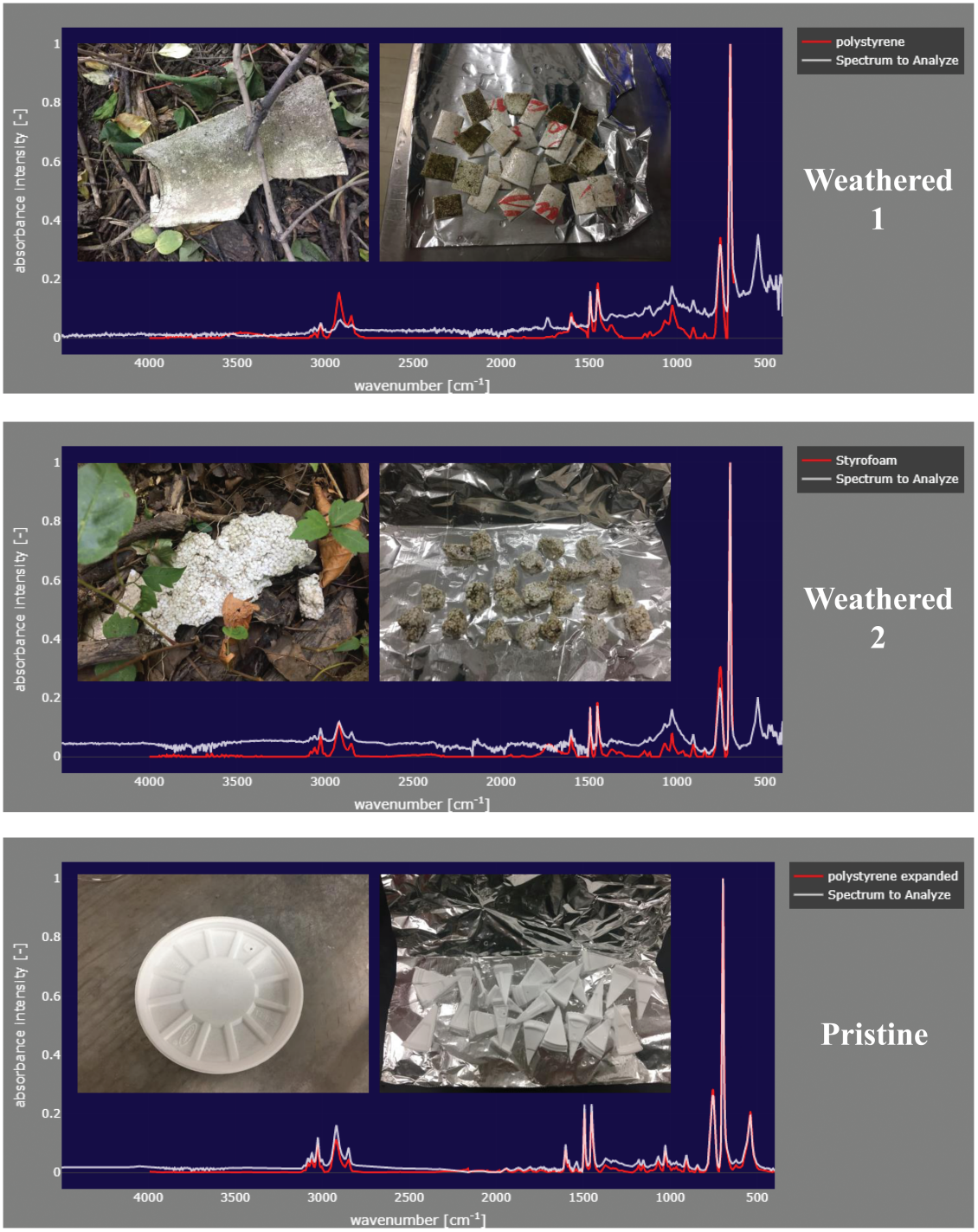


Fig. 1. Images of the original and cut pieces of the PS foam items comprising the three experimental treatments, with confirmatory ATR-FTIR spectra. Spectra in white represent our samples, spectra in red represent the closest spectral match obtained via OpenSpecy.

compare the number of fragments produced between the five plastic treatments.

Results

Four-species Experiment

The beetle larva *Zophobas morio* was most inclined to fragment the PS foam, producing hundreds to thousands of particles from all tested PS types. The isopod *Oniscus asellus* and cricket *Acheta domesticus* fragmented both types of weathered PS foam, but not the pristine PS. The snail *Cornu aspersum* did not appreciably fragment anything. Examples of produced particles are shown in Fig. 2. In general, *Z. morio* produced the largest particles, some exceeding 1 mm in size. Particles produced by *A. domesticus* and (rarely) *C. aspersum* reached up to 500 µm in size, and *O. asellus* produced the smallest particles, rarely exceeding 250 µm in size.

Invertebrate species had a significant effect on the total number of PS foam fragments found in fecal material, arena sand, and cadavers (Kruskal-Wallis rank sum test, $X^2 = 17.168$, df = 3, $P = 0.0007$), but plastic treatment did not (Kruskal–Wallis rank sum test, $X^2 = 3.176$,

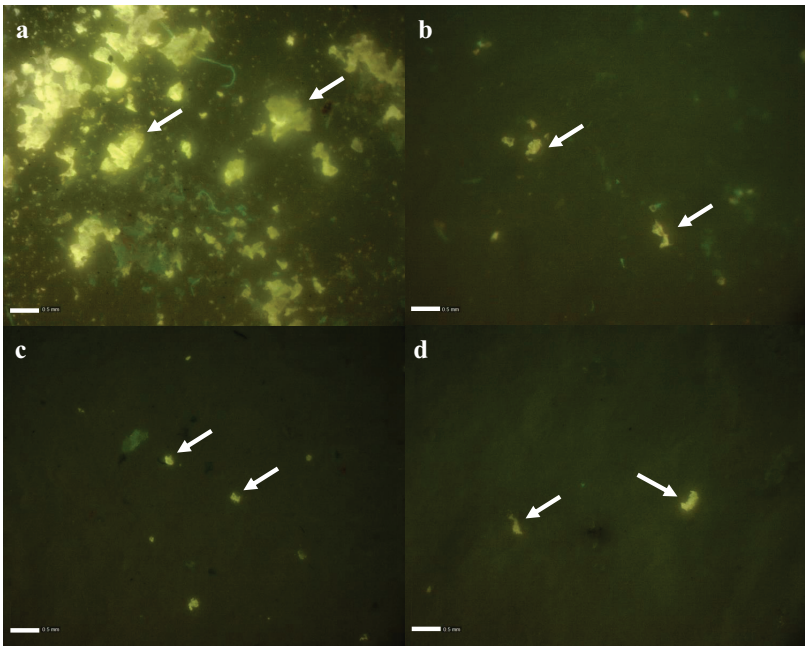


Fig. 2. Photographs of PS foam fragments produced by; a) the beetle larva *Zophobas morio*, b) the cricket *Acheta domesticus*, c), the isopod *Oniscus asellus*, and d) the snail *Cornu aspersum*. Examples of produced particles are indicated with arrows. Scale bar is 500 µm. All photographs were taken with a DinoEye microscope eyepiece camera (Dunwell Tech, Inc., Torrance, CA) and we determined scale using DinoCapture 2.0 software.

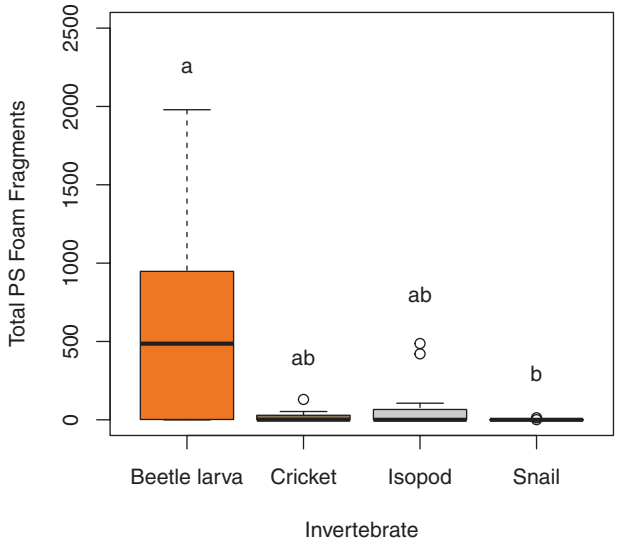


Fig. 3. Total numbers of PS foam microplastic particles found in invertebrate fecal material, arena sand, and invertebrate cadaver biomass, averaged across all three PS foam treatments (two weathered and one pristine) for the four invertebrate species. Different letters denote species producing significantly different amounts of microplastic. Lower and upper whiskers represent dataset minimums and maximums, respectively, excluding outliers.

df = 2, P = 0.2043). Due to high variance in the data, only the difference between beetle larvae and snails was statistically significant (post-hoc Kruskal Nemenyi test, P = 0.0007) (Fig. 3).

Within individual species, plastic treatment had a significant effect on fragmentation by crickets (Kruskal–Wallis rank sum test, X^2 = 7.983, df = 2, P = 0.0185) and isopods (Kruskal–Wallis rank sum test, X^2 = 6.477, df = 2, P = 0.0392), but not by beetle larvae (Kruskal–Wallis rank sum test, X^2 = 1.207, df = 2, P = 0.547) or snails (Kruskal–Wallis rank sum test, X^2 = 1.086, df = 2, P = 0.5811). Crickets fragmented one of the weathered plastics (Treatment 1) significantly more than the pristine plastic (post-hoc Kruskal Nemenyi test, P = 0.018) and isopods fragmented the other weathered plastic (Treatment 2) significantly more than the pristine plastic. (post-hoc Kruskal Nemenyi test, P = 0.041) (Fig. 4).

We found very few suspected PS foam particles on our negative control, procedural blank, and air blank filters (1 particle each on 1 procedural blank and 1 air blank out of 82 total), though air blanks contained microfibers consistent with an indoor environment. Some fluoresced under Calcofluor White excitation and were most likely cotton; others did not and could have been plastic, but were not mistakable for the PS foam particles we were counting. In addition, the air blank filters were exposed to the lab environment for 1–3 h, depending on the number of samples being filtered, whereas most individual samples were exposed for no more than 10 min each throughout filtration, staining, and particle counting.

Recovery from positive control samples was high, with a mean ± SE across all four species of 91.4 ± 3.2% (n = 11) added fragments counted in spiked feces samples. Recovery from spiked feces + sand samples was nearly identical, with 92.5 ± 2.4% of added fragments counted (n = 11). Some individual positive control samples yielded slightly more PS foam fragments than the originally added 50, which we attribute to abiotic fragmentation, perhaps due to abrasion by sand, or disentangling of particles initially counted as single fragments.

Isopod-only Experiment

Plastic treatment had a significant effect on the number of fragments the isopods produced (Kruskal–Wallis rank sum test, X^2 = 22.077, df = 4, P = 0.0002). Though isopods fragmented all types of plastic to at least a slight extent, plastics treated with only the soil suspension were fragmented significantly more than untreated plastics (post-hoc Kruskal Nemenyi test, P = 0.012) or plastics treated with UV and water (post-hoc Kruskal Nemenyi test, P = 0.022). Plastics treated with only water or with the soil suspension and UV were not significantly different from any others (Fig. 5).

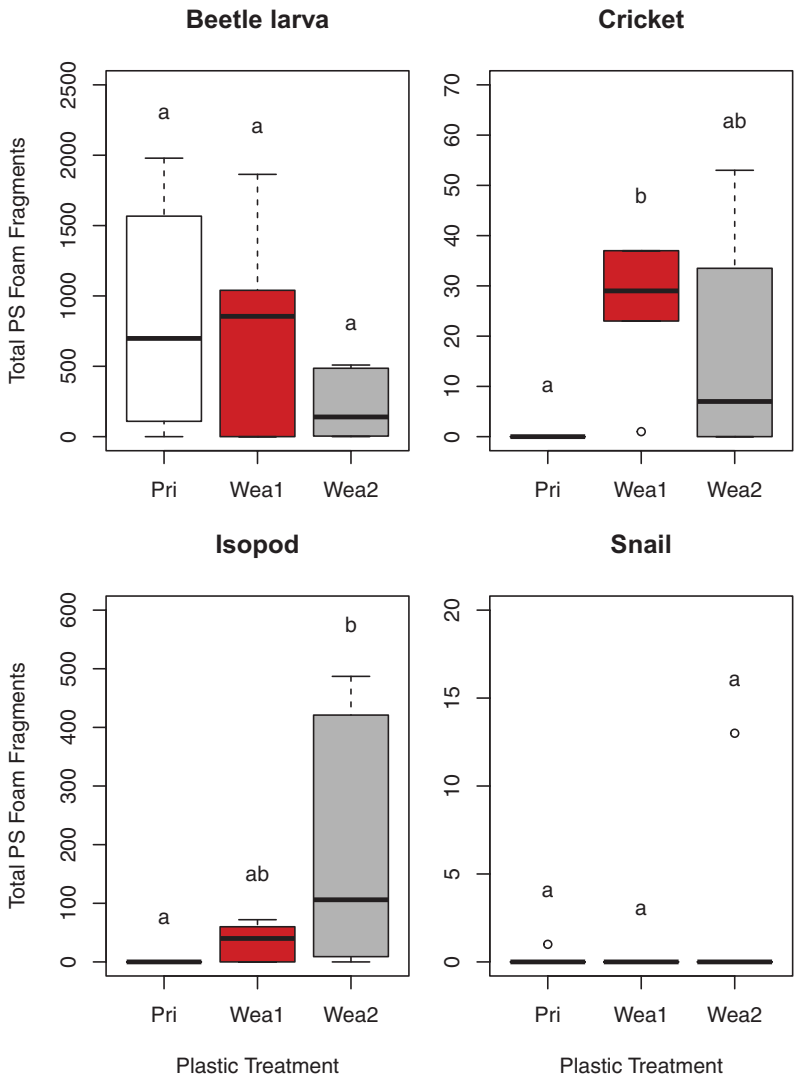


Fig. 4. Total numbers of PS foam microplastic particles found in invertebrate fecal material, arena sand, and invertebrate cadaver biomass of each species across the three PS foam treatments (Pri = Pristine, Wea1 = Weathered 1, Wea2 = Weathered 2). Different letters denote PS foam treatments from which the invertebrates produced significantly different particle numbers. Lower and upper whiskers represent dataset minimums and maximums, respectively, excluding outliers.

We again found very limited contamination by PS foam particles, detecting only 1 particle on a single isopod-only negative control and 1 particle on a single air bank, out of 68 total negative control, procedural blank, and air blank filters.

Discussion

This study demonstrates the potential for soil invertebrates to produce PS foam microplastics by fragmenting larger debris after only brief contact and provides further evidence that the environmental history of plastic affects its relative 'palatability' to decomposer organisms. *Zophobas morio*, like other tenebrionid beetle larvae (Yang et al., 2018a, Peng et al., 2019), was already known to fragment and consume PS foam (Zielińska et al. 2021), so it was no surprise to find it doing so here. Of the other three taxa investigated, isopods fragmented PS foam the most consistently, though only weathered pieces. Wood and Zimmer (2014) observed the *Porcellio scaber* ingesting starch- and cellulose-based biodegradable plastics, though this is to our knowledge the first record of terrestrial isopods fragmenting conventional plastics. Crickets have previously been shown to consume polyurethane foam (Khan et al. 2021); our study demonstrates their ability to fragment PS as well. The lack of fragmentation by snails was surprising, as Song et al. (2020), one of few other papers investigating plastic fragmentation by soil animals, observed significant microplastic production by the snail *Achatina fulica*. Our conflicting results could be due to our choice of a different snail species and/or the fact we gave the snails much less time in contact with the plastic, 24 h rather than 4 wk. Interestingly, both isopods and crickets fragmented a different one of the two weathered plastics significantly more than the pristine plastic, but not the other. The cause of this is unclear and a more thorough investigation of individual species' preferences within plastics of the same type is likely warranted.

Though UV exposure did not lead to higher plastic fragmentation in our isopod-only experiment, it is known to sensitize plastic to microbial colonization (Vimala and Mathew 2016, Wei and Zimmermann 2017). Many common soil microbes are known to colonize plastics (Kale et al. 2015), which may lead to increased palatability of plastic as it does for dead plant material (Cummins

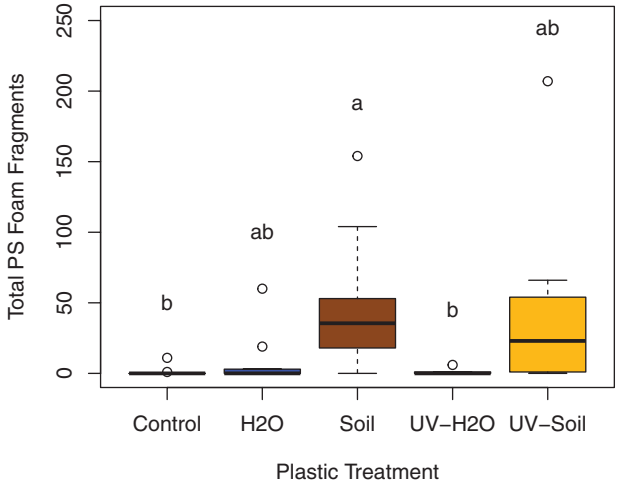


Fig. 5. Total numbers of PS foam microplastic particles found in isopod *Oniscus asellus* fecal material, arena sand, and isopod cadaver biomass, across all five PS foam treatments (untreated control, water only, soil suspension only, UV light and water, and UV light and soil). Different letters denote PS foam treatments from which the isopods produced significantly different particle numbers. Lower and upper whiskers represent dataset minimums and maximums, respectively, excluding outliers.

1974, Digel et al. 2014, Potapov et al. 2019). That said, we did not directly quantify microbial biomass on the plastic pieces in either experiment, so this remains a question for future research, both with respect to macroplastic debris and microplastic particles. Also, our plastics' immersion in the soil suspension may have resulted in microbially-colonized soil particles clinging to the plastic rather than colonization of the plastic itself, but how much this distinction matters is debatable, since rain spatter off the soil surface could replicate the effect in natural settings.

As discussed above, visual detection methods, even augmented by fluorescent staining, cannot locate microplastic particles below a certain size threshold. Our microplastic counts may thus be underestimations, if large numbers of particles smaller than 10 µm were produced. Fully characterizing the size distribution of microplastic particles produced by biotic fragmentation is an important step for future research, as size is known to affect microplastics' bioavailability and toxicity to other organisms (Lehtiniemi et al. 2018, Fueser et al. 2019). Slight overestimation may have come from us grinding the invertebrates' cadavers prior to digestion, which could have caused additional fragmentation of PS foam particles present within. However, the proportion of microplastics recovered from the cadavers as opposed to feces or arena sand was low in most cases, so production of additional fragments is unlikely to have significantly altered our total microplastic counts.

Microplastics' interactions with soil organisms are a potentially important piece to understanding the true impact microplastics have on the soil ecosystem (Helmberger et al. 2020b). Fragmentation of large plastic debris could facilitate uptake of microplastics by other organisms, perhaps especially if the newly created microplastics are passed through the gut of the fragmenting animal and chemically altered or coated with organic material. We did not conduct chemical analyses of excreted microplastics in our study, though studies with snails (Song et al. 2020) and tenebrionid larvae similar to *Z. morio* (Yang et al. 2015) demonstrated chemical changes, including depolymerization of PS molecules, following fragmentation. Along with incorporation into fecal material itself, these chemical changes could increase the microplastics' bioavailability.

Conclusions

Our results show that the propensity of soil macroinvertebrates to fragment PS foam debris and create microplastics varies by taxa, and within certain taxa such as isopods, depends on the condition of the plastic. These results add to our understanding of potential biotic sources of soil microplastics and conditions leading to their formation. Future work should assess the field prevalence of biologically-fragmented or biologically-altered microplastics as well as any unique ecological properties of these particles compared to microplastics produced via physical weathering. These properties could mediate microplastic uptake by and effects on terrestrial organisms.

Supplementary Data

Supplementary data are available at *Journal of Insect Science* online.

Acknowledgments

We would like to thank Keith Koonter, Charlotte Schuttler, Ariana Hernandez, and Robin Fischer for logistics and laboratory assistance, Caspian Bonee for providing snails, and Win Cowger for methodological and spectral analysis advice. Funding for this study was provided by the Sustainable Michigan Endowed Project and Michigan State University Graduate School.

Author Contributions

MH: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Validation, Visualization, Writing – original draft, Writing – review & editing. LT: Conceptualization, Resources, Writing – review & editing. JM: Methodology, Resources, Writing – review & editing. MG: Conceptualization, Resources, Supervision, Writing – review & editing.

Data Availability

Microplastic count data from all treatment replicates, negative and positive controls, and blanks are provided in the Supp Information (online only).

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