



## Differences in microplastics in passerine feces across species, diet, and foraging location

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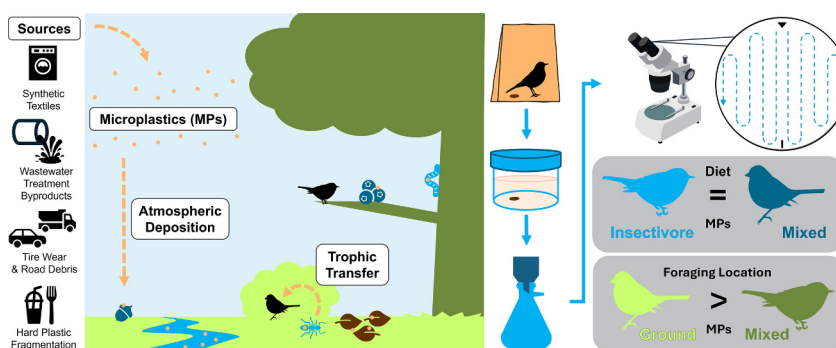
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### HIGHLIGHTS

- Microplastic (MP) exposure varies among passerine species and may be influenced by diet or foraging location.
- Ground foragers had more total MPs in their feces than birds that forage in both ground and arboreal habitats.
- MP fibers were the most common shape and clear was the most common color of MP across all species.
- Insectivores had a higher contribution of MP fragments versus fibers in their feces, as compared to birds with mixed diets.

### GRAPHICAL ABSTRACT



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### ABSTRACT

Environmental microplastics (MPs) are ubiquitous contaminants with potential to harm organisms, including birds. Birds are exposed to MPs, although the mechanisms through which they ingest MPs are currently unclear. Examining the gut contents or feces of birds has provided insight into their ingestion of MPs, but previous research has put relatively little focus on passerines, which are the largest and most diverse order of birds. We collected fecal samples from five species of passerines which vary in their diets and foraging locations: Common Yellowthroat *Geothlypis trichas*, Gray Catbird *Dumetella carolinensis*, Northern Cardinal *Cardinalis cardinalis*, Wood Thrush *Hylochichla ustulata*, and White-throated Sparrow *Zonotrichia albicollis*. We quantified and characterized MPs in fecal samples, and used procedural and field blanks to account for MP contamination. We found MPs in samples from all five species. Ground foraging birds had more MPs in their feces by both count and density. While all species had mostly fiber-shaped MPs in their feces, insectivorous birds had more fragment-shaped MPs compared to birds with mixed diets. Transparent was the most common color of MP across all species. Our results suggest that MP exposure differs between species based on characteristics of their feeding behavior, and thus certain species of passerines may be more at risk than others of the deleterious effects of MPs on fitness. Moving

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forward, bird feces could be used to monitor MP presence in the environment, but it is important to consider these differences among species when designing monitoring efforts.

## 1. Introduction

Microplastics (MPs; plastic particles less than 5 mm in size) are an emerging contaminant with the potential to expose organisms to toxic compounds and disrupt physiological processes (Thompson et al., 2024). Following the steady increase in both plastic production and plastic waste, MPs have become a ubiquitous contaminant in marine, freshwater, terrestrial, and atmospheric environments (Kallenbach et al., 2022). Environmental MPs are of concern for several reasons. The surfaces of MPs provide both a substrate and a mode of transport for potentially harmful microbes (Kirstein et al., 2016). Plastics themselves are often made with toxic compounds and can adsorb organic compounds, leading to the leaching and distribution of endocrine disruptors and carcinogens (Aib et al., 2025). These compounds can potentially be spread further and more widely on MPs than they would on larger particles.

Environmental microplastics may be categorized according to origin (Frias and Nash, 2019), shape, and color. Primary MPs are plastic particles which are intentionally manufactured at less than 5 mm in size. Secondary MPs are plastic particles that are generated via the deterioration of larger pieces of plastic as a result of chemical reactions, physical weathering, photodegradation, and degradation by biota (Zhang et al., 2021). Once plastics degrade to less than 5 mm in size, they are classified as secondary MPs. Shape and color can be helpful for inferring potential sources of MPs. Commonly reported MP shapes include fibers (long and thread-like, often with an even diameter), fragments (irregularly shaped), and beads (spherical) (Lusher et al., 2020; Rosal, 2021). Color is widely variable for MPs, but transparent (colorless) and blue are common in environmental samples (Fox et al., 2024; Martí et al., 2020).

Terrestrial biota at multiple trophic levels encounter microplastics (Thompson et al., 2024), and birds around the globe ingest and egest MPs (e.g., Carlin et al., 2020; Hoang and Mitten, 2022; Provencher et al., 2018). What remains unclear is how birds' diets (the food items they primarily feed on) and foraging locations (the areas in which they primarily forage for food items) affect the quantities, shapes, and colors of MPs they ingest. Few studies have compared differences in MPs sampled across species with different diets (e.g., Carlin et al., 2020;) or with differing foraging guilds or locations (e.g., Caldwell et al., 2020; Mylius et al., 2023). These studies tend to sample birds of prey, wading birds, and seabirds, leaving out smaller terrestrial birds.

Two potential pathways by which terrestrial birds may ingest MPs are atmospheric deposition onto food items and trophic transfer from prey. Atmospheric deposition is a natural process by which small particles or dissolved matter that have entered the atmosphere in one location get transported and later fall out of the atmosphere to the Earth's surface in another location. Atmospheric deposition is a major pathway for MP transport (Fox et al., 2024) that could lead to accumulation of MPs on trees, shrubs, and open fields. These are common foraging areas of frugivorous, granivorous, and some insectivorous birds, presenting a potential pathway for MP ingestion.

Due to their small size, MPs can be easily ingested by small animals that serve as prey for birds, including aquatic macroinvertebrates (Kolenda et al., 2020).

Macroinvertebrates, such as juvenile forms of mayflies and crane-flies, ingest MPs (Windsor et al., 2019), and retain MPs in their gut contents when they metamorphose into aerial adult forms (Simakova et al., 2022). These aerial insects are common prey items of insectivorous birds living near freshwater bodies (Gray, 1993), thereby providing a second potential pathway for MP exposure. Soil invertebrates can also contain and transfer MPs to their predators (Mustafa et al., 2025).

Detection of MPs ingested by birds has often been accomplished by sampling gut tissue (Carlin et al., 2020; Hoang and Mitten, 2022), which requires obtaining already deceased birds or sacrificing birds. This is not ideal, as bird populations worldwide are suffering declines (Lees et al., 2022). Alternatively, researchers can use the much less invasive method of sampling fecal material and regurgitate (D'Souza et al., 2020). Again, most studies of MPs in birds have included fecal samples or gut contents of seabirds, wading birds, and waterfowl (e.g. Caldwell et al., 2020; Provencher et al., 2018), which coincides with the historical focus on plastic pollution in coastal and marine ecosystems. Relatively few studies have quantified MPs in the fecal samples of terrestrial passerines (e.g. Charles et al., 2024; Sherlock et al., 2022).

Sampling from birds of the order Passeriformes (also known as passerines, songbirds, or perching birds) could be an option for monitoring MPs in terrestrial and freshwater environments (Deoniziak et al., 2022; Sherlock et al., 2022) due to their abundance and the relative ease with which fecal samples can be obtained. Passerines like the White-throated Sparrow (*Zonotrichia albicollis*) defecate several times in one hour (Birds of the World, 2025), and birds readily defecate during the banding process. There are many bird banding programs and bird observatories which already use long-term, standardized, and constant capture efforts like mist-netting (Dunn, 2016). Because of their established workflows and wide geographical distribution, bird banding stations provide a unique opportunity to quantify MPs from a variety of species non-invasively and with robust sample sizes.

Traits related to songbirds' feeding behavior, like diets and foraging locations, are likely to influence their exposure to MPs. To assess the risks of MPs to songbirds and the food webs they occupy, and to inform targeted conservation efforts, we must better understand the effect of these feeding traits on MP exposure. In this study, we quantified the MPs in the feces of passerine species to investigate whether the quantities, shapes, and colors differ based on species, diet, and foraging location. Specifically, our research sought to answer the questions:

- Q1 How does MP quantity differ between species, diets, and foraging locations?
- Q2 How do the proportions of MP shapes differ between species, diets, and foraging locations?
- Q3 How do the proportions of MP colors differ between species, diets, and foraging locations?

## 2. Methods

### 2.1. Study location & species

This study focuses on species of passerines frequently captured at Rushton Woods Banding Station, which is operated on a portion of Rushton Woods Preserve, an 86-acre preserve that is a stopover site for migratory birds in Newtown Square, Pennsylvania, USA (39.984468, -75.486236; Fetterman, 2017). The species included in this study are Common Yellowthroat ("COYE", *Geothlypis trichas*), Gray Catbird ("GRCA", *Dumetella carolinensis*), Northern Cardinal ("NOCA", *Cardinalis cardinalis*), Wood Thrush ("WOTH", *Hylocichla mustelina*), and White-throated Sparrow ("WTSP"). These species represent a variety of diets and foraging locations and readily defecate during the banding process, facilitating sample collection.

Each species was categorized by diet (Insectivore or Mixed Diet) and by foraging location (Ground or Mixed Foraging) (Table 1), based on their spring feeding behaviors reported in Birds of the World (2025). "Mixed Diet" refers to species that eat a mixture of insects and fruits/grains, and do not primarily rely on only one of them. "Mixed Foraging"

**Table 1**  
Study species and their diets and foraging location.

Species	Species Code	Diet	Foraging Location
Common Yellowthroat	COYE	Insectivore	Mixed Foraging
Gray Catbird	GRCA	Mixed Diet	Mixed Foraging
Northern Cardinal	NOCA	Mixed Diet	Mixed Foraging
Wood Thrush	WOTH	Insectivore	Ground
White-throated Sparrow	WTSP	Mixed Diet	Ground

refers to species that spend time foraging in both ground and arboreal habitats.

## 2.2. Fecal sample collection

At Rushton Woods Banding Station, birds were captured using passive mist netting. All mist nets were purchased from Avinet with a net size of 30 mm and a denier/ply of 70/2. Some mist nets were made of polyester, and some were made of nylon. Sixteen nets were stationed around forest edges, meadow edges, and hedgerows (high canopy and dense brushy understory). Nets were opened at dawn and checked every 40 min. Species were identified at the time of capture by federally permitted ornithologists using established plumage and morphometric criteria (Pyle, 2022). Captured birds that belonged to one of our study species were removed from the nets and gently placed in brown paper bags (labelled with species, date, time, net number, and band ID number) until they were processed at the banding station. Birds were kept in the bag for ~15 min to provide time for them to defecate. If they defecated in the bag, the bag with the fecal sample was transported to the research space at West Chester University (WCU), where it was transferred to a glass jar on the date of collection (Fig. 1-a). All bird handling was performed in accordance with Jennifer Uehling's WCU IACUC protocol #202403 and Rushton Woods Banding Station's

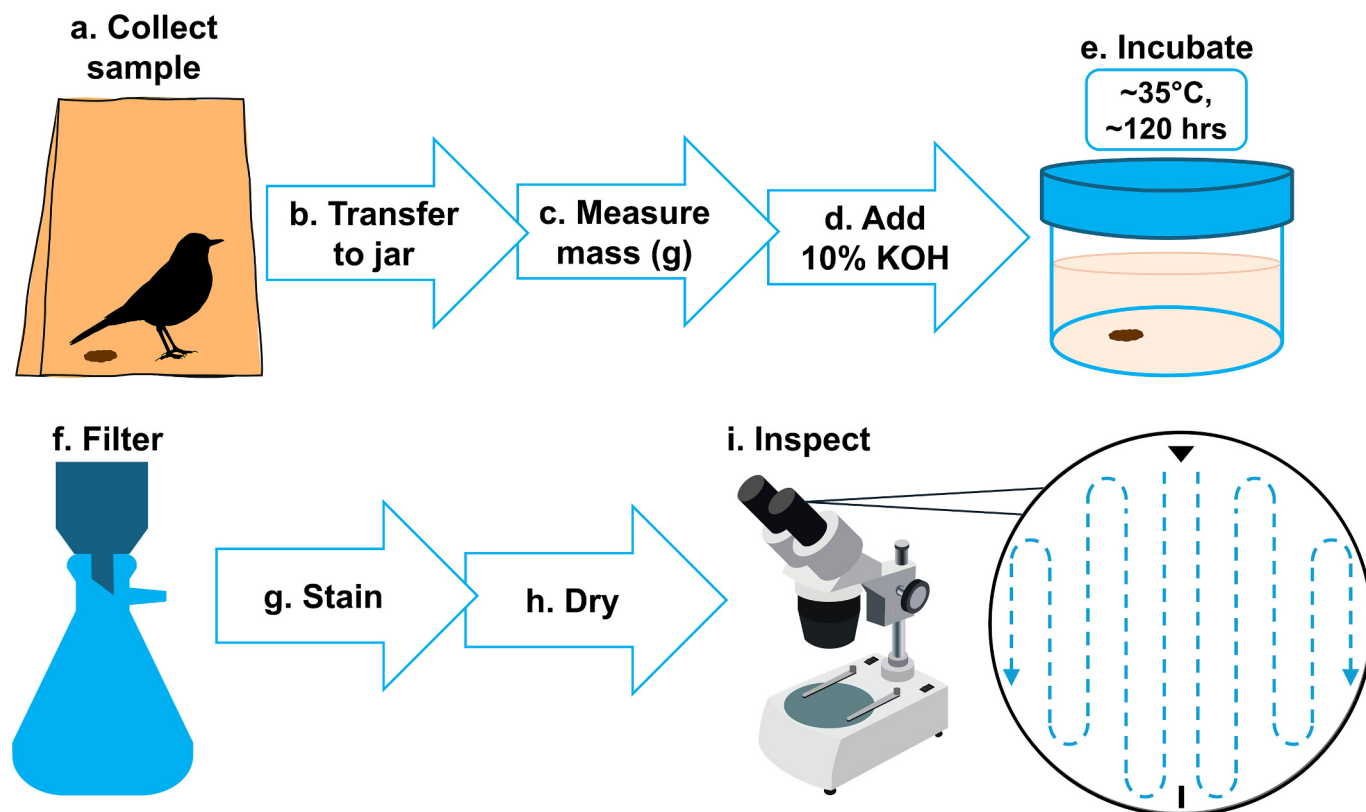
Pennsylvania state permit (#60982) and USGS federal bird banding permit (#24467).

Fecal sample collection took place during regular bird banding sessions at Rushton Woods Banding Station between April 9 and May 16, 2024 ( $n = 69$ ), the window for spring bird migration. Additional GRCA samples were collected on May 21 ( $n = 11$ ) and additional WOTH samples were collected on June 4 and June 19 ( $n = 3$ ) during the summer breeding bird banding sessions. If an individual bird was recaptured and provided more than one sample on the same day, the samples were processed individually but the data were combined before statistical analysis. If an individual bird was recaptured and provided a sample on a different day, that sample was considered a unique sample. Recaptured birds were identified using their unique band ID numbers.

## 2.3. Fecal sample processing

Upon return to the lab on the date of collection, each fecal sample was transferred to a glass jar by emptying and scraping the contents from the inside of the paper bag for 3–3.5 min using clean metal tools (Fig. 1-b). Once in jars, fecal samples were stored at  $\sim 4^\circ\text{C}$  until processing for MP extraction, which took place from May to September 2024. Samples were not dried prior to processing, therefore wet mass was used. At the start of processing, the mass of each sample was measured using a microbalance (Fig. 1-c). First, the lid of a jar was placed on the microbalance and tared. Then the lid was replaced, the jar was upturned so the sample sat in the center of the lid, and the lid was placed back on the microbalance. Mass was determined by subtracting the mass of the lid from the mass of the lid with the sample. If the resulting mass was negative, the mass was considered to be below the resolution limit of the microbalance and was assigned a value of 0.005 g.

To extract MPs, fecal samples were first digested using a 10% KOH solution. KOH is commonly used to digest organic materials, allowing for the extraction of MPs, such as from tissue samples (Pérez-Guevara



**Fig. 1.** Fecal sample processing workflow. 1-i depicts the pattern for visually scanning glass fiber filters. A triangle and line were drawn on the margins of the filter using a fine-tipped marker to denote the top and bottom, respectively. One half of the filter was systematically scanned at a time.

et al., 2021). Samples were digested at 34–37 °C for ~120 h (Fig. 1-d, 1-e). Temperatures of 40 °C or lower prevent degradation of polymers, even after long incubation periods (Thiele et al., 2019). In this study, a long incubation period was required to fully digest the often densely-packed samples. Digested samples were then vacuum filtered onto 47 mm diameter GF/F filters with a particle retention of 0.7 µm (Cytiva Whatman Binder-Free Glass Microfiber Filters GF/F Circles) (Fig. 1-f). This may have led to a loss of any MPs smaller than 0.7 µm, which would likely have been too small to be detected through stereo microscopy alone.

A 200 mg/L Rose Bengal dye solution was applied to the filter for 5 min and then rinsed with filtered DI water (Fig. 1-g). Rose Bengal is a bright pink dye which stains organic materials and can be used to differentiate them from plastic, since plastic polymers are not typically stained by Rose Bengal (Gbogbo et al., 2020). However, Rose Bengal staining has been noted to have limited reliability, with the dye either not staining organic structures like chitin and cellulose, or sometimes staining synthetic materials like polystyrene (Lares et al., 2019; Ribeiro et al., 2024). Thus, we did not rely solely upon Rose Bengal staining for identification.

Larger samples and samples with more debris were split onto two or three filters to aid in visual identification and differentiation of MP particles. Filters were dried for 24 h at 34–37 °C before identification and counting of MPs (Fig. 1-h). Filters were systematically scanned under a dissecting microscope (Fig. 1-i), and MP particles in the digested and dyed sample were identified visually based on observation of morphology (e.g., surface texture, luster, thickness), staining by Rose Bengal dye, and most conclusively by their behavior in response to a hot needle (Beckingham et al., 2023) (Fig. 1-i). In the presence of a hot needle, microplastics melt or sway towards the needle, while natural materials have no visible reaction, burn, or appear to waver randomly. We recorded the shape (e.g., fiber, fragment) and color of each MP and tallied to obtain the total number of MPs per sample (Appendix A).

#### 2.4. Quality control

Due to the ubiquity of MPs outdoors and indoors, care must be taken to avoid contamination in the field and in the lab (Pérez-Guevara et al., 2021). We wore clothing made primarily from natural fibers (e.g., cotton, wool) during bird handling and cotton lab coats during active laboratory processing to avoid MP contamination from clothing. Only non-plastic tools were used with samples, and these tools were rinsed with 0.45 µm-filtered DI water before and after each use.

Samples were processed in a lab with HEPA filtration and were kept covered except during active handling to avoid deposition of MPs from indoor air. All reagents were prepared using 0.45 µm-filtered DI water. The sample filtration period was restricted to June through August 2024, when there was less foot traffic in the lab, to reduce the amount of movement and introduction of MP contamination. At the beginning of June, the benches and floor in the lab were cleaned with a hand vacuum and wiped with a cellulose dishcloth and DI water. We also wiped the nearby lab surfaces using a cellulose dishcloth and DI water each day before and after sample filtration or MP counting.

Some amount of plastic contact or MP contamination could not be avoided. The lids of the jars used for sample storage included a synthetic lining, but fragments of this lining material were identifiable under the microscope and were excluded from MP counts. Nitrile gloves were used when filtering samples to prevent skin contact with KOH and again during MP identification. Plastic (LDPE) squeeze bottles were used to store and dispense filtered DI water for mixing reagents and rinsing tools, but introduction of MP fragments from LDPE bottles is unlikely (McNeish, pers. comm.).

We quantified rates of background contamination in the field and lab by creating procedural and field blanks. Procedural blanks were prepared by opening clean, empty jars in the lab for 3 min. They were then subjected to the same process as all other samples starting at point C

(Fig. 1). A total of 10 procedural blanks (roughly 1 for every 10 fecal samples) were created, which is common among other studies involving fecal samples (Pérez-Guevara et al., 2021). Field blanks were prepared using bags that contained a bird which did not produce a fecal sample. These bags were emptied into a jar for 3 min in the same manner as though a fecal sample were present, then subjected to the same process as all other samples starting at point C (Fig. 1).

#### 2.5. Data analysis

All analyses were conducted using R (v4.4.3; R Core Team, 2025). We analyzed both the total count of MPs per sample and the count of MPs per mg of fecal sample (i.e., density) as response variables. The means of each of these responses were calculated for each species, diet, and foraging location.

To answer Q1 (How does MP quantity differ between species, diets, and foraging locations?), we performed Kruskal-Wallis tests to detect whether mean MP count or density (count per mg of fecal sample) differed among species. For post-hoc testing, pairwise Wilcoxon tests were used to identify pairwise group differences. To detect whether mean MP count or density differed by diet or foraging location, we used Wilcoxon rank-sum tests. For Q1, MP count was corrected for each sample by subtracting the mean number of MPs in both procedural and field blanks prior to density calculations and statistical analyses, representing an overall conservative correction, since field blanks were also subject to lab contamination during processing. For any corrections that resulted in a negative value, the value was set to zero.

To answer Q2 (How do the proportions of MP shape differ between species, diets, and foraging locations?) and Q3 (How do the proportions of MP colors differ between species, diets, and foraging locations?), we performed a set of Chi-square Tests of Independence between shape (or color) and species, diet, or foraging location. We used the package “chisq.posthoc.test” to run post hoc analyses using the Bonferroni method (Ebbert, 2019).

#### 2.6. Sensitivity analysis for MP density in Q1

For 22 of the 83 fecal samples, mass was not measured before sample digestion. In these cases, an average mass was calculated for the species based on the other samples and applied to the samples lacking a mass measurement. A sensitivity analysis was performed to determine if using average mass measurements influenced the results of Kruskal-Wallis tests and Wilcoxon sum-rank tests in Q1. To perform the sensitivity analysis, we calculated the masses at the 10th, 50th, and 90th percentile for the species-specific distributions of fecal sample masses. We then calculated the MP densities using each of these representative mass values and performed each analysis using these three sets of calculated MP densities to see how results differed based on our assumptions of missing mass measurements (Table B.1, Appendix B).

### 3. Results

#### 3.1. Blanks and corrections for background MPs

Field blanks were obtained only for GRCA, NOCA, and WTSP. For those species, their respective mean MP count from field blanks were subtracted from each sample (GRCA mean = 2.0 MP,  $n = 5$ ; NOCA mean = 0.667,  $n = 3$ ; WTSP mean = 0.667,  $n = 3$ ). The count of MP in field blanks did not differ among species (Kruskal-Wallis  $\chi^2 = 3.595$ ,  $df = 2$ ,  $p = 0.166$ ). Therefore, for WOTH and COYE samples, we subtracted the overall mean MP count of all field blanks (1.11;  $n = 10$ ). The mean count of MP for procedural blanks was 1.4 MP ( $n = 10$ ).

We collected 83 fecal samples, which contained a total of 227 MPs. Mean fecal sample mass for each species, after correction, was: COYE = 6.88 mg, GRCA = 38.2 mg, NOCA = 18.9 mg, WOTH = 56.5 mg, and WTSP = 20.0 mg.

3.2. (Q1) how do MP count and density differ between species, diets, and foraging locations?

Mean MP count differed among species (Kruskal-Wallis  $X^2 = 15.127$ ,  $df = 4$ ,  $p = 0.004$ ). Specifically, the MP count in fecal samples from COYE and GRCA were significantly lower than WTSP (Fig. 2-a). We found no significant difference in MP count between birds with insectivore (mean = 0.758) versus mixed diets (mean = 1.43;  $W = 597$ ,  $p = 0.522$ ; Fig. 2-b). Birds that foraged primarily on the ground had a significantly higher count of MPs in their feces (mean = 3.49) as compared to birds that used mixed foraging locations (mean = 0.458;  $W = 973$ ,  $p < 0.001$ ; Fig. 2-c).

The patterns in MP density between species, diet, and foraging location were similar to patterns in MP count. Mean MP density (MP/mg) differed between species (Kruskal-Wallis  $X^2 = 13.195$ ,  $df = 4$ ,  $p = 0.010$ ), with MP density for WTSP significantly higher than GRCA ( $p = 0.004$ ; Fig. 2-d). We found no significant difference in MP density between insectivorous birds (mean = 0.071) and birds with mixed diets (mean = 0.100;  $W = 637.5$ ,  $p = 0.877$ ; Fig. 2-e), but samples from birds that foraged primarily on the ground had a significantly higher MP density (mean = 0.199) than birds that used mixed foraging locations (mean = 0.054;  $W = 941.5$ ,  $p = 0.001$ ; Fig. 2-f).

3.3. Sensitivity analysis for MP density results

For all representative values of fecal sample mass we compared (10th, 50th, and 90th percentile), the calculated MP density differed between the two foraging locations and did not differ between the two diets. Similarly, MP density of WTSP samples was significantly higher than GRCA samples across all representative values for fecal sample

mass.

3.4. (Q2) how do the proportions of MP shape differ between species, diets, and foraging locations?

Overall, fibers were the most abundant shape of MP particles across all species, diets, and foraging locations (Fig. 3). Specifically, 89.9% of individual MPs ( $n = 204$ ) were fibers while 10.1% ( $n = 23$ ) were fragments. We did not detect any other shapes of MPs in fecal samples.

The relative proportion of MP shapes differed among species ( $X^2 (4, N = 227) = 12.017$ ,  $p = 0.017$ ), with COYE samples containing significantly more fragments than other species' samples ( $p = 0.037$ ; Fig. 3-a).

Insectivorous birds had a significantly higher percentage of fragments in their fecal samples (19.6%) compared to birds with mixed diets (7.7%) ( $X^2 (1, N = 227) = 4.413$ ;  $p = 0.036$ ; Fig. 3-b). However, MP shape did not differ between birds that foraged primarily on the ground and birds that used mixed foraging locations ( $X^2 (1, N = 227) = 0.314$ ,  $p = 0.575$ ; Fig. 3-c).

3.5. (Q3) how do the proportions of MP colors differ between species, diets, and foraging locations?

Across all species, diets, and foraging locations, transparent was the most abundant color of MP particles, with 44.5% of MPs being transparent (Fig. 4). Another 24.7% of MPs were black and 13.2% were blue. Pink MPs were also abundant in NOCA (10.0%), WOTH (10.5%), and WTSP (8.5%) samples.

The proportion of different colors of MP did not differ among species ( $X^2 (40, N = 227) = 37.23$ ,  $p = 0.596$ ), diets ( $X^2 (10, N = 227) = 6.703$ ,  $p = 0.753$ ) or foraging locations ( $X^2 (10, N = 227) = 12.993$ ,  $p = 0.2241$ )

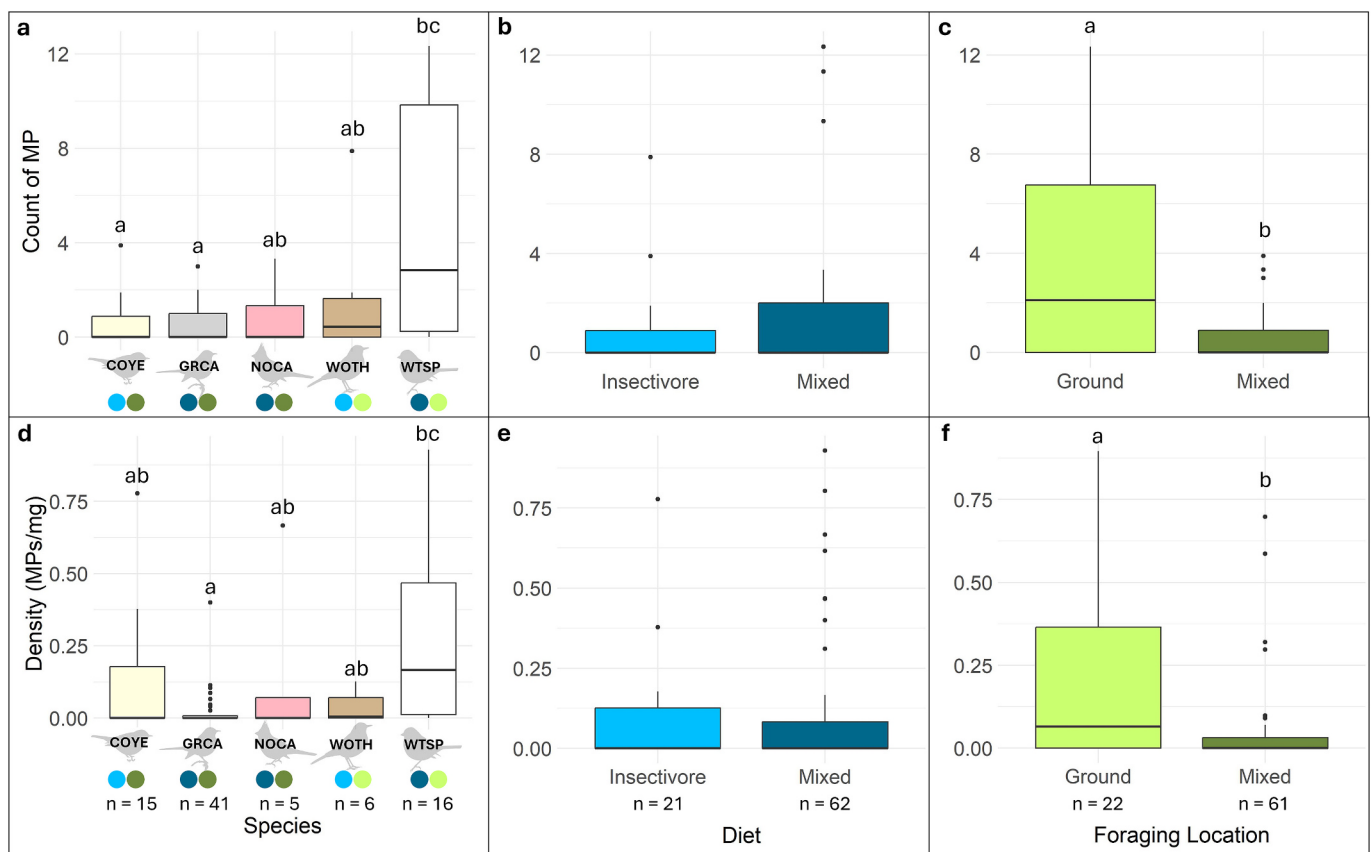


Fig. 2. Box plots comparing (a) MP count among species ( $p = 0.004$ ), (b) between diets ( $p = 0.522$ ), and (c) between foraging locations ( $p < 0.001$ ), as well as (d) MP density among each species ( $p = 0.010$ ), (e) between diets ( $p = 0.877$ ), and (f) between foraging locations ( $p = 0.001$ ). Colored dots underneath each species indicate their respective diet and foraging location.

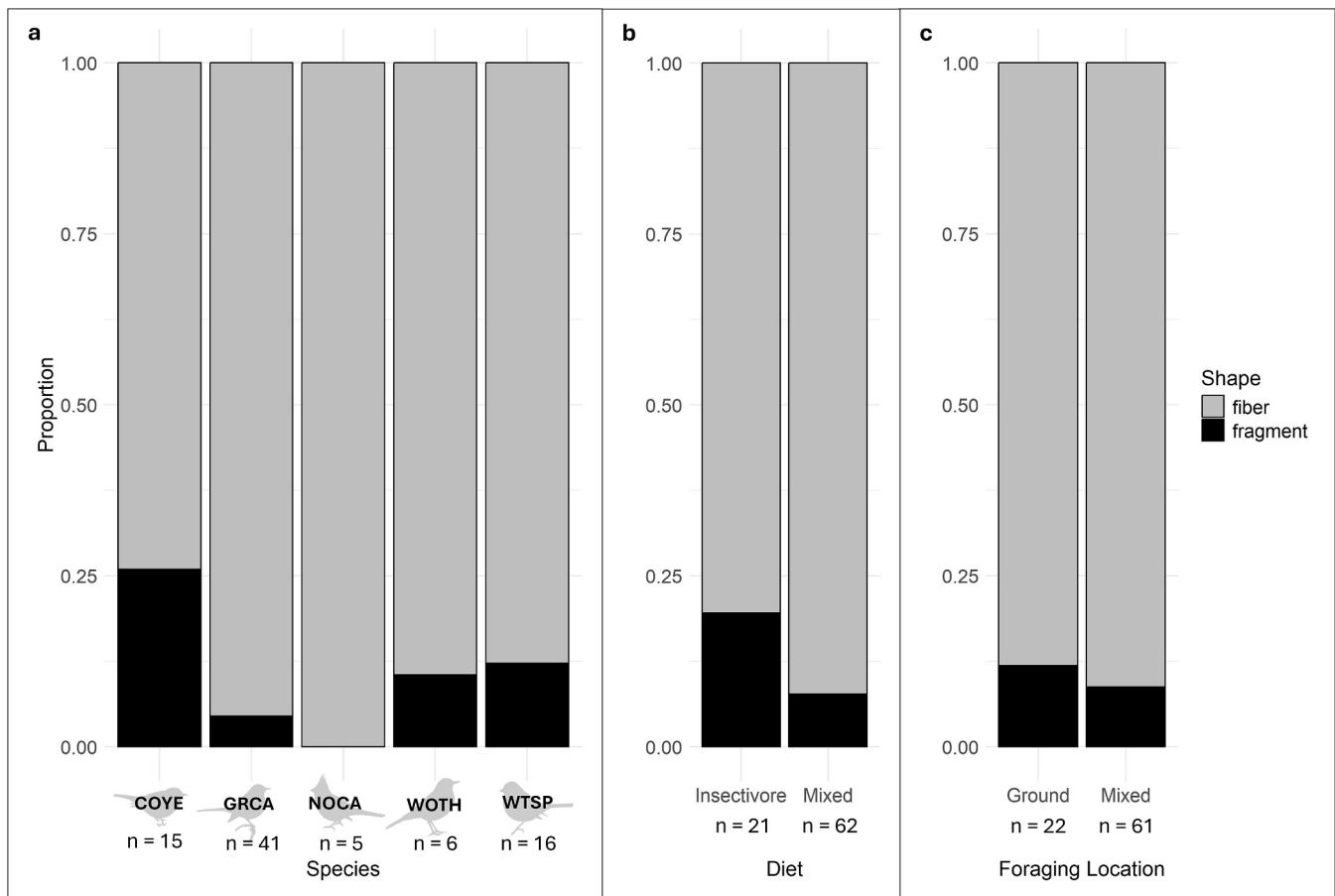


Fig. 3. Stacked bar graphs of frequency of each MP shape for (a) each species, (b) both diets, and (c) both foraging locations. Sample size (n) reports the number of individual birds in each group.

(Fig. 4-a-c).

Comparing between the two MP shapes detected, there was a larger proportion of pink fragments than pink fibers ( $\chi^2(10, N = 227) = 42.243, p < 0.001$ ) while white fragments nearly made up a larger proportion than white fibers (post hoc,  $p = 0.06$ ) (Fig. 4-d).

#### 4. Discussion

##### 4.1. Ground foraging birds have higher MP count and density

Birds who primarily forage on the ground had both a higher count and a higher density of MPs in their fecal samples, and these results were robust to assumptions we made about missing fecal masses. In this study, ground foragers were NOCA, WTSP, and WOTH. These species forage in leaf litter and expose the ground substrate to find prey by kicking (WTSP; [Birds of the World, 2025](#)) or plucking (WOTH; [Birds of the World, 2025](#)) leaves aside. It is possible that, while foraging, these species encounter and ingest MP-contaminated substrates and prey.

While few studies have quantified MPs in leaf litter in terrestrial habitats ([Mustafa et al., 2025](#)), it has been proposed that terrestrial plants may act as a temporary sink for MPs, as certain morphologies of leaves (e.g. high surface area, trichomes, many leaflets) can make them apt at retaining MPs from atmospheric deposition ([Perera et al., 2024](#)). The White-throated Sparrow prefers areas with more cover and thus forages under dense shrubs, such as those in forest edge habitats ([Birds of the World, 2025](#)). This means individuals may be spending more time in habitats that accumulate more MPs due to the high surface area of leaves.

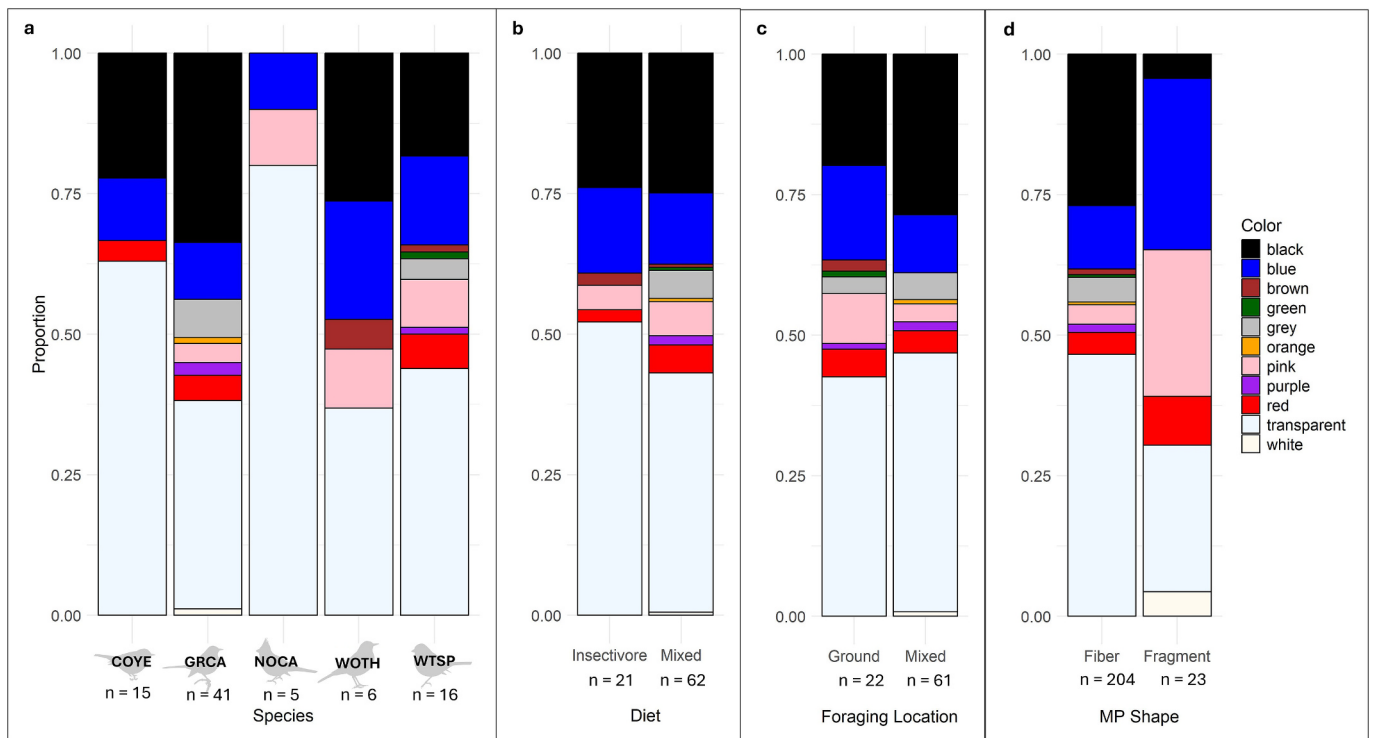
Both WOTH and WTSP have diets that consist at least partially of

insects, generally soil invertebrates they encounter on the ground. Soil invertebrates have been noted as a potential source of MP trophic transfer to their predators ([Mustafa et al., 2025](#)). However, we did not find evidence that potential trophic transfer of MPs resulted in higher MP count or density for birds whose diet consists primarily of insects versus birds with mixed diets.

##### 4.2. Fibers are the most common MP shape in bird feces

We found overwhelmingly more fibers than fragments in our samples, which is consistent with other observations of environmental MPs ([Fox et al., 2024](#); [Lusher et al., 2020](#)) and with microplastics collected over seven months in 2021 from the stream in Rushton Woods Preserve, where 95% of the MPs collected in streamwater were fibers ([McGrath, pers. comm.](#)). According to previous investigations, the ubiquity of MP fibers in the environment is, in part, because of the shedding of microfibers from synthetic clothing during laundry processes, and subsequent release of those MPs into household wastewater ([Aib et al., 2025](#)) and dryer exhaust. As part of the water treatment process, most MPs are removed from the water along with other contaminants, but they remain in the resulting sludge which may be applied to land as fertilizer ([Kallenbach et al., 2022](#)). Microplastics in land-applied sludge may then be transported to waterways in runoff or be entrained in the atmosphere (especially fibers), transporting MP pollution through the environment. These sources may be similar in our study system.

Insectivorous birds had a higher proportion of MP fragments in their fecal samples than birds with mixed diets, indicating that insectivorous birds may be encountering more fragments via their diets. Insectivores were represented by COYE and WOTH in this study, and COYE had more



**Fig. 4.** Stacked bar graphs of proportion of each MP color for (a) each species, (b) both diets, and (c) both foraging locations. Proportion of each MP color for each MP shape (d) is also shown. Sample size (n) reports the number of individual birds in each group (a, b, c) or the number of individual MPs of either shape (d).

fragments in their fecal samples than any other species. This might be explained if the insects they prey on also have a higher chance to be exposed to or to retain fragments. Previous studies have provided evidence for the trophic transfer of MPs from insects to insectivorous birds (D'Souza et al., 2020; Sherlock et al., 2022). Although studies have begun to find adverse effects of ingested MPs on insects (e.g., El-Kholly et al., 2024), research on the shapes of MPs retained by insects is still limited.

#### 4.3. MP color did not differ by species or feeding behavior and reflected typical environmental abundances

Studies of marine macroplastic ingestion show that animals are more likely to ingest items that are visually similar to typical prey items, including sharing a similar color (Martí et al., 2020). However, in our study, the color of MPs did not differ between species, diet, or foraging location. Although these results are exploratory, this may suggest that the birds in this study ingested MPs incidentally, rather than selectively by color because of similarities to prey. Fish have also been observed ingesting MPs incidentally through behaviors like feeding or breathing (Li et al., 2021). Further, if insectivores obtain MPs through trophic transfer, the color of MP is not visible to the bird since it is inside the prey item at the time of consumption.

The most abundant color of MP detected in fecal samples in this study was transparent (44.5%), followed by black (24.7%), and blue (13.2%). This is similar to the abundances of different colors of MPs in streamwater in Rushton Woods Preserve, where 26% of MPs were blue, 19% were black, and 12% were transparent (but 14% were red; McGrath, pers. comm.). Similarly, researchers have found white, transparent, or blue to be the most abundant color of marine MPs (Martí et al., 2020) and other environmental MPs subject to atmospheric transport (Fox et al., 2024). Transparent (i.e., colorless) may be a common color in plastics because plastic polymers are typically colorless before any colorants are added. In the natural environment or in water, colorless MPs may blend in the most easily, leading to higher likelihood

of unintentional ingestion by organisms. Further, differences in UV degradation among colors of plastics may age certain colors (like transparent, blue, and black) more quickly than others, producing more MPs of these colors in the environment (Zhao et al., 2022).

Among species, NOCA was one species observed to have an abundance of pink MPs. Coincidentally, these colors match with the colors of their feathers. However, our methods for differentiating natural particles from MPs, especially the hot needle test, meant that any feather fragments caught by the filters were unlikely to be misidentified as MPs. Rose Bengal, however, may have stained MPs. This could explain why there appeared to be a larger proportion of pink fragments than fibers if the dye stained the fragments more readily (Fig. 4-d).

#### 4.4. Potential limitations of this study

Studies involving MPs have inherent difficulties due to the ubiquitous nature of MP contamination in lab settings (Aminah and Ikejima, 2023). In this study, there is a possibility that MPs we detected were released from the outside of the bird (e.g., shaken from feathers while inside the bag) rather than exclusively from the fecal sample. Field blanks accounted for any shedding external MPs that may have contaminated fecal samples. External MPs could also lead to routes of ingestion other than diet, such as through preening or inhalation (Tokunaga et al., 2023). However, a higher measured count or density of MPs in this case would still reflect a higher overall exposure to MPs, regardless of how they were ingested.

Rose Bengal was used in this study to aid in distinguishing natural materials from MPs. The dye was supposed to stain organic materials, and thus allow for visual differentiation between MPs and non-MPs. However, the dye did not always consistently stain all organic materials, and possibly stained some MPs. Chitin debris fragments in filtered samples took on the Rose Bengal inconsistently, bringing into debate Rose Bengal's usefulness in differentiating MPs in this context.

While the hot needle test was very useful in identifying MP particles, it has its limitations (Beckingham et al., 2023). Thermoset plastics are

resistant to melting, which means any potential MPs encountered during this study made of a thermoset plastic might not have been identified as MP, leading to a lower overall MP count and possibly an underrepresentation of certain shapes or colors. Additionally, due to equipment and time limitations, we were not able to identify MPs by polymer type. This limited our ability to make assumptions about the sources of the MP pollution.

Chi-square Tests were chosen to compare the proportions of MPs across species, diet, and foraging location instead of Fisher's Exact Tests since the resulting contingency tables between variables were larger than two-by-two. However, Chi-square Tests may be less accurate when values in the contingency table are less than five, which was the case in some of our analyses (Franko et al., 2012). Sample sizes were uneven between species (e.g., GRCA  $n = 41$ , while NOCA  $n = 5$ ), which may have affected the accuracy of the chi-square tests for the species with smaller sample sizes. Because of these limitations, the results answering Q3 (How do the proportions of MP colors differ between species, diets, and foraging locations?) should be considered exploratory.

#### 4.5. Future research directions

Future studies should compare more foraging locations (e.g., arboreal only, aerial), or diets (e.g., frugivory, granivory). This would allow for a wider comparison of MP exposure across feeding behaviors to narrow down the routes of MP exposure. For instance, if insectivorous birds had more MPs in their feces than birds with mixed diets, and they each had more MPs than birds with granivorous diets, it may indicate a high potential for trophic transfer of MPs via insects. Further, direct environmental sampling from birds' foraging areas and food items to compare MP density would also help elucidate the routes of exposure to MPs. Understanding not only where these MPs originate (e.g., microfibers from wastewater byproducts) but how they are ingested by birds may provide insight on where to target management strategies that reduce MPs in the environment and therefore reduce MP exposure for birds.

The health impacts of MP exposure on birds are starting to be documented, including changes to gut microbiome, immune function, and growth rate in captive Japanese quail (Jing et al., 2024) and impairment of digestive and liver function in captive chickens (Yin et al., 2023). Meanwhile, studies on wild birds remain rare (Fackelmann et al., 2023). Although still understudied in terrestrial passerines, we would predict that MPs would have similar negative impacts at high concentrations.

Obtaining fecal samples can be easily integrated into the banding workflow, as it was at Rushton Woods Banding Station. Bird banding programs are typically annual efforts. If MP sampling was incorporated annually across multiple different banding stations, temporal as well as geographical trends in MP quantities could be tracked. However, sample processing, specifically MP quantification, can be time consuming, and researchers must be trained in MP identification. Emerging technologies that automate quantification (Primpke et al., 2020) may facilitate more widespread monitoring of MPs in birds and the environment.

Few studies have been conducted on MP abundance in the predators of these passerines, such as raptors (Carlin et al., 2020). Trophic transfer from macroinvertebrates to passerines is already a known phenomenon (D'Souza et al., 2020). As such, a better understanding of the MP accumulation versus egestion and gut residence time of MPs in passerines would be key in predicting trophic transfer to their predators. Aside from trophic transfer, birds themselves could be considered a vector in transporting MPs from one environment to another, especially in the case of migratory birds, like four of the five species in this study.

#### 4.6. Conclusion & implications

To our knowledge, this study is among the first to compare MP quantities, shapes, and colors in fecal samples of Mid-Atlantic passerine

birds across diets and foraging locations. Despite the stated limitations, this study provides early insights into the differences between bird species in their exposure to MPs. The species in this study differed in the amounts of MPs egested in feces, suggesting that traits relating to feeding behavior may influence MP exposure and ingestion. Future research, monitoring efforts, and conservation and management work should account for the feeding behavior of focal species and how that may influence MP exposure and risk. Microplastic exposure is ubiquitous in terrestrial birds (Carlin et al., 2020; Hoang and Mitten, 2022; Provencher et al., 2018), shedding further light on the need for stronger environmental controls on the proliferation of MPs in the environment. The species of birds included in this study, and many others frequently captured at banding stations, are considered "backyard birds." Their familiarity means they can serve as flagship species for connecting the issue of plastic pollution to the public and stakeholders interested in conservation efforts.

#### CRedit authorship contribution statement

**Victoria Moreira:** Writing – original draft, Visualization, Methodology, Funding acquisition, Formal analysis, Conceptualization. **Jennifer J. Uehling:** Writing – review & editing, Resources, Methodology, Conceptualization. **Alison Fetterman:** Writing – review & editing, Resources, Methodology. **Lisa Kiziuk:** Resources, Methodology. **Michelle A. Eshleman:** Writing – review & editing. **Abbie Ganas:** Methodology, Investigation. **Megan L. Fork:** Writing – review & editing, Resources, Methodology, Conceptualization.

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#### Declaration of competing interest

The authors declare no competing financial interests.

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#### Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scitotenv.2026.181681>.

#### Data availability

Data and code for this study are available through the Environmental Data Initiative (EDI) Data Portal at <https://doi.org/10.6073/pasta/52913f18f780071f5bdc4e019b4a824d>.

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