# Macroinvertebrate ecosystem engineering affects streambed retention of microplastics

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**Abstract:** Microplastic pollution of aquatic environments threatens human health, ecosystem processes, and biodiversity. Many existing models of microplastic movement in streams do not account for biotic effects on microplastic fate. Ecosystem engineering by net-spinning caddisflies (Hydropsychidae) has been shown to substantially affect sediment and organic matter transport as well as streambed hydrology. Caddisfly engineering may likewise affect the movement of microplastic pollution in streams. We used a controlled 11-d flume experiment to investigate the potential for caddisflies to serve as a biotic control on microplastic transport. Flumes containing a single gravel dune were randomly assigned to density treatments: control (0 caddisflies/$m^2$) or stocked with 500, 800, or 2500 caddisflies/$m^2$, incubated (d 1–10) to allow for caddisfly silk structure construction, inoculated (d 11) with PVC microplastics (333 µm–1 mm), and sampled (d 12). Microplastic was quantified as caught in a drift net (downstream transport), eaten by caddisflies (ingestion), or captured in caddisfly silk structures or settled into the gravel dune (i.e., total streambed retention). Mean downstream plastic transport was 9% lower than the control in the 800 caddisflies/$m^2$ treatment ($p < 0.001$) and 10% lower in the 2500 caddisflies/$m^2$ treatment ($p = 0.003$). Mean total streambed retention was 9% higher than the control in the 800 caddisflies/$m^2$ treatment ($p < 0.001$) and 10% higher in the 2500 caddisflies/$m^2$ treatment ($p = 0.004$). Ingestion of plastic by caddisflies was rare and highly variable (0–0.55% of plastic particles) but did increase with caddisfly density ($p = 0.002$). This work represents one of the first investigations of animal ecosystem engineering as a control on the movement and fate of microplastic particles in fresh waters and establishes a foundation for future research on biotic control of microplastic transport. Our results suggest that ecosystem engineering by net-spinning caddisflies may serve as a biotic control of microplastic transport in freshwater streams.

**Key words:** pollution, storage, fate, freshwater, benthos, transport, biotic control, hyporheic exchange, plastic cycle

Microplastics are a ubiquitous pollutant in aquatic environments, where they threaten human health, ecosystem processes, and biodiversity (Desforges et al. 2014, Sharma and Chatterjee 2017, Campanale et al. 2020). In freshwater streams, microplastics are found in surface water as well as trapped in the stream bed, an interface between surface flow, sediment, and groundwater that is crucial to our understanding of ecosystem processes and water quality (Helton et al. 2011, McCormick et al. 2016, Drummond et al. 2020). Recent research suggests that the proportion of microplastics transported downstream in the surface water of lotic systems may be overestimated by some existing models, and a greater fraction than previously thought may interact with the stream bed via hyporheic exchange (Besseling et al. 2017, Drummond et al. 2020).

Along with hyporheic exchange, biological processes are a potentially important control on microplastic fate, yet many existing models of microplastic movement in freshwater have not considered the role of biotic effects on microplastic fate (Besseling et al. 2017, Drummond et al. 2020, D'Avignon et al. 2021). Those studies that do explore biological effects on microplastic fate in freshwater often focus primarily on the bioavailability of plastics through the food web or on biofouling of microplastics. For example,

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studies of microplastic entering the food web have examined ingestion by macroinvertebrate filter feeders but focus primarily on understanding the factors driving microplastic ingestion and the corresponding adverse effects on the stress of individual organisms (Windsor et al. 2018, Silva et al. 2019, Bellasi et al. 2020, Wardlaw and Prosser 2020). Although uptake by food webs has been proposed as a part of the plastic cycle, the magnitude of this flux has not yet been thoroughly investigated, and substantial uncertainty exists about the persistence of microplastic particles in the bodies of consumers (D'Avignon et al. 2021, Hoellein and Rochman 2021). Additional studies have shown that the formation of biofilms on microplastic particles decreases their buoyancy and may lead to a higher likelihood of retention in the stream bed (Besseling et al. 2017, Kaiser et al. 2017, Leiser et al. 2020). However, although researchers have proposed that animal ecosystem engineering affects microplastic fate in freshwater systems (e.g., Larsen et al. 2021), this idea has not been thoroughly explored (though see Ehlers et al. 2020).

Ecosystem engineering by macroinvertebrates affects sediment and organic matter transport and can alter streambed hydrology (Statzner 2012, Albertson and Daniels 2018, MacDonald et al. 2021). Thus, microplastic particles that interact with the stream bed are also likely affected by macroinvertebrate ecosystem engineers, such as net-spinning caddisfly larvae (Hydropsychidae; hereafter caddisflies). Caddisflies are highly diverse, have a broad geographic distribution spanning 6 continents, and can be highly abundant where they are found (Statzner et al. 1999, Morse et al. 2019). Caddisflies may capture plastics directly through filter feeding, and their alterations to hydrology may enhance particle retention in the hyporheic zone of gravel-bedded streams (Juras et al. 2018, MacDonald et al. 2021), which may play roles in determining the fate of microplastic particles in the hyporheic zone. Understanding how ecosystem engineers affect the movement of microplastics through freshwater systems will be crucial for understanding the effects of microplastic pollution on freshwater ecology and in understanding the role freshwater systems play in transporting plastic pollution between terrestrial and marine ecosystems.

In this study, we asked how ecosystem engineering by caddisflies affects microplastic fate in freshwater systems. We hypothesized that ecosystem engineering by caddisflies increases retention of microplastic particles in the stream bed and simultaneously reduces their transport downstream. We also hypothesized that higher population densities of caddisflies would lead to higher retention and lower downstream transport due to increased ingestion of plastic by caddisflies and larger numbers of caddisfly silk structures. To test these hypotheses, we designed a laboratory experiment to quantify streambed microplastic retention and downstream transport across a range of caddisfly population densities.

## METHODS

### Flume setup

We used a randomized and controlled laboratory experiment to investigate caddisfly effects on microplastic transport. We set up 4 recirculating flumes (channel dimensions: $15 \times 22 \times 120$ cm) and ran 4 consecutive replicate trials for a total of 16 trials. Flumes were flushed with fresh water between each trial but were otherwise unaltered between replicates. In each flume, we stocked a heterogenous mixture of natural, stream-derived gravel that passed through a 45-mm sieve but was retained by a 5-mm sieve (diameter: mean = 40.5 mm, SE = 8.8 mm). The gravel formed a single dune, 75 cm long and 15 cm deep, at the apex in each flume (Fig. 1). We placed a 333-μm mesh collapsible bag underneath the base of each dune, which facilitated the removal of gravel and microplastic particles at the end of the experiment. Prior to plastic introduction, we also deployed a 333-μm-mesh drift net 25 cm downstream of the gravel dune in each flume to capture any plastics that passed through the gravel dune and returned to surface flow or that moved over the dune while suspended in the water column. The drift net allowed for measurement of the number of particles transported downstream over and through the dune and prevented particles from being recirculated to pass over the dune a 2nd time. We filled the flumes with water to cover the apex of the dune and set them to run at a velocity of 0.15 m/s, which we measured with a micro acoustic doppler

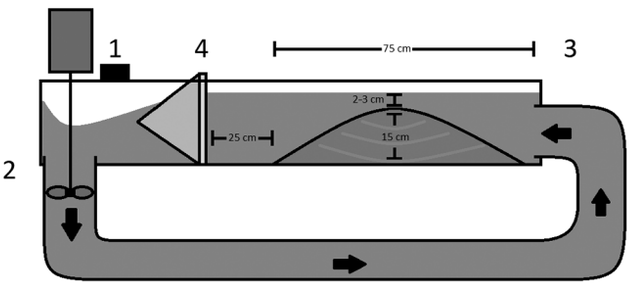


Figure 1. Diagram depicting the flume setup during an experiment to test the effects of caddisfly density on downstream transport and streambed retention of microplastics. The large rectangle, associated line, and propeller represent the flume pump. Black arrows indicate the direction of flow, and the shaded area under the curved line in the flume channel represents the gravel dune. Lighter curved lines in this region indicate hyporheic flow paths. Microplastics were distributed along the following route: 1) Plastic falls from the automatic fish feeder (represented by the small black rectangle) into the flume intake. 2) Plastic is drawn into the intake and circulated towards the outflow. 3) Plastic exits the outflow and is forced over the gravel dune. 4) Plastic not retained in the dune (i.e., not included in total streambed retention) is captured in the drift net, represented by the triangular shape in the flume channel (i.e., downstream transport). The drift net prevents the plastic from being recirculated. A color version of this figure is available online.

velocimeter (Vectrino Velocimiter; Nortek, Rud, Norway) flow tracker (3D down-looking laboratory model) at 0.6× the depth of the flume (9 cm) and 25 cm downstream of the dune. We ran flumes for 24 h after introducing gravel to allow the temperature to equilibrate and to ensure that gravel dunes were stable.

### Caddisfly stocking and flume incubation

After the first 24 h of operation, we turned off the flume pumps and randomly assigned flumes to 1 of 4 caddisfly density treatments: control (0 caddisflies/$m^2$) and 300, 800, or 2500 caddisflies/$m^2$. These densities fall within the range observed in natural streams in the Rocky Mountains of North America and can be easily maintained in the laboratory (Oswood 1979, Hauer and Stanford 1982, Valett and Stanford 1987). We collected caddisflies for this experiment by picking individuals from gravels in Bridger Creek, a 3rd-order stream located in Gallatin River Drainage near Bozeman, Montana, USA (lat 45.705925, long –111.005105). We identified all caddisflies as members of the genus *Hydropsyche* in the field and selected individuals that were of relatively uniform size (head capsule width: mean = 1.0 mm, SE = 0.03 mm, $n = 38$). The number of caddisflies collected exceeded the number required for the specified density levels, which ensured that some individuals could be retained to confirm identification in the laboratory using the key to larval Trichoptera found in the 4th edition of *An Introduction to the Aquatic Insects of North America* (Merritt et al. 2008). We returned the caddisflies to the lab, enumerated them, and evenly distributed them by hand across each gravel dune according to each flume's assigned density. Following caddisfly introduction, we returned power to the flume pumps and gradually increased the current velocity back to 0.15 m/s. We selected this velocity to fall within the range of velocities that allow *Hydropsyche* spp. to construct nets and retreats similar to those they build in natural streams (Tachet et al. 1992) and that would not result in obstruction to flow when the 333-µm-mesh drift nets were introduced to the flumes (Fig. 1). We ran the flumes for 10 d after caddisfly stocking, which allowed the caddisflies time to construct silk nets and retreats (MacDonald et al. 2021). The day after introduction (d 2), we fed caddisflies 300 mg of dried and crushed *Acer saccharum* leaves/flume to use as material for constructing retreats. We repeated this feeding on d 6 and 11. We also fed caddisflies 750 mg of powdered Algae Wafers™/flume (Hikari®, Hayward, California) on d 3, 5, 7, 9, and 11.

### Microplastic generation and characterization

We generated PVC microplastics by milling pieces of blaze orange PVC according to a modified method from Imhof et al. (2017). We selected blaze-orange-colored PVC to allow for easy differentiation of experimental microplastic particles from any potential contaminants. We initially created PVC pieces by cutting 1 × 1-cm squares from a length of PVC pipe. We then froze all pieces to –60°C overnight in falcon tubes before milling them with a metal burr-plate kitchen grinder (Cuisinart®, Stamford, Connecticut) (Imhof et al. 2017). We then sieved the resulting powder and discarded any particles >1 mm or <333 µm, restricting all particles to microplastic size (sensu Hanvey et al. 2017). We carried out microplastic production in a fume hood to reduce the possibility of aerial microplastic contamination of the laboratory.

The resulting microplastic particles had characteristics that were useful for this study. First, the variety of fragment sizes and shapes in this mixture mimicked the range of sizes and shapes found in natural microplastics more realistically than other common experimental analogues like plastic microbeads or thermoplastic pigments (Paul-Pont et al. 2018, Drummond et al. 2020). Next, the particles were negatively buoyant ($\rho = 1100–1470$ kg/$m^3$) and interacted with the hyporheic zone easily (Hanvey et al. 2017, Drummond et al. 2020). PVC microplastics are of considerably lower prevalence in streams than many other plastic polymers (e.g., polystyrene, polypropylene), but this negative buoyancy results in a high likelihood of interaction with the stream bed (Hanvey et al. 2017, Hoellein et al. 2017). Although using a mixture of many different polymers and shapes (e.g., fibers, films) would have been more environmentally realistic, the tendency of PVC to interact with streambed sediment made it ideally suited to the objectives of the experiment.

Following creation of the microplastic stock, we prepared the microplastics for introduction to the flumes. First, we weighed 3 samples on a precision balance and manually counted them under a dissecting microscope. Then, we used linear regression to estimate the number of microplastic particles introduced into a flume from their mass. To do so, we used the lm function in the base version of R (version 4.1.2; R Project for Statistical Computing, Vienna, Austria) to carry out a linear regression of the sample mass against the number of particles in the sample, with the intercept set to the origin. The regression produced a highly accurate model ($r^2 = 0.9985$, $p < 0.0001$). To simulate a stream with a plausible, documented surface-water concentration of 2.5 particles/$m^3$, we allotted each flume a total of 752 microplastic particles to be dispersed into the flume over a 24-h period (McCormick et al. 2016). We used a precision balance to weigh each sample of microplastics to ±0.00005 g. We then placed each sample in a labeled petri dish. Each petri dish was filled with 15 mL of algae-laden water, covered with a cover plate, and placed in a sunny spot on the lab bench for 10 d to facilitate the accumulation of biofilms on the outside of the particles (Parrish and Fahrenfeld 2019, Wu et al. 2019). We considered this inoculation necessary to simulate modifications to the physical properties

of microplastics, such as their buoyancy or ability to form heteroaggregates, which result from the growth of biofilms and affect the vertical transport of microplastics in natural systems (Rummel et al. 2017). The night before microplastic introduction, we used a 100-μm sieve to separate microplastic particles from the incubation solution and combined the particles with each flume's next ration of crushed *A. saccharum* leaves and algae wafers.

### Microplastic addition

On d 11, we added the mixture of microplastic particles, powdered algae wafers, and crushed *A. saccharum* leaves to each flume, all of which were in a separate room from the main laboratory to minimize the risk of contaminating samples. An automatic fish feeder added this mixture to the flumes at a constant rate over 24 h (CloserPets, Walton-On-Thames, England). The automatic fish feeder dispensed the mixture over the flume intake, allowing the mixture to be drawn in, carried by the current, and subsequently forced over the gravel dune (Fig. 1). One h before the end of microplastic introduction, we visually inspected each fish feeder and swept any minute remaining particle mixture into the flume intake with a fine-tip paintbrush.

### Microplastic sampling

Each microplastic particle recovered from the experiment could have 1 of 4 distinct fates at the time of flume disassembly: downstream transport, caddisfly capture, caddisfly ingestion, and settling samples. All plastic recovered from the drift net, which trapped plastic that passed over or through the gravel dune, was categorized as downstream transport samples. Plastic that was not transported downstream into the drift net was either trapped in caddisfly silk nets and retreats (caddisfly capture samples; Fig. 2), ingested by caddisflies (caddisfly ingestion samples), or settled out into the gravel dune (settling samples).

We disassembled and sampled flumes on d 12 after caddisfly introduction. The sole exception was the 800 caddisflies/$m^2$ flume in the $2^{nd}$ replicate, which experienced pump failure on the night of d 8, potentially leading to caddisfly mortality. We subsequently introduced microplastics to this flume on d 9 and sampled microplastics from the flume on d 10. We considered this trial to be valid because previous research has shown that most caddisfly silk in similar flumes has been constructed by the 10-d mark and silk is able to persist without caddisfly maintenance for prolonged periods (Albertson and Daniels 2016, Maguire et al. 2020).

We disassembled and sampled each flume following a standard procedure to assign the fate of each microplastic particle recovered to 1 of 4 categories: downstream transport, caddisfly ingestion, caddisfly capture, and settling. At the time of sampling, we turned off the pump powering each flume and removed the drift net. We turned the drift net inside out, washed it thoroughly over a 100-μm sieve and visually inspected it to ensure that all plastic had been removed. We then transferred the contents of the drift net to a 532-mL Whirl-Pak® bag (Nasco®, Atlanta, Georgia), which we froze at −18°C for later processing, comprising the downstream transport sample. Following the removal and washing of the drift net, we blocked both the intake and outflow openings of each flume to prevent plastic remaining in the dune from inadvertently being pushed into the recirculation pipe. We removed and individually inspected rocks from the dune to identify silk nets, retreats, and caddisflies, each of which was collected with fine-tip forceps. We used a desktop magnifier and flexible gooseneck light-emitting diode lights to assist in assessing nets, caddisflies, and microplastic particles. Caddisflies that were alive at the time of disassembly and caddisfly silk were placed in separate Whirl-Pak bags and preserved with 95% ethanol alcohol, comprising the caddisfly ingestion and caddisfly capture

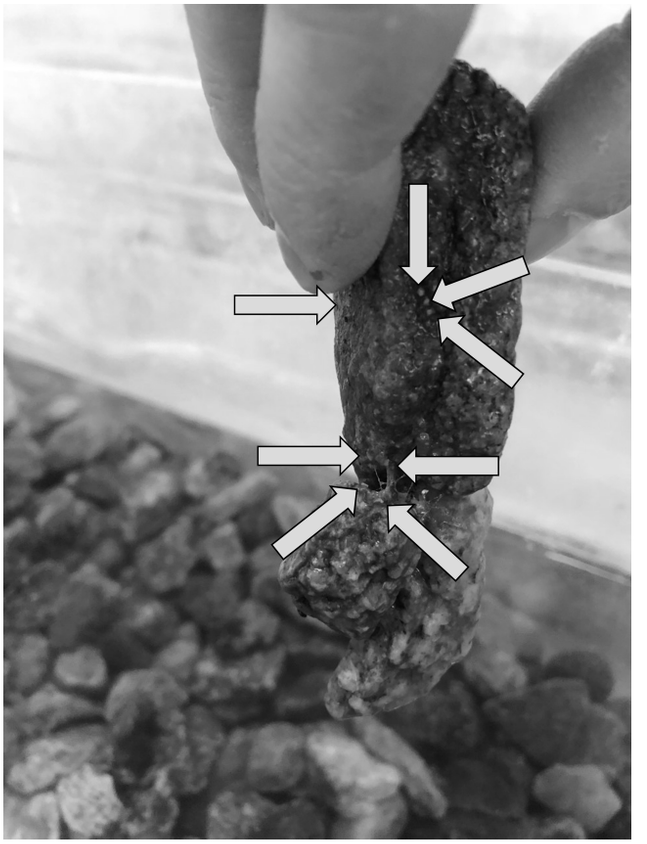

Figure 2. Caddisfly silk structures recovered from an experiment to test the effects of caddisfly density on downstream transport and streambed retention of microplastics. Several gravel particles (mean sediment size = 40.5 mm along the longest axis) are bound together by caddisfly silk. The light-colored fragments visible at the tip of each arrow are PVC microplastics. Photo: Dr Benjamin Tumolo, Department of Zoology and Physiology, University of Wyoming, Laramie, Wyoming. A color version of this figure is available online.

samples, respectively. We identified visually any microplastics that settled out within the dune but that were not attached to silk or caddisflies and removed them with the aid of forceps and a plastic transfer pipette then placed them in a Whirl-Pak bag. Following the disassembly of each dune, we used the collapsible mesh bag that had been placed under the gravel dune during flume setup to collect additional sediment and plastic that had settled to the bottom of the flume during dune removal. We then turned the mesh bag inside out, thoroughly washed it over a 100-μm sieve, and visually inspected it to ensure all plastic and sediment had been removed. We used a fish-tank net and transfer pipettes to remove any additional sediment that had not settled into the bag, then washed the resulting sample through a 100-μm sieve. We agitated and then thoroughly rinsed the gravels used for each dune to recover any plastic that had been missed during initial disassembly. We then combined the sieved sample and any additional sediment from the rinse with the plastics that had settled out of the dune and froze them at −18°C for later processing, comprising the settling sample.

Unexpectedly, we encountered frequent ambiguity concerning whether microplastics belonged in the settling sample or caddisfly capture sample. Microplastic particles were often found adjacent to caddisfly structures but not directly in nets or retreats. These particles may have been initially captured by caddisflies structures but subsequently dislodged. Furthermore, microplastic particles were sometimes found resting on caddisfly silk structures but were not tightly bound in silk. These particles may have simply settled onto caddisfly silk, rather than being actively incorporated into caddisfly retreats or captured in caddisfly nets. When classifying ambiguous particles into the settling or caddisfly capture samples, we gently probed the particles with forceps prior to removal. If there was resistance from, or movement of, caddisfly silk, we classified that particle into the caddisfly capture sample and classified the others as part of the settling sample.

## Sample processing

Following sample collection, we followed specific protocols to process each type of sample. For caddisfly ingestion and caddisfly capture samples, we removed each sample from its Whirl-Pak bag and placed the sample in an aluminum weigh boat. We counted caddisflies and carefully inspected them with a dissecting microscope to remove any microplastics that might be found on their exterior. Any plastic removed during this process was added to the caddisfly capture sample. We pooled all caddisflies from a caddisfly ingestion sample together for further analysis. We then dried both samples separately overnight at 90°C and recorded caddisfly dry mass after removal from the oven. Counts of stock microplastics dried overnight at this temperature did not show any evidence of loss or visible degradation of microplastic particles. We gently crushed the samples with a mortar and pestle then transferred them into a 15% solution of hydrogen peroxide, which was heated to 50°C overnight. Digestion in hydrogen peroxide removes organic matter from microplastic samples while leaving microplastic particle size and shape unchanged (Hanvey et al. 2017). We examined three 500-mL aliquots of the 15% hydrogen peroxide solution used for sample processing, which revealed no contamination with experimental microplastics.

To process samples not associated with caddisfly insects or structures, we removed downstream transport and settling samples from their Whirl-Pak bags, placed them directly into a 15% solution of hydrogen peroxide, and heated to them 50°C overnight. For settling samples, we repeated this digestion twice because they typically had larger quantities of leaf litter and algae than were found in other sample types. To ensure thorough separation of microplastics from sediment and debris, we washed the settling samples over a 100-μm sieve after the 2$^{nd}$ digestion and carefully transferred them to a density separator, which we constructed following the procedure of Coppock et al. (2017). The density separator was filled with a $ZnCl_2$ solution (concentration = 972 g/L, density = 1.5 g/cm$^3$), which allowed microplastic particles to float to the surface. We repeated this process 3× for each sample, with the $ZnCl_2$ solution being filtered, recovered, and reused each time. We confirmed the density of the $ZnCl_2$ solution between samples by weighing a known volume in a graduated cylinder. Blank samples were not run at the time of sample processing. Although we did not verify that the solutions themselves did not acquire microplastics during sample processing through the use of blanks, the easily identifiable nature of the orange coloring, filtration of $ZnCl_2$, absence of contamination from the $H_2O_2$ solution, and handling of microplastics under the controlled fume hood circumstances described above was considered sufficient to minimize the risk of any contamination of samples during processing.

## Sample imaging

To enumerate microplastics, we filtered each processed sample onto a GF/F (0.07-μm pore size, 4.7-cm diameter) glass micromesh filter (Whatman®, Maidstone, United Kingdom) and imaged the filtered sample under a dissecting microscope with a D60 camera (Leica, Wetzlar, Germany). In cases where substantial amounts of debris or large numbers of microplastics existed in a sample, we used multiple filters to allow clear images to be obtained. Prior to use, we gently marked a 1 × 1-cm grid with a fine-tip felt marker onto each filter used for a microplastic sample. During imaging, we photographed each section of the grid marked on the filter individually. Filters were retained in separate Whirl-Pak bags after imaging, allowing for capture of new images if the initial photographs proved unsatisfactory. We then assembled the photos of

each grid section into a single composite image of the entire filter using the GNU Image Manipulation Program (version 2.10.24; The GIMP Development Team, Bremen, Germany). Once composite photos were assembled, we enumerated microplastics using the cell counter plugin for Fiji (version 1.51; Schindelin et al. 2012) and recorded the number of particles.

### Statistical analysis

To assess the effect of caddisfly density on the amount of plastic contained in each sample type, we used grouped binomial generalized linear mixed models constructed with the glmer function from the *lme4* package (version 1.1-27.1; Bates et al. 2015) in R. Each model compared the number of particles recovered from the sample type of interest with the number of particles recovered from all other sample types combined. We assessed model assumptions by examining plots of the residuals generated with the allEffects function from the *effects* package (version 4.2-2; Fox and Weisberg 2019) on both the link and response scale (Fox 2003, Fox and Weisberg 2018).

Because of ambiguity in the assignment of particles to the proper sample, we did not conduct further statistical analysis on either settling or caddisfly capture samples individually. Instead, we combined these samples into a single category, termed total streambed retention, for further statistical analysis (but see Results for data on particle retention for each individual sampling category). For models of downstream transport and total streambed retention, we included the density of caddisflies initially stocked as a categorical fixed predictor. We chose to use the stocked number of caddisflies as opposed to final density because silk structures constructed in similar experimental flumes have been shown to persist for 60 d or longer, even after being abandoned by caddisflies (Maguire et al. 2020). However, because only caddisflies that were alive at the end of the experiment could have consumed plastic, we used the density of surviving caddisflies at the time of disassembly as a continuous, fixed predictor for the caddisfly consumption. We also included random effects for flume and replicate to control for the possibilities that differences between individual flumes, or that successive replicate trials being carried out in the same experimental setup, affected the outcome of the experiment. We omitted control observations from the caddisfly consumption analysis because control flumes contained no caddisflies. After fitting the downstream transport and total streambed retention models, we conducted pairwise comparisons between caddisfly density groups with Tukey's (HSD) honestly significant difference post hoc test with the glht function from the *multcomp* package (version 1.4-17; Hothorn et al. 2008). We used the theoretical method of the r.squaredGLMM function from the *MuMIn* package (version 1.43.17; Bartoń 2020) to generate pseudo $r^2$-values for the fixed-effect component of total streambed retention and downstream transport models. For the caddisfly ingestion model, we used the delta method to calculate the pseudo $r^2$-value.

## RESULTS

Higher densities of caddisflies corresponded to lower mean proportions of downstream plastic transport and higher mean proportions of total streambed plastic retention (Fig. 3). Specifically, our analysis provided strong evidence for a reduction in plastic transported past the dune and into the drift net in the 800 (9.0% lower mean proportion plastic recovered than control; $p < 0.001$) and 2500 caddisflies/m$^2$ treatments (10.1% lower than control; $p = 0.003$; Tables S1, S2). This difference was corroborated by Tukey's HSD test (Table S3, Fig. 4A). There was strong corresponding evidence for an increase in total streambed retention of microplastic in the 800 (8.9% higher mean proportion plastic recovered than control; $p < 0.001$) and 2500 caddisflies/m$^2$ treatments (9.9% higher than control; $p = 0.004$; Tables 1, 2). The increase in total streambed retention in the 800 and 2500 caddisflies/m$^2$ treatments was also corroborated by the Tukey's HSD test (Table S3, Fig. 4B). Although mean downstream transport was 5.4% lower than the control in the 500 caddisflies/m$^2$ treatment, and

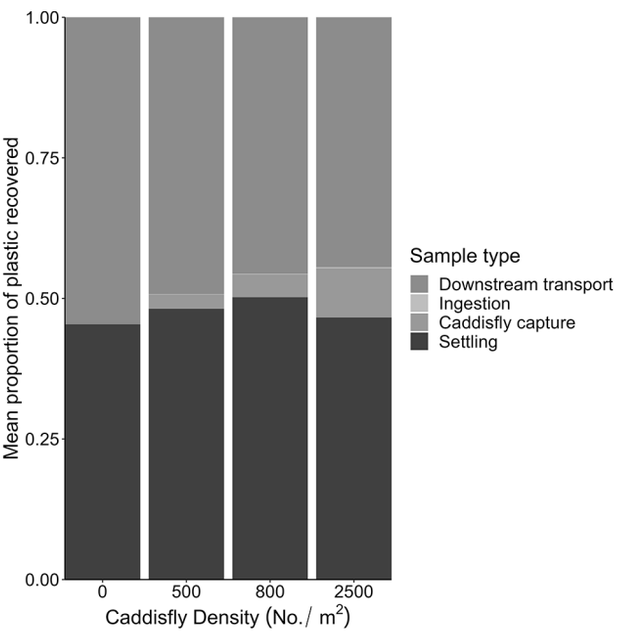


Figure 3. The mean proportion of plastic recovered from each sample type across stocked densities of caddisflies in an experiment to test the effects of caddisfly density on downstream transport and streambed retention of microplastics. Increasing stocked density of caddisflies corresponds to a lower mean proportion of plastic transported downstream and a higher mean proportion retained in the stream bed (caddisfly capture + settling). A color version of this figure is available online.

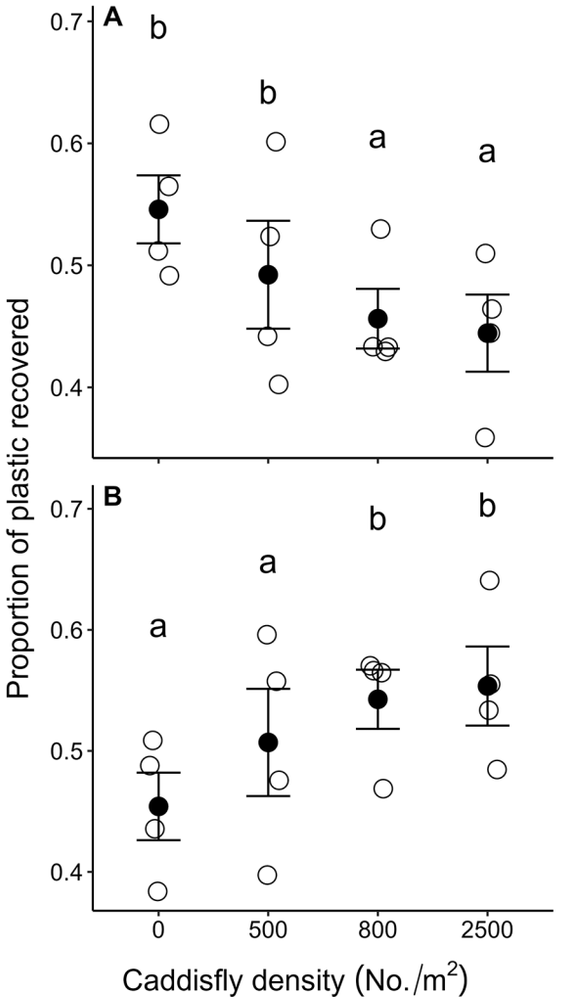


Figure 4. The proportion of plastic in downstream transport (A) and total streambed retention (B) samples in an experiment to test the effects of caddisfly density on downstream transport and streambed retention of microplastics. Lowercase letters correspond to caddisfly density treatment groups that differ (at the level of $p = 0.05$) as determined by post hoc Tukey's honestly significant difference pairwise tests following binomial generalized linear mixed modeling. Means are solid circles. Observations are open circles, which are jittered along the $x$-axis. Error bars represent SE.

total streambed retention was 5.3% higher than the control (Table S2), we cannot confidently report these differences ($p = 0.96$ and 0.97 respectively; Tables S1, S2, Fig. 4A, B). Downstream transport and total streambed retention at the 500 caddisfly/m$^2$ level were both highly variable (SD = 8.9% for both; Table S2), which likely contributed to our inability to confidently detect differences between that density level and the control.

Direct ingestion of plastic by caddisflies was also highly variable across the range of stocked caddisfly densities, and ingestion always represented an exceptionally small proportion of the total plastic recovered from each flume (0–0.54% of total plastic particles; Table S2, Fig. S1A). However, there was evidence that more plastic was consumed as final caddisfly density increased ($p = 0.002$; Table S1, Fig. 5). However, this relationship is driven in part by a large leverage point, where the highest final caddisfly density corresponds with the highest number of microplastic particles recovered. There was also a single observation of 0 microplastic ingestion at high caddisfly density. The number of microplastics consumed/dry mass of caddisfly tissue ranged from 0 to 0.03 microplastic particles/mg and from 0 to 0.06 microplastic particles/caddisfly (Table 1).

Total streambed retention was dominated by plastic retained in the streambed by settling into the dune, with a smaller contribution by capture in caddisfly silk structures (Table S2, Fig. S1B, C). Settling was responsible for estimated means of 45.4, 48.2, 50.2, and 46.6% of the total recovered plastic from the 4 density treatments (0, 500, 800, and 2500 caddisflies/m$^2$, respectively). Structures captured estimated means of 2.5, 4.1, and 8.7% of total plastic from lowest to highest caddisfly densities. These results reflect general trends, but capture in caddisfly silk and streambed settling, individually, were not statistically analyzed.

Numbers of caddisflies and microplastic particles at the end of the experiment differed somewhat from initially targeted numbers. Final counts of microplastics revealed that a larger, more variable number of particles than had been initially targeted were present in each flume (mean = 1818, SE = 92). Additionally, mortality during the experiment led to caddisfly numbers below the intended densities in all trials (Table 2).

## DISCUSSION

In this study, we hypothesized that ecosystem engineering by caddisflies increases streambed retention of negatively buoyant microplastic particles and simultaneously reduces their transport downstream. Our experimental results confirmed this hypothesis by providing evidence that caddisflies increased streambed plastic retention and decreased transport of microplastics to downstream habitats and that these effects were more pronounced in treatments with greater caddisfly density. These findings make an important contribution to the literature because they show that ecosystem engineering by an animal can affect microplastic dynamics in a laboratory experiment (Larsen et al. 2021). Though case-building caddisflies bind microplastic particles by using them to construct their cases, no previous work has investigated whether engineering by caddisflies that build net structures affects the overall amount of plastic retained in freshwater systems or transported to downstream habitats (Ehlers et al. 2020). Additionally, the magnitude of the reduction in downstream plastic transport documented here (mean = 10.1% at the highest caddisfly density) is similar to rates of plastic retention demonstrated

Table 1. The number of microplastic particles consumed by caddisflies/dry mass (mg) of caddisfly tissue and the number of microplastic particles consumed by caddisflies/no. of ind. in an experiment to test the effects of caddisfly density on downstream transport and streambed retention of microplastics. Caddisfly density reflects the stocked density of caddisflies at the start of the experiment. Max = maximum, min = minimum.

| | | Microplastic particles (no./mg) | | | | |
|---|---|---|---|---|---|---|
| Measure | Caddisfly density (no./m$^2$) | Min | Median | Max | Mean | SD |
| Dry mass of caddisfly tissue | 500 | 0 | 0.02 | 0.03 | 0.02 | 0.02 |
| | 800 | 0 | 0.02 | 0.03 | 0.02 | 0.02 |
| | 2500 | 0 | 0.01 | 0.02 | 0.01 | 0.01 |
| | | Microplastic particles (no./ind.) | | | | |
| | | Min | Median | Max | Mean | SD |
| No. of caddisfly ind. | 500 | 0 | 0.04 | 0.06 | 0.03 | 0.03 |
| | 800 | 0 | 0.03 | 0.05 | 0.03 | 0.02 |
| | 2500 | 0 | 0.01 | 0.05 | 0.02 | 0.02 |

by habitat-forming species (i.e., seagrasses, macroalgae, hard corals) in nearshore marine systems, reinforcing the plausibility and likely importance of this capture rate in natural systems (de Smit et al. 2021). Engineering by caddisflies could retain large numbers of microplastic particles in similar systems, even if caddisflies affect only a small percentage of the total downstream flux.

Some existing hydrologic models of microplastic movement in freshwaters predict that plastic particles in the size range used for this study are efficiently transported downstream in lotic ecosystems (Besseling et al. 2017). However, a variety of factors may influence the strength with which caddisfly engineering controls the number and type of microplastic particles that are either retained in or exported from freshwater systems. Caddisfly population density, diversity, and traits may determine the strength with which their engineering behavior affects microplastic particles. Additionally, the effect of caddisfly engineering on microplastic movement and fate may be contingent on seasonal or stochastic events. The influence of such events may not be apparent unless caddisfly interactions with microplastic particles are observed over broad temporal or spatial extents.

### Temporal and spatial patterns

Our laboratory experiment showed caddisfly engineering enhanced microplastic retention but was conducted over a relatively small temporal and spatial extent. Though we observed these results over only 24 h, it is possible that retention of plastic in caddisfly silk structures may provide a long-term sink for microplastic pollution in freshwater

Table 2. Stocked and final number of caddisflies recovered from replicate trials, along with the calculated % mortality, from an experiment to test the effects of caddisfly density on downstream transport and streambed retention of microplastics.

| Trial number | Stocked caddisfly density (no./m$^2$) | Stocked caddisfly count (no.) | Final count (no.) | % mortality |
|---|---|---|---|---|
| 1 | 500 | 56 | 43 | 23.2 |
| | 800 | 90 | 59 | 34.4 |
| | 2500 | 281 | 220 | 21.7 |
| 2 | 500 | 56 | 48 | 14.3 |
| | 800 | 90 | 53 | 41.1 |
| | 2500 | 281 | 236 | 16.0 |
| 3 | 500 | 56 | 43 | 23.2 |
| | 800 | 90 | 76 | 15.6 |
| | 2500 | 281 | 239 | 15.0 |
| 4 | 500 | 56 | 36 | 35.7 |
| | 800 | 90 | 50 | 44.4 |
| | 2500 | 281 | 168 | 40.2 |

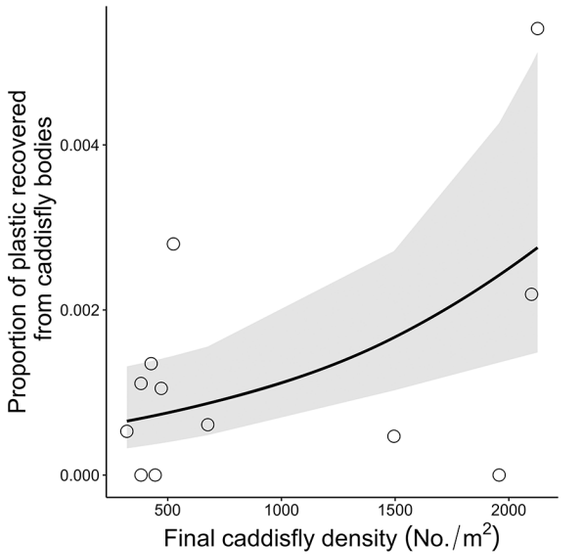


Figure 5. Proportion of plastic recovered from caddisfly ingestion samples in an experiment to test the effects of caddisfly density on downstream transport and streambed retention of microplastics plotted against final density of caddisflies (i.e., accounting for mortality over the course of the experiment). Open circles are observations, the black line is predicted values of a binomial generalized linear mixed model, and the shaded region corresponds to a 95% CI.

streams. Caddisfly retreats and cases can form large concretions that bind streambed sediments together, persisting long after such structures are abandoned by their builders, even appearing in the fossil record (Lewis 1972, Leggitt and Cushman 2001, Maguire et al. 2020). However, we acknowledge that the mean rate of streambed retention demonstrated at the highest caddisfly density would result in implausible rates of plastic capture if this storage were entirely permanent. Assuming each dune results in a 55.4% reduction to the amount of microplastic suspended in surface flow, 10 dunes would result in the capture of >99% of suspended microplastic particles:

$$1 - 0.554^{10} = 0.9972. \quad \text{(Eq. 1)}$$

Notably, this equation is based on retention rates observed for the negatively buoyant PVC fragments used in this experiment. PVC fragments are an uncommon type of microplastic in lotic environments, and it is reasonable to expect that the diverse set of microplastic shapes and polymers that exist in freshwaters would be differently affected by animal ecosystem engineering. Additionally, existing research conducted in the hyporheic zone of natural streams supports the conclusion that microplastic storage in streambed sediments is not entirely permanent. Drummond et al. (2020) found that 43% of the microplastic particles that entered a 150-m reach of hyporheic zone were resuspended in surface water within 15 d. Investigation of caddisfly control over both the rate of microplastic capture in the streambed and the rate of microplastic resuspension in surface water will be necessary to better understand how caddisflies affect the long-term fate of microplastics (Hoellein et al. 2019, Drummond et al. 2020). Given that microplastics are known to behave similarly to other types of particulate organic matter, the extensive literature that describes the spiraling of particulate organic matter will prove useful for such work (Hoellein et al. 2019).

The rate of resuspension observed by Drummond et al. (2020) was observed at base flow, but disturbance events associated with flooding (i.e., storms, spring runoff) may also mobilize streambed sediment, reduce the length of time over which microplastics are able to stay retained in the streambed, and speed the export of microplastics to marine ecosystems (McCormick et al. 2016, Song et al. 2020). The role of differing flow conditions in mediating biotic control over microplastic transport may prove to be a fruitful area for future research and may be influenced by geographic context (Windsor et al. 2018). For example, caddisfly control over microplastic movement may be of lesser importance for montane streams in western North America with a hydrograph dominated by seasonal flooding (e.g., snowmelt dominated) than for streams with more uniform annual discharge (e.g., humid, rainfall-dominated systems) typical of the southern United States. The lifecycle of caddisflies may also determine the relative importance of caddisfly control over microplastics. Seasonal changes in body size, silk net structure size, or abundance could be important predictors of caddisfly ability to retain microplastics in the stream bed (Wallace 1975, Oswood 1979, Hauer and Stanford 1982, Zuellig et al. 2004, Alexander and Smock 2005, Ogbogu and Adu 2011).

Spatial extent may also influence the effect of caddisfly ecosystem engineering on microplastic movement. The reductions in microplastic transport documented here took place over an artificial riffle with a length of only 75 cm. The effect demonstrated on this scale was strong, but plastic passing over a series of caddisfly-inhabited riffles in a natural stream may behave differently. It is worth noting some existing evidence that human-engineered structures, such as dams, do not cumulatively reduce the downstream flux of microplastic particles (Watkins et al. 2019). Whether the same is true for the structures built by animal ecosystem engineers is a promising topic for future research.

## Density dependence

Higher caddisfly densities could correspond to additional reduction in downstream transport, though it is uncertain whether this effect would continue to increase or plateau with additional density increases. Our caddisfly density treatments were within the range common in the Rocky Mountains (Oswood 1979, Hauer and Stanford 1982, Valett and Stanford 1987, McCarty et al. 2022). However, localized

densities of ≤6500 caddisflies/$m^2$ have been documented in Montana, with numbers as high as 70,000 caddisflies/$m^2$ known to occur within natural streams in other regions of the globe (Oswood 1979, Hauer and Stanford 1982, Valett and Stanford 1987, Statzner et al. 1999, McCarty et al. 2022). Although much higher densities of caddisflies are known to occur, the only increase in plastic retention in our experiment for which there was strong evidence occurred between the 500 and 800 caddisflies/$m^2$ treatment levels. The reduction in downstream plastic transport between the 800 and 2500 caddisflies/$m^2$ treatments was relatively small, and statistical support for a difference between the 2 population densities was weak, raising questions about whether caddisfly effects on microplastic transport are likely to grow stronger at higher densities. It is possible that microplastic retention increases rapidly with caddisfly density initially, but the effect of additional caddisflies on plastic movement may asymptote after a sufficient population density is reached.

### Caddisfly biodiversity and traits

The effects of ecosystem engineers on microplastic transport may differ depending on the aquatic insect assemblage present. Hydropsychid caddisflies are a diverse group, with multiple species co-occurring and building a variety of different silk structures (Morse et al. 2019). Although our experiment used a single genus of caddisfly (*Hydropsyche*), increased diversity of caddisfly assemblages at the genus level can lead to nonadditive increases in ecosystem function (see Cardinale et al. 2002, Albertson et al. 2014), which could correspond to stronger caddisfly effects on microplastic transport. These increased effects could occur given that co-occurring caddisfly genera engage in resource partitioning by constructing their nets and retreats in different microhabitats (Cardinale et al. 2002, Albertson et al. 2014). This resource partitioning prevents upstream individuals from blocking flow to the nets of downstream neighbors through current shading (Cardinale et al. 2002). Diversity-induced increases in the capture of suspended particulate matter like those documented by Cardinale et al. (2002) may translate directly to increased caddisfly capture of microplastic particles in silk structures. The diversity of caddisfly assemblages may likewise play a role in governing the proportion of microplastic particles that are incorporated into food webs because of caddisfly ingestion. Caddisflies engaging in resource partitioning can also weave nets with differing pore sizes, which are correlated with the size of their preferred food particles (Wallace 1975, Melas and Wallace 1977, Loudon and Alstad 1992, Cardinale et al. 2002). Although the proportion of plastic consumed by caddisflies during the experiment was low (always <0.55% of total plastic), the number of microplastics/mg of caddisfly dry mass closely matched mean values documented by the single previous study of microplastic ingestion in *Hydropsyche* (mean of 0.009–0.021 compared with 0.019–0.038 microplastics/mg found by Windsor et al. 2018).

It is also possible that the microplastics provided to caddisflies during the experiment were not ideally sized for ingestion. Wallace (1975) reported the traced surface area of particles from the guts of *Hydropsyche vernalis*, which ranged from a mean of 22,122 $\mu m^2$ for animal-derived particles consumed in the spring to as small as 2352 $\mu m^2$ for detrital particles consumed during the summer. Assuming these particles are roughly square, these average measurements translate to mean widths ranging from 149 to 48 μm along a single axis, much smaller than 333 μm, which were the smallest particles used for our experiment. Thus, larger caddisflies, or smaller plastics that are more palatable to *Hydropsyche*, might result in increased ingestion of microplastic.

Not only taxonomic identity and diversity of caddisflies in a system but also abiotic conditions may determine how strongly caddisflies affect microplastic movement. For example, caddisfly net structure varies across genera, but variation can also be linked to abiotic conditions such as water temperature and flow velocity (Loudon and Alstad 1992, Tachet et al. 1992). Loudon and Alstad (1992) showed that when current velocity increased from 0.05 to 0.45 m/s, some species of *Hydropsyche* produced more individual nets with greater surface area and smaller mesh size, which are all associated with increased capture rate of suspended particles (Loudon and Alstad 1992). On the other hand, the same experiment showed no relationship between changes in current velocity and either mesh size or surface area of the nets spun by *Cheumatopsyche* caddisflies (another genus of Hydropsychidae) (Loudon and Alstad 1992). Future experiments that manipulate factors such as temperature and current velocity are needed to fully understand interactions between abiotic conditions and the ability of ecosystem engineers to control microplastic fate.

### Assessing experimental conditions

Numbers of microplastics and caddisflies initially targeted in the experiment differed from final numbers, which may have affected our results. The difference between targeted and final microplastic numbers was explained by 2 primary factors. The 1$^{st}$ factor was that the mass–number regression that was used to estimate the number of microplastic particles being introduced to a flume was based on counts of microplastic particles that had not yet been exposed to water. Investigation of the microplastic stock following the experiment revealed that microplastic particles that clung together when dry, and thus were counted as a single particle, were more easily recognized as 2 separate particles when suspended in water. The 2$^{nd}$ factor was vertical stratification of microplastic sizes within the container in which they were stored. Although the microplastic stock was well homogenized when the mass–number regression

was 1st carried out, the microplastic stock was subsequently stored underneath a bench where the lab vacuum pump was in use. Vibrations from the operation of the pump appear to have resulted in large particles remaining at the top of the container, whereas smaller particles were concentrated near the bottom. Thus, the relationship between mass and number of microplastic particles differed somewhat depending on the depth a scoop was taken from within the stock container. However, the elevated number of microplastic particles that were consequently introduced into flumes still fell within the range documented from highly polluted streams, which can reach as high as 17.93 particles/$m^3$ in heavily polluted systems (McCormick et al. 2014). Furthermore, downstream fluxes of microplastic pollution $>10^6$ particles/d have been documented from natural streams (McCormick et al. 2016).

The final number of live caddisflies recovered from the experiment also differed from the introduced number of individuals. Some mortality is common when caddisflies are kept in captivity, largely because of competition between individuals for limited space and resources (Albertson et al. 2014). Although final caddisfly densities did differ from the targeted numbers, the silk structures produced by caddisflies that died after the initial introduction were likely able to persist throughout the entirety of the experiment (Maguire et al. 2020). We therefore believe that using categorical densities for the analysis of total streambed retention and downstream transport samples best reflects the nature of caddisfly ecosystem engineering and presence of silk, even though they may not have reflected the mortality observed during the experiment.

### Future directions

Our work provides a valuable proof of concept and a starting point for investigations of ecosystem engineering as a process affecting the fate of microplastic particles in freshwater systems. Additional considerations in future studies will help determine the magnitude and mechanism of caddisfly control over microplastic fates. Studies that explicitly investigate temporal and spatial context will be necessary to broaden our understanding of the results shown here. The mechanism underlying caddisfly control over microplastic movement also deserves further exploration. Separating which microplastic particles were retained in the streambed because of caddisfly structure capture and which were retained because of altered hydrodynamics and settling proved to be difficult. Despite these challenges, there was strong evidence that both caddisfly capture and settling result in an overall increase in streambed plastic retention.

Particular attention should be paid to understanding how caddisflies affect the transient nature of microplastic storage in the stream bed. Such studies will need to be carried out across a broad geographic range to elucidate the relative importance of caddisfly engineering to microplastic movement and fate in regions with a variety of different climates, macroinvertebrate assemblages, and hydrologic regimes. Examining diverse caddisfly assemblages to ascertain their ability to sequester microplastic has been previously proposed and has proven a worthwhile topic of research (see the work of Ehlers et al. 2019, 2020). Examining the effects of other ecosystem engineers, such as beavers and their impoundments, on microplastic and other pollution, as proposed by Larsen et al. (2021), may also prove a fruitful area for future work. Better understanding of the complex interactions between microplastic pollution and the biosphere will enhance the ability of researchers and managers to mitigate the negative effects of microplastic pollution on freshwater ecosystems.

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Author contributions: SFF, EJM, HCO, GCP, and LKA were responsible for conceptualization and planning of the experiment. SFF conducted setup of the experiment and monitored the flumes during the incubation period. SFF, LKA, and JLH sampled and processed the experiment. JLH conducted all microplastic image assembly and enumeration. SFF, LKA, EJM, and HCO conducted statistical analysis. SFF wrote the initial draft of the manuscript, and all authors contributed to subsequent revisions.

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Raw data and the code necessary to repeat the analysis are accessible on Dryad: https://doi.org/10.5061/dryad.2v6wwpzsz.

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