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RESEARCH ARTICLE

Transport and retention of microplastic fibers in streams are impacted by benthic algae, discharge, and substrate

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Abstract

Microplastics (particles < 5 mm) are pollutants of emerging concern in aquatic ecosystems worldwide. Streams are key sites of microplastic input, retention, and transport, and empirical measurements of microplastic movement in lotic ecosystems are needed to inform global microplastic budgets. However, factors that influence microplastic retention in lotic ecosystems are not well studied. We used particle spiraling metrics to directly measure microplastic retention following pulse releases of polyester fibers using outdoor, experimental streams lined with substrates of varying sizes. We tested the impact of benthic algae, stream discharge, and substrate type on the transport of experimentally added microplastic fibers. We also quantified microplastic retention in and release from the stream benthos after an increase in discharge to simulate a storm event. Microplastic deposition rates were significantly higher with (1) well-established benthic algal biofilms, (2) higher stream discharge, and (3) larger benthic substrate. The increase in microplastic deposition rates with elevated discharge is opposite the expected trend observed for particulate organic matter, indicating distinct retention processes for microplastics. A rapid increase in discharge in our experimental streams resulted in resuspension of retained microplastic from all substrate types, suggesting that storm events could trigger microplastic release in natural streams. The results from this study provide direct measurements of the magnitude and direction of factors that drive microplastic retention in streams, which will contribute to the parameterization of models for microplastic deposition (and release) at larger spatial and temporal scales for freshwater ecosystems.

Plastic is a versatile and ubiquitous material common in nearly all industrial sectors, including agriculture, construction, shipping, technology, and textiles. Plastic manufacturing began commercially in the 1950s and has since increased exponentially, with 8.3 billion tons of plastic produced between 1950 and 2015 (Geyer et al. 2017). A vast majority of this plastic (79%) has been disposed of in landfills or the environment (Geyer et al. 2017). Microplastics (< 5 mm) comprise a major component of plastic pollution (Rochman et al. 2019) and are ubiquitous in aquatic environments worldwide, including both freshwater (Eriksen et al. 2013; Castañeda et al. 2014; McCormick et al. 2014;) and marine ecosystems (Moore et al. 2001, 2002; Law et al. 2010; Doyle et al. 2011). Microplastics in aquatic ecosystems can originate from breakdown of larger plastic litter (Duis and Coors 2016), terrestrial runoff (Rochman et al. 2019), atmospheric deposition (Zhang et al. 2020), and release of treated or untreated wastewater (Wagner et al. 2014; Eerkes-Medrano et al. 2015; Mason et al. 2016; Kelly et al. 2021). Microplastics enter domestic wastewater through use of personal care products, including microplastic abrasives (Zitko and Hanlon 1991; Conkle et al. 2018), or through laundering synthetic textiles, which releases

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microplastic fibers (Napper and Thompson 2016; Carney Almroth et al. 2018). Fibers are often the most abundant microplastic in freshwater ecosystems (McCormick et al. 2016; Baldwin et al. 2016; Hoellein et al. 2017).

Microplastics are classified as a pollutant of emerging concern because they are persistent in the environment and enter food webs (Au et al. 2017). Microplastics can be ingested by a wide range of aquatic organisms, including fish (Lusher et al. 2013; McNeish et al. 2018), plankton (Katija et al. 2017), crustaceans, mollusks, and other taxa (Eerkes-Medrano et al. 2015). Once ingested, microplastics may negatively affect organismal health by disrupting digestion (Wright et al. 2013), reducing fecundity (Cole et al. 2015), causing physiological stress (Rochman et al. 2013; Barboza et al. 2018), and serving as a vector for harmful chemicals (Rochman et al. 2013; Barboza et al. 2018).

Streams are an important source of microplastics to marine, lacustrine, and wetland ecosystems (Dris et al. 2015; Hoellein et al. 2017), and they are also active sites of microplastic retention and transformation (Hoellein and Rochman 2021; Baldwin et al. 2016; Lenaker et al. 2019). However, the retention of microplastics in streams is a poorly understood component of global microplastic budgets (Hoellein et al. 2019). Notably, empirical measurements of instream transport and retention are time-intensive, resulting in a critical lack of data on the potential accumulation in the stream benthos relative to rates of downstream transport. Previous studies of microplastic transport in flowing waters have largely been model-based (Nizzetto et al. 2016; Kooi et al. 2018; Wagner and Lambert 2018; Petersen and Hubbart 2021); therefore, empirical measurements are required to constrain microplastic deposition and distribution in aquatic environments. As a solution to this data gap, spiraling metrics are well-developed tools for measuring retention and transport of solutes and particles in streams (Webster et al. 1999) and have been successfully applied to quantify microplastic dynamics (Hoellein et al. 2017, 2019). The concept of "spiraling" refers to the unique aspect of particle transport in streams and includes longitudinal and vertical movement in the water column, deposition to the streambed, resuspension, and additional downstream transport, which occur simultaneously with particle degradation (Webster et al. 1999; Hoellein et al. 2019). Microplastics represent a novel, manufactured form of allochthonous carbon, and using foundational stream ecology tools allows for direct quantification of microplastic retention and transport using experimental designs, terminology, and units already described for naturally occurring particles (Hoellein et al. 2019).

Previous measurements of microplastic spiraling have been generated by comparing benthic and water column concentrations along a longitudinal gradient (Hoellein et al. 2017; Vincent and Hoellein 2021) or via the addition of microplastics to experimental streams (Hoellein et al. 2019). Results from these studies show that microplastic transport dynamics are affected by properties specific to the particle, for example, polymer type and shape (Hoellein et al. 2019). However, no previous empirical studies of microplastic dynamics have assessed the influence of environmental factors, including flow conditions, streambed geomorphology, and benthic biofilm accumulation, on the retention of microplastics in streams.

Our objective was to determine how changes in benthic algal colonization, stream discharge, and benthic substrate size influence the retention and transport of microplastic fibers in experimental streams. Previous studies have shown that features such as benthic algal biofilms and macrophytes trap particulate matter in streams, which increases retention (Webster et al. 1994; Brookshire and Dwire 2003; Rovira et al. 2016; Shogren et al. 2020). Based on these foundations, we predicted that increased algal biomass would increase the retention of microplastics in streams. Based on current understanding of particulate organic matter (POM) transport, we also predicted that microplastics would be retained less effectively in streams with higher discharge (Brookshire and Dwire 2003; Larrañaga et al. 2003; Rovira et al. 2016). Lastly, we predicted that streams with larger benthic substrate size would retain more microplastics, since complexity in the stream bed increases interstitial spaces and bed roughness, creating more places for particle trapping (Jones 1997). A further goal of this study was to compare microplastic retention to the retention of other materials, which could be useful in developing models for microplastic deposition (and release) at larger spatial and temporal scales for freshwater ecosystems.

Materials and methods

Microplastic

We created microplastic fibers by cutting synthetic yarn (Discount School Supply) into segments of approximately 2 mm in length using razor blades and manually separating these yarn segments into individual fibers that we measured as approximately 10 μM in diameter using a microscope (Olympus BH2) and Image Pro Premier software (*see* Supporting Information Fig. S1 for images of yarn and microplastic fiber). We analyzed the yarn using a micro-Fourier transform infrared spectrometer (Spotlight 200i, Perkin Elmer) and identified the polymer composition of the yarn as polyester. Polyester density ranges from 1.24 to 2.30 g cm^{-3} (Guasch et al. 2022). We used six unique colors of yarn to avoid cross-contamination from replicate releases and to enable us to distinguish our experimentally added fibers from potential "background" contamination in the streams or source water. We generated a mass × fiber number regression by weighing and then counting the number of fibers in multiple subsamples using a stereomicroscope (AmScope, United Scope LLC). This regression enabled us to estimate fiber numbers based on mass. For each experimental addition (described below) we created a "microplastic slurry" containing approximately 300,000 individual fibers suspended in 400 mL of water from the field site (described below), which we briefly stored in a 0.5-L glass Pyrex bottle before each experimental release (Supporting Information Fig. S2). We created three extra

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slurries using identical methods and used subsamples of these slurries to confirm the number of fibers contained in each experimental slurry by counting the number of fibers in each subsample using a stereomicroscope, finding the average number of fibers per mL, and multiplying by the volume of the slurry.

Study site

We conducted experimental releases of microplastic fibers in four outdoor, experimental streams at the Notre Dame Linked Experimental Ecosystem Facility (ND-LEEF) located in Indiana, USA (Supporting Information Fig. S3). Streams are 60 m long, 3.5 cm deep, and 0.4 m wide, and are fed by low-nutrient groundwater that is pumped into an on-site reservoir and delivered to the streams via underground pipes. Streams are concrete-lined and can be filled with different substrates. These experimental streams have been successfully used to examine an array of particle and solute transport dynamics (Shogren et al. 2017; Shogren et al. 2019; Hanrahan et al. 2018; Hoellein et al. 2019).

We conducted microplastic releases in each of the four streams, dividing each stream into three sequential longitudinal reaches of approximately 16 m, which served as replicates for each treatment (Supporting Information Fig. S3; Hoellein et al. 2019). Before conducting experimental releases of microplastics, we estimated transport time for each reach using a conservative tracer solution (30 g NaCl in 400 mL of water) paired with conductivity measurements. Briefly, at the upstream end of each reach, we released the conservative tracer solution and measured breakthrough curves (BTCs) of conductivity (as $\mu S\ cm^{-1}$) every 15 s post-release at 5, 10, and 15 m away from the release site (Workshop 1990). We used these breakthrough curves to determine the sampling intervals for each downstream sampling station during microplastic releases.

Setup and colonization of the streams

In spring 2017, the four streams were lined with different substrates: cobble ($D_{50} = 5$ cm), pea gravel ($D_{50} = 0.5$ cm), sand ($D_{50} = 0.2$ cm), and an even mixture of the three (Supporting Information Fig. S4). Beginning in June 2017, we initiated natural colonization of the streams by benthic algae by maintaining constant baseflow discharge conditions ($0.9\ L\ s^{-1}$). Benthic algae colonized the streams under undisturbed conditions for approximately 5 months until we began our microplastic study (Supporting Information Fig. S5). During the entire colonization period and during the experimental microplastic releases, we measured ammonium, nitrate, and soluble reactive phosphorus concentrations and water temperature periodically for each of the streams at sites 10, 20, 30, 40, and 50 m downstream from the starting point of each stream (*see* Supporting Information Table S1). Ammonium, nitrate, and soluble reactive phosphorus concentrations were measured using a Lachat Quikchem 8500 Series 2 Flow Injection Analysis System (Hach Company). On the same dates, we measured photosynthetically active radiation (*see* Supporting Information Table S2) using a weather station deployed at the field site (EmNet, LLC). We characterized benthic algal coverage for each stream at the end of the colonization period (i.e., immediately prior to the start of our experiment) based on visual estimates. We scored algal coverage in streams in increments of 10%, categorizing coverage as filamentous algae, miscellaneous benthic algae, or bare substrate.

Experimental releases

We conducted a total of 36 microplastic releases in the experimental streams over 3 d in November 2017 to test the effects of three factors on microplastic retention: presence of benthic algae, stream discharge, and substrate type (Table 1). For each release, we added one microplastic slurry ($\sim$ 300,000 fibers in 400 mL of stream water) at the upstream end of the reach. Before each release, we shook the bottles to evenly disperse microplastics in the water, and we released the entire slurry as smoothly and quickly as possible (i.e., a slug addition).

On day 1, we conducted microplastic releases in each of the reaches ($N = 3$ replicate reaches) within each of the four streams, which were lined with different substrate types ($N = 4$ substrate types), at low discharge ($0.9\ L\ s^{-1}$) with benthic algae present ($N = 12$ total microplastic releases). For each release, we used different color microplastic fibers, and grab samples were collected at the 5, 10, and 15 m sampling stations downstream of the release site (Supporting Information Fig. S3). At each sampling station, we collected grab samples ($\sim$ 140 mL, $N = 20$) at regular intervals after the release (every

Table 1. Experimental design for microplastic releases*

Day	Substrates	Discharge	Benthic algae	Microplastic measurements
Day 1	Cobble, pea gravel, sand, mixture	Low	Present	Deposition length, deposition velocity, retention by benthic algae
Day 2	Cobble, pea gravel, sand, mixture	Transition from low to high	Present	Resuspension
Day 2	Cobble, pea gravel, sand, mixture	High	Present	Deposition length, deposition velocity, retention by benthic algae
Day 3	Cobble, pea gravel, sand, mixture	High	Absent	Deposition length and deposition velocity

*All releases were conducted in triplicate.

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15–30 s) timed according to results from the conservative tracer breakthrough curves.

At the end of day 1, we collected benthic samples to quantify microplastic retention within algae and substrate. We collected these benthic samples by placing 140 mL plastic specimen containers directly onto the bottom of the stream (with the open top of the container facing downward) and scooping the top layer of substrate and algae up into the container, resulting in sampling 28.3 cm^2 of the benthic surface. In each stream ($N = 4$), we took samples every $\sim$ 10 m longitudinally ($N = 5$ samples per stream). We repeated this measurement after day 2. In the lab, we measured the dry mass of the sample and quantified microplastic fibers.

On day 2, we quickly increased stream discharge to 2.7 L s^{-1} to simulate a storm event, which was a simple process of manipulating a valve, so it is a relatively sudden change. We took advantage of this manipulation and collected grab samples ($N = 36$) of approximately 140 mL from the water column at the downstream end of each of the four study streams to estimate resuspension of fibers. To do so, we visually assessed when the discharge of the stream was increasing at the downstream end and began taking samples at regular intervals (every 30 s to 1 min) until we had taken 36 samples. We then conducted a second series of microplastic releases in the four streams ($N = 3$ reaches within each of four streams; $N = 12$ total releases) at high discharge (2.7 L s^{-1}) with benthic algae present, following the protocol described for day 1.

On day 3, we manually removed benthic algae from all streams to mimic an intense biofilm scouring event (Supporting Information Fig. S5). We dislodged algae from the stream bottoms with our feet, hands, and using rakes. We worked from upstream to downstream in $\sim$ 10 m longitudinal sections. We collected the algal biomass and stored it in ziplock bags for transport to the lab. In the lab, we put the biomass in a drying oven at 55°C for $\sim$ 72 h until the weight had stabilized and recorded that weight as total dry mass per 10 m of stream length. We then conducted another set of releases in all four streams ($N = 3$ reaches within each of four streams; $N = 12$ total releases) at high discharge (2.7 L s^{-1}) in the absence of benthic algae following the protocol described for day 1.

Microplastic processing

We had two types of samples for microplastic processing: water column ($\sim$ 140 mL grab samples) and benthic. We processed water column samples by direct filtering (Masura et al. 2015; Barrows et al. 2017; McNeish et al. 2018). The entire 140 mL volume of each sample was filtered onto a 0.7-μm glass-fiber filter (MilliporeSigma) (Hoellein et al. 2019). Filters were covered loosely with aluminum foil and left to dry at room temperature for at least 48 h before counting microplastics. On each filtering day, we made at least one new laboratory control by filtering deionized water and counting those filters in parallel with the sample filters ($N = 85$). For benthic samples, we digested organic matter using wet peroxide oxidation (0.05 M Fe[II] and 30% H_2O_2), which does not dissolve polyester (Masura et al. 2015; McNeish et al. 2018). After digestion, we filtered the liquid onto 0.7-μm glass-fiber filters. We measured the dry mass of benthic samples before and after digestion to quantify organic matter dry mass from each sample. Samples were dried before and after digestion by being covered loosely with aluminum foil and placed in an oven at 75°C for at least 8 h. We counted individual microplastic fibers on each filter using a stereomicroscope (AmScope, United Scope LLC). We had at least two trained scientists check each filter to reduce errors. We counted all samples with > 100 fibers a total of three times. Due to the uniformity of color, size, and age, experimentally added microplastic fibers were readily distinguished from other microplastics that may have been present in the sample. In our controls, we found an average of 0.04 relevant (i.e., same color as release) fibers per sample.

Calculations of microplastic transport metrics

We quantified physical dimensions and discharge of study streams throughout the 3-d study. We measured stream width at 5–7 locations along each stream, spaced 5–10 m apart longitudinally. We measured stream depth at five evenly spaced points along the width of the stream. Measurements were repeated at low and high discharge. We calculated stream discharge (Q; m^3 s^{-1}) by using our conservative tracer release as a slug injection and following the formula adapted from Gordon et al. (2004):

$$Q = 1000 \frac{\forall c_t}{\int_{t_1}^{t_2} (c - c_0) dt}$$

where $\forall$ is the volume of the release solution (L), c_t is the conductivity of the solution (mS), c_0 is the background conductivity of the stream (mS), c is the changing conductivity measured downstream (mS), and t_1 and t_2 are the initial and final times of measurement (s). We calculated the water velocity (v, m s^{-1}) from Q using the formula:

$$v = \frac{Q}{d \cdot w}$$

where d is depth (m) and w is width (m).

We calculated deposition length (S_W) and deposition velocity (v_{dep}) of microplastic fibers according to methods used for particle and solute uptake (Supporting Information Fig. S6) (Tank et al. 2006; Webster and Valett 2006; Allan and Castillo 2007; Hoellein et al. 2019). We plotted the number of microplastics at each sampling site (5, 10, 15 m) through time and integrated the areas under the curves, which provided the number of microplastics that traveled across each location. We did the same for conductivity through time for the salt releases. We plotted the natural log of the ratio of microplastic concentration relative to the change in conductivity (y-axis)

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over travel distance (m; x-axis) where k is the slope of the line. The inverse of the slope ($1/k$) represents the deposition length S_W (m), or the average distance particle will travel before being deposited on the bottom of a stream. We also calculated deposition velocity (v_{dep}, mm s^{-1}) using the formula:

$$v_{dep} = 1000\frac{v \cdot d}{S_W}$$

where v is the velocity of the stream (m s^{-1}) and d is the depth of the stream. Here, v_{dep} (mm s^{-1}) is the velocity (i.e., vertical transfer coefficient) at which microplastic particles travel from the water column to the benthic surface, which allows particle transport to be compared in streams of varying discharges (Cushing et al. 1993). We used a linear regression to find the slope of the line for each microplastic deposition trial. If p-values were greater than 0.05 or R^2 values were less than 0.5, we examined the data further. For a subset of releases ($N = 2$), we removed the data from the first sampling site (at 5 m) due to incomplete mixing in stream water from 0 to 5 m that prevented accurate concentration assessment, following the approach used in previous ND-LEEF experiments (Jerde et al. 2016).

Statistical analyses

As described above, breakthrough curves and microplastic releases were conducted independently for the three experimental reaches within each of the streams, so the reaches were treated as a replicates of each of the experimental treatments. In addition, each of the reaches varied in terms of the shape of their path (Supporting Information Fig. S3) and the random organization of their substrate (Supporting Information Fig. S4). However, it should be noted that the replicate reaches for each treatment were located on the same stream channel and thus were not fully independent. For normally distributed data, we used parametric statistical tests. When data were not normally distributed, we used the most similar nonparametric tests. For all statistical analyses, we tested normality using the Shapiro–Wilks test, and $p < 0.05$ was deemed significant. We conducted a two-way ANOVA to assess the effects of substrate type and benthic algae (the independent variables) on S_W and v_{dep} (the dependent variables), as well as a two-way ANOVA to assess the effects of discharge and substrate type (the independent variables) on S_W and v_{dep} (the dependent variables). If there was no significant interaction term for a two-way ANOVA, we used the Tukey honestly significant difference (HSD) post hoc test to determine differences among substrates following a significant substrate effect ($p < 0.05$). Following a significant interaction term in the two-way ANOVA, we used one-tailed paired t-tests with Holm–Bonferroni p-value corrections (Holm 1979) to compare treatments of the same substrate type. We also assessed the effect of substrate type on S_W and v_{dep} in the absence of algae using one-way ANOVAs. We used a two-way ANOVA to compare the amount of microplastics in the benthic surface of each stream after the releases, which required a $3\sqrt{(\log + 1)}$ transformation to meet the assumption of normality since the data were strongly skewed to the right. We compared the amount of microplastics resuspended in each stream after the increase in discharge using a Kruskal–Wallis rank sum test followed by a post hoc pairwise Wilcoxon rank sum test because the data did not meet the assumption of normality. We compared the algal biomass in the streams using a one-way ANOVA followed by Tukey's HSD post hoc test, which required a ($\log + 1$) transformation to meet the assumption of normality since the data were slightly skewed to the right. We used Pearson's correlation tests to explore relationships between v_{dep} or S_W and algal biomass or percent algal coverage. We ran all statistical tests in R Studio version 1.3.1093 (RStudio Team 2020).

Results

Effect of benthic algae on microplastic deposition

Our first analysis compared microplastic deposition at high discharge with and without benthic algae across four different substrates (Fig. 1; Supporting Information Table S3). Benthic algae across all substrate types were dominated by filamentous green algae, with minor contributions from miscellaneous benthic algae for all substrates except pea gravel (Supporting Information Fig. S7). Micropastic deposition length (S_W; Fig. 1a) was significantly shorter in the presence of benthic algae (two-way ANOVA, $F_{1,16} = 9.985$, $p = 0.006$), but deposition length was not affected by substrate type ($F_{3,16} = 2.973$, $p = 0.063$), and there was no interaction (two-way ANOVA, $F_{3,16} = 0.741$, $p = 0.543$). For deposition velocity (v_{dep}; Fig. 2b) we found significant effects for algae (two-way ANOVA, $F_{1,16} = 55.831$, $p < 0.001$) and substrate ($F_{3,16} = 7.000$, $p = 0.003$), and a significant interaction ($F_{3,16} = 3.745$, $p = 0.033$), indicating the effect of benthic algae on v_{dep} varied among substrate types. The presence of algae significantly increased v_{dep} for cobble ($t_2 = -10.0$, p.adj $= 0.020$) and pea gravel ($t_2 = -5.54$, p.adj $= 0.047$). Algal presence resulted in a similar trend of higher v_{dep} for sand and mixture, but the effect of algae was not significant for sand ($t_2 = -3.19$, p.adj $= 0.086$) or mixture ($t_2 = -2.92$, p.adj $= 0.086$).

Effect of discharge on microplastic deposition

Our second analysis compared microplastic deposition at high and low discharge across stream substrate types with benthic algae present (Fig. 2; Supporting Information Table S4). There was no difference in S_W (Fig. 2a) between discharge levels (two-way ANOVA, $F_{1,16} = 1.028$, $p = 0.326$) or among substrates (two-way ANOVA, $F_{3,16} = 2.576$, $p = 0.090$), but there was a significant interaction (two-way ANOVA, $F_{3,16} = 4.351$, $p = 0.020$). There was no statistical difference in S_W during high discharge compared to low discharge for any of the substrates. In contrast, for v_{dep} (Fig. 2b) we found a significant effect of discharge (two-way ANOVA, $F_{1,16} = 62.87$, $p < 0.001$) and substrate type ($F_{3,16} = 4.47$, $p = 0.018$), and a significant interaction ($F_{3,16} = 5.76$, $p = 0.007$). Using t-test, we found

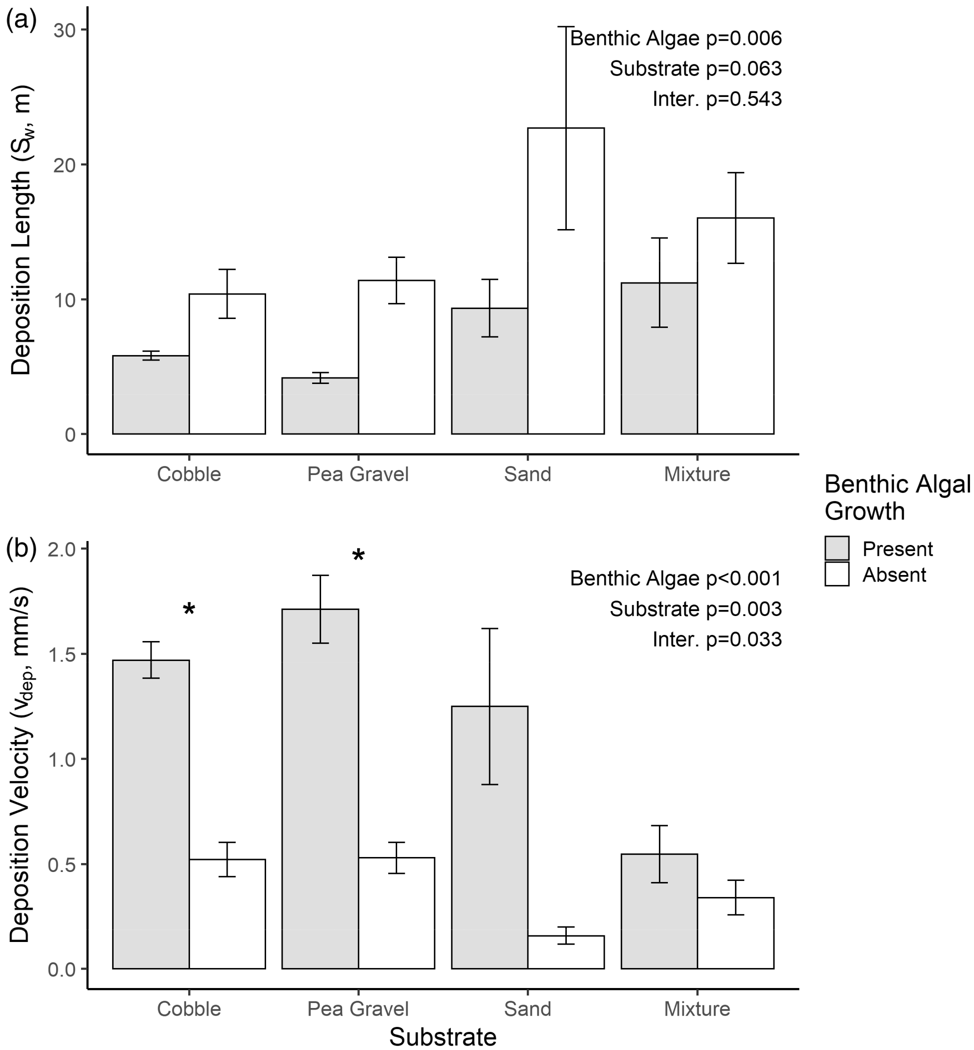

Fig. 1. Mean (± SE) values for (**a**) deposition length (S_W) and (**b**) deposition velocity (v_{dep}) of microplastics in streams with benthic algae present and absent and different substrate types. The results from two-way ANOVAs comparing values under benthic algae and substrate treatments are reported. Significant differences between discharge treatments for each substrate type based on one-tailed paired *t*-tests with Holm–Bonferroni corrections following significant interaction in ANOVA are shown with asterisks (*p.adj < 0.05).

significantly higher v_{dep} during high discharge for cobble ($t_2 = 10.9$, p.adj = 0.004) and pea gravel ($t_2 = 20.5$, p.adj = 0.002). Here we could not analyze releases for sand and mixture because these data were not normally distributed and the nonparametric test requires an $N > 3$; however, there was a similar trend of higher v_{dep} at higher discharge for both sand and mixture.

Effect of substrate on microplastic deposition

The direct effect of substrate on microplastic deposition was assessed by comparing S_W and v_{dep} among streams when algae were absent (Fig. 1a,b). One-way ANOVA indicated a significant effect of substrate on v_{dep}, with cobble and pea gravel having a significantly higher v_{dep} than sand, and mixture having an intermediate v_{dep} (Supporting Information Table S5). Although not statistically significant (Supporting Information Table S5), S_W followed the same, but inverse, pattern, indicating that microplastic travels less far with larger substrate size.

Microplastic retention and resuspension

Microplastic was retained in all of the benthic samples (substrate plus algae) collected after the releases during both low and high discharge (Supporting Information Fig. S8). However, variation was high across treatments and among replicates, and there was no significant effect of discharge (two-way ANOVA, $F_{1,32} = 2.645$, $p = 0.064$) or substrate ($F_{3,32} = 2.677$, $p = 0.114$), and no interaction ($F_{3,32} = 0.919$, $p = 0.443$). Retained microplastic fibers were resuspended from all substrate types when stream discharge was rapidly increased (Fig. 3a) with sand releasing significantly more fibers

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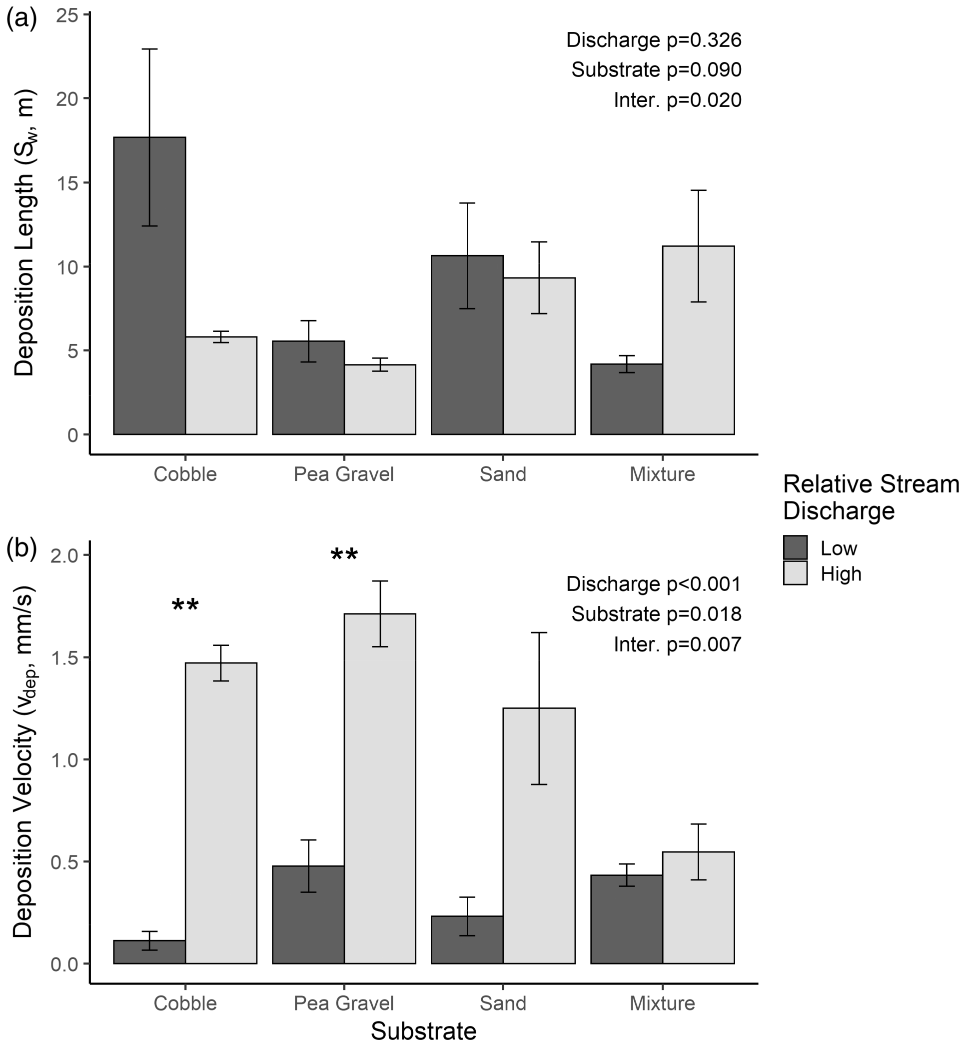

Fig. 2. Mean (± SE) values for (**a**) deposition length (S_W) and (**b**) deposition velocity (v_{dep}) of microplastics in streams of differing discharges and substrate types. The results from two-way ANOVAs comparing values under discharge and substrate treatments are shown in the figure. Significant differences between discharge treatments for each substrate type based on one-tailed paired *t*-tests with Holm–Bonferroni corrections following significant interaction in ANOVA are shown with asterisks (**p.adj < 0.01).

than other substrates (Kruskal–Wallis, $\chi 2 = 28.034$, $p < 0.001$; post hoc pairwise Wilcoxon rank sum, $p < 0.01$).

Relationships between algal biomass and coverage and microplastic deposition

Algal biomass and coverage varied by substrate type (Fig. 3b; Supporting Information Fig. S7). Algae on cobble had significantly lower biomass (275.2 g 10 m^{-1}) relative to mixture (786.3 g 10 m^{-1}), pea gravel (1399.0 g 10 m^{-1}), and sand (1411.4 g 10 m^{-1}; one-way ANOVA, $F_{3,16} = 14.670$, $p < 0.001$). Benthic algal colonization was also assessed based on visual estimates of surface coverage (as %). Algal coverage was high (> 75% across all substrates) but followed a similar pattern to biomass, with pea gravel having the highest coverage and cobble the lowest (Supporting Information Fig. S7). We compared S_W and v_{dep} of microplastic to algal biomass (Fig. 4a,4b) and found no significant correlations (Kendall rank correlation; S_W $p = 0.213$, tau = 0.19; v_{dep} $p = 0.316$, tau = 0.15). In contrast, when we compared microplastic S_W and v_{dep} to algal coverage (Fig. 4c,d), we found a significant negative correlation between S_W and percent coverage (Kendall rank correlation; S_W $p = 0.012$, tau = −0.41), but still no correlation with v_{dep} (Kendall rank correlation; v_{dep} $p = 0.330$, tau = 0.16).

Discussion

In this study, we tested the effects of three factors on microplastic retention in artificial streams: presence of benthic algae, stream discharge, and substrate type. As discussed in more detail below, microplastic deposition rates were significantly higher with well-established benthic algal biofilms, higher stream discharge, and larger benthic substrate. We also

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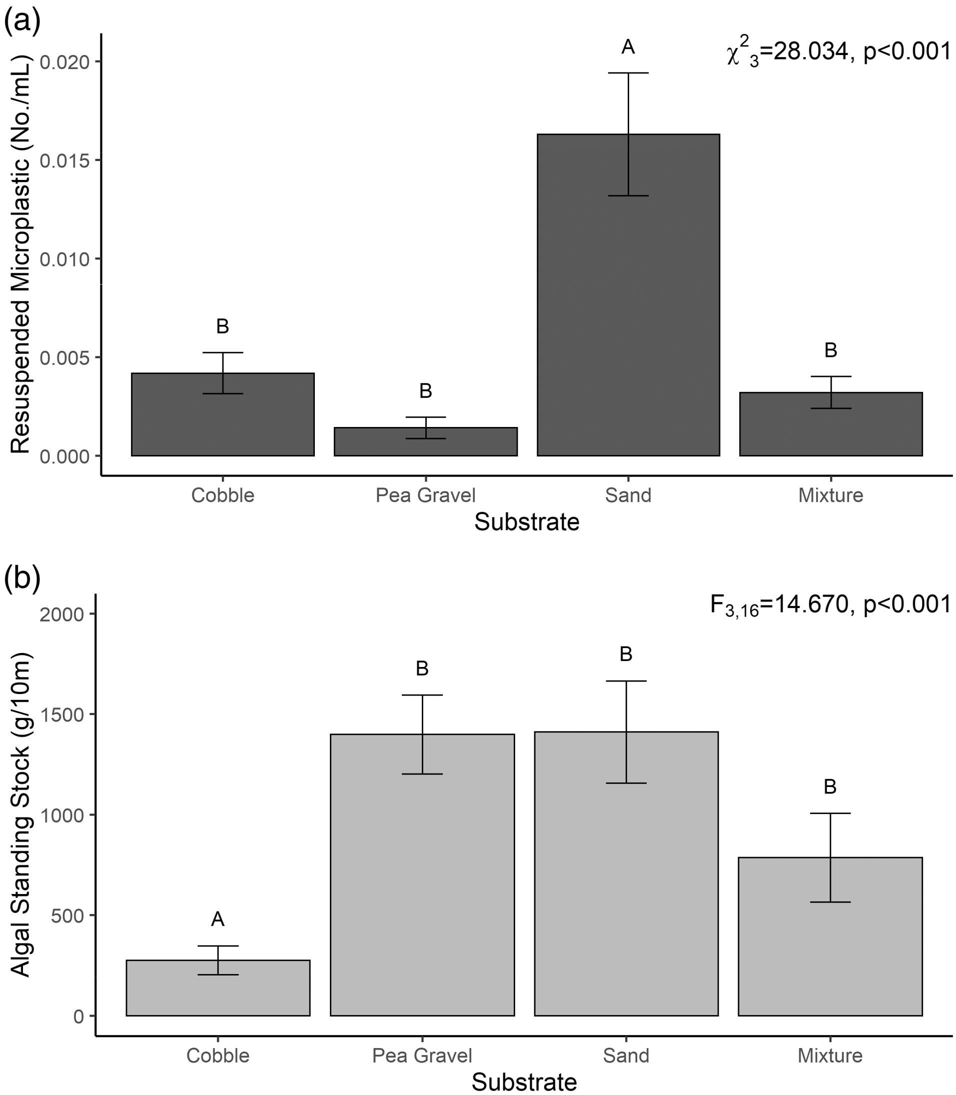

Fig. 3. Mean (± SE) concentration of resuspended microplastics resuspended in streams with differing substrates following the increase from low to high discharge (**a**). The results from the Kruskal–Wallis rank sum test are shown. A Shapiro–Wilk normality test showed data were not normally distributed ($p < 0.001$) and were not normally distributed after being transformed using typical methods. Uppercase letters represent differences from post hoc pairwise Wilcoxon rank sum test $p < 0.01$. Mean (± SE) algal standing stock (g dry mass 10 m^{-1}) for streams with differing substrate types (**b**). The results from a one-way ANOVA with (log + 1) transformed data are shown. A Shapiro–Wilk normality test showed that data were normally distributed ($p = 0.058$) after a (log + 1) transformation to account for right-skewed data. Uppercase letters represent differences from post hoc Tukey HSD $p < 0.05$.

found that a rapid increase in discharge resulted in resuspension of retained microplastics from all substrate types, suggesting that storm events could trigger microplastic release in natural streams.

Role of algae

The presence of benthic algae significantly enhanced the retention of microplastics, resulting in a shorter deposition length (S_W) and a higher deposition velocity (v_{dep}). More specifically, algae reduced S_W by ~50% and increased v_{dep} by ~220% compared to the same streams post algal removal (i.e., to simulate scouring); these results suggest that the algae are retaining microplastics from the water column. This general pattern is commonly observed for naturally occurring particles in streams (Webster et al. 1994; Brookshire and Dwire 2003; Rovira et al. 2016; Shogren et al. 2020) and in laboratory experiments where microplastics adhere to macrophytes (Mateos-Cárdenas et al. 2019). We also observed a significant negative correlation between microplastic S_W and percentage algal coverage of benthic substrate, that is, higher algal coverage correlated with a shorter transport distance, but found no correlation between retention metrics and direct measures of algal biomass. These results suggest that algal coverage of benthic substrate is the critical factor in streams,

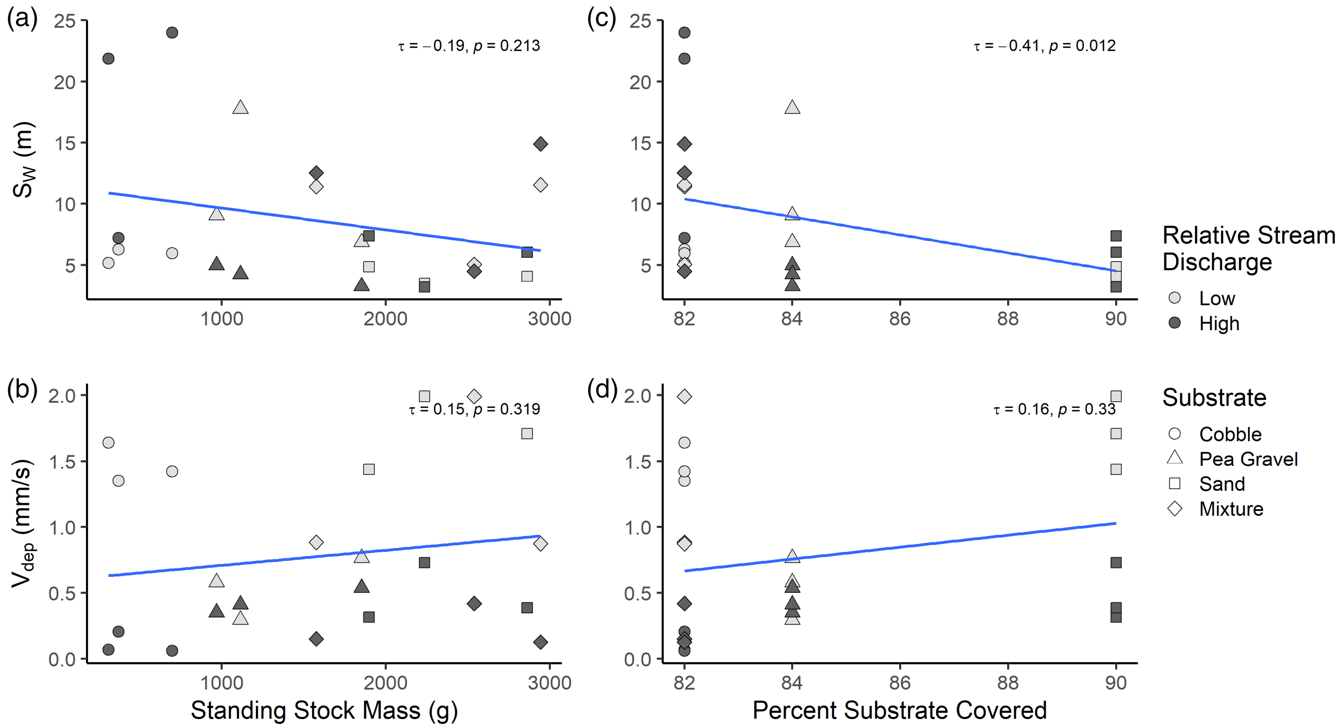

Fig. 4. Correlations between the standing stock of algae and (**a**) deposition length (S_W) and (**b**) deposition velocity (v_{dep}) and between the percent substrate covered by algae and (**c**) S_W and (**d**) v_{dep} in streams during low and high discharge. The results from the Kendall rank correlation are shown in the figure.

creating a surface that retains microplastics, but the depth or biomass of the algae did not drive microplastic retention.

Microplastic fibers were found in benthic algal samples from all treatments, further supporting the conclusion that algae reduced transport by physically trapping microplastics. However, the number of microplastics in the benthic samples was extremely variable, both across streams of different substrate types and within the same stream at different reach locations. This indicates that microplastic deposition is not uniform; even within a short stream reach, microplastic accumulation sites can be spatially heterogeneous. In natural streams, retention of POM is also patchy, with particles often accumulating around dense plant beds where localized water velocity is slowed (Sand-Jensen and Mebus 1996; Kleeberg et al. 2010; Rovira et al. 2016). Considering that we found no correlation between algal biomass and v_{dep} or S_W, the final location of retained microplastic is more complex than solely the mass of algae present.

Microplastic retention by algae is likely to be affected by their physical structure. Most of the benthic algae in our study streams were classified as filamentous green algae, which grew in thick, stringy mats of intertwined fibers. The filamentous nature of this algae likely played an important role in retaining microplastic fibers, given its high surface area, which facilitates the trapping of small particles (Sand-Jensen and Mebus 1996; Rovira et al. 2016). Increased contact between flowing water and a retentive structure increases drag forces, which decrease water velocity in that area and leads to fine particulate deposition (Riis and Sand-Jensen 2006; Alnoee et al. 2016; Rovira et al. 2016). In future studies, it would be beneficial to study microplastic retention in streams with algae of differing morphologies to parse out some of the complexity in microplastic transport dynamics.

Microplastic retention in benthic algae is a concern because it may enhance the uptake of microplastics into aquatic food webs since benthic algae are an important food resource for a variety of aquatic organisms. For example, the experimental addition of microplastics to laboratory mesocosms resulted in their adherence to periphyton and ingestion by tadpoles feeding on periphyton (Boyero et al. 2020). A comparison of microplastic abundance within the digestive tissue of fish collected from tributaries of Lake Michigan found the highest microplastic concentrations in zoobenthivorous species; trophic transfer has been suggested as the route of plastic entry into these fish (McNeish et al. 2018). Uptake of microplastic into aquatic organisms can have negative impacts on the health of these organisms by disrupting digestion, reducing fecundity, causing physiological stress, and serving as a vector for harmful chemicals (Wright et al. 2013; Rochman et al. 2013; Cole et al. 2015; Barboza et al. 2018).

While the effect of benthic algae on microplastic retention is strong, this storage is likely temporary. Since streams often have seasonal patterns of biofilm growth, and even more

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variable flow (e.g., storms), microplastic transport may also display seasonal dynamics that correspond with the seasonal growth, decomposition, and sloughing of natural biofilms. Seasonal senescence can resuspend stored organic and synthetic particulates and transport them downstream in pulses (Alnoee et al. 2016, 2021; Rovira et al. 2016). Some past studies have documented seasonal patterns in microplastic pollution (Veerasingam et al. 2016; Lebreton et al. 2017), while others have not observed such patterns (Rodrigues et al. 2018; Constant et al. 2020; Stanton et al. 2020), and no general consensus has been drawn regarding seasonal microplastic transport in rivers (Shahul Hamid et al. 2018). Therefore, future studies examining microplastic pollution in lotic systems should consider seasonal changes in biological communities (i.e., algae and beyond) as an explanatory factor for seasonal variations in microplastic concentrations.

Role of discharge

Stream discharge also had a significant effect on the deposition of microplastic fibers in our study, with higher discharge leading to significantly higher deposition velocity in streams with larger substrates (cobble and pea gravel), while streams with smaller substrates showed a similar but nonsignificant trend. The magnitude of the discharge effect was approximately a fourfold increase in deposition velocity. A plausible explanation for this result is that higher discharge resulted in more microplastics flowing through the biofilms and becoming entrained in filamentous algae or entering the hyporheic zone where they became trapped. A previous study using the ND-LEEF streams indicated that water that entered the hyporheic zone returned to the water column very slowly (Aubeneau et al. 2016), suggesting that entry into the interstitial spaces in the benthic substrates could be an important mechanism of microplastic retention. The increase in deposition velocity with higher discharge that was observed for microplastics in our study contradicts previous reports of POM transport in streams, in which higher discharge increased transport distances and lowered deposition velocity (Brookshire and Dwire 2003; Larrañaga et al. 2003). These contrasting results suggest that our microplastic fibers behave differently in streams than POM, perhaps due to differences in buoyancy or other physical traits. However, we note that other common shapes of microplastics in streams, such as fragments, are highly variable in size and particle density, and may show greater similarity to POM movement patterns. Overall, research on microplastic deposition in flowing waters is at an early stage, and more work is needed to improve the capacity to predict how changes in discharge affect particle transport across a diversity of microplastic particles and shapes, and across stream sizes.

The rapid increase in stream discharge that occurred in our study when the stream flow was switched from low to high resulted in resuspension of microplastic from the benthos of all substrate types. These results suggest that in natural streams, events that cause a sharp increase in discharge (e.g., heavy rainfall) will initially lead to resuspension of formerly settled microplastics, which is consistent with field studies on microplastics in streams (Kleeberg et al. 2010; Hurley et al. 2018; Constant et al. 2020). This can be especially important in flashy systems, including urban streams, which have been identified as sites of microplastic inputs (McCormick et al. 2016). The "flushing" pattern, where particles are quickly suspended and exported after sharp increases in discharge, is also congruent with previous studies of how POM stored in aquatic plants moves with changing hydrology (Kleeberg et al. 2010) and that flooding periods during rainfall or snowmelt are key moments of POM export from streams (Brookshire and Dwire 2003; Larrañaga et al. 2003; Rovira et al. 2016), suggesting that these could also be important events for microplastic export.

Role of substrate

Benthic substrate size also had a significant effect on microplastic retention, where we found the fastest deposition velocity in the streams with larger substrates (cobble and pea gravel) and the slowest deposition velocity in the stream with small substrate (sand). The effect of large substrates on microplastic retention is further supported by the positive effects of benthic algae and discharge on deposition velocity, which were strongest in the streams with larger substrates. Furthermore, the larger substrates retained more microplastic during the rapid increase in discharge, while sand released the most microplastic. All of these results indicate that stream reaches with larger benthic substrates retain more microplastics, which will impact microplastic export to downstream ecosystems. A plausible explanation for this finding is that larger substrates will have more interstitial space, creating more opportunities for microplastics to enter the hyporheic zone, whereas the smallest substrate provides a smooth benthic surface with fewer interstitial spaces (Webster et al. 1994; Brookshire and Dwire 2003; Larrañaga et al. 2003). These findings could be used to predict rates of retention in streams with different benthic substrates or within different reaches of the same stream. These results also suggest that resuspension of microplastic from the benthos will be particularly significant in urban streams, which tend to have high microplastic concentrations (McCormick et al. 2014) as well as sandy substrates and flashy discharge (Paul and Meyer 2001).

Retention of microplastic vs. other materials

Microplastic v_{dep} values measured in our study can be directly compared to previous measurements of v_{dep} for microplastics and other materials, offering support for the reproducibility of the method we used to quantify deposition and suggesting specific directions for future research. The v_{dep} of 0.53 mm s^{-1} measured in the current study for polyester fibers in pea gravel lined streams without benthic algae corresponds well with v_{dep} values reported in a previous study by our team, which used the same methods and measured v_{dep} values of 0.50 mm s^{-1} for acrylic fibers, 0.19 mm s^{-1} for polypropylene pellets, and 0.77 mm s^{-1} for polystyrene fragments in pea gravel–lined streams without benthic algae at the same ND-LEEF field site (Hoellein et al. 2019). Comparison of data from these two studies supports the reproducibility of the results and suggests some consistency for different microplastic types. These v_{dep} values for microplastic are also in the same range as those reported for corn pollen in a natural stream (0.094–0.31 mm s^{-1}; Georgian et al. 2003). In contrast, our v_{dep} values for microplastic were one to two orders of magnitude lower than those reported for larger organic matter in natural streams, for example, 25.6 mm s^{-1} for leaves and 30.8 mm s^{-1} for sticks (Webster et al. 1999), and one to two orders of magnitude higher than values reported for environmental DNA (0.00213 mm s^{-1}; Jerde et al. 2016), leached proteins (0.037 mm s^{-1}; Shogren et al. 2019), and fine POM (0.048 mm s^{-1}; Shogren et al. 2020) in pea gravel lined streams at ND-LEEF. Thus, the v_{dep} values for microplastics fall within the broad range of values for naturally occurring organic materials but are distinct, necessitating further study over a wider range of conditions and stream sizes. This quickly growing dataset will allow for follow-up meta-analyses to generate new insights on the role of particle properties on deposition dynamics (Hoellein et al. 2019).

Limitations

A few limitations of our study should be noted. Microplastics are a diverse suite of materials and shapes (Rochman et al. 2019) but our study only looked at polyester fibers. While fibers are often the dominant form of microplastics in freshwaters (McNeish et al. 2018; Grbić et al. 2020), other particle types (e.g., fragments) and polymers (e.g., polyester, nylon, etc.) also merit attention. In addition, extrapolating our results to natural streams requires careful consideration of the unique features of the outdoor experimental streams used in our study. It is possible that the benthic algae played a larger role in the experimental streams than would occur in real-world streams because our experimental streams are very shallow (3.5 cm), and in some places, the benthic algae at late colonization covered the entire wetted channel. In addition, the conditions in the experimental streams were extremely favorable to benthic algae growth: limited macroinvertebrate grazers, high light conditions (i.e., are surrounded by grasses), and no hydrologic disturbance for a 5-month period. These conditions allowed the benthic algae population to flourish to a greater extent than typically possible in a natural stream. However, the obvious advantage of the artificial stream array is the ability to study experimental additions of materials such as microplastics in a controlled environment that does not contribute to pollution.

Moreover, our results provide more in-depth deposition metrics than any previous study and present a framework for conducting follow-up studies which vary the intrinsic (i.e., particle specific) as well as extrinsic (i.e., stream specific) factors that drive microplastic dynamics. Advancements in research on deposition rates for microplastics and other particles will need to include analyses across a broader range of stream sizes, and the techniques demonstrated here may prove a useful model for larger-scale studies.

Conclusions

Streams are key sites of microplastic input, retention, and transport. Our study demonstrated the importance of benthic algae, stream discharge, and benthic substrate size on microplastic retention in streams, and the potential for a rapid increase in stream discharge, such as might occur during a storm, to cause resuspension of retained microplastic. These results significantly advance our understanding of factors influencing microplastic transport in stream ecosystems, which have important implications for management and remediation of microplastic contamination in streams and downstream ecosystems, including lakes and oceans.

Author Contributions

Elizabeth M. Berg: Conceptualization; funding acquisition; methodology; investigation; data curation; formal analysis; project administration; writing – original draft preparation; writing – review and editing. Shannon Speir: Investigation; writing – review and editing. Arial J. Shogren: Investigation; writing – review and editing. Martha M. Dee: Investigation; writing – review and editing. Anna E. S. Vincent: Investigation; writing – review and editing. Jennifer L. Tank: Conceptualization; funding acquisition; methodology; resources; writing – review and editing. John J. Kelly: Funding acquisition; investigation; supervision; writing – original draft preparation; writing – review and editing. Timothy J. Hoellein: Conceptualization; investigation; formal analysis; methodology; supervision; writing – original draft preparation; writing – review and editing.

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Conflicts of Interest

The authors declare no conflicts of interest.

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