

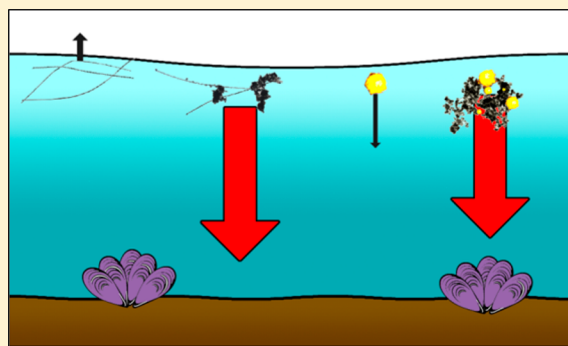
## Role of Marine Snows in Microplastic Fate and Bioavailability

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### Supporting Information

**ABSTRACT:** Microplastics contaminate global oceans and are accumulating in sediments at levels thought sufficient to leave a permanent layer in the fossil record. Despite this, the processes that vertically transport buoyant polymers from surface waters to the benthos are poorly understood. Here we demonstrate that laboratory generated marine snows can transport microplastics of different shapes, sizes, and polymers away from the water surface and enhance their bioavailability to benthic organisms. Sinking rates of all tested microplastics increased when incorporated into snows, with large changes observed for the buoyant polymer polyethylene with an increase in sinking rate of 818 m day<sup>-1</sup> and for denser polyamide fragments of 916 m day<sup>-1</sup>. Incorporation into snows increased microplastic bioavailability for mussels, where uptake increased from zero to 340 microplastics individual<sup>-1</sup> for free microplastics to up to 1.6 × 10<sup>5</sup> microplastics individual<sup>-1</sup> when incorporated into snows. We therefore propose that marine snow formation and fate has the potential to play a key role in the biogeochemical processing of microplastic pollution.



### INTRODUCTION

Microplastic particles (pieces of plastic <5 mm) are ubiquitous and pervasive pollutants of the marine environment globally,<sup>1,2</sup> having been recorded from the poles to the tropics and from surface waters to the seafloor.<sup>3</sup> They have also been found in the guts of over 300 different marine species,<sup>4</sup> prompting widespread concern over their environmental impact. Global microplastics sampling efforts to date have heavily focused on the oceanic gyres and the floating portion of plastic debris, leading to a global estimate of 93–236 thousand metric tonnes<sup>3</sup> of microscopic plastic debris currently floating on the sea surface. However, there is a vast discrepancy between the amount of plastic estimated to enter the marine environment and what is being recorded in these surface monitoring efforts.<sup>5,6</sup> In 2010 alone, 4–12 million metric tonnes of plastic is thought to have entered the oceans, vastly outstripping this sea-surface data.<sup>6</sup>

It is becoming increasingly apparent that microplastics are not just present on the sea surface and that, somehow, these particles eventually make their way down to the seafloor. Concentrations on the deep seafloor are estimated from limited sampling efforts to be as high as 4 × 10<sup>9</sup> fibers km<sup>-2</sup>, with an average around 1 × 10<sup>9</sup> km<sup>-2</sup>.<sup>7</sup> It is even being argued that microplastics may already form part of a stratigraphic signal of the Anthropocene due to their accumulation into sediments.<sup>8</sup> Microplastics of buoyant polymers such as polypropylene and polyethylene, which should float as virgin (unfouled) particles, have now been reported at depths down to 5000 m in ocean

sediments<sup>9–12</sup> and in the guts of deep sea organisms.<sup>1</sup> This, together with the “missing” surface plastic, suggests that environmental transformations of microplastics must occur that alter their densities and fates after entering the marine environment. Modeling approaches have started to look at how processes such as biofouling and fragmentation of plastic particles might alter particle buoyancy and hence lead to net sedimentation,<sup>13,14</sup> based on a series of assumptions regarding these interactions in a water column. This has led to a recent estimation that 99% of plastic entering the oceans will eventually reach the ocean floor, including buoyant polymers.<sup>15</sup> A key transport route for organic matter to the benthos that is not accounted for in such models is the formation of marine snows.

Marine snows are organic-rich aggregates (distinct particles >200 μm<sup>16</sup>) made up of fecal pellets, larvacean houses, phytoplankton, microbes, particulate organic matter (POM), and inorganics brought together by shear forces and Brownian movement.<sup>17</sup> Marine snows have much higher settling rates than their individual particle components (following Stokes’s Law)<sup>18,19</sup> and are primarily responsible for the mass flux of organic material from surface waters to the deep ocean<sup>17</sup> forming a key component of the biological carbon pump.<sup>20</sup>

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Marine snow concentrations generally range from <1 to 100 aggregates L<sup>-1</sup><sup>21</sup> but can be as high as 5300 aggregates L<sup>-1</sup>.<sup>22</sup> Other transport mechanisms could be in fast sinking fecal pellets, but these are usually retained in the upper few hundred meters of the water column as they are recycled by coprophagy. Much of the global oceans vertical flux of particulate material is therefore dictated by the movement of marine snow.<sup>20</sup> Unlike biofouling, marine snow formation is not light limited.<sup>13</sup> Hence it can be hypothesized that marine snows have the potential to provide an important pathway by which microplastic particles can be rapidly transported vertically downward.

Here, we investigate this potential for marine snow to form a transport mechanism to move buoyant microplastics away from the sea-surface, through the water column, and ultimately to the seafloor. As incorporation of microplastic into marine snows might increase the effective particle size of microplastics, they may also enhance their bioavailability to invertebrate consumers.<sup>23</sup> Here, we focus on a bioavailable fraction of what are termed “microplastics” using plastics ranging from 7–3000 μm. Using a laboratory simulation of marine snow formation, we determine whether a range of different microplastic polymers, shapes, and sizes will incorporate into marine snows, how this influences their behavior in the water column, and ultimately how it influences their uptake into a model benthic filter feeder. Given the important role that marine snow plays in the downward flux of organic material in global oceans, understanding its potential role in the movement of microplastics is key to understanding the fate of microplastics in marine ecosystems and quantifying the potential risk that they pose to marine biota and ultimately to human health via our consumption of benthic fisheries species.<sup>24</sup>

## MATERIALS AND METHODS

**Production of Marine Snows and Plastic Contaminated Snows.** Marine snows were produced using the modified protocol of Shanks and Edmunson<sup>25</sup> to include 10 μg L<sup>-1</sup> hyaluronic acid as recommended in Ward and Kach.<sup>26</sup> Natural seawater was collected 1 h before high tide from the same location for each exposure (Starcross, Devon, UK. Lat: 50.628204, Lon: -3.4477383) between February 2016 and March 2017, filtered to 200 μm to remove any large particles and plankton, and placed in 1 L Nalgene bottles. For the plastic contaminated snows, a range of plastic sizes, shapes, and polymer types was used in order to determine if shape, size, or polymer type influenced incorporation into marine snows. Test microplastics included polyamide fibers (10 × 50 μm) made according to Cole 2016,<sup>44</sup> polystyrene beads (7–30 μm), polyethylene beads (9–11 μm), polyvinyl chloride fragments (115–156 μm), polyamide fragments (6–30 μm), and polypropylene fibers (23 × 3000 μm) (all bar polypropylene fibers fluorescently labeled, further details in the SI, summarized in Table S1). These plastics are polymers commonly found in the environment. Polypropylene fibers are the most common fiber found in water and sediment samples with polyamide fibers being the third most common in water and sediment samples,<sup>27</sup> and polyethylene, polystyrene, and polyvinyl chloride are in the five major commodity plastics commonly encountered.<sup>28</sup> These were added to the roller bottles before rolling, to aggregate into the marine snow matrix, and the bottles with and without microplastics were placed on a roller table for 3 days at 14 rpm.

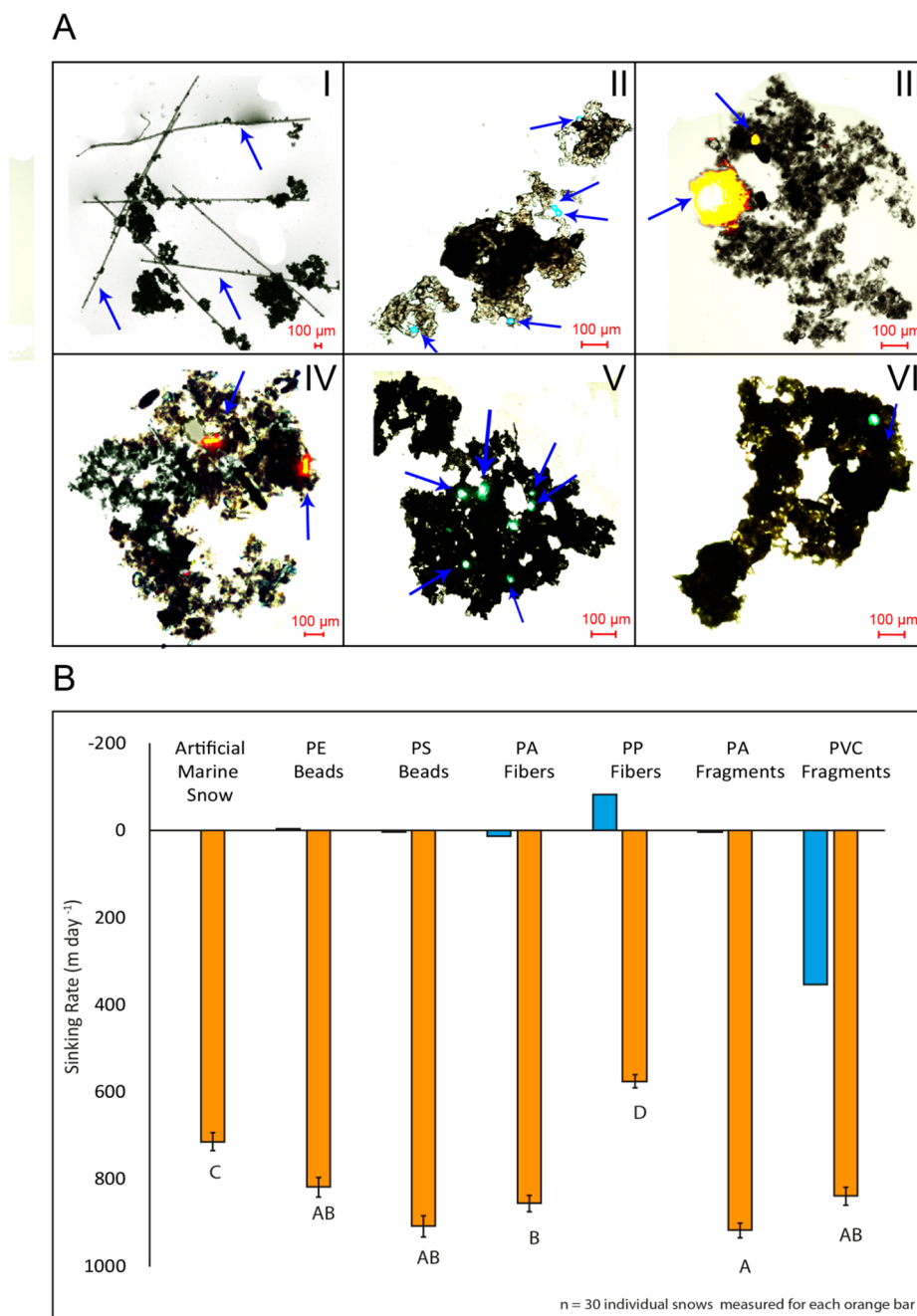
**Incorporation Index Calculations.** To establish how readily the different microplastic types and shapes incorporated

into marine snows, an incorporation index was calculated according to Doyle et al.<sup>19</sup> Microplastics were added to the 1 L roller bottles at a concentration of 50 particles mL<sup>-1</sup> or 0.1 mL<sup>-1</sup> in the case of the polypropylene fibers (the fibers were much larger and so using their weight, the concentration was decreased by 500 times as their weight was 500 times that of the smallest bead). This affected a final concentration in our biological exposures of 2.5 particles mL<sup>-1</sup> and 0.05 mL<sup>-1</sup> respectively. This process was repeated 4 times with different seawater to account for natural variations between water collections such as variations in particulate matter and transparent extracellular polymers (TEP) concentration. The snows, once formed, were allowed to settle, and then all aggregates were pipetted into a separate falcon tube for each treatment. The snows were resuspended by gently rolling the falcon tubes, a subsample of aggregate filled seawater was then put in a Petri dish under a Leica inverted fluorescence microscope (Leica DMI4000 B using UV (360 nm), Green (515–560 nm), and Blue (450–490 nm) filters), and every aggregate >300 μm was counted to give a number of aggregates per milliliter. The total number of aggregates in each 1L bottle was then calculated with this information. Subsequently, 30–40 snows per treatment were imaged, and fluorescence was used to identify the number of microplastic particles bound into the aggregate matrix. The ImageJ software package<sup>29</sup> was used to measure the maximum calliper length of each snow. An average number of microplastics per snow was then calculated, and this number was multiplied by the number of snows calculated to be in the original 1 L of aggregate seawater to give a final number of microplastics in marine snows for each treatment. This then allowed the incorporation of plastics to be calculated using a modified equation from Doyle et al.<sup>19</sup>

$$\text{Incorporation (\%)} = \left[ \frac{\text{Concentration in snows}}{\text{Input concentration}} \right] \times 100$$

We confirmed that our calculations were not affected by the small numbers of plastics that adhered to the bottle surfaces during the snow formation step. These losses were minor and hence did not impact our results.

**Measuring Sinking Rates.** Sinking rates were calculated as “relative sinking rates” given the variety of factors including vessel size and shape (wall effects), temperature and salinity, air/water interface size, and air flow that can alter these rates.<sup>30</sup> The sinking rates were calculated for all marine snow polymer types with 30 individual snows being measured per replicate, all made from the same seawater, and repeated three times giving a total of 90 measurements per polymer type. To be able to compare the sinking rates of marine snows against the respective free microplastic particles, which were too small to be measured visually, the sinking rates for all plastics were calculated using Stokes’s Law to calculate terminal velocity and using a modified version for cylindrical fibers.<sup>31</sup> To do so a 1 L measuring cylinder was filled with artificial seawater at a fixed salinity and temperature (15.6 °C, 27.1 ppt, 8.07 pH) to a water height of 360 mm and then left to settle for 30 min. A white nonreflective card was used to aid visualization, and a subsample of each plastic contaminated snow treatment was pipetted using a serological pipet (Drummond Portable Pipet-Aid XP2). The snows were allowed to sink through the pipet and released from the pipet under gravity, just under the surface of the water. Marine snows then sank for 142 mm to achieve a constant velocity and then were filmed sinking through a 36.5



**Figure 1.** A) Images of marine snows with each of the six polymers incorporated and sinking rates plotted. I) polypropylene fibers  $23 \times 3000 \mu\text{m}$  (note the change in scale bar for all other microplastics); II) polyethylene beads  $9\text{--}11 \mu\text{m}$ ; III) polyvinyl chloride fragments  $115\text{--}156 \mu\text{m}$ ; IV) polyamide fibers  $10 \times 50 \mu\text{m}$ ; V) polyamide fragments  $6\text{--}30 \mu\text{m}$ ; VI) polystyrene beads  $7\text{--}30 \mu\text{m}$ . Blue arrows indicate incorporated microplastics that are colored by their fluorescence in the figure. B) Modeled sinking rates of microplastics (blue bars) and measured sinking rates of marine snows with plastics incorporated (orange bars). Artificial marine snow, polyethylene (PE) beads, polystyrene (PS) beads, polyamide (PA) fibers, polypropylene (PP) fibers, polyamide (PA) fragments, and polyvinyl chloride (PVC) fragments were all measured using a Canon SD MKIII. Bars with different letters are significantly different (One-Way ANOVA,  $F_{6,622} = 3001$ ,  $p \leq 0.01$ , Tukey's Post-Hoc Test).

mm window using a Canon DSLR set at 720 p, 50 fps (720 p = frame size of  $1280 \times 720$  pixels, 50 fps = shot at 50 frames per second). The time the snows took to sink through the window was calculated using the following equation

$$\text{Time} = \frac{\# \text{Frames}}{\text{Frame Rate}}$$

where the number of frames is derived by subtracting the frame number the marine snow entered the window from the frame

number when it exited the window. Speed was then calculated using the time and distance data in  $\text{m day}^{-1}$ .

**Mussel Uptake Experiment.** The blue mussel, *Mytilus edulis* (shell length:  $53.7 \text{ mm} \pm 4.6 \text{ mm}$ ), was collected from a local source at Starcross, Devon adjacent to where the seawater was collected (Lat: 50.618945, Long:  $-3.4462054$ ) 3 days prior to exposure. Their shells were scrubbed to remove organisms and underwent two water changes in a temperature controlled aquarium setting to allow them to depurate and were fed a concentrated blend of microalgae (Shellfish Diet 1800, Reed

Mariculture). Mussels were then transferred to a flow-through aquarium tank and kept in treatment seawater conditions for at least 3 days to acclimate before being added to any exposure. The mussels were then starved 12 h before exposure and removed 2 h pre-exposure to ensure they ventilated promptly during the feeding exposure.

Nine mussels per treatment for polystyrene and polyethylene and 16 mussels per treatment for polypropylene (where uptake was lower therefore more replicates were required for statistical power) were exposed in individual tubes to the following for 60 min at 15 °C:

1. Controls – natural seawater that had not been rolled (i.e., no marine snow or microplastics present).
2. Marine snows with incorporated microplastics referred to as plastic contaminated snows (PCS) – Rolled seawater with microplastics incorporated into the aggregates through the rolling process.
3. Marine snows with added microplastics referred to throughout as plastic and snows (PAS) – Rolled seawater with no microplastics. Plastics were then added to the exposure at time 0 (T0) to differentiate between active feeding on the snows and passive plastic uptake from free beads in the water.
4. Seawater with microplastic spheres, referred to as a “free plastic” treatment (FP) – artificial seawater (ASW) with freely suspended plastics added. The PAS and FP treatments had PS and PE added to the 1 L bottles at a concentration of 50 particles mL<sup>-1</sup>, and the larger PP fibers were added at a concentration of 0.1 particles mL<sup>-1</sup>. Control mussel exposures were undertaken to assess laboratory contamination and the quality control of the protocol. No microplastics were recovered in any of the control treatments, and so controls were eliminated from the analysis.

To assess the ability of marine snows to transport plastics from the surface to the benthos, vertical transport chambers (VTCs) (Figure S2) were made to ensure a head of water above the mussel (water height of 194 cm equal to ≈20 L of water in a 116 mm interior diameter tube) to mimic a sublittoral environment and to help test sinking. Mussels were placed in the VTCs, and once every mussel was visibly ventilating each treatment was added at T0. The artificial seawater was filtered to 0.2 μm, acclimated to the aquarium temperature, and diluted to the salinity of the natural seawater taken to produce the marine snows. At T0 the aggregates making up the treatments, plastic contaminated snows (PCS) and plastic and snows (PAS), were transferred to VTCs using a serological pipet. The snows sank through the pipet and were released just under the surface of the water to ensure that the snows remained intact and that eddy formation was minimized which could prevent uniform sinking. Microplastics at the required concentrations were added to PAS and FP treatments at T0 also. The exposure was run for 60 min (T60) based on preliminary feeding trials using uncontaminated marine snows ensuring that significant uptake occurred within this time period and to ensure that slow sinking microplastics had the requisite time to reach the mussels in the experiment to be compared with the sinking marine snows.

**Microplastic Recovery and Quantification.** At T60 mussels were removed by a rapid inversion of the VTCs, causing them to cease ventilating and therefore feeding, and were then dried off with blue roll. Mussels were snap-frozen

prior to dissection from their shells and dried at 60 °C for 48 h, and a dry tissue weight was calculated. Mussels were then rehydrated by placing each dried mussel in a conical flask with 20 mL of 0.2 μm filtered deionized water (DI). The mussels were left for 2 h to rehydrate, transferred to a 50 mL falcon tube with the DI water, and then homogenized using a Stuart SHM1 Homogenizer. Once a smooth homogenate was achieved, 6 × 20 μL of homogenate was viewed under an inverted fluorescent Leica microscope in a clear bottomed well plate, and the beads per well were counted. The number of beads per mussel was calculated based on the dry weight plus 20 mL of DI using the following equation

$$\frac{\text{Total Sample Volume } (\mu\text{L})}{20 \mu\text{L}} \times \text{Well average } (\# \text{beads}) \\ = \text{Total beads per mussel}$$

where total sample volume (μL) is the total mussel dry weight (1 g = 1 mL) added to the DI volume (mL) used to rehydrate the mussel.

**Quality Control.** Microplastics were fluorescently labeled where necessary to ensure that contamination from the laboratory environment could not be mistaken for an experimental particle. The large polypropylene fibers were not labeled as they were easily visible, but each sample was inspected fully in this case and the fibers were very distinct (see Figure S1).

Similarly the homogenization step was inspected to ensure that the plastics were not damaged in the homogenization process. Visual assessment of all plastics found no evidence of fracturing or surficial damage, even in our largest plastics, the 23 × 3000 μm polypropylene fibers.

## RESULTS AND DISCUSSION

We found that all of the microplastic polymer types, shapes, and sizes that we tested readily incorporated into laboratory made marine snows (Figure 1, details of plastics used in Table S1, images in Figure S1). Using a calculated incorporation index, we found that for the buoyant polymer (density lower than seawater) polyethylene (PE), 79% of beads incorporated into the aggregate matrix. For polyamide (PA) fibers, PA fragments, polystyrene (PS) beads, polypropylene (PP) fibers, and polyvinyl chloride (PVC) fragments, we found incorporation values of 100%.

For all polymer types and microplastic shapes tested, we measured enhanced sinking rates when these microplastics were incorporated into marine snows (Figure 1) compared to their calculated sinking rates as free particles. This relative change in sinking rate from that as a free microplastic particle to particles incorporated into snows varied according to polymer type. Critically, buoyant polymers became negatively buoyant once incorporated into marine snows and hence sank during the observation period rather than remaining on the surface. For example, buoyant PP fibers had calculated sinking rates of −82 m day<sup>-1</sup> and float on the surface when added to the vertical transport chamber. Once incorporated into marine snows, PP fibers became negatively buoyant, sinking at a rate of 576 m day<sup>-1</sup> an increase of 658 m day<sup>-1</sup> (Figures 1AI and 1B). Similarly, PE beads had a negative calculated sinking rate of −0.19 m day<sup>-1</sup> as free particles (i.e., floated on the surface) but had sinking rates of 818 m day<sup>-1</sup> when incorporated into marine snows, a reversal from slightly buoyant to rapidly sinking (Figures 1AII and 1B).

For polymers denser than seawater, sinking rates were calculated to be  $0.39 \text{ m day}^{-1}$  for PS beads,  $1.49 \text{ m day}^{-1}$  PA fragments, and  $12.15 \text{ m day}^{-1}$  for PA fibers (Figure 1B) as free microplastic particles. Due to its high density, PVC had the greatest sinking rate as free microplastic of  $354 \text{ m day}^{-1}$  and exhibited a relatively small increase to  $839 \text{ m day}^{-1}$  when incorporated into snow (an increase in sinking rate of  $485 \text{ m day}^{-1}$ ) (Figures 1AIII and 1B). PA fibers when incorporated into marine snows sank at a rate of  $855 \text{ m day}^{-1}$ , an increase of  $843 \text{ m day}^{-1}$  compared to free PA fibers (Figures 1AIV and 1B). Marine snows contaminated with PA fragments had the fastest sinking rates of  $917 \text{ m day}^{-1}$ , an increase of  $916 \text{ m day}^{-1}$  compared to its sinking rate as free microplastic (Figures 1AV and 1B). PS beads exhibited an increase in sinking rate of  $908 \text{ m day}^{-1}$  when incorporated into marine snow from  $0.39 \text{ m day}^{-1}$  as free plastic to  $908 \text{ m day}^{-1}$  in marine snows (Figures 1AVI and 1B).

The sinking rates for the free microplastic particles are based on simple models for a static water column and the laboratory based observations for the plastics incorporated into marine snows made under similarly static conditions. As such these values cannot be taken as representative of true particle sinking rates under more turbulent, real-world oceanic conditions, which will vary in space and time according to a number of oceanographic processes and that act as a large force on sinking processes (although the net flux in the global ocean is downward). Our measured marine snow sinking rates are therefore higher than those generally reported for marine snows in the natural environment (reported as  $1\text{--}280 \text{ m day}^{-1}$ )<sup>23</sup> where turbulent mixing acts to slow this rate.<sup>32</sup> Additionally, the water used to generate the snows in this study was collected from an estuary high in lithogenic material, potentially adding denser material to the aggregate mix than might occur in open ocean conditions and which would be expected to enhance sinking.<sup>33</sup> Zooplankton fecal pellets have been found to sink faster than  $820 \text{ m day}^{-1}$ <sup>33</sup> however, suggesting that our rates are not beyond the realms of what is conceivable for POM in the open ocean.

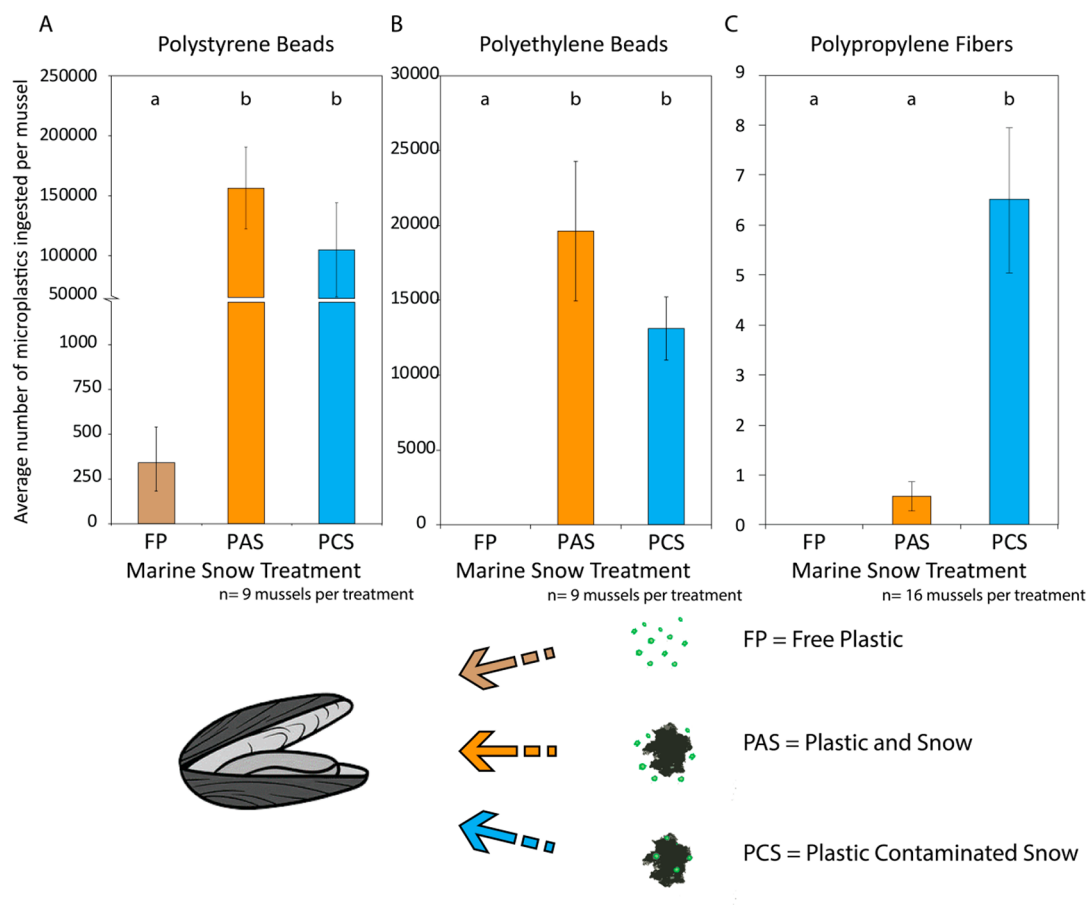
The benefit of using this controlled static system is that it allows relative sinking rates to be compared for our range of test microplastics against modeled sinking rates for free plastic particles (which are also devoid of real world perturbations), without the complex confounding factors of oceanic conditions, thus allowing us to test our hypothesis. This comparative data demonstrates a clear relative increase in sinking rates of the plastic particles when they are incorporated into marine snows for all microplastics tested. Even with an obvious attenuation in sinking speed, and within a complex system of fragmentation and coagulation, the magnitude of these relative changes in sinking rates provides strong evidence that the process of incorporation into marine snows dramatically changes the behavior of the microplastic particle in a water column. Hence this data provides empirical support for the paradigm that marine snow represents an environmentally relevant, viable pathway for microplastics to be transported from the sea surface to the seafloor, including buoyant polymers which would otherwise float as virgin particles.

The aggregation of microplastics into marine snows not only altered the microplastics behavior within the water column but also altered the sinking rates of the marine snows themselves. Marine snows with PP fibers incorporated had slower sinking rates compared to uncontaminated snows ( $714 \pm 25 \text{ m day}^{-1}$ ) and had the slowest sinking rate of  $576 \pm 16 \text{ m day}^{-1}$ . This

would be equivalent to a reduction of  $138 \text{ m day}^{-1}$  (Figure 1), which over the average depth of the ocean ( $\approx 4000 \text{ m}$ ) equates to 1.3 days longer to reach the benthos. This reduction is likely due to the greatly increased size of the plastic/snow parcel formed with these large fibers (as can be seen in Figure 1AI). Polypropylene fibers formed groups of snows and fibers which would likely experience significant drag while sinking, slowing them down. All other microplastic contaminated snows sank significantly faster than the uncontaminated snows (One-Way ANOVA,  $F_{6,622} = 3001$ ,  $p \leq 0.01$ , Tukey's Post-Hoc Test) (Figure 1B), equivalent to an increase of  $153 \text{ m day}^{-1}$  ( $\text{SE} \pm 19 \text{ m day}^{-1}$ ). This would theoretically cause POM to reach the benthos 1 day before an uncontaminated snow might.

Altered POM sinking rates as a result of microplastic incorporation has previously been demonstrated in the laboratory using polystyrene beads for zooplankton fecal pellets<sup>34</sup> and for cultured algal aggregates<sup>18</sup> further adding to the evidence that microplastics have the potential to interact with important aspects of the oceans' biological pump. Slower sinking would potentially allow more grazing, fragmentation, and microbial degradation of marine snows<sup>20,34</sup> and possibly lead to rerelease of buoyant microplastics to the surface, whereas a faster sinking POM could result in higher rates of accumulation of plastic debris in the benthic realm. Of course, there are a whole suite of environmental factors that will affect marine snow sinking rates in addition to the concentration, type, and shape of microplastics, for example the amount of POM present and abiotic processes such as turbulence and homogeneity of the water column, grazing on snows as they sink potentially rereleasing plastics, temperature, salinity, and viscosity of the water.<sup>20</sup> However, the relative change between our plastic and marine snow sinking rates are, in most cases, orders of magnitude different (polystyrene beads increased from a free plastic sinking rate of  $0.39 \text{ m day}^{-1}$  to a sinking rate of  $908 \text{ m day}^{-1}$  when in marine snows and even the smallest increase was relatively large for PVC fragments from  $354 \text{ m day}^{-1}$  to  $839 \text{ m day}^{-1}$ ), and therefore even with the aforementioned attenuations in sinking rates, even sinking at environmentally measured rates, the plastics will still be traveling from the sea surface to the seafloor at a much enhanced rate than they would as individual particles and indeed the buoyant polymers would have to wait for the much slower process of biofouling to occur to overcome their positive buoyancy. A study by Zhao et al.<sup>16</sup> provides evidence that this process of microplastics incorporating into marine snows occurs in the field, finding marine snows with buoyant plastics incorporated in the top 2 m of the water column. Our study goes further to explore the sinking dynamics and implications of marine snows as a transport vector to the deep ocean for a range of microplastic polymers and shapes.

**Uptake of Microplastics in *Mytilus edulis*.** Finally, we demonstrate that the incorporation of microplastics into marine snow acts to increase their bioavailability to a model benthic filter feeder, the blue mussel (*Mytilus edulis*), when mussels were separated from the surface by a distance of 2 m. We selected the PS and PE beads and the PP fibers for use in the mussel exposures so as to have two size comparable beads with differing densities and the PP fibers as they were much larger particles, much less dense, and represented fibrous material, which can make up a large proportion of real world samples from microplastic trawls. We found that for all three microplastics tested, mussels ingested significantly more microplastics when they were incorporated into marine snows



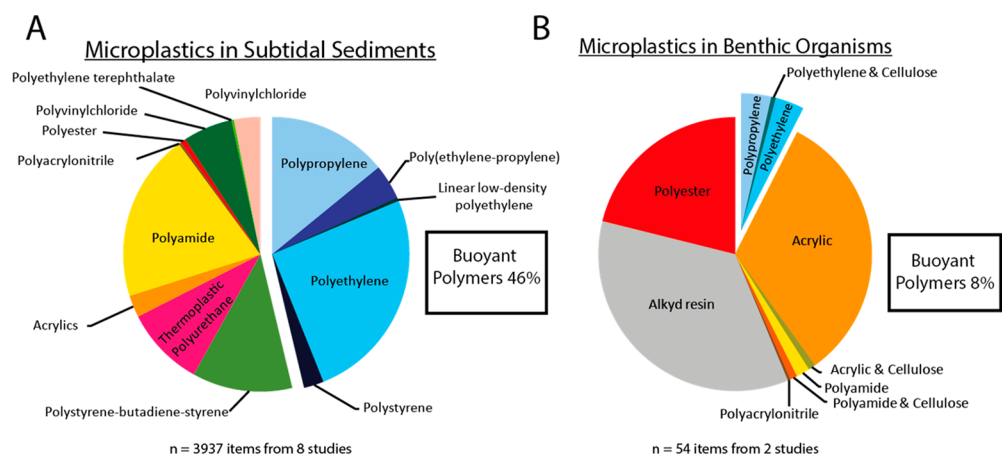
**Figure 2.** Uptake of microplastics into *Mytilus edulis* in the absence of marine snow (“free plastic” = FP), in the simultaneous presence of marine snow at the time of the uptake experiment (“plastic and snow” = PAS), and after preincorporation into marine snow matrix (“plastic-contaminated snow” = PCS) for A) polystyrene beads (7–30  $\mu\text{m}$ ), B) polyethylene beads (9–11  $\mu\text{m}$ ), and C) polypropylene fibers (23  $\times$  3000  $\mu\text{m}$ ). Significant differences are highlighted by differing letters (Tukey’s HSD Test). Infographic below gives a visual descriptor of the three treatment types and codes.

(plastic contaminated snows (PCS)) than when added as free plastic (FP) (Figure 2). For PS beads, uptake over an hour’s exposure increased  $\approx 300$  times from an average of 340 ( $\pm 158$  SE) beads per mussel when freely suspended to  $\approx 105,000$  ( $\pm 3,900$ ) beads per mussel when incorporated into marine snows (ANOVA:  $F_{3,32} = 13$ ,  $p \leq 0.01$ ) (Figure 2A). For PE, beads were only taken up by the mussels when marine snow was present (ANOVA:  $F_{3,32} = 12.38$ ,  $p \leq 0.01$ , Figure 2B). The difference in uptake of PS compared to PE when fed to mussels is likely due to the buoyant nature of the PE beads and their reduced incorporation rate into marine snows of 79% compared to 100% for PS. For the PP fibers, the ingestion rate was significantly greater (ANOVA:  $F_{3,52} = 18.66$ ,  $p \leq 0.01$ , Figure 2C) when incorporated into the marine snow ( $6.5 \pm 1.5$  fibers per mussel) compared to when fibers were mixed with snows at the start of the exposure ( $0.6 \pm 0.3$  fibers per mussel). As with PE no fibers were ingested when input as free plastics (Figure 2).

An increased uptake of freely suspended microplastics by the mussels was also detected when they were added to the vertical transport chambers at the same time as previously formed marine snow (plastic and snow treatment (PAS)) (Figure 2B). This is likely due to a combination of factors; first, that the process of incorporation is happening *in situ* as the plastic and marine snows are mixed at the start of the experiment such that some plastics are collected as the snows fall. Second, the

downdraft of the sinking snows is likely to be enough to carry down plastics in their wake as a large body of particulate matter with high densities sink. Even with buoyant PE this is plausible as the polymer is only just less dense than the surrounding water and so an energy flow moving downward may be enough to overcome the buoyancy of the particle by itself. Indeed a number of oceanographic processes including saline subduction, offshore convection, and dense shelf water cascading have been hypothesized as routes of microplastic transport to deeper waters.<sup>7</sup>

Interactions between microplastics and biota are observable throughout marine ecosystems globally.<sup>35</sup> Plastic debris has been documented to have entangled or been ingested by at least 557 species, including marine mammals, seabirds, and many benthic organisms.<sup>4</sup> Mussels, used here as representative filter feeders since this is a common feeding mode in benthic ecosystems, are efficient at the capture of small particulate matter,<sup>26</sup> readily ingest microplastics,<sup>36</sup> and obtain 5 to 10 times more nitrogen from marine snows than from dissolved organic matter and particulate detritus.<sup>37</sup> Our findings suggest that not only do marine snows redistribute microplastics by drawing them downward but they also, potentially increase the uptake of microplastics via a bioconcentration process. This concentration process has been recognized in studies looking at marine snow and pathogen interactions<sup>38</sup> whereby organisms have increased exposure to a pathogen due to their aggregation



**Figure 3.** Microplastic polymers identified in subtidal environmental samples from 1–2700 m. Data was collected from a literature search of microplastic studies that both listed and quantified the polymer types found in A) subtidal sediments (8 studies) and B) deep sea organisms (2 studies) (see Table S2).

within a food source. Marine snows may well be enhancing the bioavailability of microplastics to invertebrate consumers, causing bioaccumulation in mussels on an as yet unknown scale and potentially causing biomagnification through the food chain and potentially to humans also.<sup>38</sup>

To examine the wider relevance of our findings, we conducted a review of the existing literature on microplastic pollution in benthic samples (see Table S2 for references and details). This reveals that a variety of different polymer types, shapes, and sizes of microplastics, including buoyant polymers, have been found in sediments and the guts of benthic species. Microplastics have been found in benthic sediments at depths ranging from 1 to 2700 m<sup>7,10</sup> (Figure 3A) and in the guts of organisms at depths of 334–2200 m<sup>1,7</sup> (Figure 3B). Buoyant polymers PE and PP are reported in deep sea organisms at similar concentrations to those ingested in our study (2 to 5 fibers per individual in Taylor et al.)<sup>1</sup> compared with 0 to 21 fibers per individual (mean  $\pm$  SE of  $6.5 \pm 1.45$  per individual) in the present study. Of all the plastics recovered from these studies buoyant polymer types comprised 46% of all plastics in sediments. In benthic organisms buoyant polymers made up 8% of the total number of plastics recorded.

Overall, our results demonstrate that the formation of marine snow represents an environmentally relevant, viable pathway for microplastics to be transported from the sea surface to the seafloor and into benthic fauna by ingestion. This mechanism has the potential to fill in the gap between what we know is entering the marine environment and the relative fraction found in sea surface trawls and adds further evidence to the prediction that plastic contamination of the benthic habitat is occurring at much greater volumes than first thought. The soft sediments that cover much of the ocean floor are dynamic and productive habitats, supporting many ecologically and economically important species and playing key roles in ecosystem functioning,<sup>39</sup> raising questions as to the impact that microplastic pollution is having on these important communities.<sup>40–43</sup> There is however a multifaceted issue to disentangle: first, that marine snows have the potential to be a highway for plastic transport to the benthic realm and second, that plastics, in theory, have the ability to influence the fluxes of POM in the marine water column. We also show that mussels ingest more plastics when they are incorporated into marine snows potentially leading to adverse effects as yet unseen with

standard feeding models based on free plastics. This mechanism of plastic delivery to benthic organisms is also potentially important as plastics have been repackaged into a food source and concentrated, opening questions regarding whether this will influence the effects of plastics on these organisms.<sup>16</sup> Addressing the paucity of data relating to the presence of midwater and benthic microplastics, evidence of the transport pathways, and the understanding of their fate upon reaching the benthos is of paramount importance when taking a global ocean view of microplastic pollution. The transformations of plastics that occur during their journey from source to sink will be critical in their distribution and thus our understanding of risk of microplastics to marine systems. This study is an important step in understanding the fate and sinking dynamics of microplastics in global oceans and highlights the potential for microplastics to affect more than just the sea surface.

## ■ ASSOCIATED CONTENT

### 📄 Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.8b01000.

Additional Materials and Methods, Figures S1 and S2, and Tables S1 and S2 (PDF)

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### Notes

The authors declare no competing financial interest.

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## REFERENCES

- (1) Taylor, M. L.; Gwinnett, C.; Robinson, L. F.; Woodall, L. C. Plastic microfibre ingestion by deep-sea organisms. *Sci. Rep.* **2016**, *6*, 33997.
- (2) Van Cauwenberghe, L.; Vanreusel, A.; Mees, J.; Janssen, C. R. Microplastic pollution in deep-sea sediments. *Environ. Pollut.* **2013**, *182*, 495–499.
- (3) van Sebille, E.; Wilcox, C.; Lebreton, L.; Maximenko, N.; Hardesty, B. D.; van Franeker, J. A.; Eriksen, M.; Siegel, D.; Galgani, F.; Law, K. L. A global inventory of small floating plastic debris. *Environ. Res. Lett.* **2015**, *10* (12), 124006.
- (4) Kühn, S.; Bravo Rebollo, E. L.; van Franeker, J. A. Deleterious Effects of Litter on Marine Life. In *Marine Anthropogenic Litter*; Bergmann, M., Gutow, L., Klages, M., Eds.; Springer International Publishing: Cham, 2015; pp 75–116, DOI: 10.1007/978-3-319-16510-3\_4.
- (5) Cózar, A.; Echevarría, F.; González-Gordillo, J. I.; Irigoien, X.; Úbeda, B.; Hernández-León, S.; Palma, Á. T.; Navarro, S.; García-de-Lomas, J.; Ruiz, A.; Fernández-de-Puelles, M. L.; Duarte, C. M. Plastic debris in the open ocean. *Proc. Natl. Acad. Sci. U. S. A.* **2014**, *111* (28), 10239–10244.
- (6) Jambeck, J. R.; Geyer, R.; Wilcox, C.; Siegler, T. R.; Perryman, M.; Andrady, A.; Narayan, R.; Law, K. L. Plastic waste inputs from land into the ocean. *Science* **2015**, *347* (6223), 768–771.
- (7) Woodall, L. C.; Sanchez-Vidal, A.; Canals, M.; Paterson, G. L. J.; Coppock, R.; Sleight, V.; Calafat, A.; Rogers, A. D.; Narayanaswamy, B. E.; Thompson, R. C. The deep sea is a major sink for microplastic debris. *R. Soc. Open Sci.* **2014**, *1* (4), 140317.
- (8) Waters, C. N.; Zalasiewicz, J. A.; Williams, M.; Ellis, M. A.; Snelling, A. M. *A stratigraphical basis for the Anthropocene?* Geological Society: London, Special Publications 2014, 395, DOI: 10.1144/SP395.18.
- (9) Munari, C.; Infantini, V.; Scoponi, M.; Rastelli, E.; Corinaldesi, C.; Mistri, M. Microplastics in the sediments of Terra Nova Bay (Ross Sea, Antarctica). *Mar. Pollut. Bull.* **2017**, *122* (1–2), 161–165.
- (10) Vianello, A.; Boldrin, A.; Guerriero, P.; Moschino, V.; Rella, R.; Sturaro, A.; Da Ros, L. Microplastic particles in sediments of Lagoon of Venice, Italy: First observations on occurrence, spatial patterns and identification. *Estuarine, Coastal Shelf Sci.* **2013**, *130*, 54–61.
- (11) Graca, B.; Szewc, K.; Zakrzewska, D.; Dołęga, A.; Szczerbowska-Boruchowska, M. Sources and fate of microplastics in marine and beach sediments of the Southern Baltic Sea—a preliminary study. *Environ. Sci. Pollut. Res.* **2017**, *24* (8), 7650–7661.
- (12) Peng, G.; Zhu, B.; Yang, D.; Su, L.; Shi, H.; Li, D. Microplastics in sediments of the Changjiang Estuary, China. *Environ. Pollut.* **2017**, *225*, 283–290.
- (13) Kooi, M.; van Nes, E. H.; Scheffer, M.; Koelmans, A. A. Ups and Downs in the Ocean: Effects of Biofouling on Vertical Transport of Microplastics. *Environ. Sci. Technol.* **2017**, *51* (14), 7963–7971.
- (14) Kaiser, D.; Kowalski, N.; Waniek, J. Effects of biofouling on the sinking behavior of microplastics. *Environ. Res. Lett.* **2017**, *12* (12), 124003.
- (15) Koelmans, A. A.; Kooi, M.; Lavender Law, K.; van Sebille, E. All is not lost: deriving a top-down mass budget of plastic at sea. *Environ. Res. Lett.* **2017**, *12* (11), 114028.
- (16) Zhao, S.; Danley, M.; Ward, J. E.; Li, D.; Mincer, T. J. An approach for extraction, characterization and quantitation of microplastic in natural marine snow using Raman microscopy. *Anal. Methods* **2017**, *9* (9), 1470–1478.
- (17) Newell, C. R.; Pilskaln, C. H.; Robinson, S. M.; MacDonald, B. A. The contribution of marine snow to the particle food supply of the benthic suspension feeder, *Mytilus edulis*. *J. Exp. Mar. Biol. Ecol.* **2005**, *321* (2), 109–124.
- (18) Long, M.; Moriceau, B.; Gallinari, M.; Lambert, C.; Huvet, A.; Raffray, J.; Soudant, P. Interactions between microplastics and phytoplankton aggregates: Impact on their respective fates. *Mar. Chem.* **2015**, *175*, 39–46.
- (19) Doyle, J. J.; Palumbo, V.; Huey, B. D.; Ward, J. E. Behavior of Titanium Dioxide Nanoparticles in Three Aqueous Media Samples: Agglomeration and Implications for Benthic Deposition. *Water, Air, Soil Pollut.* **2014**, *225* (9), 1–13.
- (20) Turner, J. T. Zooplankton fecal pellets, marine snow, phytodetritus and the ocean's biological pump. *Prog. Oceanogr.* **2015**, *130*, 205–248.
- (21) Simon, M.; Hans-Peter, G.; Bernd, S.; Helle, P. Microbial ecology of organic aggregates in aquatic ecosystems. *Aquat. Microb. Ecol.* **2002**, *28* (2), 175–211.
- (22) Riebesell, U. Particle aggregation during a diatom bloom. I. Physical aspects. *Mar. Ecol. Prog. Ser.* **1991**, *69* (3), 273–280.
- (23) Jackson, G. A.; Checkley, D. M.; Dagg, M. Settling of particles in the upper 100m of the ocean detected with autonomous profiling floats off California. *Deep Sea Res., Part I* **2015**, *99*, 75–86.
- (24) Wright, S. L.; Kelly, F. J. Plastic and Human Health: A Micro Issue? *Environ. Sci. Technol.* **2017**, *51* (12), 6634–6647.
- (25) Shanks, A. L.; Edmondson, E. W. Laboratory-made artificial marine snow: a biological model of the real thing. *Mar. Biol.* **1989**, *101* (4), 463–470.
- (26) Ward, J. E.; Kach, D. J. Marine aggregates facilitate ingestion of nanoparticles by suspension-feeding bivalves. *Mar. Environ. Res.* **2009**, *68* (3), 137–142.
- (27) Gago, J.; Carretero, O.; Filgueiras, A. V.; Viñas, L. Synthetic microfibers in the marine environment: A review on their occurrence in seawater and sediments. *Mar. Pollut. Bull.* **2018**, *127*, 365–376.
- (28) Andrady, A. L. The plastic in microplastics: A review. *Mar. Pollut. Bull.* **2017**, *119* (1), 12–22.
- (29) Schneider, C. A.; Rasband, W. S.; Eliceiri, K. W. NIH Image to ImageJ: 25 years of image analysis. *Nat. Methods* **2012**, *9*, 671.
- (30) Bach, L. T.; Riebesell, U.; Sett, S.; Febiri, S.; Rzepka, P.; Schulz, K. G. An approach for particle sinking velocity measurements in the 3–400  $\mu\text{m}$  size range and considerations on the effect of temperature on sinking rates. *Mar. Biol.* **2012**, *159* (8), 1853–1864.
- (31) Komar, P. D. Settling Velocities of Circular Cylinders at Low Reynolds Numbers. *J. Geol.* **1980**, *88* (3), 327–336.
- (32) Alldredge, A. L.; Silver, M. W. Characteristics, dynamics and significance of marine snow. *Prog. Oceanogr.* **1988**, *20* (1), 41–82.
- (33) Trull, T. W.; Bray, S. G.; Buesseler, K. O.; Lamborg, C. H.; Manganini, S.; Moy, C.; Valdes, J. In situ measurement of mesopelagic particle sinking rates and the control of carbon transfer to the ocean interior during the Vertical Flux in the Global Ocean (VERTIGO) voyages in the North Pacific. *Deep Sea Res., Part II* **2008**, *55* (14), 1684–1695.
- (34) Cole, M.; Lindeque, P. K.; Fileman, E.; Clark, J.; Lewis, C.; Halsband, C.; Galloway, T. S. Microplastics Alter the Properties and Sinking Rates of Zooplankton Faecal Pellets. *Environ. Sci. Technol.* **2016**, *50* (6), 3239–3246.
- (35) Galloway, T. S.; Cole, M.; Lewis, C. Interactions of microplastic debris throughout the marine ecosystem. *Nature Ecology & Evolution* **2017**, *1*, 0116.
- (36) Van Cauwenberghe, L.; Claessens, M.; Vandegehuchte, M. B.; Janssen, C. R. Microplastics are taken up by mussels (*Mytilus edulis*) and lugworms (*Arenicola marina*) living in natural habitats. *Environ. Pollut.* **2015**, *199*, 10–17.
- (37) Alber, M.; Valiela, I. Incorporation of organic aggregates by marine mussels. *Mar. Biol.* **1994**, *121* (2), 259–265.
- (38) Shapiro, K.; Krusor, C.; Mazzillo, F. F. M.; Conrad, P. A.; Largier, J. L.; Mazet, J. A. K.; Silver, M. W. Aquatic polymers can drive pathogen transmission in coastal ecosystems. *Proc. R. Soc. London, Ser. B* **2014**, *281*, 20141287.
- (39) Thrush, S. F.; Dayton, P. K. Disturbance to Marine Benthic Habitats by Trawling and Dredging: Implications for Marine Biodiversity. *Annu. Rev. Ecol. Syst.* **2002**, *33* (1), 449–473.
- (40) Courtene-Jones, W.; Quinn, B.; Gary, S. F.; Mogg, A. O. M.; Narayanaswamy, B. E. Microplastic pollution identified in deep-sea



water and ingested by benthic invertebrates in the Rockall Trough, North Atlantic Ocean. *Environ. Pollut.* **2017**, *231*, 271–280.

(41) Woodall, L. C.; Gwinnett, C.; Packer, M.; Thompson, R. C.; Robinson, L. F.; Paterson, G. L. J. Using a forensic science approach to minimize environmental contamination and to identify microfibrils in marine sediments. *Mar. Pollut. Bull.* **2015**, *95* (1), 40–46.

(42) Coppock, R. L.; Cole, M.; Lindeque, P. K.; Queirós, A. M.; Galloway, T. S. A small-scale, portable method for extracting microplastics from marine sediments. *Environ. Pollut.* **2017**, *230*, 829–837.

(43) Mistri, M.; Infantini, V.; Scoconi, M.; Granata, T.; Moruzzi, L.; Massara, F.; De Donati, M.; Munari, C. Small plastic debris in sediments from the Central Adriatic Sea: Types, occurrence and distribution. *Mar. Pollut. Bull.* **2017**, *124* (1), 435–440.

(44) Cole, M. A novel method for preparing microplastic fibers. *Sci. Rep.* **2016**, *6*, 34519.