

## Article

# Influence of Sediment Mixing Induced by the Gallery-Diffusor *Hediste diversicolor* on the Burial of Different Microplastic Particles: A Comparative Tracer Study

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## Abstract

Although marine sediments are recognized as a sink for marine microplastic particles, investigations into the fate of once deposited particles have received relatively little interest so far. Through interactions with sediment-dwelling organisms, these particles are subject to potential burial in the wake of bioturbation. In this study, we analyzed the effect of sediment reworking by the polychaete *Hediste diversicolor* O.F. Müller, 1776, using different particle tracers—luminophores, polyethylene (PE), and polyamide (PA). Transport induced by *H. diversicolor* was observed to extend to sediment depths up to 14 cm with similar biodiffusive and advective transport rates for all tracer types. Local transport ( $Db$ ) was  $1.09 \text{ cm}^2 \text{ yr}^{-1}$  for luminophores; microplastics showed slightly lower local transport with  $Db$  of  $0.81 \text{ cm}^2 \text{ yr}^{-1}$  (PA) and  $0.57 \text{ cm}^2 \text{ yr}^{-1}$  (PE). Non-local transport ( $r$ ) was comparably low for all particle types, ranging from  $0.69 \text{ yr}^{-1}$  for luminophores and  $0.95 \text{ yr}^{-1}$  (PA) and  $1.27 \text{ yr}^{-1}$  (PE) for microplastics. Food addition did not significantly increase bioturbation activity of *H. diversicolor*. Despite differences in size and density, the particle tracer types in this study showed no significant differences in modeled bioturbation coefficients or burial depth, emphasizing that bioturbation of microplastics is not governed by particle properties alone but also by the functional ecology of the bioturbator.

**Keywords:** polyethylene; polyamide; luminophores; bioturbation; modelling

## 1. Introduction

The contamination of numerous natural habitats with solid plastic waste has been recognized as an emerging environmental hazard for decades. The impacts of plastic pollution are particularly evident in the marine environment, which, due to substantial terrestrial inputs, is one of the major global sites for plastic distribution and accumulation [1]. In this context, microplastics (hereafter referred to as MPs), generally defined as particles  $\leq 5 \text{ mm}$  in diameter [2], have significant potential for long-distance transport and have been found to be ubiquitous in marine systems, including surface waters [3], coastal shelf sediments [4], and deep-sea sediments [5,6]. Over time, the majority of MPs in marine systems undergo vertical export to the seafloor [7], either by size-dependent particle export [8], density-altering processes such as biofouling of surfaces [9,10], incorporation into marine aggregates [11] or zooplankton fecal pellets [12,13], rendering marine sediments a sink for MPs [5,8,14]. The fate of deposited MPs on the seafloor has received increased attention, particularly emphasizing the role of fauna-induced MP transport. These transport



Academic Editor: Francesco Tiralongo

Received: 30 January 2026

Revised: 28 July 2026

Accepted: 3 August 2026

Published: 5 August 2026

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processes, especially in the context of bioturbation, may enhance the vertical transport of deposited MPs into the sediment matrix [15,16].

Bioturbation, defined as “all transport processes carried out by animals that directly or indirectly affect sediment matrices” [17], influences both solid and solute transport, profoundly impacting the ecological functioning of marine sediments by altering sediment geochemistry and particulate matter exchange processes. In this context, particle reworking can result in the burial of freshly deposited organic material into sub- or anoxic zones, inhibiting organic matter degradation and associated nutrient remineralization [18,19]. Conversely, upward transport of buried material to oxic sediment layers can stimulate remineralization processes, sometimes by up to one order of magnitude [20]. Particle reworking encompasses a set of numerous processes that, often in complex interaction with each other, are affected by species composition, abundance and feeding mode [21]. To address this complexity, particle reworking is frequently classified into sub-processes based on transport step length and direction [22,23]. To quantify particle mixing with respect to these factors, the vertical distribution of particle tracers is analyzed within the framework of deterministic bioturbation models which describe the respective bioturbation coefficients  $Db$  (local transport) and  $r$  (non-local transport) [24,25].

MPs are widely present in benthic habitats, and there is increasing evidence of MP redistribution due to infaunal bioturbation activity at both the species [26,27] and community level [28]. In this context, non-local transport may contribute to vertical MP export by bypassing upper, well-mixed sediment layers [18,29] and depositing MP particles at deeper sediment strata. The assumption that MPs are affected during bioturbation in the same manner as regular sediment particles appears plausible. Artificial plastic particles have already been used as tracers in both in situ and laboratory experiments to assess sediment reworking rates [30,31]. Experimental and in situ studies have demonstrated that MPs can be transported into considerable sediment depths by bioturbation [26,32] and that MP redistribution strongly depends on feeding modes and bioturbation types of the organisms involved [33,34]. Since most MP bioturbation follows biodiffusive patterns, especially at the community level [16], differences in particle properties compared to ambient sediment grains may become relevant for MP distribution in sediments. Most plastic polymers are less dense and often considerably smaller than sediment grains—properties which might induce a differential transport behavior, especially under random, diffusion-analogous mixing, hypothetically leading to separation effects similar to those observed in other mixed substrates [35,36], a potential influence that has not yet been addressed.

In this study, the ragworm *Hediste diversicolor* O.F. Müller, 1776 was chosen to address these questions. Widespread along European and North American coasts, this species constitutes a major faunal component in many estuaries [37]. This polychaete is known for constructing complex burrow networks [38], which can extend to depths of up to 20 cm [39]. These burrows typically consist of multi-branched structures near the sediment surface (the so-called gallery) combined with vertically oriented sections that reach to deeper sediment layers [40]. Due to this distinctive burrow morphology, *H. diversicolor* is classified as a gallery-diffuser, facilitating both local and non-local mixing processes [17].

Local mixing is limited to the gallery, representing the sum of activities, with feeding by dragging food particles into the burrow being the dominant process [41]. Locomotion and burrow construction also contribute to local mixing in upper sediment layers [22]. In contrast, non-local transport occurs along vertically oriented burrow sections, primarily through gravity-driven particle deposition, often resulting in particle accumulation zones in deeper sediment strata [22,42]. Based on its characteristic bioturbation behavior, *H. diversicolor* was selected to investigate potential effects of differential MP burial depth and burial rate on local and non-local transport processes. A bioturbation experiment was

conducted to simultaneously assess the reworking rates of MPs and sediment particles (i.e., luminophores). Luminophores have been used in MP research previously, either to assess the bioturbation performance of invertebrates in MP-contaminated sediments by formulating bioturbation coefficients [43] or to evaluate related ecosystem functions such as organic matter accumulation and associated aerobic respiration [44].

In our study, we quantified MP mixing as  $Db$  and  $r$  parallel to luminophores using an experimental approach for the first time to reveal possible differences in bioturbation-induced transport between MPs and sediment grains due to varying particle sizes and densities. Furthermore, a food addition treatment was included, as enhanced food availability is known to stimulate the sediment reworking activity of *H. diversicolor* [45]. We hypothesized that food addition could increase non-local transport processes, thereby further highlighting potential effects of differential MP mixing caused by particle transport due to preferential food ingestion or gravity-driven particle export into burrows.

The aims of this study are (I) to characterize the transport of MPs induced by the gallery-diffuser *H. diversicolor* within the context of different functional bioturbation transport processes (local and non-local); (II) examine the effect of pulsed food input on MP burial and its consequences on differential particle mixing processes; and (III) to provide new insights into whether MPs are subjected to differential burial rates compared to ambient sediment grains due to differences in particle properties as size or specific gravity.

## 2. Materials and Methods

### 2.1. Sediment and Polychaete Sampling

Sediment, polychaetes and seawater were collected in a shallow water area at the eastern shore of the Breitling, the lagoon-like opening of the Warnow River near its outlet to the southern Baltic Sea at Rostock, Germany, in March 2017 (54.1728° N, 12.1420° E).

The collected sediment was sieved through 1000 µm mesh to remove all macrofauna. Approximately 90 specimens of *H. diversicolor* were collected during sieving and transferred to several 20 L plastic barrels containing sediment and habitat water for transport. In the laboratory, polychaetes were kept in aerated and temperature-controlled glass aquaria (20 × 25 × 20 cm) filled with several cm of sediment and habitat water (15 °C) until use in experiments.

Sediment sorting, porosity and distribution of sediment particle sizes were assessed according to Folk & Ward [46]. Organic matter content was evaluated as loss on ignition (LOI) at 500 °C for 12 h [47]; particular organic carbon (POC) content was derived from LOI as described by Leipe et al. [48].

C and N content were determined using a NC 2500 C/N analyzer (CE Instruments, Hindley Green, UK).

### 2.2. Experimental Setup

The influence of *H. diversicolor* bioturbation on MP distribution was assessed using a setup of nine glass aquaria (20 × 25 × 20 cm, volume 10 L; area 500 cm<sup>2</sup>), filled with 16 cm sediment and overlain by several cm of habitat water (salinity 12). Sediment was allowed to settle for 13 d (control mesocosms C1 and C2) and 27 d (all other mesocosms). Mesocosms were maintained at a constant temperature of 15 °C and equipped with portable aerators to ensure water aeration. Two aquarium lamps were used to simulate a 12 h light/dark cycle (Nano light, 11 W, Dennerle, Münchweiler, Germany). To remove small fauna and other bioturbators that might not have been eliminated by previous sieving, aeration was stopped for three consecutive days to induce anoxia. Before starting the experiment, polychaetes were kept overnight in aerated plastic trays filled with habitat water for gut clearance and were subsequently weighed. Ten individuals of *H. diversicolor* with a total blotted

fresh mass (FM) of circa 4.0 g were introduced to each of six mesocosms, representing an abundance of 200 ind.  $\text{m}^{-2}$  per mesocosm. The remaining three aquaria served as azoic control setups (C1–C3). After introduction, polychaetes were allowed to acclimate for 7–9 days. Specimens lying motionless on the sediment surface during this period were removed and replaced by polychaetes of similar mass; this procedure was applied to mesocosm F<sup>−</sup>2 (one individual, 3 days acclimatization) and F<sup>−</sup>3 (two individuals, 3 and 1 days acclimatization, respectively). Dead animals that were encountered during the experiment were carefully removed but not replaced.

### 2.3. Tracer Calibration and Quantification of Bioturbation Activity

For the application in the MP treatments, commercially available polyamide (PA) particles with a diameter of 0.5 mm and a density of  $1.14 \text{ g cm}^{-3}$  (Monofil-Technik GmbH, Hennef/Sieg, Germany) and UV-fluorescent polyethylene (PE) particles (diameter 125–150  $\mu\text{m}$ , density  $1.03 \text{ g cm}^{-3}$ ; Cospheric, Santa Barbara, CA, USA) were used. To further investigate potential differential mixing effects between microplastic particles and ambient sediment grains, luminophores in the form of fluorescently labelled sediment particles (diameter 130  $\mu\text{m}$ ; Partrac Ltd., Glasgow, UK) were used as a third particle tracer [49]. Tracer calibration was carried out by particle count-to-mass ratio determination for PA particles and luminophores. Particle samples (luminophores:  $n = 148$ ; PA:  $n = 142$ ) of varying masses (luminophores: 0.09 to 2.3 mg; PA: 0.14 to 27.0 mg) were weighed (Pro 11, Sartorius AG, Göttingen, Germany), photographed (D7100, AF-S NIKKOR 60 mm 1:2.8 lens, Nikon, Tokyo, Japan) and quantified using ImageJ v1.54g [50] with the software's cell counter plugin. For PE particles, calibration data provided by the manufacturer were used.

PA particles were incubated for seven days in seawater before experimental use to decrease surface hydrophobicity and induce biofilm formation, thereby improving particle suspension [51,52]. PE particles were preconditioned in 0.1 w% polysorbate solution (Tween<sup>TM</sup> solution) according to the protocol "Preparing Tween Solutions" [53] to ensure proper suspension in seawater. At the start of the experiment, particle tracers suspended in 15 mL of seawater were added to each polychaete treatment (luminophores: 8.0 g per mesocosm; PA: 1.3 g per mesocosm; PE: 2.0 g per mesocosm) using a plastic syringe. The absolute tracer quantities added to each mesocosm are shown in Table S1 (Supplementary Information).

Polychaete treatments were divided into two groups, each consisting of three mesocosms. No food was added to the non-feeding treatment (F<sup>−</sup>1, F<sup>−</sup>2, F<sup>−</sup>3) during experimental incubation; the feeding treatment (F<sup>+</sup>1, F<sup>+</sup>2, F<sup>+</sup>3) received 0.7 g chopped spinach (deep-frozen, fresh mass) per mesocosm weekly, representing the addition of 289 mg C and 35 mg N per feeding event with a C/N ratio of 8.25.

In total, 250 mL of water was exchanged weekly from each mesocosm using a syringe. Fresh habitat water was added gently to avoid sediment disturbance and tracer resuspension. Water losses due to evaporation were compensated by adding deionized water. Salinity was adjusted during every water exchange or addition using a HQ40d portable 2-channel multimeter (Hach, Düsseldorf, Germany). Specimens of *H. diversicolor* found on the sediment surface showing no movement during the experiment and unable to retreat back into the sediment within 12 h were removed from the mesocosms.

After 28 days of exposure, mesocosm aeration was stopped, and the overlying water was carefully removed. The sediment was then further dried with a maximum of 19 h using a 100 W heat lamp, as this method proved effective in removing residual water while keeping polychaetes alive until sediment sectioning. Subsequently, the sediment from each mesocosm was scraped off in 16 layers, each with 1 cm thickness, and was carefully removed using plastic scoops with depth markings. Additional markings were drawn

on the aquaria walls to aid the process. Specimens retrieved alive were stored overnight in aerated, water-filled plastic trays for gut clearance before determining fresh mass, dry mass, and ash-free dry mass according to van der Meer et al. [54]. The sectioned sediment layers were further dried at 60 °C, thoroughly homogenized and stored in plastic containers in darkness at room temperature until further analysis. Subsamples of dried sediment (1.5 mL,  $n = 3$ ) were taken from each layer of each mesocosm for luminophore and PE particle quantification and photographed with a single-lens reflex camera (D7100, AF-S NIKKOR 60 mm 1:2.8 lens, Nikon, Tokyo, Japan) under UV light (emission maxima at 254 and 365 nm). Sample images were subsequently processed with ImageJ by splitting channels and applying binary thresholding of the color channels in question (luminophores: red channel; PE: green channel). Binary images were analyzed using the particle count function of the ImageJ software.

For PA particle quantification, the remaining sediment was sieved thoroughly through 500  $\mu\text{m}$  mesh. PA content was determined by photographing the PA-containing sieves and counting particles using the ImageJ cell counter plugin. For PA quantities too large to count accurately on-sieve, density separation was performed as described in [55]: particles were transferred to 1 L of saturated NaCl solution, the suspension was vigorously shaken and allowed to settle for 5 min. The supernatant was then sieved through a 500  $\mu\text{m}$  mesh. This separation step was repeated twice. Recovered PA particles were dried and weighed using a BA 210 S balance (Sartorius AG, Göttingen, Germany). Particle numbers were determined based on particle mass. After density separation, all sieves were inspected for PA particles potentially trapped in the mesh. These sieves were photographed and particles were quantified from the images using the ImageJ cell counter plugin.

#### 2.4. Bioturbation Modeling

To describe the polychaete-induced particle transport, the one-dimensional gallery-diffusion model by François et al. [56] was used and implemented in MATLAB 7.5 (R2007B). This model allows for the description of two different main mechanisms for particle transport, typically mediated by *H. diversicolor*: (1) diffusion-analogous (local) mixing, which is usually associated with shallow sediment depths exhibiting high burrow density, and (2) advective (non-local) transport, which occurs along burrow shafts extending into deeper sediment layers. Using the translocation of conservative particle tracers such as luminophores, the model formulates two particle mixing coefficients—the biodiffusion coefficient ( $Db$ ) and the non-local coefficient ( $r$ ) by minimizing a weighted sum of squared differences between observed and modeled depth-dependent particle tracer concentrations [56]. For modeling, the maximum depth of biodiffusive mixing was set to 2 cm, reflecting a conservative estimate of the biodiffusive mixing depth for *H. diversicolor* bioturbation [22,57].

#### 2.5. Statistical Analysis

To assess the influence of treatment (control,  $F^-$ ,  $F^+$ ) and particle type (luminophores, PE, PA) on  $Db$  and  $r$ , a two-way ANOVA, along with the Games–Howell test, was performed. The latter was also applied to evaluate the influence of feeding treatment on particle maximum penetration depth (MPD) and on tracer inventories below sediment depths of 1 cm, 2 cm and 10 cm. Student's  $t$  test was used to assess the effect of feeding regime (control,  $F^-$ ,  $F^+$ ) on polychaete mortality and biomass. Multidimensional scaling analysis (MDS) was conducted to indicate differences in tracer quantities below sediment surface levels and at sediment depths of 10 cm. A three-way permutational multivariate analysis of variance (PERMANOVA, [58]) was performed to compare the effects of the factors feeding regime (controls,  $F^+$ ,  $F^-$ ), particle type (luminophores, PE, PA) and sediment depth (16 respective depth layers, nested into feeding regime to account for depth

layer dependency) on particle quantities across all sediment layers. Homogeneity of factor dispersion was tested using the PERMDISP test. Prior to analysis, assumptions of normality and homogeneity of variances were evaluated with the Shapiro–Wilk test to verify the assumptions for ANOVA and *t* test. As the data did not meet assumptions for ANOVA, they were log<sub>10</sub>-transformed. All results are given as means with standard deviations. Statistical analyses were conducted using IBM SPSS Statistics for Windows (version 23.0; Shapiro–Wilk test, Games–Howell test) and PRIMER v.6 (MDS, PERMANOVA (using the add-on package PERMANOVA+).

### 3. Results

#### 3.1. Sediment Properties

Sediment median grain size was 184 µm with a corresponding sorting of 0.78. The sediment was thus classified as moderately sorted fine sand. Sediment organic matter content was determined to be  $0.46 \pm 0.02\%$  DM (*n* = 5); correspondingly, POC content was  $0.18 \pm 0.01\%$  DM (*n* = 5). Mean porosity was measured as  $0.36 \pm 0.004$  (*n* = 5).

#### 3.2. General Observation

After several days of incubation, a thin yellowish-brown layer appeared at the sediment surface, with an underlying layer of yellow oxidized sediment extending to a depth of 0.5–1 cm. This contrasted with deeper sediment strata exhibiting a dark grayish color, indicating anoxic or suboxic conditions. Numerous small burrow structures, approximately 1 mm in diameter near the sediment surface, were found in all mesocosms, suggesting the presence of smaller fauna. This indicates that sieving and mesocosm anoxia were insufficient to remove all fauna.

Characteristic polychaete food-searching behavior, involving prospecting the sediment surface and the formation of mucus-lined trails in star-like patterns around most burrow openings, was frequently observed. In this context, the immobilization of all particle tracer types by mucus was visible by the naked eye, resulting in the formation of numerous mucus-particle aggregates and leading to an increasingly patchy distribution of tracers at the sediment surface over time. Upon food addition, polychaetes exhibited a strong and immediate response, characterized by intense sediment surface searching behavior. Particle transport as a consequence of food searching and feeding was frequently observed. Particle tracers adhering to polychaete bodies or spinach fragments were translocated during feeding and dragged into burrows.

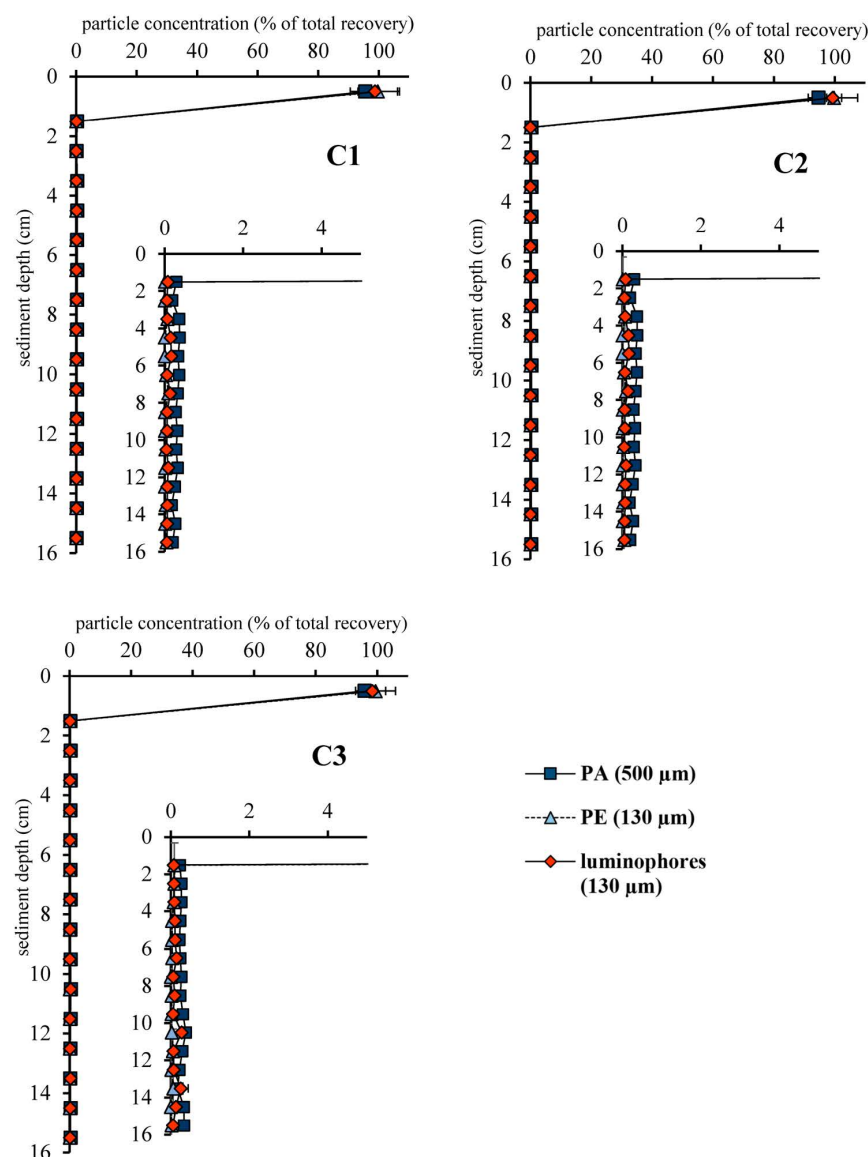
#### 3.3. Mortality and Biomass Change

Eighteen polychaetes were found lying on the sediment surface with little to no movement and did not retreat into the sediment within 12 h and were removed, indicating a mortality rate of 30% (Table S2, Supplementary Information). Mortality occurred in every mesocosm except F<sup>−</sup>1 and reached up to 60% of individuals originally introduced into a mesocosm (F<sup>+</sup>1). The feeding treatment had no significant effect on mortality (Student's *t* test).

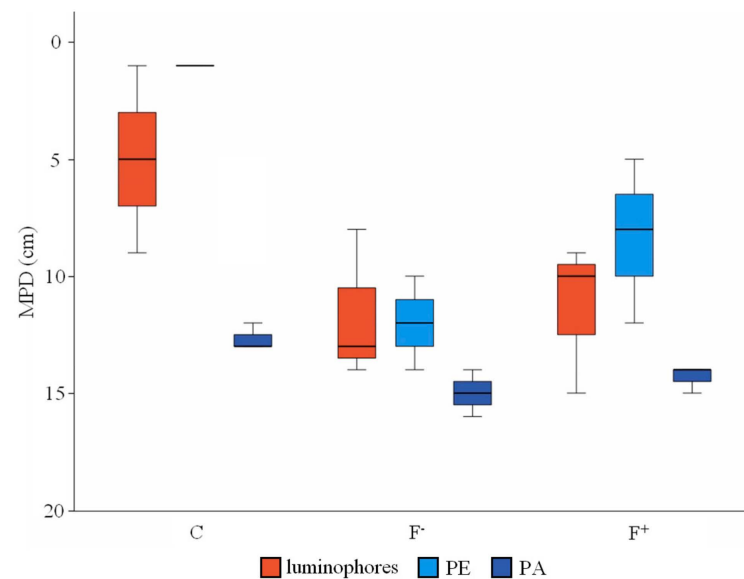
A net loss of biomass (Table S2, Supplementary Information) was observed in all mesocosms where polychaete mortality occurred, with a biomass decrease ranging from 0.7 g to 3.0 g (FM) by the end of the experiment. The greatest loss coincided with the highest mortality observed in F<sup>+</sup>1. Almost no net biomass change was detected in F<sup>−</sup>1. Biomasses for each feeding treatment did not differ significantly at either the start or end of the experiment (Student's *t* test).

### 3.4. Particle Tracer Distribution

Particle recovery rates were high,  $\geq 95\%$  for each tracer in 11 out of 15 mesocosms, with lowest recovery observed for PA particles in the F+1 treatment (83.01%, Table S3, Supplementary Information). In control treatments, the vast majority of particle tracers were found in the top 1 cm of sediment at the end of the experiment (luminophores:  $98.82 \pm 0.52\%$ ,  $n = 3$ ; PE:  $99.63 \pm 0.16\%$ ,  $n = 3$ ; PA:  $95.42 \pm 0.65\%$ ,  $n = 3$ ) (Figure 1). Low and uniform particle quantities were detected in all subsurface layers down to 16 cm sediment depth. PA concentrations in the control treatment were slightly elevated across all depth layers ( $0.31 \pm 0.03\%$ ,  $n = 15$ ) compared to luminophores ( $0.08 \pm 0.02\%$ ,  $n = 15$ ) and PE particles ( $0.02 \pm 0.02\%$ ,  $n = 15$ ). Maximum penetration depth (MPD), defined as the depth at which 99% tracer recovery occurs [33], was highest for PA particles (Figure 2) and differed significantly from the MPD of PE particles ( $p < 0.01$ ; Games–Howell test), but not from luminophores.

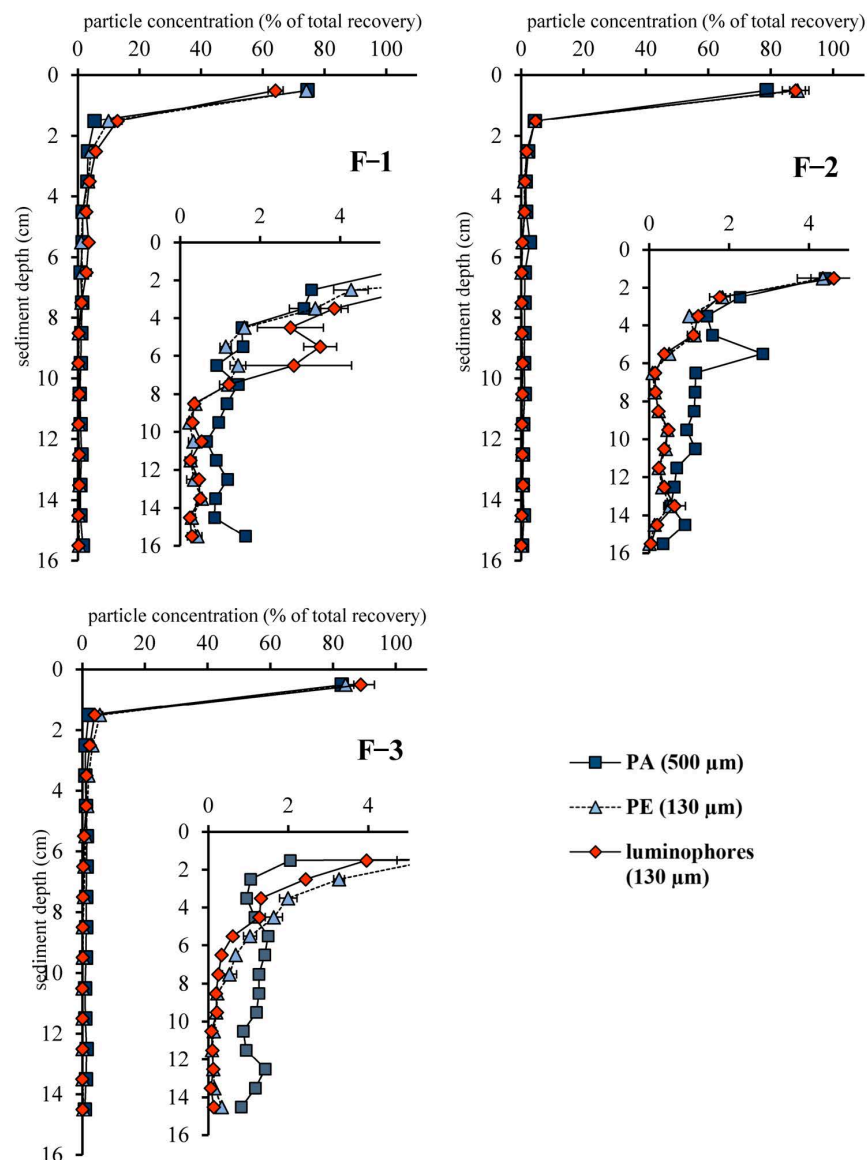


**Figure 1.** Distributions of luminophore and microplastic (PA, PE) particles in the control treatment. Error bars represent analytical replicates for luminophore concentration determination (mean  $\pm$  SD;  $n = 3$ ). Insets depict the low concentration range (0–4%).



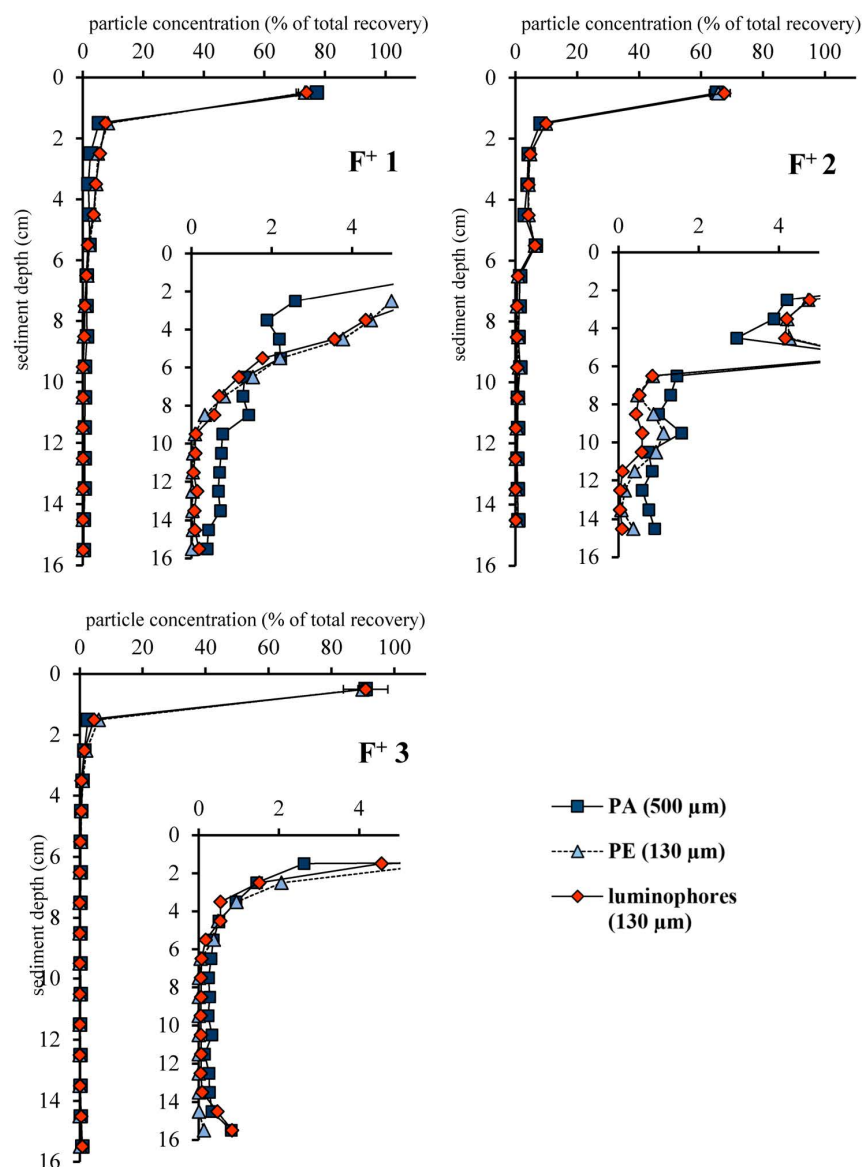
**Figure 2.** Maximum penetration depth (MPD) of all tracer types used in control and feeding treatments. MPD is defined as the depth at which 99% of tracers are recovered. Boxplots ( $n = 3$ ) indicate median and 25th and 75th percentiles.

In polychaete treatments, particle removal from the sediment surface due to sediment reworking was similar for all tracer types used, with recovered sediment surface tracer quantities of  $78.88 \pm 11.82\%$  (luminophores,  $n = 6$ ),  $79.25 \pm 9.72\%$  (PE,  $n = 6$ ) and  $78.27 \pm 8.58\%$  (PA,  $n = 6$ ). From the sediment surface to a depth of 5 cm, all tracer concentrations exhibited a moderate exponential decrease ( $R^2$  values ranging from 0.51 to 0.76 for  $F^-$  and 0.54 to 0.60 for  $F^+$ , respectively; Table S4, Supplementary Information) with increasing sediment depth, reaching control treatment background concentrations at approximately 10 cm depth (Figures 3 and 4). A subsurface luminophore and MP concentration peak was observed in the  $F^+$  mesocosm in the depth range of 4–6 cm with maximum concentrations of circa 10% of particle quantities added (Figure 4). Luminophores and PE particles generally penetrated deeper into the sediment in polychaete treatments, with MPDs around or below 10 cm (Figure 2). Penetration depth of PE particles was significantly greater in  $F^-$  treatments compared to controls and the  $F^+$  treatment. No significant difference in MPD between feeding treatments was found for any tracer type. MPD of PA particles was high in all treatments but was significantly greater than control penetration depths only in  $F^-$  treatments. Cumulative particle concentrations below sediment depths of 1 cm, 4 cm and 10 cm were determined as measures of particle export from the sediment surface (0–1 cm) to below the zone of biodiffusive mixing (4 cm) or near the bottom zone of polychaete burrows (10 cm), respectively. Similar to MPD, significant differences in particle transport were found only between control and  $F^-$  treatments for PA particles at all three depth thresholds ( $p < 0.05$ , Games–Howell test). Using these depth threshold data, an MDS analysis was performed, which allowed for a rough distinction between control and HD mesocosms; however, a clear distinction between the two feeding treatments could not be made due to high variability in all three tracer depth distributions (Figure 5).



**Figure 3.** Distributions of luminophore and microplastic (PA, PE) particles in the no-feeding treatment ( $F^-$ ). Error bars represent analytical replicates for luminophore concentration determination (mean  $\pm$  SD;  $n = 3$ ). Insets depict the low concentration range (0–4%).

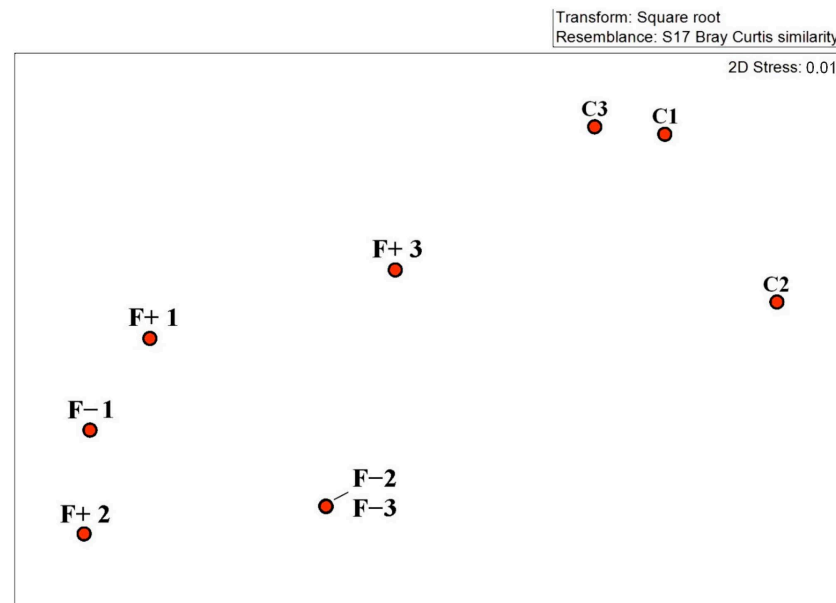
PERMANOVA analysis revealed significant effects of the factors particle type, treatment, and depth on particle distribution across every sediment depth layer ( $p < 0.01$ ). Interactions were observed between particle type and treatment, particle type and depth, as well as between treatment and depth, but not among all three factors simultaneously. The PERMDISP test indicated significant differences in dispersion homogeneity for all three factors ( $p < 0.01$ ). Particle-dependent mixing was detected only in control mesocosms, with PA concentrations significantly exceeding those of luminophore and PE particles in most depth layers ( $p < 0.05$ , Games–Howell test; Table 1). PA concentrations were significantly increased in some sediment layers below 8 cm depth in the  $F^-$  treatment (8–10 cm, 11–12 cm, 14–15 cm;  $p < 0.01$ ; Games–Howell test). No differential particle mixing was observed for the  $F^+$  treatment at any depth.



**Figure 4.** Distributions of luminophore and microplastic (PA, PE) particles in the feeding treatment ( $F^+$ ). Error bars represent analytical replicates for luminophore concentration determination (mean  $\pm$  SD;  $n = 3$ ). Insets depict the low concentration range (0–4%).

**Table 1.** Results from a two-way PERMANOVA analysis of particle tracer quantity for the factors particle type (PE, PA, and luminophores), treatment (control, no feeding, and feeding) and depth (respective depth layers from 1 to 15 cm). Significant effects ( $p < 0.05$ ) are indicated by asterisks.

| Source                                          | df  | SS      | MS     | Pseudo-F | $p$<br>(Perm) | Unique<br>Perms |
|-------------------------------------------------|-----|---------|--------|----------|---------------|-----------------|
| Particle type                                   | 2   | 58,088  | 29,044 | 79.929   | 0.001 *       | 997             |
| Treatment                                       | 2   | 100,530 | 50,266 | 138.33   | 0.001 *       | 998             |
| Depth                                           | 15  | 226,240 | 15,083 | 41.508   | 0.001 *       | 998             |
| Particle type $\times$ treatment                | 4   | 18,579  | 4644.7 | 12.782   | 0.001 *       | 999             |
| Particle type $\times$ Depth                    | 30  | 16,338  | 544.6  | 1.4987   | 0.006 *       | 998             |
| Treatment $\times$ Depth                        | 30  | 49,647  | 1654.9 | 4.5543   | 0.001 *       | 999             |
| Particle type $\times$ Treatment $\times$ Depth | 60  | 17,025  | 283.75 | 0.78087  | 0.968         | 998             |
| Residuals                                       | 265 | 96,294  | 363.37 |          |               |                 |



**Figure 5.** Two-dimensional MDS plot of controls and feeding treatments ( $F^-$ : no feeding;  $F^+$ : feeding) showing square-root-transformed luminophore and microplastic (PA, PE) concentrations at sediment depths of less than 1 cm, 4 cm and 10 cm based on Bray–Curtis similarities.

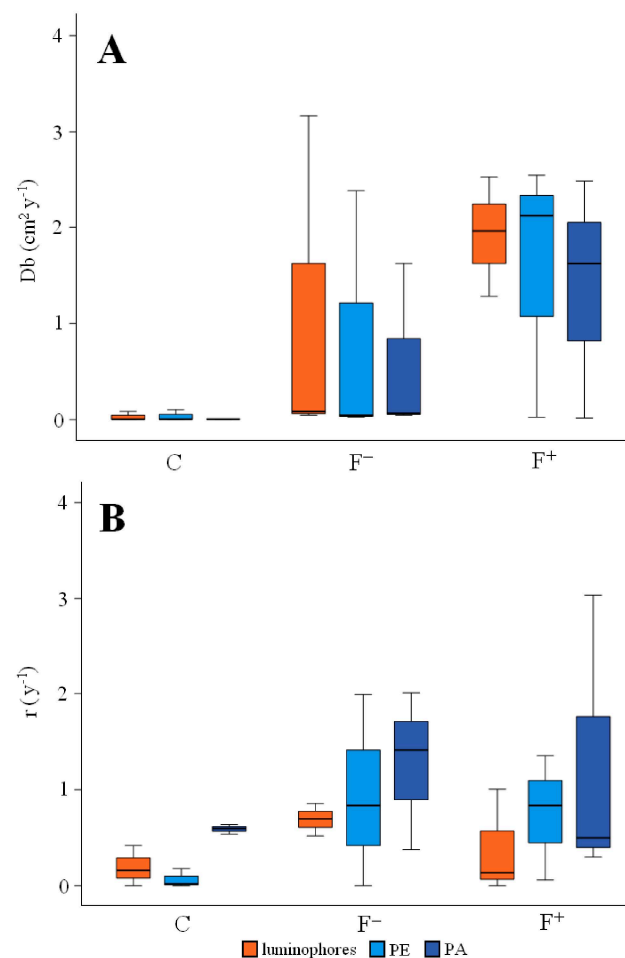
### 3.5. Modeled Particle Transport

Polychaete bioturbation resulted in increased local and non-local transport of all particle types. Control mesocosms exhibited minimal particle transport, with  $Db$  values close to zero for luminophores ( $0.03 \pm 0.05 \text{ cm}^2 \text{ yr}^{-1}$ ,  $n = 3$ ) and PE particles ( $0.03 \pm 0.06 \text{ cm}^2 \text{ yr}^{-1}$ ,  $n = 3$ ). No bioturbative transport was observed for PA particles. Small non-local transport rates ( $r$ ) were detected for all particle tracers, with higher values for luminophores ( $0.19 \pm 0.21 \text{ yr}^{-1}$ ,  $n = 3$ ) and PA particles ( $0.59 \pm 0.05 \text{ yr}^{-1}$ ,  $n = 3$ ) compared to PE particles ( $0.07 \pm 0.1 \text{ yr}^{-1}$ ,  $n = 3$ ; Figure 6A).

Local and non-local transport coefficients increased in polychaete treatments compared to controls, with the highest  $Db$  values observed for luminophores and the lowest for PA particles (luminophores:  $1.51 \pm 1.28 \text{ cm}^2 \text{ yr}^{-1}$ ; PE:  $1.19 \pm 1.28 \text{ cm}^2 \text{ yr}^{-1}$ ; PA:  $0.97 \pm 1.07 \text{ cm}^2 \text{ yr}^{-1}$ ;  $n = 6$ ). In contrast to  $Db$ , for  $r$  values the pattern was reversed (luminophores:  $0.54 \pm 0.40 \text{ yr}^{-1}$ ; PE:  $0.85 \pm 0.77 \text{ yr}^{-1}$ ; PA:  $1.28 \pm 1.10 \text{ yr}^{-1}$ ,  $n = 6$ ; Figure 6A,B). Observed  $Db$  and  $r$  were not dependent on particle type; however,  $r$  was dependent on treatment (two-way ANOVA,  $p < 0.05$ ), while  $Db$  was not. The interaction between factors particle type and depth showed no significant effect on bioturbation coefficients.

Modeled bioturbation coefficients exhibited considerable variation among treatments, with  $Db$  values close to zero but detectable  $r$  transport in two mesocosms ( $F^-2$ ,  $F^-3$ ) and the reversed pattern in the remaining four mesocosms ( $F^-1$ ,  $F^+1$ ,  $F^+2$ ,  $F^+3$ ).  $Db$  and  $r$  values were similar for all tracer types in all mesocosms except for  $F^+3$ , which showed luminophore  $Db$  values of  $1.28 \text{ cm}^2 \text{ yr}^{-1}$ , contrasted by MP  $Db$  values near zero (PE:  $0.02 \text{ cm}^2 \text{ yr}^{-1}$ , PA:  $0.01 \text{ cm}^2 \text{ yr}^{-1}$ ).

PA  $r$  values were significantly higher than PE  $r$  values in the control mesocosms ( $p < 0.05$ ). Bioturbation coefficients for all other particle tracers showed no significant differences between the two feeding groups, most likely due to high variability. Additionally, the feeding treatment had no significant effect on particle transport for any of the tracer types.



**Figure 6.** Modeled biodiffusion coefficients ( $Db$ ) (A) and non-local coefficients ( $r$ ) (B) of all particle tracers for control and feeding treatments ( $F^-$ : no feeding,  $F^+$ : feeding), assessed using the gallery-diffuser model. Boxplots ( $n = 3$ ) indicate the median and the 25th and 75th percentiles.

## 4. Discussion

### 4.1. Luminophore and Microplastics Transport

The presence of MPs potentially impacted the bioturbation activity of *H. diversicolor*, as MPs are known to reduce the bioturbation performance of polychaetes [44,59] and to affect ecological functions linked to bioturbation, such as increased ammonia fluxes [60] and reduced downward transport of organic matter [44]. PE particles of different sizes have been shown to induce neurotoxic effects in *H. diversicolor*, but did not affect the energetic values of the polychaetes [61]. The implications of these findings for the bioturbation performance of *H. diversicolor* still remain unclear; however, some effects cannot be ruled out completely.

Tween is known to induce harmful effects in some invertebrates, such as the inhibition of larval development in sea urchins [62]. However, data about any effects of Tween on *H. diversicolor* are lacking. Since juvenile stages of invertebrates are generally more susceptible to effects of Tween surfactants, and toxic concentrations occur far below toxicity limits known for mammals [63], any effect of Tween on the condition of adult polychaetes can be considered negligible.

Particle distributions in all polychaete treatments exhibited exponential decreases near the surface layer, indicating diffusion-analogous (local) particle transport. The observed particle export down to sediment depths greater than 10 cm, along with the presence of particle peaks in mesocosms  $F^-2$  and to some extent in  $F^+2$ , suggests non-local transport.

The simultaneous occurrence of local and non-local particle transport is characteristic of the bioturbation behavior of the gallery-diffuser *H. diversicolor* [64,65]. However, no significant differences in particle transport were found between controls and polychaete treatments, primarily due to high variability in particle concentration within the biodiffusive layer (sediment depth  $\leq 4$  cm) and generally low particle concentrations below this layer.

Given the relatively low number of mesocosms, which potentially limits the statistical power of the analysis, and the highly variable sediment reworking observed among mesocosms within the same treatment, interpreting particle transport results is challenging. The MDS results for vertical particle transport (Figure 5) allow a rough distinction between control and  $F^-/F^+$  mesocosms, indicating a measurable effect of polychaete activity on particle redistribution. Modeled  $Db$  values range between  $0.57 \text{ cm}^2 \text{ yr}^{-1}$  (PA;  $F^-$ ) and  $1.97 \text{ cm}^2 \text{ yr}^{-1}$  (luminophores,  $F^+$ ), which fall well within the range reported by other studies conducted under similar temperatures and abundances [22,65]. In contrast to biodiffusion,  $r$  values were generally low, with minimum values of  $0.27 \text{ yr}^{-1}$  (luminophores,  $F^+$ ) and maximum values of  $1.23 \text{ yr}^{-1}$  (PA,  $F^-$ ), which are 3 to 10 times lower than those reported in other studies [65,66]. Nevertheless, similarly low biotransport values for *H. diversicolor* were reported by Cournane et al. [64] and notably by Nogaro et al. [45]. The latter study stimulated the bioturbation activity of *H. diversicolor* through food addition, in a manner comparable to the approach used in this study (see also Section 4.2).

Various factors are known to influence the sediment reworking activity of *H. diversicolor*, with abundance and burrow morphology likely playing the most pivotal roles [42,56]. Mortality rates in both polychaete treatments were relatively high ( $F^-$ : 17%;  $F^+$ : 43%), likely due to the onset of reproduction in some individuals. This is supported by the observation of several polychaetes exhibiting a distinctive greenish coloration during the experimental incubation, indicating a recent spawning event. Baltic Sea populations of *H. diversicolor* are known to spawn in early spring [67], which coincides with the polychaete sampling date.

The strong territorial behavior of *H. diversicolor* may also influence abundance in the mesocosms, as polychaetes are known to defend burrows and openings against intruders [68]. The observed mortality might therefore be attributed to intraspecific competition. Abundances in both feeding treatments were  $200 \text{ ind. m}^{-2}$ , which is well below the maximum densities recorded in the field, where populations can reach several  $1000 \text{ ind. m}^{-2}$  [38,69]. At the abundances used in this experiment, long-term mortality is reported to remain low, at about 5% [70], suggesting that the observed mortality does not reflect an abundance-related effect.

Another possible effect of territoriality is the decoupling of bioturbation and abundance. The individual contribution to overall bioturbation performance decreases with increasing abundance [42], reflecting changes in burrow geometry and a reduction in the individual searching area at the sediment surface. A small number of polychaetes can cover comparably large sediment areas in their search for food and thus mediate particle transport rates comparable to those carried out by larger communities of *H. diversicolor* [65], potentially compensating for mortality effects in terms of particle transport. This effect is likely reflected in bioturbation coefficients that do not differ significantly between feeding treatments, despite the  $F^+$  treatment exhibiting mortality rates 2.5 times higher than the  $F^-$  treatment. Since burrows established by different polychaetes never come into contact with each other [38], the sediment volume available for constructing a dense gallery network in the upper burrow sections is much smaller at high abundances, leading to more vertically oriented galleries and burrows [38], which may promote non-local transport processes. At lower abundances, gallery networks of single burrows with extended horizontal sections can extend farther, favoring local transport in the upper sediment layers. The slightly higher  $Db$  values in the  $F^+$  treatment, which has reduced abundances due to higher mor-

tality, might indicate this effect, although differences between feeding treatments are not statistically significant.

#### 4.2. Feeding Effect

Increased food availability is known to significantly enhance particle transport mediated by *H. diversicolor*, as demonstrated in other studies [45,57]. This species is known to drag food into the gallery sections of its burrow for safe ingestion [41], thereby promoting local particle transport. However, this study found no significant effect of feeding on either local or non-local particle transport. As previously discussed, the effect of food addition on particle reworking may be obscured by the strong individual variability observed. A non-linear impact of enhanced food availability on bioturbation performance was also reported by Murray et al. [57], with the greatest increases in particle transport occurring only at moderate food additions. Furthermore, Nogaro et al. [45] demonstrated that food addition caused no increase in local, but only in non-local transport, further emphasizing that particle burial is likely governed by multiple factors.

The rapid and selective uptake of freshly deposited food items observed in the F<sup>+</sup> treatment has been previously documented, with freshly deposited particles reaching maximum burial depths in less than one day [66]. This suggests that feeding-induced particle burial can occur over short time scales. Despite such rapid responses following food addition, increased food supply was found to have no significant effect on particle transport for *H. diversicolor* [45,57], indicating that polychaete activity cannot be directly correlated with food availability. Mucus secretion at the sediment-water interface may hinder particle burial by immobilizing particulate material, even when polychaete activity is high. Selective ingestion of freshly added material [71] might also reduce particle transport by limiting the individual sediment surface area that is prospected for food.

As these short-term responses represent a pathway for the rapid removal of freshly deposited material [72], this selective uptake may, in contrast, serve as an effective mechanism for the ingestion of deposited MPs incorporated into marine aggregates [11,73]. *H. diversicolor* is known to preferentially ingest MPs incorporated into biodeposits of the mussel *Mytilus galloprovincialis* [74], potentially facilitating the subsequent burial of MPs into marine sediments.

#### 4.3. Particle Selective Transport

Differences in fauna-induced particle transport between MPs and ambient sediment grains may result from the distinct physical properties of MP particles, particularly those that are smaller or have a lower specific density than the surrounding sediment. These two parameters are known to influence differential particle transport in the context of bioturbation, specifically as a consequence of animal locomotion, burrow construction or the selective ingestion of particles. Differential gravitational particle migration that occurs during sediment disturbance by animal movement can cause a stronger vertical transport of smaller particles [75,76]. Similarly, selective ingestion can promote the burial of smaller particles, as these particles have a greater surface area-to-volume ratio and thus provide more food per ingested volume in habitats where most available food is associated with particle surfaces. Additionally, many surficial deposit-feeding animals tend to preferentially ingest particles of lower specific gravity, which is also explained in the context of the optimal foraging theory, since less dense particles are often associated with higher nutritional value. Based on these considerations, the selective uptake of polystyrene particles over ambient sediment grains has been demonstrated for several taxa of surficial deposit-feeding invertebrates [77].

The particle tracers used in this study differed in size and specific density, making differential transport of MPs plausible. PERMANOVA results indicated a significant influence of particle type on tracer burial, as well as significant interactions between particle type and treatment. This interaction is most likely attributable to significantly increased  $r$  values and MPDs for PA compared to PE in the control treatment. This observation may represent an artifact that occurred during mesocosm sectioning, as no further differences in particle transport or burial were observed in any other treatment. The formation of a subsurface tracer peak in F<sup>+</sup>2—a typical result of gallery diffuser bioturbation—was observed for all three tracer types, indicating that luminophores and MPs were subjected to non-local transport similarly. Despite high variability in bioturbation coefficients and limited statistical power due to a low number of treatment replicates, these characteristic features of gallery-diffuser bioturbation support the conclusion that the distribution of MPs and sediment grains is governed by the functional ecology of *H. diversicolor*, despite the particles exhibiting different physical properties.

Size- and density-driven differences in particle transport, as described by Wheatcroft [76] and Taghon [75], are primarily stochastic processes governed by differential gravitational forcing or by the probability of contact between feeding appendages and sediment particles. Passive tracer transport by adhesion to polychaete bodies during food search may operate similarly, likely favoring the transport of small, low-density particles such as PE and luminophores. However, no increased transport of PE particles or luminophores was observed in this study. Conversely, active particle ingestion by *H. diversicolor* may result in directed tracer transport. Ingestion of PA particles similar in size to those used in this study has been reported for *H. diversicolor* [71]. Interestingly, the same study describes a preferential ingestion of natural eelgrass seedlings over PA particles of comparable size and density, suggesting that polychaetes can distinguish between particles, likely based on differing surface properties. Furthermore, the uptake of relatively large particles of both natural (eelgrass seedlings) and anthropogenic origin (PA particles, paint fragments; [78]) indicates that particle size may influence the ingestion behavior of *H. diversicolor*. Larger particles may more closely resemble detritus or organic matter aggregates, representing a preferential food source for *H. diversicolor*. However, no significant effect of particle size on local or non-local transport was detected in this experiment. Preferential particle transport and burial through ingestion may be canceled out by fecal pellet deposition at the sediment surface, resulting in no net transport of ingested particles over longer time scales. In this context, effective uptake and egestion of differently sized PE particles (63–355  $\mu\text{m}$ ) has been demonstrated for *H. diversicolor* [61].

Differential transport of particles with varying sizes and specific gravities may also be influenced by other factors as well, such as feeding mode, mucus secretion, and burrow morphology. Consequently, these factors can cause spatial and temporal variations for a given community of *H. diversicolor*.

Strong differential MP transport based on size-selective feeding could also be observed in polychaetes representing other bioturbation types [79], highlighting the importance of functional characteristics of macrozoobenthos in MP ingestion and transport [45,80]. Unlike the single-species approach used in this study, assessing in situ MP bioturbation often requires considering sediment reworking activity at the community level, where bioturbation performance results from various mixing types with differing burial step lengths and frequencies that interact with one another. Although species that promote strong non-local transport can influence particle distributions, many depth-dependent particle profiles observed in situ can be adequately described using biodiffusion models alone—a phenomenon referred to as the “biodiffusion paradox” [23,24]. In this context, the distinction between local and non-local transport often depends on the time scales considered for

interpretation, as the number of transport events increases, particle distributions tend to conform to diffusion-analogous mixing patterns [81]. This process can smooth out particle peaks associated with discrete transport events. Therefore, the distinctive pattern of MP burial caused by *H. diversicolor*, which includes both local and non-local transport characteristics, may only be evident in specific regions with high abundances of *H. diversicolor*, where the species represents the dominant faunal element of a benthic community, such as shallow brackish waters or estuaries along the European coast [38,82].

## 5. Conclusions

In this study, we demonstrated the burial of MP particles as a result of bioturbation activity of *H. diversicolor* to sediment depths of 10 cm and beyond. Linking MP bioturbation to other index parameters, such as bioturbation potential [26] or modeled transport coefficients [43], is a relatively novel approach. To our knowledge, expressing MP bioturbation through modeled *Db* and *r* values has not been previously done and provides new insights into this field of research. Our results demonstrate that MPs undergo both biodiffusive and advective transport as a consequence of gallery-diffuser bioturbation. Although polychaetes exhibited strong behavioral responses to feeding, the addition of food had no significant effect on tracer burial, likely obscured by the high variability in sediment reworking activity of *H. diversicolor*. Despite differences in density and size, the distribution of particle tracers used in this study showed no evidence of differential bioturbation-induced transport, contrasting with findings from studies focusing on other bioturbation types, such as conveyor-belt feeders, and further emphasizing the influence of the functional composition of benthic communities on MP burial. The consistency of MP and luminophore mixing observed here may allow for the derivation of MP burial potentials from existing bioturbation data in habitats dominated by biodiffusive mixing, thereby complementing established MP monitoring approaches.

**Supplementary Materials:** The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/jmse14151433/s1>, Table S1: Tracer quantities added to each mesocosm in the experiment. The shown particle concentrations represent the MP load integrated over the total mesocosm sediment dry mass (DM); Table S2: Individual biomass (g fresh mass, FM) of all polychaetes added to the mesocosms. Net biomass change per mesocosm is shown as the difference between the start and end of the experiment; Table S3: Recovery rates (% of the total amount of added tracers) for all mesocosms and particle types used in this study; Table S4: Exponential regressions of all F treatment tracer distributions. Regression parameters correspond to the model  $y = a \cdot e^{bx}$ . “x”: sediment depth. “R<sup>2</sup>”: coefficient of determination. To optimize model fit, regression analysis performed using sediment depths where no particle tracer peaks occurred (0–5 cm).

**Author Contributions:** Conceptualization, C.G.; methodology, C.G. and M.B.; software, C.G. and M.B.; validation, M.B.; formal analysis, C.G. and M.B.; investigation, M.B.; resources, S.F.; data curation, C.G. and M.B.; writing—original draft preparation, C.G.; writing—review and editing, M.B. and S.F.; visualization, C.G.; supervision, S.F.; project administration, S.F.; funding acquisition, S.F. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research was supported by the WGL-PAKT project “The role of microplastic as vector for microbial populations in the ecosystem Baltic Sea” (MikrOMIK), funded by the German Leibniz Association (grant number SAW-2014-IOW-2) to Leibniz Institute for Baltic Sea Research Warnemünde (IOW).

**Data Availability Statement:** The original data presented in the study will be made are openly available until publication of the study.

**Acknowledgments:** We wish to thank M. Powilleit (University of Rostock) for helpful suggestions and advices on experiment setup and sample preparation and Elke Meier (University of Rostock) for the processing of C/N samples.

**Conflicts of Interest:** Michael Beckers was employed by Sonnis Energy GmbH. The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

## Abbreviations

The following abbreviations are used in this manuscript:

|     |                            |
|-----|----------------------------|
| DM  | dry mass                   |
| FM  | fresh mass                 |
| MDS | multidimensional scaling   |
| MP  | microplastics              |
| MPD | maximum penetration depth  |
| PA  | polyamide                  |
| PE  | polyethylene               |
| POC | particulate organic carbon |

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