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Benthic fauna contribute to microplastic sequestration in coastal sediments

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ABSTRACT

Microplastics are ubiquitous in the marine environment, however, the mechanisms governing their uptake by, and burial within, seabed habitats are poorly understood. In this study, microplastic burial and its impact on fauna-mediated sedimentary processes was quantified at three coastal sites, and the potential contribution of burrowing faunal communities to this process assessed via functional trait diversity analysis of field data. In addition, laboratory exposures were used to assess whether sediment-processing undertaken by the brittlestar *Amphiura filiformis,* a key species in the sampled area, could explain the burial of microplastic fibres. Field observations confirmed broad-scale burial of microplastics across the coastal seabed, consistent across sites and seasons, with microplastic sequestration linked to benthic-pelagic exchange pathways, driven by burrowing fauna. Brittlestars were observed to bury and line their burrow walls with microfibres during experiments, and their burial activity was also modified following exposure to nylon fibres, relative to controls. Collectively, these results indicate that biodiverse and functionally important seabed habitats act as microplastic sinks, with burrowing fauna contributing to this process via well-known benthic-pelagic pathways, the rates of which are modified by plastic exposure.

1. Introduction

Microplastic debris (plastic 1 μm – 5 mm) is a globally recognised pervasive pollutant (Thompson et al., 2004), affecting the health of marine species and ecological processes (Galloway et al., 2017, 2008/56/EC Marine Strategy Framework Directive, Descriptor 10, United Nations Sustainable Development Goal 14 target 14.1.1). The majority of studies reporting marine plastic pollution stem from measurements at the sea-surface (Cózar et al., 2014), yet microplastics have been shown to accumulate in benthic sediments (Bergmann et al., 2017; Ling et al., 2017; Woodall et al., 2014). Though the processes regulating sedimentary loading are much less understood than in the water columns (Underwood et al., 2017), existing data suggest there is currently a mismatch between expected and reported microplastic concentrations in surface waters (Eriksen et al., 2014; Lindeque et al., 2020; Thompson et al., 2004), and concentrations in sediments can exceed overlying waters by more than four orders of magnitude (Bergmann et al., 2017; Kane et al., 2020; Woodall et al., 2014). Benthic sediments may therefore serve as a final sink for microplastics, and the global inventory of microplastic litter in the ocean may be much larger than expected from more widely reported water column observations. Addressing the impacts of plastic litter on ocean health may thus be significantly more complex and impacts longer lasting than previously assessed, and our understanding limited by the challenge of sampling plastics from sediments (Coppock et al., 2017).

Microplastic transport through the water column to the seabed reflects wider benthic-pelagic exchange pathways, including biologically mediated transport via biofouling (Kooi et al., 2017), incorporation into organic matrices, including marine snow (Porter et al., 2018) and faecal pellets (Coppock et al., 2019), and by physical processes such as gravitational sinking, wind and current and tidal advection (Chubarenko et al., 2016). These hydrodynamic processes are the dominant transport drivers over the water column; however, in productive, organically rich and biodiverse coastal sediments, which include valued conservation habitat supporting a wealth of ecosystem service delivery, diffusion and other types of local transport dominate benthic-pelagic exchange

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pathways, driven by fauna-mediated processes including bioturbation, i.e. sedimentary particle and pore water exchanges (i.e. bioirrigation) (Kristensen et al., 2012). Bioturbation mediates fundamental benthic-pelagic processes including organic matter exchanges with the water column, and nutrient remineralisation (Queirós et al., 2019, 2015; Zhang et al., 2015). Bioturbating animals significantly alter the structure and pore-water content of soft sediment through foraging, feeding, dispersal, mating behaviours, burrow flushing and aerobic respiration. These activities enhance sedimentary habitat complexity and mixed layer depth, promoting oxidising conditions within marine sediments that are essential drivers of global ocean biogeochemical ecosystem function (Boyle et al., 2014; Kristensen and Kostka, 2004; Teal et al., 2008). This impact on benthic-pelagic exchange makes it extremely likely that bioturbators affect sedimentary plastic burial in natural environments. Indeed, recent laboratory-based studies have demonstrated that benthic fauna can bury microplastic which may not be returned to the sediment surface (Näkki et al., 2019, 2017). To date, no studies have investigated the role of burrowing fauna on microplastic burial in natural seabeds; such interactions will be especially important in coastal and shelf seas where co-occurrence of microplastics and fauna are predicted to be high (Clark et al., 2016).

1.1. Aims and hypotheses

This two-component study quantifies microplastic burial in coastal marine sediments and explores the potential role of marine benthic macrofauna on its mediation. First, field observations investigated the role that benthic faunal communities play in the burial of plastic in coastal systems. A field program was devised to test the hypotheses that bottom seawater adjacent to the sediment surface ("fluff layer") presents a viable repository for microplastics to enter the benthos and that microplastics are buried in natural coastal habitats, mediated by marine benthic fauna. Secondly, a microcosm experiment investigated the mechanisms underpinning plastic burial potential by benthic species, focusing on the widely studied and locally abundant brittlestar *Amphiura filiformis* (Calder-Potts et al., 2018; Queirós et al., 2015; Widdicombe et al., 2004). These experiments test the hypotheses that microfibres are buried through bioturbation/bioirrigation activities; and microfibres in sediments alter normal bioturbation activity and oxygen uptake by a key benthic species.

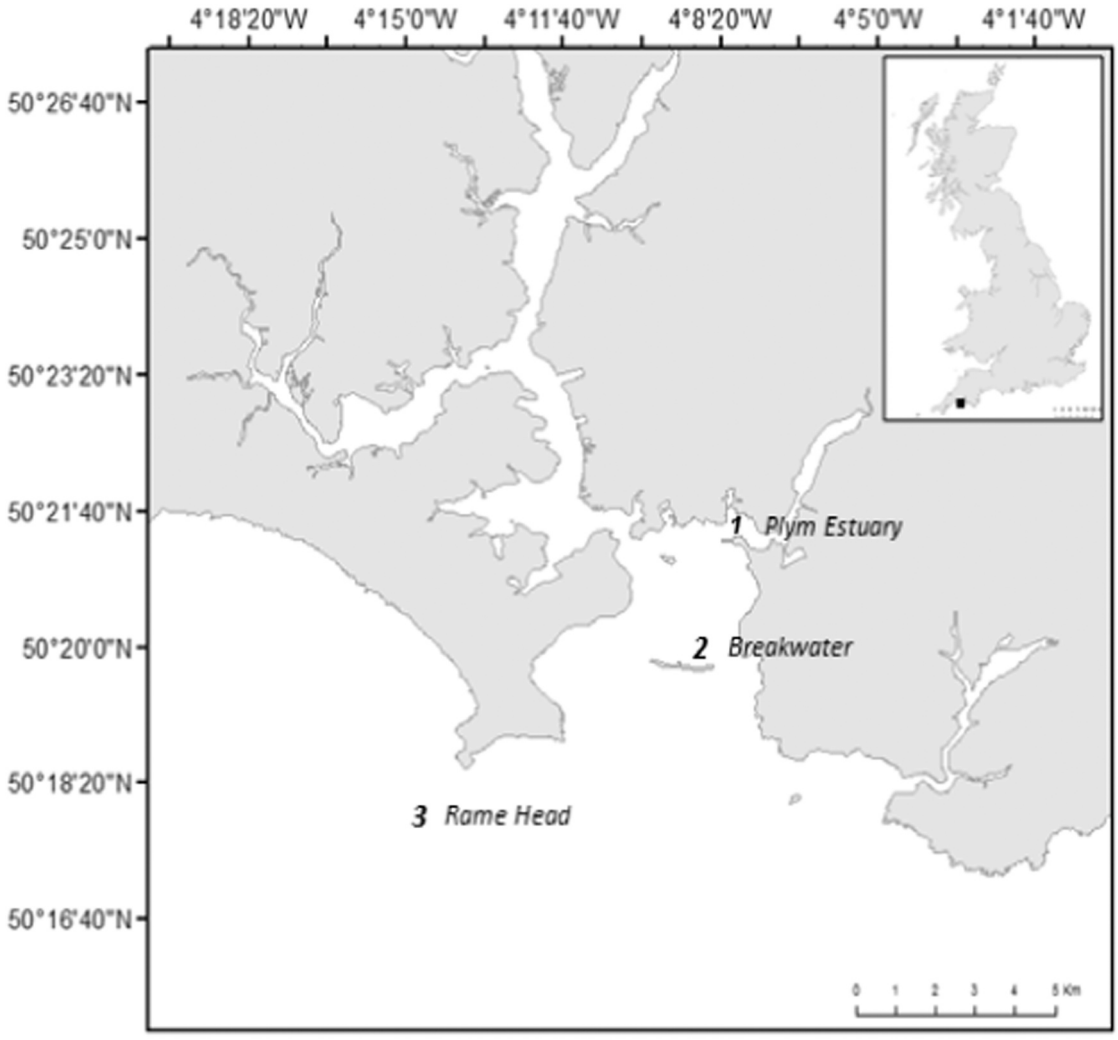

Fig. 1. Site map detailing benthic sample locations from 1) entrance to Plym Estuary (N50°21.716'; W4°08.073'), 2) inside Plymouth Sound breakwater (N50°20.174'; W4°08.605') and 3) off Rame Head (N50°17.925'; W4°15.057').

2. Methods

2.1. Environmental study

2.1.1. Site selection

Samples were collected from 3 sites (Plym, Breakwater and Rame) in the Western English Channel (Smyth et al., 2015) (Plymouth, UK; Fig. 1). Site selection was guided by past studies into the hydrodynamics of Plymouth Sound (Uncles et al., 2015), and model simulations of particle transport and dispersal (Chen et al., 2003). Sites were selected from a number of modelled possibilities; 1: The "Plym" site is located at the mouth of the Plym Estuary, and was selected as it receives direct inputs from the River Plym which flows at a long-term mean rate of 1 m^3 s^{-1} alongside the city of Plymouth where it receives industrial, maritime and wastewater inputs (Natural Environment Research Council, 2003), including the sewage works located approximately 3 km up the estuary; 2: the "Breakwater (BW)" site is located inside the Plymouth Sound breakwater, an artificial barrier that reduces hydrodynamic flow and is therefore a likely deposition zone; 3: the "Rame" site is located 2.5 km off Rame Head and is one of the stations routinely sampled as part of the Western Channel Observatory benthic sampling programme. This site has been intermittently used as a disposal site for over 100 years, initially used for munitions disposal but subsequently used for dredged material from the nearby ports, harbours and navigation channels (Bolam et al., 2011) and is thus likely rich in anthropogenic debris.

2.1.2. Sediment and fauna collection

Sampling was conducted using the Plymouth Marine Laboratory's RV Plymouth Quest. All sites were sampled during summer (June 2016), while in addition the Plym site was sampled at multiple points throughout the year (January, April, June and September 2016). Sediment samples ($n = 3$ per site, season and depth; Fig. 2) were collected using a benthic multicorer, housing four cylindrical polycarbonate corers (length: 50 cm x diameter: 10 cm) that collect sediment and bottom waters preserving sedimentary structure, including the integrity of the sediment-water interface.

2.1.3. Fluff layer collection and processing

The 'fluff layer' (organic rich (Queirós et al., 2019) layer immediately above the sediment-water interface), was gently syphoned from each core into a pre-rinsed 500 mL Nalgene bottle using pre-rinsed silicone tubing and syringe, avoiding resuspension of the sediment surface. The water sample was filtered onto new 10 μm membrane filters (Whatman Nuclepore Track-Etch) and transferred into a new, previously sealed lidded Petri dish.

2.1.4. Sediment processing - field

Each sediment core was sliced into three layers using a custom-made plywood core slicer (Section 1: top 2 cm; Section 2: 2–6 cm; Section 3: 6–10 cm depth, Fig. 2) and a stainless steel plate (25 cm × 20 cm), which was rinsed clean with MilliQ between slices and replicates. The top Section (1) was immediately placed into a pre-rinsed 1 L lidded pot (Kartell™) for processing at the laboratory. For the remaining Sections 2 and 3, 3 × 10 mL subsamples (30 mL per section) were taken randomly from each slice for quantification of microplastic abundance using a pre-rinsed 20 mL syringe with the end sliced off. Subsamples were extruded into pre-rinsed foil trays, sealed and transported to the laboratory. The remainder of each section was then sieved on deck using a 1 mm stainless steel sieve (Endcotts) to retain macrofauna, which was fixed in 4% buffered formaldehyde.

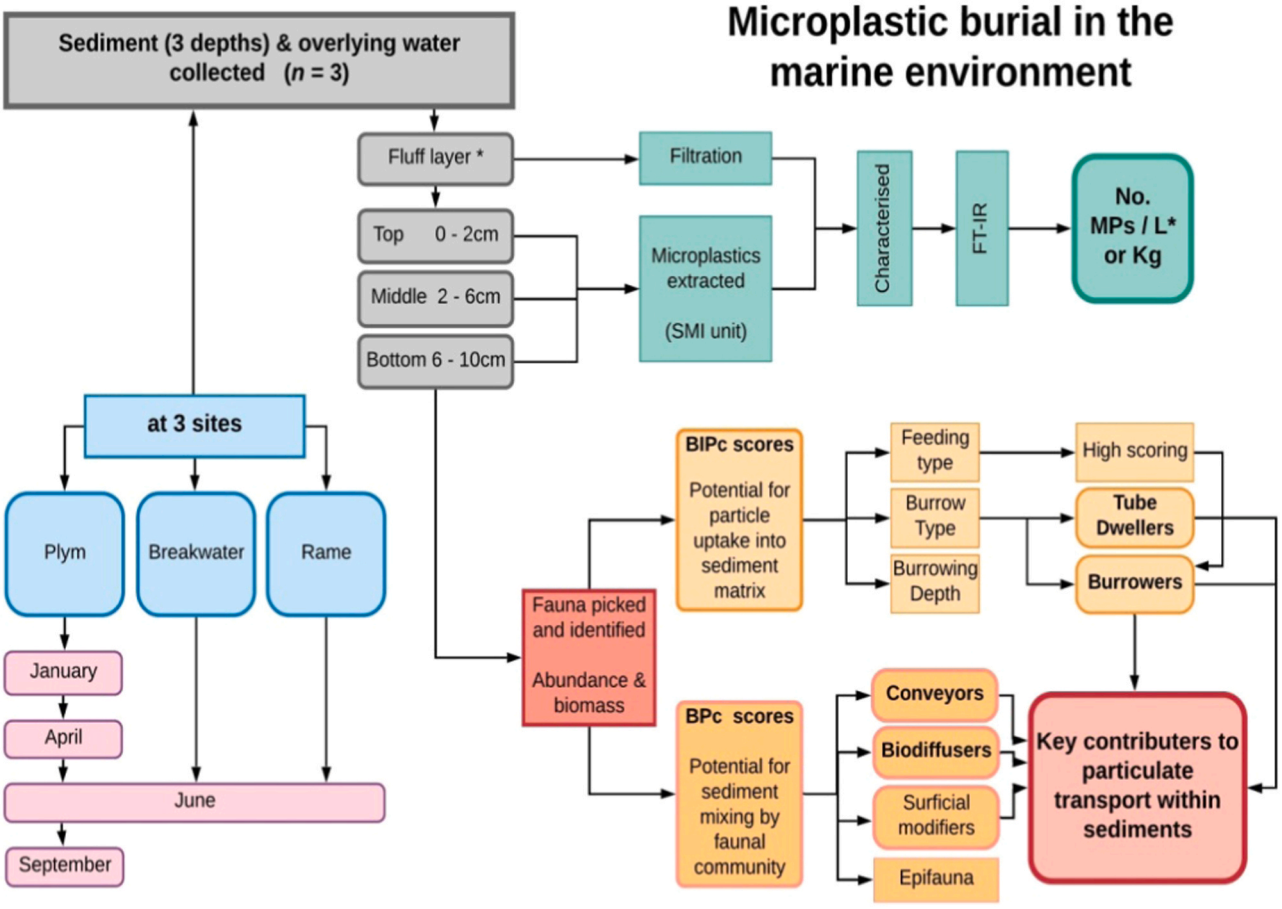

Fig. 2. Flow chart illustrating sample collection on the left and onward sample processing and functional trait analyses to the right.

2.1.5. Sediment processing - laboratory

Section 1 was sieved (1 mm stainless steel sieve) into a glass dish to remove fauna > 1 mm, which were fixed in 4% formaldehyde for a minimum of 48 h before transferring animals to 70% ethanol prior to identification. The sieved sediment was transferred to a foil tray, covered and dried (72 h; 50 °C) at the same time as the 30 mL subsamples collected during sampling from Section 2 & 3. Water content was estimated in sediment samples from each site (June 2016) by comparing sediment fresh and dry weight, after placing them in the oven at 60 °C until weight remained stable. Sediment grain size was determined from a surface sediment sample collected at each site in June 2016, using a laser particle size analyser (Coulter LPS 230).

2.1.6. Microplastic extraction, characterisation and identification

Microplastics were extracted from sediment samples using Sediment-Microplastic Isolation (SMI) units as per Coppock et al. (2017), precisely following protocols therein. Samples retained on 30 μm nylon meshes were transferred immediately to lidded Petri dishes. Samples were then inspected (Olympus SZX16, x25 magnification) and suspected anthropogenic particles (see Noren, 2007) were photographed and characterised, recording size, colour and type (fibre, fragment, film, bead). All isolated particles were chemically identified using Fourier Transform Infrared Spectroscopy (FT-IR; Appendix A). FT-IR analyses were conducted on Section 1 particles for June 2016 using Bruker Vertex 70 with Hyperion 1000 microscope, all other samples using Perkin Elmer Spotlight 400 FT-IR/NIR system; macroATR mode for particulates, μATR reflectance for fibres; all spectra obtained were visually inspected and compared with the Bruker or Perkin Elmer library databases. Matched spectra exceeding a confidence level of 70% were visually verified and accepted; matches between 60% and 70% prompted further consideration before accepting; matches below a 60% threshold were recorded as unknown. Extracted particles lost during the identification process were also recorded as unknown.

2.1.7. Fauna processing

Fauna were identified to the lowest taxonomic level. Taxa abundances were recorded and blotted fresh biomasses determined using a fine balance (Sartorius R200D).

2.1.8. Estimating faunal-mediated burial processes

Due to the complexities of benthic processes, a functional trait approach is commonly used to describe fauna-mediated ecosystem processes such as bioturbation (Norling et al., 2007; Queirós et al., 2015; Solan et al., 2004). Metrics have been developed and widely adopted to estimate whole community bioturbation potential (BP_c; referring to particle mixing (Queirós et al., 2015; Solan et al., 2004) and bioirrigation potential (BIP_c; referring to solutes (Renz et al., 2018). Both provide biomass and abundance weighted categorical scoring systems and include functional and life-history traits that are deemed important in calculating each. In both cases, the metric is summed for the whole community to estimate their potential effect on sediment mixing and bioirrigation.

2.1.9. Community bioturbation potential (BP_c)

This trait-based approach was used to estimate faunal community bioturbation potential (BP_c) for each sample. The BP_c index (Queirós et al., 2013; Solan et al., 2004) characterises the abundance and biomass weighted effect of macro faunal community assemblages on sediment mixing. Scores are assigned (Table 1) to each taxon in each sample ($_i$) for sediment reworking mode (R_i) and mobility (M_i). Trait scores were attributed based on Queirós et al., (2013) and followed the scoring guidance based on the life history and ecology of the animal for additional taxa.

2.1.10. Community bioirrigation potential (BIP_c)

Community bioirrigation potential (BIP_c) for each sample was calculated as per Renz et al. (2018), characterising the faunal community's potential for benthic-pelagic water (and solute) exchange, which has been shown to be highly important in the uptake of particulates into the sediment matrix (Kristensen and Kostka, 2004). Biomass (B_i) is calculated using individuals m^{-2}, abundance (A_i) converted to ash-free dry weight from wet weight (Ricciardi and Bourget, 1998), and scores assigned (Table 2) using ecologically driven faunal traits that affect ventilation and bioirrigation: feeding type (FT_i), burrow morphology (BT_i), and effective burrowing depth (L_{eff}; Table 2) of each species. BIP_c scores were assigned using a range of literature and online trait databases (Appendix B). Where information for exact species was not available, scores were based on the closest related species or next taxonomic level. Effective burrowing depth (L_{eff}) was determined from the mean faunal environmental position from the data (ie; 2 cm, 6 cm or 10 cm). Where the population of a species was found in approximately equal abundance at multiple depths, the effective depth was deemed the maximum of those depths.

Table 1
Trait scores and abbreviations used to calculate community bioturbation potential (BP_c) as per Queiros et al. (2013).

$$BP_c = \sum_{i=1}^{n} \frac{\sqrt{B_i}}{A_i} \, x \, A_i \, x \, M_i \, x \, R_i$$

Mi Score for traits		*Ri* score for sediment reworking mode		*Fti* code for reworking types	
1	Organisms live in fixed tubes	1	Epifauna	E	Epifauna
2	Limited movement	2	Surficial modifiers	S	Surficial modifiers
3	Slow, free movement through sediment matrix	3	Upward/ downward conveyors	UC/ DC	Upward/ downward conveyors
4	Free movement, via burrow system	4	Biodiffusers	B	Biodiffusers
		5	Regenerators	R	Regenerators

2.1.11. Contamination controls

Strict contamination controls were implemented during field sampling and sample processing, as per Coppock et al. (2017) (Appendix C). Laboratory sample preparation and analysis was conducted using either a laminar flow hood (Bassaire A4HF with Camfil HEPA filter), or

Table 2
Trait scores and abbreviations used to calculate community bioirrigation potential (BIP_c), L_{eff} was determined from the environmental position that each species was found.

$$BIP_c = \sum_{i=1}^{n} \frac{\sqrt{B_i}}{A_i} \, x \, A_i \, x \, FT_i \, x \, BT_i \, x \, L_{eff}$$

Trait	Model	Score
***FT* (Feeding type)**	Predator (P), Scavenger (S), Herbivore (H), Omnivore (O)	1
	Deposit feeder (DF)	2
	Facultative Deposit/Suspension feeder siphon (fDF/SF I)	2
	Suspension feeder siphon (SFI)	3
	Facultative Deposit/Suspension feeder (fDF/SF II)	2
	Suspension feeder (SF II)	4
	Subsurface deposit feeder (SDF)	5
	Funnel feeder (FF)	6
***BT* (Burrow type)**	Attached, Epifauna	0
	Free living	1
	Living in a fixed tube	2
	Living in a burrow	3
	Epifauna	0
L_{eff} (effective depth; cm)	0–2 cm	2
	2–6 cm	6
	6–10 cm	10

Adapted from Renz et al. (2018)

positive pressure laboratory fitted with HEPA filters, and cotton lab coats worn throughout. All equipment was thoroughly rinsed twice with ultrapure water (0.2 μm). All Petri dishes and foil trays were new and previously sealed before use. Control glass fibre filters (Whatman GF/C) were exposed to the air on board RV Quest, in the drying oven and in the laboratory and inspected (x10 magnification) for any airborne contamination. Zinc chloride solution used with SMI units was filtered using a 20 μm nylon mesh before first use and between samples and blank procedural controls were carried out for every 3 uses of each SMI unit. Samples were kept covered, open to the air only when necessary. All equipment was thoroughly rinsed between samples during both sample collection and processing. Sampling equipment and laboratory consumables were analysed using FT-IR and resulting spectra were compared to those obtained from particles isolated from samples to prevent inclusion of external contamination.

2.1.12. Data analyses

Correction factors for each contamination risk (SMI procedural blanks and air contamination for boat, laboratories and drying oven) and positive FT-IR identification were calculated and applied to all data prior to conducting analyses (Appendix C). Functional classifications arising from BP_c and BIP_c index calculations were used to explore microplastic loading at each depth (Tables 1 and 2). All data were analysed using R statistical software (R Core Team, 2019). Kruskal-Wallis tests were performed where data distribution did not conform to normality. ANOVA and simplified general linear models were validated by visually inspecting error distributions and homogeneity of variances relative to linear model assumptions (Appendices D and F.1).

2.2. Experimental study

2.2.1. Study species

Amphiura filiformis are burrowing brittlestars, reinforcing the burrow walls with mucus (Woodley, 1975). Their functional group within the sediment is "Gallery-diffuser", a special case of "Biodiffuser" in that in addition to random mixing, they also transport particles vertically whilst forming and maintaining their burrows. Aside from short rests, *A. filiformis* is in continual motion feeding, maintaining or ventilating its burrow. Populations of *A. filiformis* live in muddy to fine sandy habitats and typically occur in aggregations of ~200 ind. m^{-2} (Queirós et al., 2015) but have been reported at > 3000 ind. m^{-2} (Josefson, 1995). *A. filiformis* activity has considerable influence on ecosystem functioning; oxygenating sediments, nutrient recycling and creating niches for other macrofauna, earning them the reputation of 'key' species (Bowmer et al., 1986).

2.2.2. Animal and sediment collection

Sediment was collected from Cawsand Bay (50°19.81N 4°11.50W) on board RV Plymouth Quest using a Day grab in September 2018. The sediment was kept submerged with overlying bottom water and transported to Plymouth Marine Laboratory (PML) mesocosm laboratory (Findlay et al., 2008), where it was kept aerated in the dark at bottom water temperatures recorded at Cawsand Bay at the time of sampling (15 °C). *Amphiura filiformis* were collected from the same site the following week, using the same equipment. On deck, sediment was gently sluiced to minimise damage to individuals, which were then transferred to a shaded bucket of aerated local seawater, and transported back to the laboratory within 2 h of collection, where they were left in the PML mesocosm (15 °C) overnight, in the dark.

2.2.3. Sediment preparation

Sediment was defaunated using a 1 mm stainless steel sieve within 48 h of sampling. Sediment was then homogenised using a wooden stick over the course of a week, leaving the sediment to settle between mixing. The overlying water was aerated, covered and maintained in the dark at 15 °C. The homogenised sediment was added to 12 aquaria (h:40 cm x w-12 cm x d:12 cm) to a depth of approximately 15 cm, topped with local seawater (salinity 35.5 psu), aerated and left to settle for 48 h before the addition of brittlestars. Seawater was supplied to each aquarium via a re-circulating system and peristaltic pump (Watson Marlow 323), exchanging water at a rate of 11 mL min^{-1}. This did not cause sediment resuspension.

2.2.4. Microplastic preparation

Nylon microfibres (Goodmans, 19 ø x 300 μm) were produced and fluorescently stained with Nile Red to aid visualisation, as per the protocols of Cole (2016). Fibres were quantified using a Sedgwick Rafter counting chamber and microscope (x20 magnification; Wild, M5–49361). Fibre length was quantified using scaled photographs in ImageJ.

2.2.5. Experimental set up

The blotted fresh weight of individual brittlestars was recorded using a fine balance (Ohaus AX223) prior to assembly of experimental units. Ensuring even biomass distribution across replicates, five intact brittlestars were introduced to each of the 12 aquaria; one placed at the edge of each side plus one placed centrally, to a density of 357.14 ind. m^{-2}, in line with natural field densities (Queirós et al., 2015; Solan and Kennedy, 2002). Brittlestars were fed 8% dry-weight Instant Algae® Marine Microalgae Shellfish Diet 1800, every second day. Dilutions were prepared at 20% of estimated dry-weight of brittlestar abundance based on appropriate husbandry conditions for invertebrates (Ricciardi and Bourget, 1998). Animals were left to acclimate for 5 days before the addition of nylon microfibres at an environmentally relevant concentration (Bergmann et al., 2017; Ling et al., 2017) of 10,000 fibres kg^{-1} of sediment, mass concentration of 0.001 g kg^{-1}. The microfibres were then suspended in seawater in a glass beaker, continually mixed to avoid settling, and delivered to the sediment surface of 6 treatment tanks using an electric pipette, ensuring even coverage. All aquaria were kept covered and maintained at 15.0 ± 0.07 °C (mean ± SE), salinity 35.9 ± 0.03 psu in the dark throughout the experiment.

2.2.6. Estimating bioturbation

After 7 days of microplastic exposure, burial behaviour was quantified in experimental aquaria using 2D particle tracing methods (Mahaut and Graf, 1987) and the setup described in Queirós et al. (2015). 0.10 g cm^{-2} of fluorescent sediment tracer particles ("luminophores", Partrac Ltd) were added to each aquarium to form an even layer, approximately 0.2 cm thick, on the sediment surface. Luminophores were custom-made to match the sediment particle distribution at the Cawsand sampling site. Aeration and water circulation were interrupted for 1 h to allow the luminophores to settle on the sediment surface. Individual aquaria were placed at one end of a black box (h:90 cm x w-35 cm x d:64 cm) which allowed for images to be recorded under UV light, using a digital SLR camera (Canon EOS 500D; 15.1 MP) mounted at the opposite end of the box. Images were captured using a 10 s exposure, f = 5.6, ISO100 and remotely controlled via a PC using GB Timelapse software (V3.6.1). All four sides of each aquaria were imaged within 3 h of luminophore addition to capture the initial luminophore profile at the sediment surface, and then again after 8 days to capture the tracer burial profiles. Images were stitched together in ImageJ for each time point, resulting in one image per replicate, per time point. Luminophore burial was estimated from the stitched images using image segmentation methods described in Queirós et al. (2015), using R statistical software (R Core Team, v3.4.1) and ImageJ (v1.46). Luminophore profiles were calculated from a flat sediment surface (Maire et al., 2006; Fig. 3). Bioturbation was estimated from profiles via a number of parameters: 1) maximum burial depth; 2) overall bioturbation activity, quantified by calculating the percentage of luminophore tracer left at the sediment surface in the final compared to initial image (ie; 100% - % remaining (Queirós et al., 2015)); and 3) luminophore profiles (count

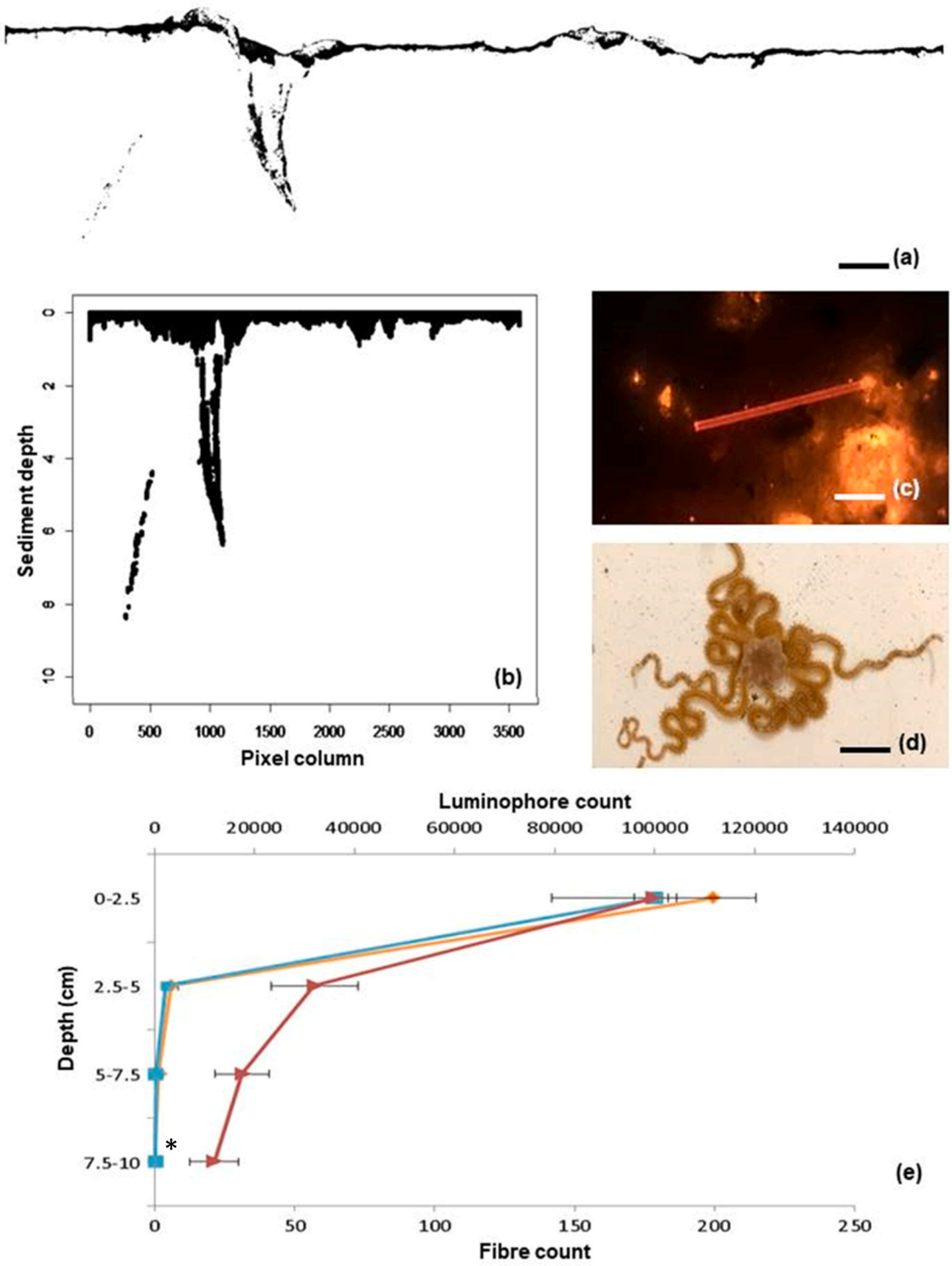

Fig. 3. (a) Threshold set for luminophores touching front of aquarium, at sediment-water interface buried (scale bar = 2 cm) and (b) XY coordinates plotted when surface flattened to quantify burial activity and depth from luminophore profiles. (c) Images of a fluorescing nylon fibre dyed with Nile Red (scale bar = 100 μm) and (d) a specimen of Amphiura filiformis (scale bar = 5 mm). (e) Plot *profiling mean* (± SE) luminophore (blue square = control, orange circle = plastic treatments) and fibre (red triangle) burial at 2.5 cm. * denotes significant difference. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

per depth) binned at 2.5 cm intervals to compare burial activity at different depths with plastic burial (see Section 2.2.8).

2.2.7. *Oxygen uptake*

Sediment community oxygen consumption was measured to establish potential implications of plastic loading to sedimentary function mediated by brittlestar bioturbation. Water in each aquarium was gently siphoned down to a 5 cm water layer and an oxygen optode sensor disc (5 mm, World Precision Instruments) was glued onto the inside of the tank using low toxicity silicon adhesive (KWIK-SIL™). Each aquarium was then carefully refilled with the same water and topped up to the brim. An initial temperature compensated dissolved oxygen reading was

taken using the Oxy-mini fibre optic logger (World Precision Instruments). Custom made Perspex lids with motorised vanes were used to create a gentle flow (13.1 ± 0.1 L min^{-1}; mean ± SE) and then sealed onto each aquarium using non-toxic aquarium silicon sealant. Incubations were carried out in sealed tanks and maintained in the dark at 15 °C. Further oxygen measurements were taken after 6 h. Sensors were batch calibrated using the manufacturer 2 point calibration method, using 0% (0% Oxygen solution, Hannah Instruments) and 100% oxygen saturation, corrected for salinity and temperature. Percent oxygen measurements were converted into concentration as mg L^{-1} and then scaled to *A. filiformis* biomass per aquarium as mg^{-1} L^{-1} g^{-1}.

2.2.8. Quantifying plastic burial

Triplicate syringe cores (6 cm^2) were taken from burrows in each treatment tank to a depth of 10 cm and frozen at − 20 °C. Cores were sliced 0–2.5 cm, 2.5–5 cm, 5–7.5 cm and 7.5–10 cm, and fibres extracted using Sediment-Microplastic Isolation (SMI) units, using the same method employed in field sample analysis. Fibres were enumerated (Olympus IMT2 inverted microscope, x40 magnification) using fluorescence (480–550 nm).

2.2.9. Quantifying plastic ingestion

After sediment sampling, *A. filiformis* were recovered from experimental aquaria, rinsed with seawater and preserved in 10% formaldehyde. Only wholly intact brittlestar discs were used to quantify fibre ingestion. To eliminate potential external fibre adherence, brittlestar arms were removed and the central disc was rinsed with water prior to dissection. The discs were then placed onto a glass Petri dish, dissected to reveal the gut, flushed with water and nylon fibres quantified (Olympus SZX16, x25 magnification).

2.2.10. Statistical analyses

All data analyses were conducted using R statistical software (R core Team, 2019; Renz et al., 2018; Shim, et al., 2018), v3.4.1, Appendix F.2).

3. Results

3.1. Environmental study

3.1.1. Sediment characteristics and plastic prevalence

Sediment at the Plym site was categorised as fine clay/silt with a particle size of 10.25 ± 3.02 μm (mean ± SD). At the breakwater site, sediment particle size was 20.78 ± 3.05 μm and categorised as fine, silty mud and sediment at the Rame site was also categorised fine, silty mud with a particle size of 21.33 ± 3.29 μm. No significant relationship was found between microplastic concentration in the sediment and sediment particle size ($F_{1,25} = 1.144$, $p = 0.295$, $R^2_{adj} = 0.005$). Microplastics were present in the fluff layer (Figs. 4 and 5) with abundances ranging 0—13.1 particles L^{-1} and a mean abundance of 5.2 ± 1.0 L^{-1} (± SE). There was no significant difference in mean microplastic abundance in the fluff layer between the three study sites (Kruskal-Wallis; H = 2.526, $df = 2$, $p = 0.283$) or at different times of the year (Plym site, Kruskal-Wallis; H = 2.408, $df = 3$, $p = 0.492$). Plastic particle sizes ranged 80 μm–5 mm in length, with a mean length of 1.6 ± 0.17 mm and diameters of 69 ± 35 μm. Of the particles found in the fluff layer, 90% were fibres with the remaining 10% being fragments. A range of colours were observed; blue (44%), black (22%), red (16%) and transparent (10%), with white, pink and grey also found. Polyester, polyethylene, nylon, acrylic and rayon were identified via FT-IR in the fluff layer (Appendix A).

Sedimentary microplastic abundances, (after adjustment for

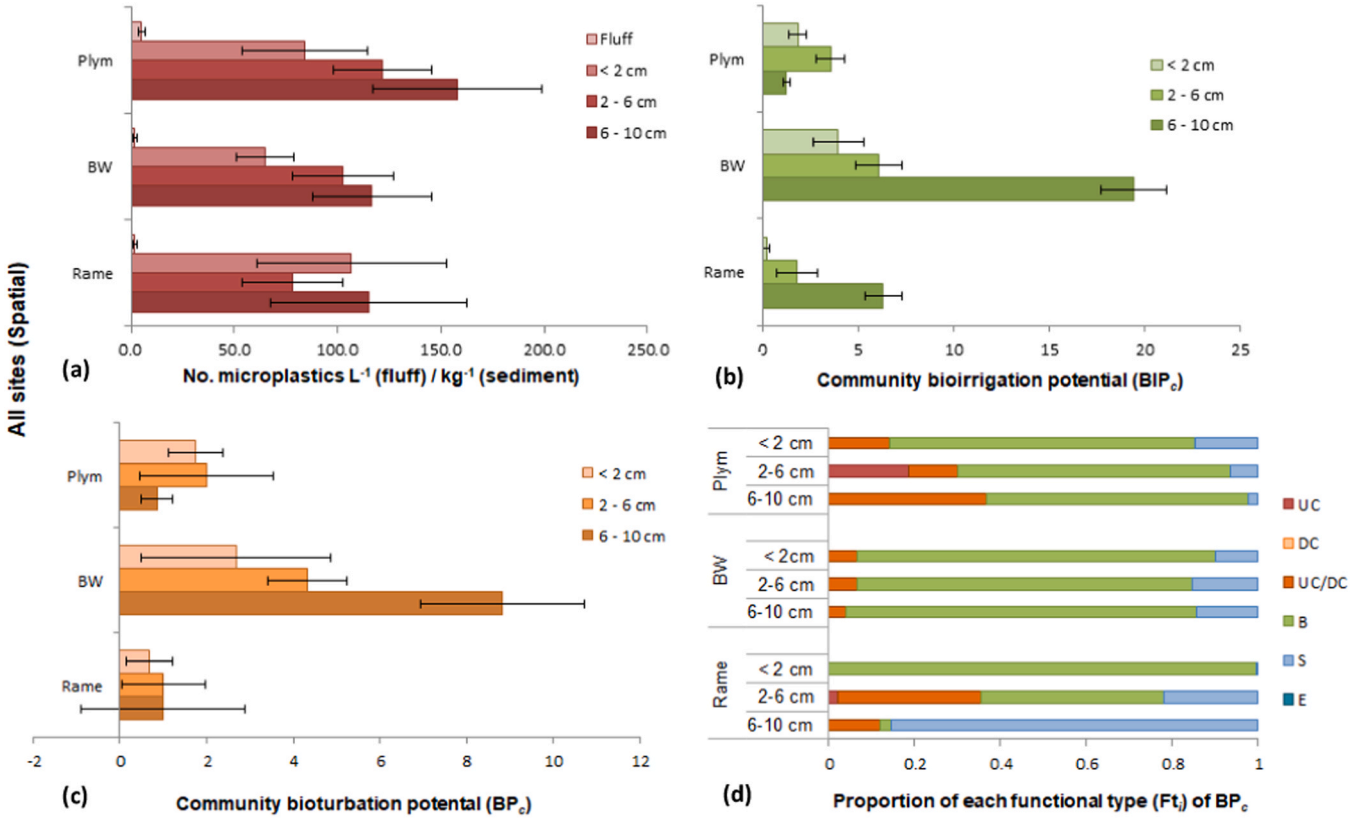

Fig. 4. (a) Mean (± SE) microplastic loading in the fluff layer and at each depth at all sites in June, (b) community bioirrigation potential (BIPc), (c) community bioturbation potential (BPc) and (d) the proportion of each functional group of the whole BPc at each depth. UC: strict upward conveyors; DC: strict downward conveyors; UC/DC: both upward and downward conveyors; B: biodiffusers; S: surficial modifiers; E: epifauna. (For interpretation of the references to colour in this figure, the reader is referred to the web version of this article.)

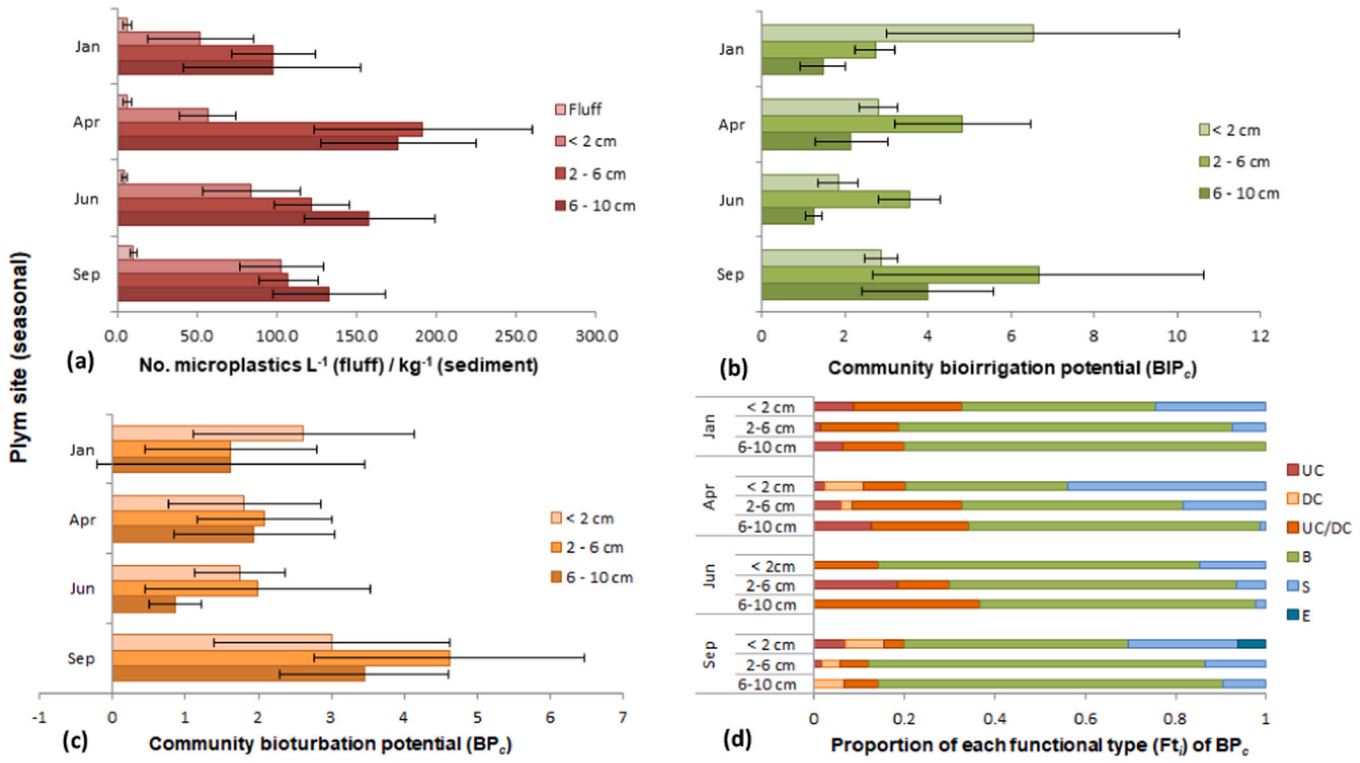

Fig. 5. (a) Mean (± SE) microplastic loading in the fluff layer and at each depth throughout the year at the Plym site, (b) community bioirrigation potential (BIPc), (c) community bioturbation potential (BPc) and (d) the proportion of each functional group of the whole BPc at each depth. UC: strict upward conveyors; DC: strict downward conveyors; UC/DC: both upward and downward conveyors; B: biodiffusers; S: surficial modifiers; E: epifauna. (For interpretation of the references to colour in this figure, the reader is referred to the web version of this article.)

contamination and Fourier Transform Infrared (FT-IR) analysis corrections; Appendix C) ranged 0–314 particles kg^{-1} of dry sediment, mean ± SE = 109 ± 8.7 kg^{-1} (Figs. 4 and 5). Fibres comprised 73% of extracted particles, 18% were fragments, 16% films and a single bead was observed. Whilst a variety of colours were present, blue and black contributed to 52.3% of all particles (Appendix A). Mean particle sizes were 1.6 ± 0.08 mm long and 0.19 ± 0.03 mm wide. FT-IR analyses indicated that the dominant polymer types were polyester, polyethylene, polypropylene, acrylic, nylon and semi-synthetic rayon. Overall, microplastics were found in greater numbers in the deepest of the three sediment layers sampled (6–10 cm, Figs. 4 and 5) compared to the top-most layer (ANOVA; $F_{2,51} = 3.815$, $p = 0.029$; TukeyHSD $p = 0.026$). Microplastics occurred at all three depths at all sites sampled in the Summer (June), however concentrations were highly variable and no significant difference was found between sites or depths (MP abundance ~ depth; ANOVA $F_{2,24} = 1.641$, $p = 0.215$; Fig. 4, Appendix A). Microplastics were present throughout the year at the Plym site and were found in significantly greater numbers at depth compared to the surface layer (microplastic abundance ~ depth; ANOVA $F_{2,33} = 3.696$, $p = 0.036$; TukeyHSD $p = 0.041$; Fig. 5). These abundances were variable within the sampling period, therefore no significant variation was found throughout the year.

3.1.2. *Faunal contribution to microplastic burial in natural sediments*

As no difference between sites or between seasons was found, all data for the deepest sedimentary layer sampled were aggregated to assess faunal contribution to plastic burial. Neither the whole community bioturbation potential "BP_c" (MP abundance ~ BP_c; $F_{1,16} = 1.093$, $p = 0.311$, $R^2_{adj} = 0.005$; Appendix D) or community bioirrigation potential "BIP_c" (MP abundance ~ BIP_c; $F_{1,16} = 0.376$, $p = 0.548$, $R^2_{adj} = -0.038$) indices had an overall effect on microplastic burial. However, significant patterns emerged when the contributions of the functional groups that contribute to those indices were assessed individually. No significant BIP_c functional guilds could explain microplastic burial (MP abundance ~ Tube dwelling + Burrowing fauna). However, marked effects were identified between BP_c functional guilds: upward and downward conveyors ("UC/DC") had a positive effect on microplastic loading into deeper sediments, whilst strict upward conveyors ("UC") and biodiffusers ("Biodiffuser") had negative effects on that transport pathway (MP abundance ~ 303.202 + 94.32 * UCDC −555.312* UC −30.867* Biodiffuser + site; $F_{5,10} = 6.7$, $p = 0.005$, $R^2_{adj} = 0.655$).

3.2. *Experimental study*

Nylon fibre burial by *Amphiura filiformis* occurred in all replicates and fibres were detected in all sampled sediment depths during plastic exposures. 55.6% of fibres were recovered from the top 2.5 cm of sediment, reducing to 8.3% in the deepest sampled layer (7.5–10 cm). The plastic distribution matched that of the luminophore profiles used to assess bioturbation (Fig. 3).

There was no overall difference in the burial activity of *A. filiformis* between the plastic exposure and control treatments when the whole sediment core sampled was considered ($F_{1,10} = 0.01$, $p = 0.921$; Fig. 3). However, there was an effect of plastic exposure on sediment burial activity in the deepest layer (7.5–10 cm; $F_{3,40} = 3.378$, $p = 0.027$; EMM joint-test, $p = 0.007$). Maximum sediment burial depth was shallower in fibre treatments (mean ± SE = 8.29 ± 3.66 cm) compared to control (9.24 ± 0.45 cm), but this was not statistically significantly different ($F_{1,10} = 2.676$, $p = 0.133$).

Oxygen consumption was marginally higher in fibre exposed brittlestars, at 0.105 ± 0.012 mg L^{-1} h^{-1} g^{-1} biomass, compared to 0.088 ± 0.011 mg L^{-1} h^{-1} g^{-1} biomass, but this difference was not statistically significant ($H = 1.32$, $df = 1$, $p = 0.251$).

Out of 30 brittlestars, 25 were wholly intact post exposure in the plastic treatment, and 48% of these had nylon fibres in their discs, with an average of 1.9 ± 0.29 fibres recovered per individual.

4. Discussion

This study indicates that microplastics are being buried in natural marine sediments of high biological value, and that benthic faunal activity plays a role in its mediation. The fluff layer, at the interface between the water column and seabed, was identified as a consistent reservoir of microplastics for uptake into the sediment matrix. Microplastic loading at depth appeared to be pervasive across sites and throughout the year and our results indicate that this loading is anchored in well-known functional traits of sedimentary infauna, expressed by their feeding and mobility ecology. This is the first time that biotic-driven microplastic burial in sediments has been shown in a natural environment.

Reported microplastic abundances in marine environments are highly variable, due in part to the natural heterogeneity of water bodies but also to differences in sampling, methods used and differences in reporting between studies (Lindeque et al., 2020; Shim et al., 2018). We observed a mean concentration in the fluff layer which is higher but in the same order of magnitude than the estimated global mean for marine surface waters (2.4×10^3 m^{-3} (Shim et al., 2018). A higher relative abundance of fibres was recorded in the fluff layer than sediments, suggesting that microfibres may be more prone to resuspension and lateral movement than other plastic particulates reaching the marine benthos, or that they are less likely to be taken up by benthic organisms mediating their burial. This perspective is consistent with the understanding that hydrodynamic forcing, and wave exposure specifically, exert a strong influence in the settling of microplastics (Ling et al., 2017).

High within-site microplastic abundance variability was observed, in line with previous studies, and as would be expected if benthic biological mediation is indeed a key driver of burial (Kendall and Widdicombe, 1999). Microplastic abundance was higher at the Plym site in Spring (April), during which increased phytoplankton and zooplankton abundance and faecal flocculation, and milder hydrodynamic conditions, may have contributed to higher trapping of plastics from the water column and facilitated sinking to the seabed (Uncles et al., 2015; Zhang et al., 2015). Correspondingly, a sharp increase in chlorophyll *a* was recorded at the Western Channel Observatory, the local benthic-pelagic monitoring station (Smyth et al., 2015), three days prior to our April sampling and indicates the occurrence of a phytoplankton bloom. Resulting organic rich aggregates can transport high concentrations of microplastics down through the water column to the fluff layer at the sediment-water interface, thus enhancing microplastic availability to benthic organisms (Guinder et al., 2015; Porter et al., 2018). Indeed, evidence of strong benthic-pelagic coupling in our study region, a temperate, coastal system, has been reported, where the composition of settled material on the seabed, taken up by the benthos through the seasonal cycle, reflects the composition of plankton and other particulate organics in the water column (Queirós et al., 2019; Tait et al., 2015). Our study region is subject to riverine input and we observed the lowest quantity of plastic loading in January which coincided with heavy rainfall that occurred the week prior to sampling, where a maximum mean flow of 125 m^3 s^{-1} was recorded in the River Tamar that also flows into Plymouth Sound (UK Environment Agency, Appendix E). This slightly lower plastic loading may indicate that the plastic particles may be washed out towards the sea during high riverine flow events (Hurley et al., 2018).

Once deposited onto the seabed, different biotic and abiotic processes may influence whether a particle is re-suspended into the water column or incorporated into the sediment matrix. Benthic-pelagic exchange pathways are a complex series of biological, physical and chemical interactions, the net product of which results in sedimentation (Rühl et al., 2020). Whilst these processes do not act in isolation, given the dynamic nature of the shallow coastal sites in this study (Uncles et al., 2015) the observed microplastic burial is unlikely to be driven by sedimentological processes (ie; deposition or erosion). Benthic macrofaunal communities can dominate sediment stability and erosion thresholds (Montserrat et al., 2008; Sgro et al., 2005) and depending on the community composition, alter the sediment structure, granulometry, cohesion and biogeochemical cycling (Montserrat et al., 2009). Faunal mediated bioturbation and bioirrigation activity are vitally important in ecosystem functioning, mediating benthic-pelagic processes such as nutrient cycling (Volkenborn et al., 2007). Different sediment reworking modes, or functional groups of burrowing benthic fauna have different effects on the vertical distribution of particulates within the sediment matrix (Kristensen et al., 2012). Biodiffusers, such as the polychaete worm *Nephtys* sp., randomly move particulates through burrowing and feeding activity; whereas conveyors, such as the lugworm *Arenicola marina,* offer local transport pathways vertically between the surface and deeper sedimentary layers, affecting burial and resuspension of sediment and nutrients to the water column (Kristensen et al., 2012). Collectively, these reworking modes substantially alter the chemical, physical and biological environment within the sediment, generating a highly dynamic and heterogeneous environment which depends on, and in turn affects, the resident species composition, varying spatially and temporally. When investigating the effect of the benthic community on microplastic burial, we found no relationship between microplastic abundance at depth and overall community bioturbation potential (BP_c) or bioirrigation potential (BIP_c). However, when we refined the parameters of our model to investigate the contribution of different bioturbator functional guilds to microplastic burial, we found that strict upward conveying fauna and biodiffusers had a negative effect on microplastic abundance in the deepest layer sampled (6–10 cm), whereas animals that contributed to both upward and downward conveying specifically had a positive effect. An in situ study in South Africa of the sand prawn *Callianassa kraussi*, found chlorophyll *a* in greater concentrations at depths of 15–25 cm than at the sediment surface, due to the conveying and bioirrigation activities of these animals burying benthic surface diatoms (Branch and Pringle, 1987). In contrast, sediment egestion and ejection from burrow openings can make non-cohesive sediment available to bottom currents and re-suspend particles, as indicated by the negative effect of upward conveying animals in our results. For instance, in situ observations of the echiuran worm *Maxmuelleria lankesteri* in a Scottish sea loch, found a mean sediment ejection rate of 2.75 kg $burrow^{-1}$ $year^{-1}$ (Hughes et al., 1999), which substantially contributed to sediment resuspension. It is likely that this process also leads to the re-suspension of buried microplastics back into the water column. Based on our results of pervasive microplastic loading at depth both seasonally and spatially, the cumulative effect of macrofauna communities is the net burial of microplastics.

The role sediment-dwelling biota play in the burial of microplastics was confirmed using a targeted exposure study. Our experimental data revealed that the brittlestar *Amphiura filiformis* buried nylon fibres to a depth of at least 10 cm in experimental cores, following the same profile as the sediment tracer particles used to track its burrowing activity (Fig. 3). This same trend has been reported in a study investigating microplastic transport using *A. marina* (Gebhardt and Forster, 2018). And whilst we detected no overall change to bioturbation activity from exposure to plastics, we found that sediment mixing by *A. filiformis* was reduced in the deepest parts of their burrows during microplastic exposure. Similarly, *A. filiformis* have shown reduced sediment reworking when exposed to North Sea oil drilling cuttings (Trannum, 2017), and in an experiment utilising nylon filaments as seagrass mimics (Valdemarsen et al., 2011) a higher than expected number of inactive *A. marina* was observed. Reduced burrowing depth is a common stress response of benthic macrofauna to changes in environmental conditions, such as lowered oxygen environments (Diaz and Rosenberg, 1995), fluctuating salinities (Haider et al., 2018), warming and reduced food availability (Przeslawski et al., 2009). Given that *A. filiformis* can reach densities of $>$ 3000 ind. m^{-2} (Josefson, 1995), such changes in faunal behaviour could result in substantial impacts on sediment

characteristics and mediated biogeochemistry (Przeslawski et al., 2009; Volkenborn et al., 2007). *A. filiformis* are an active and dominant member of European benthic macrofauna communities, and the bioturbating and bioirrigation activities of such key species significantly increases sediment oxygenation in the sedimentary layers around their galleries (Woodley, 1975). These indirect anthropogenic impacts may uncouple animal-sediment interactions in natural systems. Inhibition of deeper burial activity may reduce the flux of oxygen rich water and nutrients at these depths, reducing their facilitating effect on macrofauna community abundance and diversity (Solan et al., 2004).

In our exposure study, we used microplastic concentrations of 10,000 fibres kg^{-1}, and whilst these concentrations were an order of magnitude greater than found at our study sites, such concentrations have been observed elsewhere. For example, Ling et al. (2017) reported a regional average of 3400 microplastics L^{-1} around Australian coasts, with the highest individual concentration reported at 12,500 microplastics L^{-1}, 6600 microplastics kg^{-1} have been reported for Arctic sediments (Bergmann et al., 2017) and a recent study reported hotspots of 1.9 million microplastics m^{-2} on deep sea sediment surfaces, channelled by thermohaline-driven currents (Kane et al., 2020). We did not detect any change in oxygen consumption by *A. filiformis* after exposure to microplastics at the end of 6 h incubations, in line with prior studies on *A. marina* which only found changes at much higher concentrations (10% sediment volume) of polyvinyl chloride (PVC) (Green et al., 2016). However, we did observe that *A. filiformis* ingest microfibres, suggesting that plastic burial by this species does not just result from passively transporting the microplastics downwards whilst maintaining their burrows, but also actively through ingestion and egestion. Whilst not quantified here, other experimental studies have reported adverse health effects after exposure to microplastics. For example, *A. marina* suffered a reduction in feeding and depletion of energy reserves when exposed to PVC for 4 weeks (Wright et al., 2013), energy that is required for important functions such as reproduction and growth; further, nylon particulates (mass concentration 90 g kg^{-1}) significantly reduced reproduction in terrestrial Annelid worms (Lahive et al., 2019). *A. filiformis* undergo frequent arm regeneration following loss of limbs owing to predatory behaviour of demersal fish and invertebrates (Sköld and Rosenberg, 1996). Adults regenerate on average, 22% of their total biomass annually (Loo and Rosenberg, 2003), constituting a substantial proportion of energy allocation; further research is needed to better understand the physiological and ecological implications of microplastic ingestion in key benthic, invertebrate species.

Collectively, data from this study, combining field and laboratory based observations, indicates that coastal benthic sediments sequester microplastic pollution. Microplastic burial was ubiquitous both spatially and temporally, with the fluff layer being a consistent reservoir of microplastic for uptake into the sediment matrix. Microplastic burial was readily apparent from the environmental data, with the highest concentration in the deepest sediment layer. This is in contrast to other studies (Martin et al., 2017; Wang et al., 2019), however these differences may be related to shallower sedimentary sampling and coarser depth resolution in those studies. No large scale bioturbation events were noted in one of those studies (Martin et al., 2017), contrasting with the high biological activity recorded at all sedimentary depths in this study. This difference highlights that faunal contribution towards the elevated microplastic loading at depth within sediments may be especially important in highly biologically active coastal benthic environments, as also noted in previous work (Wang et al., 2019). Similarly to the present work, sediments in the Northern Baltic Sea have also been proposed as sinks for microplastics, after an experimental study found microplastic buried at a depth of 5 cm were rarely brought back to the surface by common Baltic benthic fauna (Näkki et al., 2019).

If current plastic production continues to increase and global waste infrastructure remains unchanged, an estimated 100–250 million tonnes of plastic waste are projected to enter the oceans annually by 2025 (Jambeck et al., 2015), with microplastic accumulation in coastal sediments expected to increase also. If, as our results suggest, microplastics are being sequestered in coastal sediments, due to the lack of any thermal or photo degradation of plastic within sediments (Andrady, 2011), once buried, microplastics could remain with little degradation for millennia, contributing irrevocably to the geological age of the Anthropocene (Zalasiewicz et al., 2016). A deeper understanding of microplastic residence times in marine sediments will require longer term studies encompassing whole communities and a wider range of sediment matrices, with burial and resuspension rates quantified at that scale. There is currently a paucity of research into the physiological effects of microplastics on benthic animals. Elevated microplastic loading in sediments will invariably increase encounter rates by benthic fauna, posing a heightened health risk. In already stressed ecosystems, this additional anthropogenic stressor, set to increase annually, will likely exhibit important pressure on ecosystem health in a multi-stressor ocean.

CRediT authorship contribution statement

RLC, PKL, MC, AMQ & TSG designed the studies. RLC carried out field sampling. RLC, PN and SR processed field samples. RLC conducted experiments using methods and image analysis scripts written by AMQ. RLC & HB processed experimental samples. RLC conducted statistical analysis, guided by AMQ. RLC wrote the manuscript, all authors contributed to editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jhazmat.2021.125583.

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