i An update to this article is included at the end

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Factors influencing microplastic abundances in the sediments of a seagrass-dominated tropical atoll[★]

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ABSTRACT

Seagrass meadows are one of the world's most diverse ecosystems offering habitats for an extensive array of species, as well as serving as protectors of coral reefs and vital carbon sinks. Furthermore, they modify hydrodynamics by diminishing water flow velocities and enhancing sediment deposition, indicating the potential for microplastic accumulation in their sediments. The build-up of microplastics could potentially have ecological impacts threatening to ecosystems, however little is known about microplastic abundance and controlling factors in seagrass sediments. Here we investigated microplastic characteristics and abundances within sediments underlying four seagrass meadow sites on the Turneffe Atoll, Belize. Sediment cores were collected and sub-sampled to include a range of replicate surface sediments (0-4 cm) and depth cores (sediment depths 0-2, 2-5, 5-10, 10-20 and 20-30 cm). These were analysed using 25 µm resolution µFTIR, with spectral maps processed using siMPle software. Microplastics were prevalent across the sites with an abundance range (limit of detection (LOD) blank-corrected) of < LOD to 17137 microplastics kg⁻¹ dw found on the east side of the atoll. However, their abundances varied greatly between the replicate samples. Polyethylene and polypropylene were the most commonly detected polymers overall, although the dominant polymer type varied between sites. There were no differences in the abundance of microplastics between sites, nor could abundance distributions be explained by seagrass cover. However, abundances of microplastics were highest in sediments with lower proportions of fine grained particles (clay, <4 µm) suggesting that hydrodynamics override seagrass effects. Additionally, no patterns were seen between microplastic abundance and depth of sediment. This suggests that microplastic abundance and distribution in seagrass meadows may vary significantly depending on the specific geographical locations within those meadows, and that more complex hydrodynamic factors influence spatial variability at a localised scale.

1. Introduction

Microplastics (plastic particles: $1 \, \mu m$ - $5 \, mm$) are a widely recognised and globally widespread contaminant. Due to their durable and longlasting nature, microplastics can persist in the environment for decades or even centuries (Thompson et al., 2004). The volume of plastics manufactured and discarded is continuing to rise, with a predicted greater than four-fold increase in plastic waste lost to the environment by 2030 if efforts are not made to reduce these losses (Borrelle et al., 2020). Nonetheless, even with concerted efforts to improve waste management, releases are anticipated to double (Borrelle et al., 2020). These releases, combined with the degradation of existing macroplastic debris (>5 mm) in the environment into microplastics, suggest that microplastic contamination, by number and by mass, will increase in coming years.

surveys have now been carried out across a range of environments and matrices: terrestrial, aquatic, and atmospheric, including soil, water, sediment, and air. To date, the highest abundances of microplastics have been observed within sediments, both in rivers, where abundances >138,000 microplastics kg⁻¹ dw were observed in the River Tame, UK (Woodward et al., 2021), and in marine sediments at water depth of 500–1000 m, where abundances of up to 1,900,000 microplastics m^{-2} (equivalent to 3800 microplastics $kg^{-1} dw$) were observed (Kane et al., 2020). Although many common polymers are buoyant in water (e.g., polyethylene and polypropylene), others are denser than water (e.g., PET and PVC) and will sink. Furthermore, natural processes such as aggregation with organic matter or biofouling lead even supposedly buoyant polymers to become dense and sink. Therefore, sediments across a wide range of water depths and environments will receive and

Efforts in microplastic research have intensified in recent years, and

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retain microplastics and are recognised as sinks for microplastics (Matsuguma et al., 2017; Woodall et al., 2014). Especially within low-energy environments with low flows and high rates of deposition, microplastics on the seafloor can become rapidly buried by deposited sediments and thus become trapped (Harris, 2020). As sediment and organic detritus continues to be deposited at the sediment surface, microplastics will be buried deeper and become sequestered within the sediment (Turner et al., 2019; Willis et al., 2017).

Seagrass meadows are vital ecosystems, acting as carbon sinks, protecting coral reefs, and providing habitats for a wide range of species, especially in the form of fish nurseries (Chittaro et al., 2005; Duarte et al., 2010; Unsworth et al., 2019). Seagrasses are known to alter hydrodynamics, slowing water flow, and often facilitating the deposition of fine-grained sediments, thus protecting adjacent coral reefs from the smothering effects of land-derived sediments (Gacia et al., 2003; Ward et al., 1984). It is therefore suggested that seagrasses will lead to the entrainment of microplastics in the same way as sediment particles (Unsworth et al., 2021). Sediment accretion has been shown to increase with seagrass density, with a shift in particle size distribution to smaller grain sizes (Bos et al., 2007). Like many other ecosystems globally, seagrass meadows have been observed to be contaminated with microplastics, both on the seagrass leaves themselves and within the sediments, often enriched compared to environments without seagrass (Boshoff et al., 2023; Goss et al., 2018; Huang et al., 2020; Kreitsberg et al., 2021). The implications that these plastic trapping abilities may have for seagrass productivity, or the organisms that rely on them, have yet to be investigated. Furthermore, while surveys have looked at surface sediments, none have yet investigated the retention of microplastics within sediments deeper beneath seagrass meadows using core sampling. Given that previous studies have suggested seagrasses as suitable bioindicators for anthropogenic pollutants (e.g., trace metals (Govers et al., 2014)), the same may be true for microplastics (Farias et al., 2018). This is of particular importance as, despite their significant role in the capture, storage, and sequestration of carbon in coastal ecosystems ('blue carbon'), seagrass meadows generally exist in coastal zones which are under serious threat from anthropogenic actives and a range of pollution (Small and Nicholls, 2003).

This study was carried out at Turneffe Atoll, Belize, a highly dynamic tropical atoll system. A previous study on microplastics has been carried out in this location before, however only microplastics adhered to seagrass leaves were sampled, and only at one location (Goss et al., 2018). Our hypotheses for this study were: 1) Different sites will have different microplastic quantities and compositions based on varying local sources of plastic from anthropogenic activity; 2) Microplastic accumulation in sediments will depend on the hydrodynamics of the area, with lower currents leading to an increased grain size and higher sediment deposition rates; 3) Higher percentage seagrass cover will lead to greater abundances of microplastics in surface sediments; 4) Microplastics will occur in greater numbers in surface sediments compared to deeper layers as a result of more recent deposition, in line with temporally increasing microplastic abundances in the environment.

2. Methods

2.1. Study area

The Turneffe Atoll in Belize is a designated marine reserve since 2012, located approximately 32 km off the east coast of mainland Belize. The atoll is mostly uninhabited with the exceptions of four small dive resorts and a field research station. On the eastern side, the Atoll is exposed to the Caribbean Sea, while on the western side the Atoll is sheltered by the mainland, in close proximity to Belize City (Fig. 1). The Turneffe Atoll is of high ecological importance comprising mangroves, seagrass meadows and coral reefs.

For the purposes of this study, the atoll was broadly divided into zones relating to coastal energetic regime, with Zone C being the least



Fig. 1. Map of the study area, Turneffe Atoll, Belize. Energetic zones A-C and study sites A-C are highlighted.

energetic (landward side), Zone B exposed to a higher energetic regime (oceanward side but protected by reef) and finally Zone A, being exposed to the open ocean and thus being the most energetic of the three environments. These energetic regimes were confirmed by grain size analysis of sediments, with the lowest energy zone (C) containing the highest proportion of fine-grained sediments (<4 µm in size, as per the Wentworth scale, Table S5 and Fig. S1). Site A (located in the high energic Zone A) is often subjected to strong prevailing winds, wave action, and storm events. Site A is located near the Calabash field station and is the site with the highest levels of direct anthropogenic activity as a result of proximity to the field station and coastguard station. In Zone B on the north-eastern site of the atoll two sub-sites. B1 and B2, were selected on opposite sides of the Northern Bogue Inlet, which connects the Central Lagoon to the eastern margin of the atoll. Although <200 m apart, the sub-sites have very different characteristics: Site B1 is located in close proximity to a holiday resort and subject to prevailing flows from the inner atoll; at Site B2 the shore is characterised by mangrove forest and is less subject to flow conditions. Site C (Zone C) has the least dynamic conditions, on the side of the atoll closest to the mainland and there is no active anthropogenic activity visible. The sampling sites covered areas dominated by seagrass beds of three species: turtle grass (Thalassia testudinum; B1 and B2) and shoal grass (Hadoule wrightii; site C). Site A was equally colonised by turtle grass and manatee grass (Syringodium filiforme).

2.2. Sample collection

Samples were collected in January 2019. At each of the four sampling sites surface sediment samples were taken by pushing a PVC pipe (diameter 6 cm) into the sediment to a depth of up to 30 cm and extracting using a rubber bung to seal the end of the core. Slices of 2 cm thickness were cut from the surface (0-2 cm depth) using a stainless steel spatula. Longer sediment cores were also taken using a larger PVC pipe (1.5 m length, 10 cm internal diameter). From all these cores the surface layer sample (0-2 or 0-4 cm depth) were taken for sediment depth profile analysis, alongside additional deeper samples (2 cm thickness) from the maximum depth available (34-74 cm dependent on the core) to use as field process blanks. Carbon date analysis of the cores from these sampling sites indicated that these deep sediment samples pre-date plastic production, i.e., samples from depths below 30 cm are at least 100 years old, and in the majority of cases are >1200 years old (Unpublished results). These deep sediment samples are also below the depths expected to be affected by the majority of recent bioturbation (Wust, 2011). Altogether for surface sediment analysis 11 cores were collected at site A, six at site B1, six at site B2 and four at site C. For all samples, any large pieces of plant material were removed before the samples were placed into polyethylene bags. Samples were kept as cool as possible, using ice packs and cool boxes, during transport back to the UK at which point they were stored at 4 °C. For each surface sediment sample, the water depth, the estimated seagrass cover as a mean of three independent estimates (%) and the seagrass species were recorded. In addition, the particle size distribution was measured using a Malvern Mastersizer 3000 laser microgranulometer and percentage clay (<4 µm) determined as a proxy for hydrodynamic conditions at each site (Madsen et al., 2001).

In addition to the surface and field blank samples, one separate additional long core was collected from each site (0.68–1.5 m) for depth analysis, kept intact and transported in PVC storage boxes to BOSCORF (British Ocean Sediment Core Research Facility). They were sliced lengthways into two halves and wrapped in clingfilm for storage. In the laboratory, samples were collected at 0–2, 2–5, 5–10, 10–20 and 20–30 cm depth from one half core per site. An additional field blank sample was taken at a depth of 60–65 cm to account for contamination to account for any laboratory-derived contamination. For subsampling of the cores, the sediment samples were taken from the inner part of the sediment to avoid sediment that was in contact with the PVC core liner, clingfilm, or any other equipment and to minimize oxygen exposure.

2.3. Contamination control

Contamination that may derive from field sampling and laboratory contamination would be accounted for by the field blanks (sliced in the field) and process blanks (only handled in the laboratory) and subsequent blank correction (subsection 2.6). Stringent contamination measures were taken throughout processing and analysis to prevent contamination in the laboratory. Sediment processing was conducted in an ISO-5 clean laboratory in a laminar flow cabinet (Felcon) and nonshreddable Tyvek suits (Dupont, IsoClean) were worn at all times. Unless stated otherwise, all processing equipment was made of glass or stainless steel and was washed thoroughly prior to use with MilliQ water, with glassware also being acid washed. Stainless steel filters were combusted at 500 °C in a glass Petri dish for 9 h to burn off any potential particulate contaminants. All reagents, including the canola oil, were filtered prior to use over a 1.2 µm glass-fibre filter (Whatman GF-C). The front and the sample stage of the FTIR microscope were surrounded with the Spotlight atmospheric enclosure made of Plexiglas.

2.4. Sample processing

All sediment samples were oven dried at 50 $^\circ$ C for 7 days or longer until dry. A subsample of 17.7–56.7 g dry weight of the homogenised

sediment, depending on the mass of sediment available, was then used for oil extraction of the microplastics (Crichton et al. (2017) and modified by Courtene-Jones et al. (2020)). Oil flotation is a density-independent process based on the oleophilic properties of microplastics and has been shown to be more effective than flotation using NaCl and ZnCl₂ solutions (Radford et al., 2021). In brief, samples were placed in 250 mL pre-cleaned glass beakers (400 mL beaker if sample >35 g) swirled and left to soak for 1 h. If any sediment lumps remained after this time, the back of a stainless-steel spoon was used to gently disaggregate the lumps and ensure full dispersion of the sediment in the water. At this point 5 mL of canola oil was added to samples and mixed again using the stainless-steel spoon. If the sample was >25 g, 7 mL of canola oil was used as recommended by Courtene-Jones et al. (2020). The spoon was then rinsed with MilliQ water into the beaker using a PTFE wash bottle and the sample topped up with more MilliQ water until approximately a 1 cm gap was left between the top of beaker and the water/oil mixture. The sample was then covered with foil and left to settle. After 2 h, the top layer of oil/water (approximately 1/3 of the mixture) was gently poured out of the initial beaker into a 150 mL beaker

This flotation was then repeated in the same way, with the top layer poured into the same 150 mL beaker containing the initial overflowed mixture. This beaker was then left overnight (20 h) to settle. The top layer of this beaker was then poured off and filtered onto a 10 μ m stainless steel mesh filter using a vacuum pump over. The filter was then placed into a 100 mL beaker, covered with 30 mL hydrogen peroxide, and left overnight in a shaking incubator (50 °C, 100 rpm) to digest any organic material in the sample.

The filter was removed and rinsed with MilliQ water into the beaker using a PTFE wash bottle. The same filter was then used to vacuum filter out the hydrogen peroxide. Residues were washed back into the same beaker which was topped up with 30 mL Decon90 to remove any oil residues. After 48 h, the Decon90 was filtered out using a new 10 μ m stainless steel filter which was rinsed thoroughly until no further bubble formation occurred. The filter residues were then passed through a 1 mm stainless steel filter to separate the <1 mm and >1 mm fractions. Each fraction was then finally filtered over a 10 μ m stainless steel filter and rinsed with ethanol (50% v/v) into a 20 mL glass vial with an aluminium lined storage cap for storage.

2.5. FTIR analysis

Identification of microplastics was conducted using μ FTIR spectroscopic analysis (PerkinElmer Spotlight 400). Samples vials (<1 mm only) were mixed thoroughly by pipetting up and down in the vial to homogenise the sample before using a glass volumetric pipette to take a representative sample from corresponding glass vials which was then deposited into the centre of a silicone ring (10 mm diameter) onto a silver filter (10 mm diameter, 3 μ m pore size, Sterlitech, Washington USA) using vacuum filtration. At this stage it was necessary to subsample to ensure particles were present but not overloaded on the filter; based on differing particle abundance the subsample volume therefore varied between samples. The liquid samples were weighed to two decimal places (Ohaus, pioneer balance) before and after depositing to determine the weight of the subsample taken (linking this back to the weight of sediment processed for each sample thus enables later scaling to microplastics kg⁻¹).

The imaging μ FTIR spectrometer was then used to scan an area of 11 mm \times 11 mm to cover the entire filtered area. Parameters were set to collect at 25 μ m pixel size at a resolution of 8 cm $^{-1}$, with 2 scans per pixel and an interferometer speed of 2.2 cm s $^{-1}$. Spectra were collected in the range of 4000 and 700 cm $^{-1}$ wave numbers and a background spectrum was collected from a clean area of the silver filter to be removed from the collected spectra. Therefore only microplastics in the size range 25 μ m–1 mm were analysed in this study.

Spectral maps were then analysed using siMPle open-access software

(As described by Primpke et al. (2020), available at www.siMPle-pl astics.eu) to obtain information on microplastic polymer types, shapes and sizes. Mass is also provided as an output and is calculated based on measured 2D particle dimensions, polymer density, and an assumed ellipsoid 3-dimensional shape to calculate particle volume. Polymers were identified using the accompanying reference database (version 1.0.1), which contains 26 reference polymer types and six natural materials, some of which are grouped based on similarity (e.g., Acrylates, polyurethanes, and varnishes, classified as one polymer category: APV. See Table S1 for full list).

2.6. Blank correction

Sediment from the deep layers of the cores from the different sites (as described above) were used as field blank and field process blank samples (See Table S2), and were processed in exactly the same way as all other sediment samples. Additional lab process blanks were conducted in the laboratory without sediment but carrying out the same steps with all reagents, glassware, and filtering to establish contamination in the microplastic extraction steps.

As there was no significant difference ($\gamma 2 = 2.58$ (2), p > 0.05, Kruskal Wallis test) between the number of microplastic particle across all blanks (field process blanks (cut in the field), field blanks (cut in the lab) and lab process blanks), the results were averaged for calculation of LOD. All data were blank corrected before data processing, using an LOD procedure. In brief, the mean contamination and standard deviation for each polymer was calculated based on a total of 19 blanks. Lab process blanks were able to analyse the whole sample using FTIR, while field blanks relied on subsampling of sediment. Therefore, for field blanks the number of microplastics detected was scaled back up to the whole original sample volume to ensure any contamination in the whole processed sample was represented. The LOD per polymer was then calculated as the mean of all the blank samples +3 * SD of the blanks (Table S2). The number of microplastics quantified using the FTIR subsample were then scaled up to the volume of the full vial (if a subsample was taken) and a LOD-blank correction was carried out by polymer type for each sample. This figure was then scaled up to MP kg⁻ values based on the mass of sediment represented per vial. If the resulting value was > LOD the value was reported, if not the value was stated as < LOD.

2.7. Statistical analysis

All statistical analyses were conducted in R Studio (April 1, 1106). Data were checked for normality using a Shapiro Wilk test. For the purpose of statistical testing, all data < LOD were treated as 0. Microplastic size data is included for descriptive purposes and was not blank corrected so should be treated with caution. Polymer diversity was also calculated for individual samples using Shannon's Diversity index (Sun et al., 2021). Categorical data (site) were analysed using a Kruskal Wallis test with a Dunn post hoc test, with significance set at p = 0.05. Continuous data (percent seagrass cover and grain size) were analysed with a Spearman's rank correlation. Due to insufficient replicates, the depth-profile data from the long cores is not included in the statistical analysis.

3. Results

3.1. Surface sediments

Site A had the highest number of microplastics in surface sediments with a maximum of 17137 microplastics kg⁻¹ dw in one sample, and an average of 3360 microplastics kg⁻¹ dw (±5266 SD) across the 11 samples (Fig. 2, Table S3). The other sites had a maximum of 2793 (Site B1), 3542 (Site B2) and 476 (Site C), with averages of 1061 microplastics kg⁻¹ dw (±1114 SD, Site B1, n = 6), 827 microplastics kg⁻¹ dw (±1404



Fig. 2. Average number of microplastics detected in sediment samples, shown as microplastics kg-1 dw (MP/kg), from four sites around Turneffe Atoll seagrass beds shown by polymer type, based on several replicates (variable depending on site; Site A n = 11, Site B1 n = 6, Site B2 n = 6, Site C n = 4).

SD, Site B2, n = 6) and 171 microplastics kg⁻¹ dw (±226 SD, Site C, n = 4). Although abundances appear to vary between sites (Fig. 2), due to high variability between replicates, there were no significant differences in average number of microplastics between sites ($\chi 2 = 6.97(3)$, p > 0.05, Kruskal Wallis test), the mass of plastic at the different sites ($\chi 2$ =2.21 (3), p > 0.05, Kruskal Wallis test) or the average size of plastic particles ($\chi 2$ =1.587, p > 0.05, Kruskal Wallis test).

Site A had the greatest variety of polymer types, with a total of nine different polymers found, of which polypropylene and chlorinated polyethylene made up the majority (36.8% and 47.7% respectively). Polycarbonate, oxidised polyethylene, polypropylene, polystyrene, and rubber were only found at Site A. The other sites contained fewer polymer varieties, particularly Site C where only APV and polyester were found. Polyester was not observed at any of the other sites (Table S3). The Shannon diversity indices were generally low across sites (mean 0.29 \pm 0.37 S.D.), suggesting samples had relatively uneven polymer distribution in terms of relative abundance of individual polymers. However, this diversity was significantly different between sites ($\gamma 2 = 12.57$ (3), p < 0.01, Kruskal Wallis test) with Site A having significantly higher diversity than sites B1, B2 and C (p < 0.05 for all. Dunn's test). The size distribution of microplastics found in samples varied across the different sites. Site A, B2 and C generally had a greater proportion of smaller particles, while Site B1 generally had an even size distribution (Fig. 3). The blank samples also had a greater proportion of smaller particles, with the dominant size being between 50 and 100 μ m (data not shown).

3.2. Sediment characteristics

The particle size of sediments was significantly different between sites ($\chi 2 = 19.94$ (3), p < 0.001, Kruskal Wallis test). Site A had coarser sediment (measured as percentage of clay-sized particles, <4 µm) than the three other sites (p < 0.05 for all, Dunn's test), supporting this site being the most hydrodynamically energetic. Sediment particle size significantly negatively correlated with the number of microplastics found across all samples (r = -0.51, p < 0.01, Spearman's rank correlation) meaning the higher proportion of fine grained (clay, <4 µm) particles the lower the abundance of microplastics found (Fig. 4; see



Fig. 3. Particle size distribution of microplastics found at the four sites on Turneffe Atoll, shown as average particle count per size category at each of the sites. Note different Y axis scaling for clearer visualisation of data per site.



Fig. 4. Number of microplastics by sediment particle size as percentage of clay sized particles, $<4 \mu m$ (r = -0.51, p < 0.01, Spearman's Rank correlation).

Figs. S2 and S3 for sand and silt correlations). This was also true for the polymer types polycarbonate, chlorinated polyethylene and polypropylene where more particles were seen when the sediment grain size was smaller (r = -0.47, r = -0.64, r = -0.55, respectively. P < 0.05 for all, Spearman's rank correlation). There was no significant correlation between the proportion of fine grained (clay, $<4 \mu$ m) particles and the mass of plastic found in sediments (r = -0.32, p > 0.05, Spearman's rank correlation).

3.3. Seagrass cover

There was no relationship between the total number or mass of microplastics and the percent cover of seagrass (number: r = -0.22, p > 0.05; mass: r = -0.15, p > 0.05, Spearman's rank correlation) nor the average size of plastics and percent cover (r = -0.253, p > 0.05, Spearman's rank correlation). This was also true when accounting for individual polymers.

3.4. Depth profile cores

Due to limited sample numbers at depth (one core per site), it was not possible to statistically analyse differences between sites. However, none of the depth profiles showed any patterns between number of microplastics and depth of sediment (Fig. 5). In line with the surface sediments, Site A had the highest microplastic abundances, with a F. Radford et al.



Fig. 5. Sediment profile of total microplastic abundances in sediment at depth based on one core per site across the four sites (A–C). N.B. Where there are no bars present, microplastic concentrations were recorded as < LOD.

maximum of 1979 microplastics kg⁻¹ dw at 5–10 cm depth, more than five times higher than the maximum at any other site (Fig. 5). Site A was the only site where microplastics were found at every burial depth, and also had the greatest diversity in polymer types, with seven different polymers found across the different depths, whereas site B1 only had two, site B2 had three and site C only had one polymer type present.

4. Discussion

Relatively high abundances of microplastics were found within this study, particularly in surface sediments at Site A (max. 17137 microplastics kg⁻¹ dw, compared to max. 3542 microplastics kg⁻¹ dw at site B2). The maximum abundance is higher than those previously reported in seagrass sediments which are generally <5000 microplastics kg⁻¹ dw maximum (Huang et al., 2020; Kreitsberg et al., 2021). These high abundances may have been a result of proximity to anthropogenic activities on the atoll, particularly a field station and coastguard station, which are situated in the south-eastern area, where Site A is located (Fig. 1). A study in Faafu Atoll, Maldives, showed microplastic abundances to be higher in areas of high anthropogenic activity (Saliu et al., 2018).

One factor that may lead to higher observed abundances in our study could be the use of μ FTIR, which identified particles to a minimum size of 25 μ m. This automated method removes the operator bias and overlooking of small particles associated with more common visual stereomicroscopy based identification methods. The robustness of the blankcorrection methods used here lead us to exclude data where polymers were observed, but were not measured above the LOD. Whilst many studies have begun to use blank correction methods to microplastic quantification (Kreitsberg et al., 2021; Boshoff et al., 2023), the majority of microplastic studies to date have not used this LOD correction method and therefore will be reporting higher numbers (with the possibility of false positives). The LOD correction method is more conservative and therefore more reliable than an average-blank correction (Dawson et al., 2023). Therefore, where higher numbers were observed here, they can confidently be determined to be higher than those of many other studies.

It should also be noted that a subsampling method was used to ensure quality spectral output from the FTIR analysis. While necessary, and a commonly utilised method for microplastic quantification in environmental samples (Brandt et al., 2021), this may lead to extrapolation error when scaling up to larger volumes. Caution should therefore be exercised when considering values expressed in microplastics kg⁻¹.

While noticeable differences in surface microplastic abundances were observed between sites, these were not significant, due to huge variability between replicate samples within sites and limited sampling number at some of the locations (six, six and four at sites B1, B2 and C, respectively). The lack of significant difference between sites was contrary to expectations, given the variable energetic natures of these sites. This suggests that microplastic abundances may be localised on an even smaller scale due to specific proximity to sources or local hydrodynamics, which may be indicated by the variety of microplastic types found at the different locations, even within sites (Boshoff et al., 2023). Although there were no significant differences in number of microplastics between sites, the polymer compositions did vary. Site A showed the most variability (closest to anthropogenic activity, Fig. 1), with a total of nine polymers found, while Sites B1 and B2 both contained the same four polymers (Fig. 2). At Site C only two polymers were found, one of which was polyester, which was only present at this site. This site-specific variation in polymer type suggests that different sources of plastics may be influencing microplastic abundances and composition at each site. For example, polyethylene and polypropylene (found at site A) are common single-use packaging materials and thus may have been derived from degraded litter, correlating with local human activities on land. Additionally, chlorinated polyethyene is a common co-polymer used often in plasticizing of PVC (Zou et al., 2020). These polymers are consistent with the most common types of microplastics found in sediments globally (Yao et al., 2019). In contrast, polyester and APV at Site C may be derived from local fishing activities that are known to occur around this site in the vicinity (attributed to fishing nets/lines and boat paints respectively).

Previous studies suggest that in addition to microplastic abundances, polymer diversity is higher in sites with closer proximity to anthropogenic activity (Boshoff et al., 2023; Sun et al., 2021), as also observed in this study. This may be influenced by the site-specific sediment dynamics: Hope et al. (2021) found that mean grain size was one of the best predictors of microplastic diversity matrices. It is important to note that using traditional (biological) diversity indices is an emerging measure of microplastic diversity, although these diversity indices have been suggested to be a good measure of 'microplastic communities' (Li et al., 2021; Sun et al., 2021). This gives insight into the potential sources of microplastics, as the higher numbers of polymers, and generally proportionally higher contamination, at Sites A, B1 and B2 may also correspond with their exposed west-facing locations, exposing them to the Atlantic Ocean, and thereby potentially receiving the deposition of plastics transported from further afield. In contrast, Site C in the east is more protected by the mainland on the landward site of the Atoll and may thus be influenced more local inputs. This points to the importance of both site characteristics (exposure) and local anthropogenic activity in influencing the abundance and types of plastics found within environmental samples.

Broadly speaking, finer-grained sediments tend to be deposited in lower energy environments (Madsen et al., 2001), and the significantly lower proportion of fine-grained sediment at Site A supports our understanding that Site A is the highest energetic environment. Sediment grain size appeared to influence microplastic abundance, although in contrast to our prediction that higher numbers of microplastics would be deposited in areas of low energy (Harris, 2020). Instead, a proportional increase in finer grained sediments (clay-sized particles $<4 \mu$ m), and thus a lower energy environment, was significantly linked to lower microplastic abundances. Our results therefore suggest that as grain size and thus energy increases, more microplastics tend to be deposited and/or retained. It should be noted that this increased abundance may in part be down to the characteristics of coarse-grained sediment retaining microplastics more effectively than fine-grained sediment rather than, or in addition to, increased deposition. This result implies that in these samples, the local hydrodynamics (at a smaller scale than site alone) override the other contributing factors when it comes to microplastic deposition and sequestration, and also that this is more spatially dependent than simply defining broad energetic zones. A study by Enders et al. (2019) similarly found that, when compared with other environmental and hydrographic conditions, sediment grain size (using proportion of clay-sized particles as a descriptor of grain size) was the most significant factor influencing microplastic abundance. However, they observed the opposite relationship between microplastic abundance and sediment grain size, reporting an increase in particles with a decrease in sediment grain size. Additionally, the size of microplastics detected should be further considered in future studies, as smaller microplastics can remain suspended in the water column for extended periods, allowing them to be transported far from their original sources (Shiravani et al., 2023). Although it has been suggested that microplastics may behave in a similar way to natural particles (Kane and Clare, 2019), the relationship between infiltration of microplastics and natural sediments is complex, particularly for lower density polymers (e. g., polypropylene and polyethylene) and those with diverging particle shapes (e.g. fibres) (Enders et al., 2019; Pohl et al., 2020).

It has previously been suggested that seagrass meadows may act as a sink for microplastics, given that seagrasses alter hydrodynamics and reduce water flows, leading to the fall-out of particles from the water column to the sea floor (Sanchez-Vidal et al., 2021; Navarrete-Fernández et al., 2022; Ward et al., 1984). Despite this assumption, few studies on seagrasses and microplastics have proven that seagrasses enhance this trapping effect within local sediments. For example, Cozzolino et al. (2020), Unsworth et al. (2021) and Boshoff et al. (2023) all found no correlation between microplastic abundances in sediment and seagrass cover. We did not analyse samples from any sites where seagrass was completely absent, therefore we cannot comment on the influence of simply the presence or absence of seagrasses on microplastics retention within underlying sediments. However, no trend was seen here with percentage seagrass cover. Contrary to previous assumptions this combined evidence suggests that seagrasses do not significantly affect the deposition and sequestration of microplastics.

With respect to vertical distribution of microplastics in sediments in general, regardless of seagrass presence, it is commonly observed that microplastic abundances increase with proximity to the sediment surface, in line with recent sediment deposition correlating with high plastic use. Within seagrass sediments in the Mediterranean, Dahl et al. (2021) found a pronounced increase in microplastic abundance with decreasing depth in the last 50 years, with the highest abundances found at the surface of sediment cores (3819 microplastics kg^{-1} dw). Deeper sediment layers reflect lower volumes of societal plastic use and thus less environmental contamination (Pervez and Wang, 2022; Turner et al., 2019). In the depth profiles here, it would therefore have been expected that abundances of microplastics would decrease with depth, as sediments increase in age. Age data for these sediments were only available for sediments >20 cm depth, and all were >100 years old (unpublished results) therefore not providing the resolution required to interpret recent microplastic deposition. Regardless in the depth profiles, microplastic abundances did not appear to be related to sediment depth at any of the four sites, with the highest abundance found at varying depths depending on the site. However, the lack of replication precludes the ability to statistically analyse any trends.

While this study showed no evidence that seagrasses are sequestering microplastics at different rates in the different sites around the Turneffe Atoll, microplastics were nonetheless present, and in some instances in very high abundances. This poses the question as to whether the presence of microplastics may influence the health and growth of seagrasses as a result of physical or chemical toxicity. Recent studies suggest the presence of microplastics may reduce growth and increase oxidative stress in seagrasses (Menicagli et al., 2022). As seagrasses are

rhizomatous plants, this leads them to be potentially more susceptible to damage from microplastics than rooted plants (Ge et al., 2021), although spermatophytes can be sensitive in different ways, with microplastics affecting timing of seed germination and plant growth (Bosker et al., 2019). Effects of microplastics may be a result of adsorption onto plant surfaces or absorption into plant cells, leading to oxidative stress and the production of reactive oxygen species (ROS) (Ge et al., 2021). Despite some preliminary knowledge, research of the effects of microplastics on plants, especially aquatic plants, is in its infancy, and long-term implications are unknown. A more holistic study which includes the different environmental compartments (microplastics associated with surrounding water, leaves, sediment, and rhizome) is required to fully assess potential exposure and likely impact of microplastics on seagrasses.

The variability presented in this study and previous studies highlights that microplastics in seagrass meadow are highly spatially specific. The presence and abundance of microplastics in seagrass systems also exhibit variability on a global scale, with varying abundances observed across different regions and locations around the world (Zhou et al., 2023). Therefore, future studies should consider this when designing sampling campaigns and choosing sampling locations and number of replicates, to ensure that both large and small-scale differences are considered. Overall, our results suggest that while there were some observed differences in microplastic abundance in sediments, for example depending on sediment grain size, the factors we initially considered to be important, including site location, energetics, and seagrass cover, cannot alone explain any differences between samples. Given the variability between abundances in samples within sites, more complex factors must be influencing spatial variability on a smaller scale, which requires further investigation. The high levels of microplastics found in the different Turneffe Atoll sites suggests that there could be a potential environmental risk to seagrasses and their associated communities, particularly with the predicted increase in plastic production. It is therefore paramount that we better understand these systems and the impacts microplastics pose to seagrasses and their associated communities and ecosystem services, to prevent future harm.

5. Conclusion

In conclusion, this study reveals high abundances of microplastics in seagrass sediments in a sparsely populated atoll (maximum 17137 microplastics kg⁻¹ dw). These abundances are highly spatially variable and highlight the need for a localised sampling strategy informed by site hydrodynamics and proximity to sources to capture the high inter-site variability. The large range of polymer types found here indicates a wide variety of sources and the potential for these plastics to have travelled long distances. This, combined with the lack of significance of seagrass cover, suggest that local and regional sources are a more significant factor influencing microplastic abundance and polymer types present. This implies the need for further source identification to inform the development of targeted waste management improvements in proximity to vulnerable seagrass ecosystems, to reduce the input of further microplastic contamination. The presence of microplastics at all locations within these systems suggests a plausible environmental concern for blue carbon ecosystems, warranting further research to proactively mitigate potential consequences.

CRediT authorship contribution statement

Freya Radford: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. Alice A. Horton: Writing – review & editing, Writing – original draft, Supervision, Methodology, Investigation, Formal analysis, Conceptualization. Stacey Felgate: Writing – review & editing, Project administration, Methodology, Formal analysis, Data curation. Anna Lichtschlag: Writing – review & editing, Writing – original draft, Investigation, Conceptualization. James Hunt: Writing – review & editing, Supervision, Formal analysis. Valdemar Andrade: Resources. Richard Sanders: Project administration, Funding acquisition. Claire Evans: Writing – review & editing, Supervision, Project administration, Methodology, Funding acquisition, Conceptualization.

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.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

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<u>Update</u>

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Corrigendum to 'Factors influencing microplastic abundances in the sediments of a seagrass-dominated tropical atoll' Environmental Pollution (2024) 357, 124483

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The authors regret that during the article revision process, one of the numbers in the highlights text was not updated accordingly with the revised blank-correction analysis. This has led one number to be incorrect.

Highlight one currently states:

'Maximum concentration 19342 microplastics $kg\!-\!1$ far exceeds other seagrass studies'.

It should read:

'Maximum concentration 17137 microplastics $kg\!-\!1$ far exceeds other seagrass studies'.

Many apologies for this oversight. The number is correct in the abstract and throughout the manuscript.

The authors would like to apologise for any inconvenience caused.

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