The effects of polyester microfibers on functionally important microphytobenthos and sediment-dwelling infauna.

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Abstract: Microplastics are accumulating in coastal soft sediments, the majority of which are fibres. Despite this, little is known about the potential ecological effects of fibrous material on functionally important benthic organisms. For instance, the microphytobenthos (MPB) and deposit-feeding bivalves which are critical for soft sediment ecosystem functions such as nutrient cycling. Red polyester microfibers (1.8 ± 0.9mm) were added at varying concentrations (0% - XX% DW sediment) to the surface 1cm of sediment in the chambers. The effects of increasing microfiber concentrations on microphytobenthic (MPB) biomass (chl a) and composition (fatty acid (FA) biomarkers) were evaluated after a total exposure period of XX days. Half the chambers were exposed to a 12 h light/dark cycle, to allow photosynthesis to occur, while the remaining chambers were exposed to extremely low light levels (XX PAR level) that would inhibit photosynthesis. After an initial 35 day MPB growth period, four deposit-feeding bivalves, *Macomona liliana,* were added to each chambers. *M.liliana is a dominant and functionally important bivalve in New Zealand sediments. These were added after the initial MPB growth to determine* whether any effects of microfibers on their food resource (the MPB) affected the burrowing behavior and energy levels of these grazers. After a further XX days (total duration XX days), sediment porewater nutrient concentrations (a proxy of ecosystem function) were evaluated and related to changes in the MPB and *M.liliana*Results suggest that microfibers additions influenced both the quantity (biomass) and quality (FA biomarkers) of the MPB. Fewer diatoms and an increase in phycocyanin pigments associated with cyanobacteria, emphasized the potential for shifts in the MPB community with increasing microfiber concentrations. The change in MPB quality coincided with up to 75% reductions in bivalve energy reserves, and reduced *M.liliana* burrowing activity. . . Under light conditions (which allowed the MPB to photosynthesise), nitrate + nitrite (together as NO_x) and ammonium (NH_4^+) concentrations were elevated at the highest microfiber concentrations. When the light was blocked (dark conditions) only NH_4^+ concentrations increased. The difference in porewater nutrient stores suggests that photosynthesis in the MPB together with *M.liliana* burrowing moderates the effect of microfibers on soft sediment nutrient cycling. These findings demonstrate the potential for microfibers to alter soft sediment ecosystems and influence ecological functions through complex feedbacks at the base of the benthic foodweb.

Introduction

Waste water[1], runoff[2] and fishing gear[3] are all significant sources of microplastics (particles <5mm), with this debris contributing to the accumulation of microplastics in coastal soft sediments. Microplastic particles have now been detected in sediments and waters in freshwater[4], marine[1], estuarine[5,6], and deep-sea[7] ecosystems and have even been detected in remote Arctic[8] and Antarctic[9] waters, far from urban sources. The extent and ubiquity of microplastics emphasizes the need to understand the ecological effects it may have, particularly in soft sediments that are a potential sink for this contaminant[7,10].

Despite growing concerns about the quantity and diversity of microplastics in marine sediments, we have limited information on the potential ecological effects of their accumulation. While microplastics are a diverse suite of contaminants rather than a single entity, we need to better characterize the effects of different morphologies, sizes and chemical compositions both in the field and in controlled laboratory studies with specific classes as these properties may affect their influence on organisms and ecological processes[11]. Although microfibers often dominant marine samples[12], representing up to 95% of microplastics found in marine waters[8,13,14], sediments[10,15] and organisms[16] in some cases, the majority of uptake or exposure experiments in the laboratory have used microplastic fragments or beads[17–19]. Polyester, the majority of which is composed of polyethylene terephthalate (PET), is often the most prevalent fiber type detected in marine systems[10]. However, polyester microfibers are under represented in ecological experiments. We therefore know relatively little regarding the effects of microfibers on functionally important sediment dwelling organisms[20,21] and ecosystem function despite their prevalence. Similarly to many microplastic fragments, different microfibers can leach toxic additives[22–24] as well as adsorb other environmental contaminants[1,25,26]. Polyester microfibers therefore have the potential to affect marine organisms through ingestion or changes to the biochemical environment, [20,21] and deserve greater attention.

Functionally important benthic organisms. Microplastic ingestion has recently been explored in marine worms[27,28] and shellfish[17,29], and freshwater phytoplankton[30]. Several studies have been conducted on benthic filter feeders[19,31] and zooplankton[32,33], due to the potential role of these organisms in filtering microplastics from the water column. However, once on the seafloor, microplastics will interact with benthic organisms that have different feeding behaviours[34]. Intertidal deposit-feeding bivalves are functionally important[35], contributing to ecosystem productivity, nutrient cycling and water quality. Deposit-feeders graze on microphytobenthos (MPB) inhabiting the surface layers of sediment and as these surface layers are where sediment microplastics accumulate[36,37], deposit feeders and the MPB may be particularly vulnerable to microplastics[38]. Nonetheless, there remains a lack of information on interactions between these benthic organisms and microplastics[39].

When bivalves are exposed to contaminants or other stressors, their burial capacity[40], activity levels[41,42] and feeding behaviours[17,19] may be affected. These behavioral changes are likely associated with changes in their energy reserves, growth and weight, as documented for other invertebrates[30,43–45]. A number of mechanisms have been proposed to explain the depletion of energy reserves during stress. Firstly, stressful conditions can increase the energy demands of an organism, thus reducing energy reserves[46,47]. Alternatively, a decrease in food or nutrient intake may limit the synthesis of lipids, carbohydrates, proteins as the organisms redirect metabolic processes to counteract toxicity effects[47]. Reduced intake of energy may also result from the ingestion of these comparatively low quality particles compared to food[44], as well as gut blockage and irritation due to ingestion[43]. As infaunal energy and activity levels change, grazing pressure and nutrient release are altered. This feeds back to the MPB, with potential effects on MPB biomass[27,35] and composition[48]. These changes may also lead to the loss of oxidized microhabitats[49] further altering nutrient cycles[27,50] with knock on effects on ecosystem productivity.

MPB can account for up to 90% of estuarine primary productivity[51,52] with highly nutritious diatoms typically dominating soft sediment habitats[53,54]. While other habitats may be dominated by less nutritious cyanobacteria these have functionally different roles to diatoms[55] therefore a shift in these taxa can alter ecosystem function. MPB such as diatoms act as an efficient nutrient filter on the sediment surface, mediating the flux of dissolved inorganic nitrogen at the sediment-water interface preventing eutrophication[56,57,58]. Conversely, cyanobacteria often benefit from stressors like nutrient enrichment[59,60] and they often utilize less nutritious carbon sources such as oil and microplastics[61]. MPB and deep-dwelling deposit feeders are vital for ecosystem function, yet there is a lack of information on the effects of microplastic contamination on these organisms or ecosystem functions such as nutrient cycling.

The effects of various microplastic on primary producers is still widely debated[62]. Decreases in algal biomass and photosynthesis associated with microplastic contamination have been observed with a number of planktonic primary producers[18,62,63]. Others have detected little or no effects[64–66] and there are just a few passing observations of the impact on MPB[27]. These studies have been critical to assess the potential effects of this emerging contaminant on marine life, however variable plastic types, unrealistic concentrations, and the use of algal monocultures has contribute to the divergent conclusions in the literature. Further complicating this picture, is growing evidence that synthetic polymers can provide a substrate that benefits various microbes[48,67,68]. Microplastics could therefore modify interactions and feedbacks associated with the MPB that are vital for soft sediment ecosystem structure and function[69].

Energy reserves and fatty acid biomarkers. Together with total lipids and glycogen reserves, fatty acids are a source of metabolic energy and nutrients to all organisms[70,71] including bivalves. Fatty acid (FA) biomarkers are useful indicators of general ecosystem health[72], sources of organic matter[73] and can reveal trophic links[74]. FAs are also valuable for assessing organisms' responses to environmental stressors like changes in salinity and temperature[71], heavy metal contamination[75] and chemical stressors[76] and therefore could be useful in assessing the potential stress of microplastics in the marine environment. While individual FAs cannot be assigned to specific organisms, changes in the presence and ratios of these biomarkers can reflect changes in the taxonomic or functional groups in sediment communities[77,78] as well as the dietary intake[79] or metabolism[80] of MPB and bacteria in consumers. The essential fatty acids, Eicosapentaenoic acid (EPA, 20:5ω3) and Docosahexaenoic acid (DHA, 22:6ω3) are synthesized by many primary producers but are primarily associated with diatoms and dinoflagellates, respectively.

EFAs cannot be efficiently synthesized by bivalves de novo[81,82] and the relative importance of DHA or EPA can be species specific[83]. However, variation in the ratio between EFAs can indicate a shift between different taxa available to the consumer, the dietary intake of primary producers or the metabolism of energy reserves due to stress[77]. The 'diatom index' of Antonio & Richoux[84] is one such useful indicator to determine the dominance of diatoms over other taxa. This index utilizes multiple FAs to determine compositional shifts in the MPB community as well as change to the dietary intake or metabolism of EFAs (Supp. table 1). The metabolism of EFAs during periods of stress can also be species-specific, with one often selectively retained over another depending on the organisms current requirements for growth and reproduction[81].

Methods

Experimental design. We investigate the effects of long-term exposure to varying concentrations of polyester microfibers, on the quality & quantity of MPB in the sediment, using FA biomarker and pigment analysis. The effects on the burrowing behavior and energy reserves of a functionally important deposit-feeding bivalve *Macomona liliana* were also assessed, as well as the FA biomarkers present in the bivalves. We hypothesize that increasing microfiber contamination could negatively influence the lipid energy reserves in deep-dwelling deposit feeding bivalves and subsequently alter their burrowing capacity. We anticipate that as the complex feedbacks between bivalves and MPB are altered, ecosystem functions will be modified.

Few studies have examined the effects of environmentally relevant microfiber concentrations[20]. Instead, the majority of studies, to date, have exposed organisms to microplastic fragments or beads at exceptionally high concentrations to assess chronic effects[85]. In the present study, microfibers were added at relatively low concentrations (1-50mg kg^{-1} WW sediment), with the potential effects assessed after a relatively long exposure period instead. This allowed the evaluation of the benthic community changes associated with long-term exposure to increasing microplastic concentrations. Sediment mesocosms containing red polyester fibers (6 levels of microplastic additions, 2 light conditions, 3 replicates) were incubated for 35 days in light and dark conditions to allow the MPB and biofilm to develop. Four adult *M. liliana* (20-30mm shell length) were added to the sediment surface of each mesocosm at a density of 90 individual m^{-2} after 35 days. Any bivalves remaining on the surface after the initial 12 h were replaced with fresh, healthy bivalves. Only one bivalve emerged from the sediment and died during the incubation experiment, which was removed within 12 h. The chambers were incubated for a further 40 days before sampling.

Materials & organisms used in the experiment. Sediment (D_{50} = 220μm) was collected from Waiwera harbour on 17th November 2017 and sieved to 500μm to exclude large infauna and shell fragments. Red, polyester (PET) microfibers were collected by washing new polyester fleece blankets multiple times in a pre-cleaned washing machine. The machine was fitted with an external 25μm filter sock on the outflow pipe to collect shed fibers. Additionally fibers were also collected from dry blankets using a fabric shaver. Microfibers were sieved through a 5mm sieve to exclude macrofibers (>5mm) then air dried prior to use. A subset of the fibers were visually inspected under a Leica MS5 microscope with a 40 x magnification to confirm only microfibers (<5mm) were used. The mean length of measured fibers was 1.8 ± 0.9mm (n=40). The chemical composition of the microfibers was confirmed to be polyester (PET) by Fourier Transform Infrared Spectroscopy (FTIR) with spectra compared with the database from Primpke and others[86]. Full details of the method are available in the supplementary material alongside an example spectra match (Supp Fig 1).

M. liliana is a common tellenid bivalve found in intertidal soft sediments throughout New Zealand[35]. Their deep position in the sediment bed (5-10cm depth) and deposit feeding behavior can facilitate coupled N-cycling processes by increasing the interface of oxic-anoxic sediment[87]. These functionally important bivalves were selected as they extract and feed on MPB and detritus on the sediment surface, by extending their inhalant siphon to the sediment-water interface[88]. As they move around and feed, *M. liliana* rework the sediment stimulating nutrient regeneration[89] and excreting inorganic nitrogen, both of which stimulate the MPB[35]. Often this results in complex interactions between the MPB and *M. liliana*, with positive effects of nutrient remineralization often counteracting grazing pressure[90].

Experimental set up. Sieved and homogenized sediment was added to 36 chambers (300mm (dia.) x 360mm (h)), to a total depth of 11cm. Red polyester fibers were mixed and evenly distributed into individual 1kg batches of wet sieved sediment at the selected concentrations (0, 10, 30, 100, 300 and 500mg fibers kg^{-1} wet weight sediment). These sediments were added as a surficial layer (1cm) to each mesocosm. Controls were prepared separately, without the addition of PET fibers to reduce risk of cross contamination.

Each chamber was carefully filled with filtered seawater (25µm) so as not to resuspend fibers and the chambers allowed to overflow gently at a rate of ~0.05L sec^{-1} throughout the experiment. Slow flow velocities limited the loss of microplastics into the overlying water while preventing nutrient or oxygen depletion. To evaluate the interaction between microplastic contamination and MPB photosynthesis and biofilm development, and infaunal activity, half the sediments were incubated under a diurnal (12 h/12 h) light regime and half in 24 h darkness (n=18). Cotton shade cloth was used to reduce the incident light reaching the sediment surface in dark chambers (>90% reduction). Chambers were randomly distributed under four double Aqua One Reflector Fluroglow T8 (40W) units suspended 30cm above the sediment surface. Each unit was fitted with 2 x 1.2m T8 sunlight fluorescent bulbs. Photosynthetically active radiation (PAR, 400-700nm) was measured using a Li-Cor LI-190R quantum sensor coupled with a Li-Cor data-logger (Li-Cor, USA) to ensure all light chambers received adequate light (ambient light of ~200 µmol photons m^{-2} s^{-1}) at the sediment surface. External sources of light and contamination were excluded from the experimental area using black-out curtains.

Post exposure sample collection. Duplicate sediment core samples (2.6cm ID, 2cm depth) were collected from each chamber for porewater nutrient analysis, with four small core samples (1cm ID, 0-1cm depth) were pooled and frozen immediately for biochemical analysis. Sediment for biochemical analysis was freeze-dried and homogenized then sub-sampled for various bio-molecular analysis. To visualize the dominant MPB present across treatments, surface scrapes of the sediment were collected, and fixed in 2.5% Gluteraldehyde solution.

After sediment core samples were extracted, individual bivalves were carefully removed, intact, from each chamber by gentle sieving. One bivalve from each chamber was placed on clean control sediment to measure bivalve reburial rates over a 20 h period following Cummings & Thrush[40]. At each time interval (0.5, 2, 4, 12 and 20 h) the number of bivalves that were fully reburied into the sediment were recorded. Any remaining on the surface after 24 h were assumed to be 'immobile'. *M. liliana* from the mesocosms were immediately frozen in liquid nitrogen for biochemical analyses and to quantify the number of ingested fibers.

Biochemical and sediment property analysis. Sediment porosity, organic matter and sediment grain size were determined from homogenized and freeze dried sediments (see supplementary materials). Determination of chlorophyll a followed Lorenzen[91] using a 90% acetone extraction. Porewater was extracted and filtered through GF/F filters and Nitrate (NO_3^-) and nitrite (NO_2^-) together as NOx, ammonium (NH_4^+) and phosphate (PO_4^{3-}) concentrations determined using a Lachat QuickChem 8500+ FIA (Zellweger Analytics Inc. Milwaukee, Wisconsin, 53218, USA).

Diatom cells were sonicated, digested in 30% H_2O_2, and mounted on permanent slides using naphrax. No quantitative analysis of the community was attempted, but the dominant taxa were examined by light microscopy across the microplastic treatments. As permanent slides destroy some MPB taxa (cyanobacteria, green algae), only diatoms were visualized. A phycocyanin assay was also adopted to quantify any changes in the cyanobacteria community[92].

M. liliana were freeze-dried, the tissue extracted from shells and homogenized for microscopic analysis. One full bivalve from each chamber was digested in 10% KOH[93] for 48 h after an initial heating of the sample to 40ºC for 6 h. Samples were gently vacuum filtered through GF/F filters before red microfibers were quantified and measured by light microscopy. During all steps, atmospheric contamination was determined from the presence of microfibers on clean dampened filter papers and procedural blanks were run with each new batch of samples[93]. Total lipid contents were extracted from bivalve tissue using a modified Bligh & Dyer method[94] and contents determined using the sulfo-phospho vanillin (SPV) spectrophotometric method[95]. The total fatty acid (TFA) composition was determined for control and high treatments only following a one-step direct transesterification method[96,97]. Full details are in available in the supplementary methods. Due to limited time and resources, and the interest in the role of photosynthesizing MPB, FAs were only processed for sediments and bivalves incubated under light conditions. Subsequently, bivalve total lipid contents were only assessed for those held under light conditions.

Identified FAs were first expressed as a percentage of the total FAs identified in each sample and designated as X:YωZ, where X in the number of carbons, Y is the number of double bonds and Z is the position of the ultimate double bond from the terminal methyl. The ratio of DHA/EPA[77] and the 'diatom index' of Antonio & Richoux[84] were employed as diatom and food quality indicators for sediment and animals to assess the effects of microplastic contamination in addition to some other indicator FAs (Supp. table 1).

Data analysis. The effects of microplastic additions and light on biochemical properties and FA biomarkers of the sediment and bivalves were assessed by separate two-way PERMANOVAs (v.7, PRIMER-E, Ivybridge, UK) based on Euclidean distances (Table 1). Euclidean distance matrices of biochemical sediment properties, nitrogen stocks and bivalve reburial rates were used to assess the effects of microfiber additions and to determine if the effects were modulated by the light conditions of the experiment (light/dark). Relationships between MPB quality indicators and sediment properties (Supp. Table 1 and Table 1) were then explored and visualized using principal components analysis (PCO,[98]). All data used in PCO analyses were normalized using a fourth-root transformation. No FA biomarkers were included in the multivariate analysis, as data were only available for the control and highest microplastic additions (0g & 0.5g treatments).

Results & discussion

Effects on sediment microbial communities. Sediments are a known sink for microplastic[7,10], and MPB communities will undoubtedly interact with microplastics depositing on soft sediments due to their position at the sediment-water interface. Nonetheless, few studies that have investigated the influence of microplastics on soft sediment MPB communities, although a number of studies have noted infaunal ingestion of microplastics can affect MPB biomass[19]. While up to 95% of microplastics detected in soft sediments are fibers[8,15,16] there are only a few studies on the influence of microfiber ingestion[20,21]and none that investigate the effects of microfibers on various compartments of benthic ecosystems including the MPB.

In the present study, microfibers were added to surface sediments and incubated the sediments over a relatively long experimental period. Multivariate analysis on the Euclidean matrix of biochemical traits suggested that the light conditions of the incubation experiment and the microfiber additions resulted in interacting effects on MPB and sediment properties, infauna behavior and condition and sediment nutrient stocks (Table 1). The observed results were reinforced by principal components ordinations (Fig 1). The ordination illustrates a clear separation between the microfiber treatment groups with differences modulated by the light regime. Porewater NO_x (-84%), sediment organic matter content of the sediment surface (-52%) and *M. liliana* burrowing activity (-51%) were highly correlated to the first PCO axis (72% variance explained).

Table 1: Results of univariate PERMANOVA tests for differences in sediment and biochemical properties using light regime and microplastic concentration as predictors.

Compartment	**Parameter**	**data**	**Factor**	**Pseudo-F**	**p (perm) or p (mc)**
	Euclidean matrix	all	L * M	37.0	<0.001
Sediment	MPB biomass (chl a)	all	L	3.96	<0.01 + <0.001
			M	110.72	
	OM%	all	L x M	4.85	<0.05
	P (µM)	all	-	-	-
	NH_4^+ (µM)	all	M	3.56	<0.05
	NO_x (µM)	all	L x M	21.85	<0.001
	Cyano biomass (phycocyanin)	all	L	4.79	<0.05
			M	2.73	0.05
	BaFAs (C15:0+C17:0)	all	-	-	-
	Diatom index (sed)	0g & 0.5g L only	M	10.73	<0.05 (mc)
	% EPA	0g & 0.5g L only	M	63.38	< 0.01 (mc)
	EPA/DHA	0g & 0.5g L only	M	25.63	<0.01 (mc)
M. liliana	Reburial rate	all	M	47.1	<0.001
	Bivalve biomass	all	-	-	-
	Lipid content	L only	M	14.65	<0.001

ω3:ω6 ratio (quality indicator)	0g & 0.5g L only	-	-	-
Diatom index (biv)	0g & 0.5g L only	-	-	-
DHA/EPA (biv)	0g & 0.5g L only	-	-	-

Significant ($P<0.05$) main effects or interactions are displayed together with PERMANOVA Pseudo-F (number) and significance levels (p (perm) and p (mc) when monte carlo permutation tests were performed.

The second PCO axis (13%) was correlated to the overall MPB (r=-0.40) and cyanobacteria (r=-0.40) biomass (Fig 1), with the overall MPB biomass decreasing with microfiber additions in the light (Fig 2A). In control sediments, however, the MPB biomass increased significantly from 2µg g^{-1} at the start of the experiment to 14µg g^{-1} at the end under light conditions (Fig 2A), indicating MPB growth under these conditions. Fatty acid biomarkers associated with diatoms were only processed for the extreme ends of the treatment gradient; 0g (control) and 0.5g (highest) microfiber treatments respectively. However, these indicated a reduction in the proportion of diatoms with microfibers compared to controls (Fig 2B).

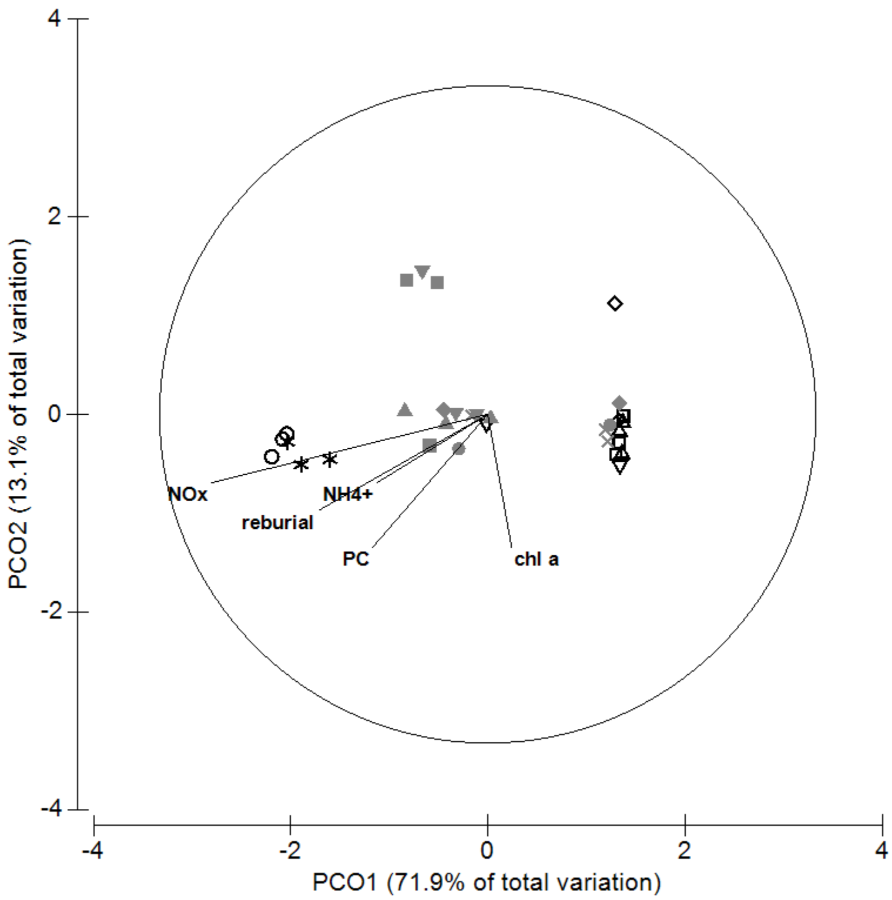


Figure 1: Principal components ordination (PCO) of biochemical variables. PCO1 explained 71.9% of the variation, PCO2 explained 13.1% while PCO3 (not presented) explained an additional 7.3% of the variation. Symbols: Black open symbols – light conditions; grey closed symbols – dark conditions. Shapes represent microfiber additions; triangles – 0g; inverted triangle – 0.01g; squares – 0.03g; diamonds – 0.1g; circles – 0.3g and stars – 0.5g microfiber additions. The correlation circle overlays measured variables that were influencing the dissimilatory between the samples. All data were fourth-root transformed prior to analysis. Chl_a – MPB biomass; C-phyco – Cyanobacteria biomass; NH_4^+ – porewater NH_4^+ concentration (µM). NO_x – porewater NO_x (NO_2^- + NO_3^-) concentration (µM). Reburial – reburial rate of *M. Liliana.* OM – organic matter has been removed for clarity of the plot but lay in the same trajectory as NO_x.

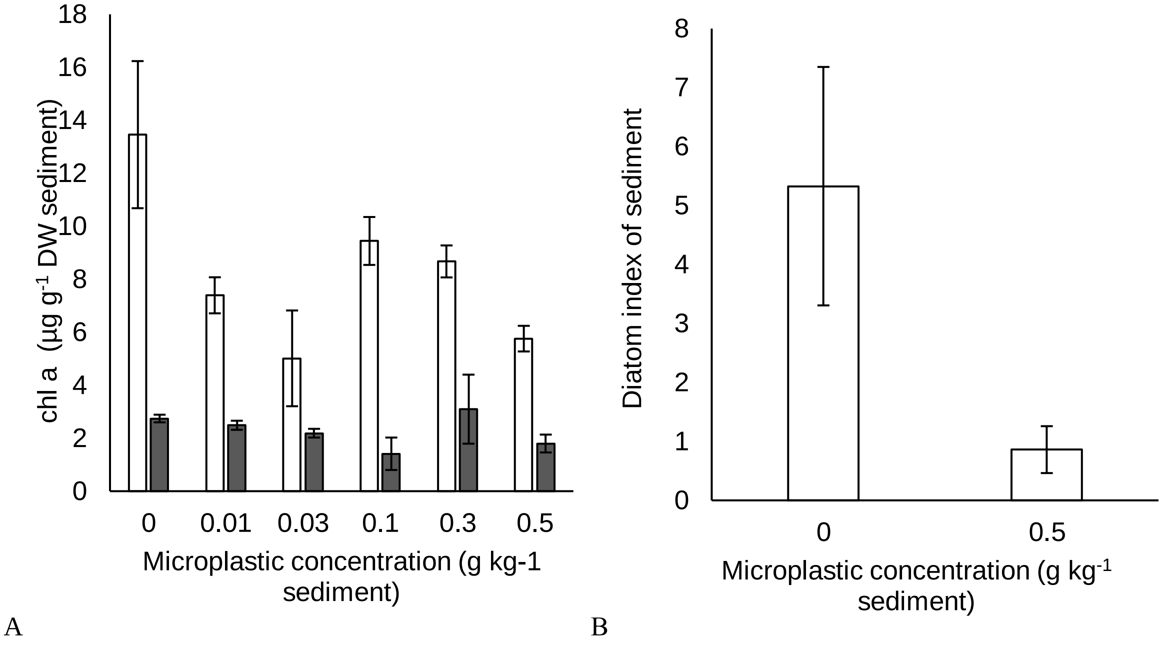
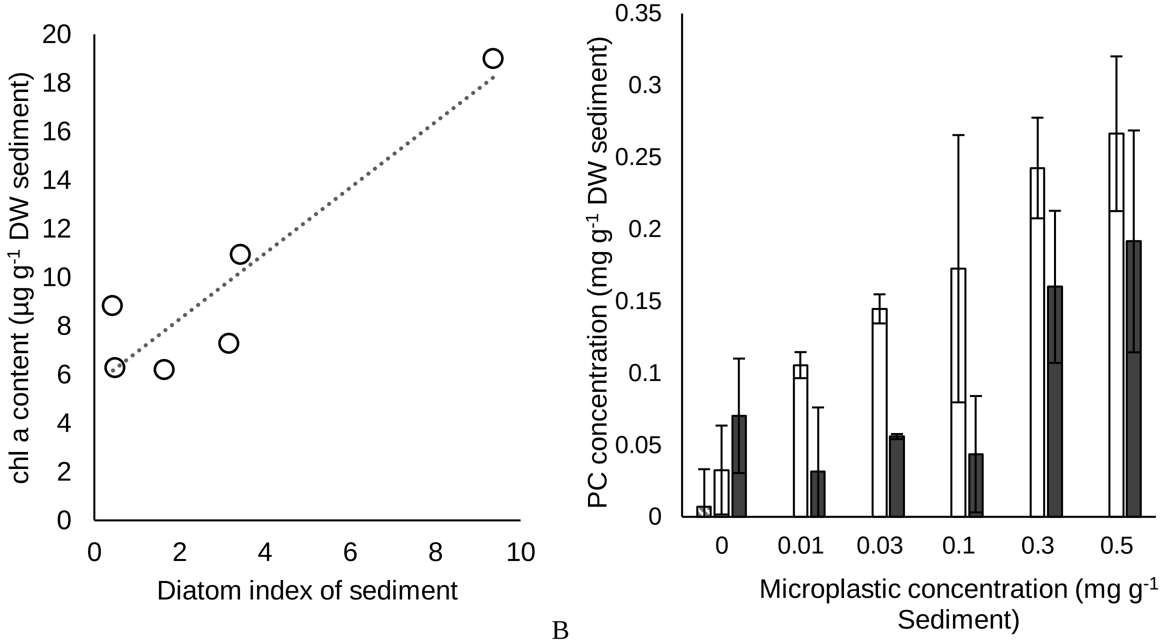


Figure 2: A) Mean (±SE) chlorophyll a content (MPB biomass) of the sediment surface for all microplastic treatments in light chambers (white bars) and dark chambers (grey bars). B) Mean (± SE) diatom index of the sediment surface for control (0g) and high (0.5g) microplastic treatments (n=3).

MPB biomass and the proportion of diatoms were correlated with one another, so the reduction in overall biomass was related to the reduction in the diatoms (Fig 3A). This coincided with a small increase in the pigment, phycocyanin, associated with cyanobacteria, with microfiber additions (Fig 3B). This increase was apparent under both light and dark conditions, with higher microfiber additions (Fig 3B). These results suggest that increasing microfiber contamination has the potential to alter the MPB community composition and consequently the functional role of the MPB. For example, less nutritious diatoms which are a preferred food resource for benthic fauna, and more cyanobacteria will alter the nutritional quality of the basal food resource[54] with implications for the marine foodweb.



A B

Figure 3: A) Correlation between the diatom index and chlorophyll a content of the sediment surface ($r_s^2 = 0.71$, $P<0.05$, n=3). B) Phycocyanin content (cyanobacteria biomass) of sediment as a function of microplastic additions. The concentrations are displayed for pre-incubated sediments (striped bars), and sediments incubated under light (white bars) and dark (dark bars) conditions.

Changes to sediment nitrogen stocks were detected (Fig 4A-B). Porewater NO_x was detectable in the dark, control sediments and remained close to the detection limits regardless of microfiber treatment (Fig 4B). Conversely, while porewater NO_x in light sediments were within the detection limits of the auto-analyzer at the end of the experiment in controls, NO_x was elevated with microfiber additions (Fig 4A). Furthermore, porewater NH_4^+ increased with microfiber additions regardless of the light conditions (Fig 4B). Altered nutrient uptake by the MPB can be induced by other stressors and this can shift a system towards greater heterotrophy[99], alter functional roles and restructure foodwebs[100]. Shifts in the microbial community (phycocyanin content) were correlated with porewater DIN concentrations (Fig 1 & Supp. Fig 2). The changes to nitrogen stocks supports the findings of Cluzard et al.[50], who observed elevated NH_4^+ during clam/microplastic incubations. Furthermore, shifts in the MPB community will alter their relationship with bacteria in the sediment[101], with subsequent feedbacks to the MPB and nutrient pathways. Cluzard et al[49] proposed that the elevated NH_4^+ detected in their study was due to a reduction in denitrifying bacteria or denitrification rates in the presence of microplastics, so this is warrants further investigation.

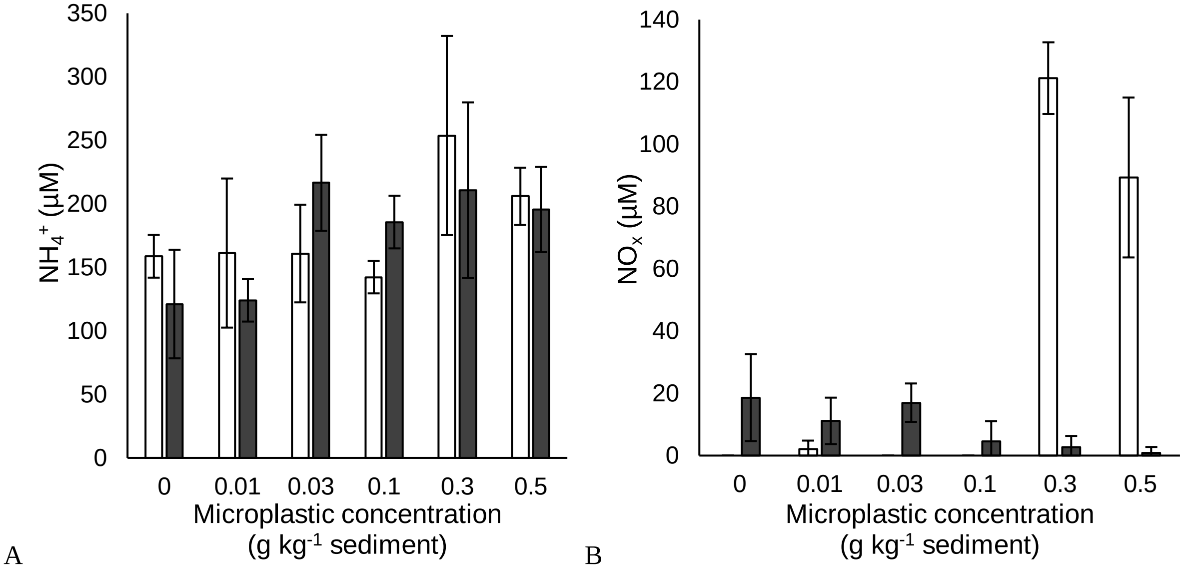


Figure 4: A) Mean (±SE) porewater NH_4^+ (µM) with increasing microplastic contamination (n=3). White bars – light conditions; grey bars – dark conditions. B) Mean (±SE) porewater NO_x concentration (NO_2^- & NO_3^-, µM) with increasing microplastic contamination (n=3).

Both autotrophs and heterotrophs have been shown to exploit microplastics as a carbon source[102,103], therefore it seems plausible that cyanobacteria, and perhaps heterotrophic bacteria, were benefiting over diatoms, from the addition of microfibers. Blue-green algae can survive and even maintain growth in darkness under anaerobic, or reduced conditions[104,105]. In our dark conditions, over 90% of the light was blocked for several weeks. In addition, our light conditions were on a 12 h light:dark cycle, resulting in a 12 h dark period. Mimicking natural light cycles restricts MPB oxygen production periods, while excluding it entirely in 24 h dark conditions. Cyanobacteria can turn sediments anaerobic within in minutes in the dark[106], therefore it is plausible that cyanobacteria were benefiting both from the light regimes and the microfiber additions. Our results advocate that microplastics have the potential to influence the net stocks of NH_4^+, and NO_x in sediment, with consequences for nutrient cycling in soft sediment habitats. These effects may not only be isolated to coastal sediments in the photic zone, however, with microplastics increasingly recorded in deep-sea sediments[7,37,107]. The presence of microfibers were influencing benthic communities that are important players for various biogeochemical processes[108–110] and altering sediment nutrient stocks in both light and dark conditions. This could have profound consequences for biogeochemical processes from coastal waters to shelf sea sediments. We therefore recommend further investigation of these interactions.

The results of the univariate and multivariate analyses suggest that light conditions influence the interaction between photosynthesizing MPB, infaunal burrowing, nutrient pools and microfibers in the sediment. UV weathering is an important mechanism by which plastics degrade in the natural environment[111] and previous studies have observed oxidative stress in cell-based bioassays due to the leachates from weathered polyethylene terephthalate (PET)[112]. UV weathering of the plastic fibers can result in the liberation of chemicals from the plastic into the surrounding environment[112]. Microfibers used in the present study were composed of PET, therefore the effects of fibers on MPB community changes, nutrient stores could potentially be the result of chemicals leaching from the fibers under UV lights.

While no visual quantification or identification of MPB taxa was attempted, fixed diatom slides were inspected and indicated a shift towards smaller cells at higher microfiber concentrations

(Pers. Obs). Smaller diatom cells typically have lower nutrient requirements, turnover quicker and exhibit lower net productivity than larger cells[113]. This was likely related to the stress of the microfiber additions and/or the shift in competition between cyanobacteria, microbes and diatoms for available porewater nutrients. Due to the digestion of the MPB in H_2O_2, no visual assessment of cyanobacteria was possible from the slides but as noted above phycocyanin pigments associated with cyanobacteria increased. Higher turnover of small MPB cells and higher degradation rates would help explain the elevated sediment organic matter (OM) content observed at the highest microfiber additions with OM positively correlated to porewater NH_4^+ ($r_s^2 = 0.56$), NOx ($r_s^2 = 0.54$) and cyanobacteria biomass ($r_s^2 = 0.44$). MPB are the primary source of labile organic matter in soft sediment systems[114,115]. Changes to the quality and quantity of this OM source has been previously been demonstrated to shift the balance between nitrogen recycling and nitrogen release processes[116,117]. Therefore, the detected changes in the quantity and quality of MPB during the present study, and the changes to nitrogen pathways that this caused was reflected in our elevated sediment nitrogen stocks. Both heterotrophic bacteria and cyanobacteria are able to fix nitrogen in low nitrogen systems in the absence of oxygen[106,118] and as nitrogen fixers can utilize a wide range of carbon sources including those of lower quality [119]. These organisms therefore have the potential to outcompete diatoms if biogeochemical processes were altered by increasing microplastic contamination. Adjustments to diatom-bacteria interactions can lead to taxonomical shifts in the MPB community as well as modifying biogeochemical processes[101,114]. Our results suggest this is particularly likely if the movement of deep-dwelling infauna was reduced, and the transport of nutrients to the MPB at the sediment-water interface is limited.

Effects on deep dwelling deposit-feeder. Bioturbation can influence MPB communities and biogeochemical gradients by altering the transfer of sediment nutrients across the sediment-water interface and stimulating biogeochemical processes[120,121]. In the present study, the burrowing activity of *M. liliana* was reduced, after long-term exposure to high microfiber additions regardless of the light regime (Fig 5A). The number of fibers ingested varied from 0 to 11 fibers per bivalve, with the length varying between 50 and 1400µm (Supp. Fig 3A-B). Less active bivalves from high microfiber treatments (0.3-0.5g), also exhibited reduced lipid energy reserves (up to 75% less) (Fig 5B). This supports growing evidence that microplastics can decrease energy reserves in a variety of marine organisms[21,39,44,45]. *M. liliana* with lower energy reserves coincided with treatments containing lower quality and quantity of primary producers (Supp. Fig 4A, $r^2=0.81$, $P<0.05$). As diatoms can dominate sediments that are moderately to highly bioturbated[122], changes to the quantity and quality of MPB and an increase cyanobacteria could also be feedbacks caused by modified bivalve behavior which would reduce the transfer of porewater nitrogen up to the MPB on the sediment surface[123].

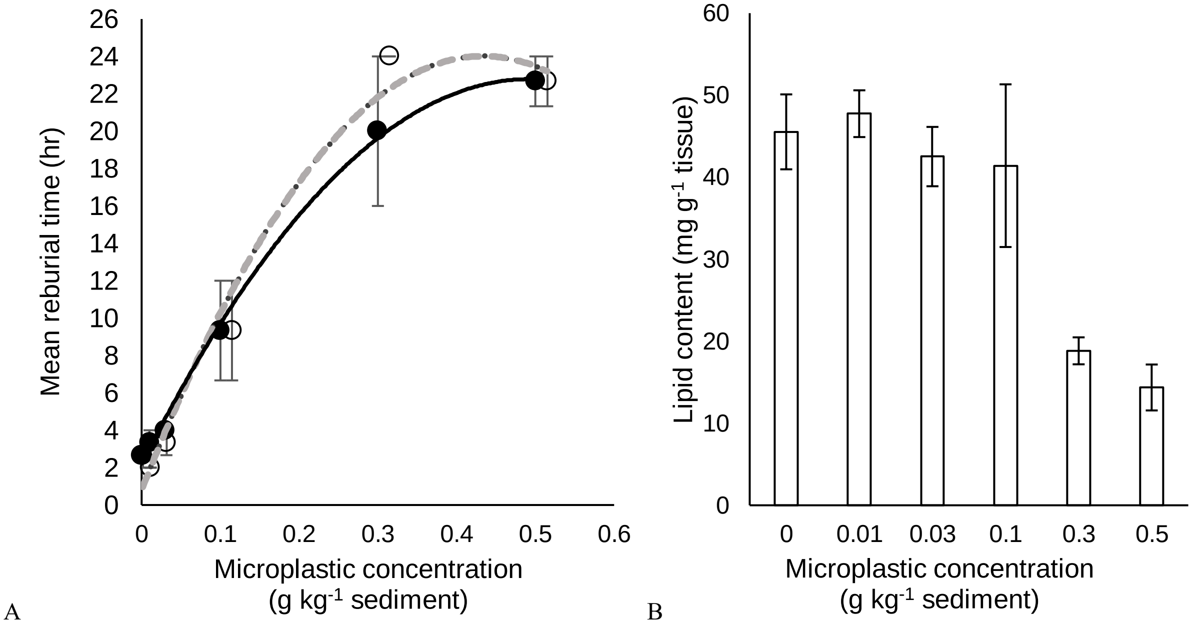


Figure 5: A) Mean (±SE) reburial time of *M. liliana* at increasing microplastic concentrations (n=3). No significant differences were observed between light (open circles) and dark (filled

circles) treatments across each microplastic concentration. Time >20 h represent organisms that remained on the sediment surface for the duration reburial trials. Polynomial curves were fitted to the light (dashed line) ($y = 123.36x^2 + 107.02x + 0.8058$, $r^2 = 0.98$) and the dark (solid line, $y = -84.52x^2 + 83.41x + 2.21$, $r^2 = 0.99$) treatments and illustrate the mean reburial times increased with increasing microplastic contamination. B) Mean (± SE) of total lipid energy reserves in *M. liliana* tissue across increasing microplastic concentrations.

FA biomarkers from bivalve tissue such as the diatom index and DHA/EPA ratio are often used to assess the nutritional status of consumers[124,125]. Despite lower bivalve energy reserves and changes to the quality of the MPB community, these ratios were preserved in *M. liliana* tissues (Supp. Fig 4B). This suggests that although basal food quantity and quality were altered by the presence of microfibers, the quality of the bivalves was not affected over the timescale of the experimental exposure (40 days). However, the selective uptake or depletion of particular FAs over others may not occur over this short period. It is also likely that feeding activity of the bivalves was reduced as activity levels were lower. Similar Tellenid bivalves in Europe, *Macoma balthica,* modulate their dietary intake if food quality is low in order to conserve energy[126] and it is likely that *M. Liliana* would conserve the essential FAs associated with diatoms over other lipids and FAs over this experimental period if their feeding was reduced.

Adverse microplastic-effects on feeding activity has been demonstrated previously (Wegner et al., 2012). Through various feedbacks, we anticipate that these potential effects on the nutritional quality of the primary food resource may lead to long-term effects on the nutritional quality of the bivalves for higher trophic levels. We emphasize the need to investigate this area further with greater knowledge of both trophic and non-trophic interactions required to fully understand the potential implications. Despite a lack of changes in the FA quality of *M. liliana*, this study has illustrate a reduction in the basal food quality and quality and a depletion in the overall lipid energy stores of the bivalves. Observed changes to the MPB community were related to lower overall energy reserves of the bivalves as well as the behavior of this functionally important deposit-feeder. Changes to bivalve behavior feeds back to the quantity and quality of MPB[123], which in turn leads to even less nutritious food resources for the bivalves and further depleting energy reserves and so forth. In addition to the influence of bioturbation on MPB, changes in grazing pressure can modify the MPB[127]. *M. liliana* are functionally similar to other tellenid bivalves found in sediments in the northern hemisphere such as *Macoma balthica* and *Macomona arenaria* (Hayward et al., 1996). We therefore stress the need to further explore the influence of microplastics on functionally important benthic organisms in these complex ecological networks.

While the majority of studies to date have focused on the impact of microplastic ingestion in marine suspension feeding bivalves[29,128], there is increasing evidence that deposit-feeding bivalves are also susceptible to microplastics pollution[38,39]. This is sensible given that deposit feeders graze at the sediment-water interface, and sediments are the ultimate sink for marine microplastics[7,10]. Changes in MPB[19] and phytoplankton biomass[62] have previously been noted but evidence of the complex feedbacks between functionally important organisms at the base of the benthic foodweb, caused by microplastics contamination is lacking. The direct and indirect effects of microfiber pollution and the feedbacks and interactions between functionally important organisms and processes requires further exploration. This is a relatively new area of research and therefore we must continue to increase the complexity of the systems we study in the laboratory in order to detect potential shifts in ecosystem structure and functions that underpin ecosystem service delivery.

Our results suggest that microfiber additions may influence the interactions between the MPB, microbes and infauna with ramifications for ecosystem functions such as nutrient cycling and productivity if the MPB community is altered. This suggests that over and above issues related to ingestion such as gut blockage, false satiation and bioaccumulation in higher organisms, the structure and function of soft sediment ecosystems and the foundation of our marine foodwebs could potentially be influenced. We know that MPB and infauna play significant roles in elemental cycling due to their interactions with the microbial community[121,129] and our observations stress that microplastics have the potential to alter the interactions and feedbacks that involve MPB, infauna and N-cycling microbial communities[55,120]. We suggest that future investigations quantify changes to both nutrient and gas fluxes, as well as determining compositional changes to the microbial community in addition to MPB, as we believe this is an attractive avenue of future research.

Soft sediment systems around the world are under pressure from not only microplastic contamination but increasing nutrient and sediment loads [130,131]. We must comprehend the potential influence of microplastic accumulation on soft sediment ecological networks. In particular, the interactions between microplastics, soft sediment ecological communities and ecosystem functions such as nutrient cycling in the face of multiple anthropogenic pressures.

ASSOCIATED CONTENT

Supporting information.

The supporting information is available free of charge on the ACS publications website at DOI:

Additional information as noted in the text (PDF)

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The manuscript was written through contributions of all authors. JAH conceived the paper and produced the first draft of the manuscript. JAH carried out the experiment, collected and processed samples. JAH analysed data with advice from SFT and GC. All authors contributed to the ideas presented in this paper, drafts of the manuscript and gave final approval for publication. All authors have given approval to the final version of the manuscript.

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Disclosures

The authors declare no competing financial interest.

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