Organic enrichment can increase the impact of microplastics on meiofaunal assemblages in tropical beach systems[☆]

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ARTICLE INFO

ABSTRACT

The cumulative impact of microplastic and organic enrichment is still largely unknown. Here, we investigated the microplastic contamination, the organic enrichment and their effects on meiofaunal distribution and diversity in two islands of the Maldivian archipelago: one more pristine, and another strongly anthropized. Field studies were coupled with manipulative experiments in which microplastic polymers were added to sediments from the non-anthropized island (i.e., without organic enrichment) to assess the relative effect of microplastic pollution on meiofauna assemblages. Our results reveal that the impact of microplastic contamination on meiofaunal abundance and taxa richness was more significant in the anthropized island, which was also characterized by a significant organic enrichment. Meiofauna exposed experimentally to microplastic contamination showed: i) the increased abundance of opportunistic nematodes and copepods and ii) a shift in the trophic structure, increasing relevance in epistrate-feeder nematodes. Based on all these results, we argue that the coexistence of chronic organic enrichment and microplastics can significantly increase the ecological impacts on meiofaunal assemblages. Since microplastic pollution in the oceans is predicted to increase in the next decades, its negative effects on benthic biodiversity and functioning of tropical ecosystems are expected to worsen especially when coupled with human-induced eutrophication. Urgent actions and management plans are needed to avoid the cumulative impact of microplastic and organic enrichment.

1. Introduction

Reef ecosystems worldwide are under increasing threat due to the combination of local impacts (e.g. coastal development, overfishing, microplastic contamination, sewage pollution) and global climate change, which can act synergistically (Ellis et al., 2019).

Macro (>5 mm) and micro-plastic (<5 mm) contamination is of increasing concern and is now spread at all latitudes and even in the most remote oceanic regions (Munari et al., 2017; Sfriso et al., 2020). It has been estimated that at least 5.25 trillion plastic particles equivalent to 268,940 tons are floating in the sea (Eriksen et al., 2014), and a much larger fraction is deposited in the sediments (Patti et al., 2020). In reef ecosystems, such a contamination mainly originates from land-based sources but is also transported and accumulated from oceanic currents

(Connors, 2017; Imhof et al., 2017; Saliu et al., 2018). Recent information reveals that beach sediments in Maldivian islands contain dozens to hundreds of microplastic particles per m^2 of sediment (Imhof et al., 2017; Saliu et al., 2018; Patti et al., 2020). However, data available in the literature are still largely inconsistent due to the discrepancies in the methodological and sampling protocols used, the different particle sizes analyzed and units of the measure adopted (Paul-Pont et al., 2018), therefore it is difficult to make a reliable quantitative estimate of the microplastic contamination in the Maldives.

The impact of microplastic contamination can cumulate with those due to other contaminants and eutrophication (organic enrichment) often linked to the sewage from urbanized areas or touristic resorts, which are increasingly evident as emerging causes, together with global climate change, of tropical ecosystem degradation at global scale

(Downing et al., 1999; Wear and Thurber, 2015; Duprey et al., 2016) and known to alter benthic biodiversity and ecosystem functioning (Danovaro et al., 2000; Pusceddu et al., 2014). Organic enrichment in reef sediments could represent an additional source of plastic contamination (Rolsky et al., 2020) and possibly exacerbate its impact on marine organisms, but information on their cumulative effects is practical non-existent.

So far, microplastics effects have been investigated on phytoplankton, zooplankton, corals, fish and large marine organisms (Mascarenhas et al., 2004; Hall et al., 2015; Allen et al., 2017; Saliu et al., 2019; Rochman et al., 2016; Law, 2017; Kögel et al., 2019; Hui et al., 2020). Multiple adverse effects on marine organisms related to microplastics have been reported, including the production of reactive oxygen species, cellular apoptosis, impacts on reproduction, development, immunity system, feeding activity and biodiversity (Rodrigues et al., 2019; Browne et al., 2013; Sharifinia et al., 2020; Corinaldesi et al., 2021). Such effects can be associated with chemical additives (i.e., persistent organic pollutants and heavy metals) absorbed onto microplastics (Ashton et al., 2010; Sun et al., 2019; Jiménez-Skrzypek et al., 2021). However, information about the extent to which microplastics can alter meiofauna assemblages is still too limited, especially in reef sediments (Gusmão et al., 2016; Haegerbaeumer et al., 2019; Fueser et al. 2020 a, b).

Meiofauna (i.e., benthic metazoans smaller than macrofauna) includes 30 out of 35 existing animal Phyla from the very abundant Nematoda to exclusive meiofaunal phyla such as Kinorhyncha, Gastrotricha, Gnathostomulida, Tardigrada, and Loricifera and represent a key benthic component in all marine environments. Due to their small size, short life span and life cycle spent entirely within the sediments, meiofauna is very sensitive to environmental alterations and represent an important indicator of ecosystem health (Giere, 2009; Danovaro et al., 2000; Bonaglia et al., 2014; Schratzberger and Ingels, 2018). Previous studies reported that reefs' carbonate sediments and coral rubbles promote habitat heterogeneity that sustains a high meiofaunal diversity (Semprucci et al., 2013, 2014; 2018).

Over the past decades, the Maldives have become a popular destination for about one million foreign tourists every year, with significant development of coastal infrastructures and consequent increase in anthropogenic impacts (Moritz et al., 2017), including those due to microplastic contamination and organic enrichment induced by sewage discharge (Nepote et al., 2016; Miller and Sluka, 1999).

The aim of this study is to investigate microplastic contamination, organic enrichment and their possible cumulative effects on meiofauna assemblages inhabiting reef sediments from two islands of a Maldivian atoll (Lhaviyani atoll). In particular, we assessed the responses of meiofauna assemblages (in terms of abundance, biomass and diversity) from the reef sediments of Naifaru, an urbanized island characterized by untreated wastewater and from those of Vavvaru, a virgin and pristine island. We hypothesized that the reef sediments of Naifaru and benthic communities are more affected by higher organic loads and microplastics than those of Vavvaru. To better define the relative effect of microplastic pollution on meiofauna assemblages, we also performed an additional experiment to test the effects of acute contamination by the plastic polymers most commonly found in natural marine environments. Our findings add new insights into understanding benthic community's responses to the impact of microplastics and other sources of anthropogenic stressors in tropical ecosystems.

2. Materials and methods

2.1. Study area and sampling strategy

Maldives are an archipelago formed by about 1200 islands, most of which are non-anthropized belonging to 7 provinces and 20 administrative atolls, whose population depends on the coral reef resources and tourism (Jaleel, 2013). Our investigation was conducted in the Vavvaru

and Naifaru islands in the Lhaviyani atoll from 2015 to 2016, (Fig. 1).

Vavvaru is an uninhabited island and was accessible only through the research vessel provided by the Korallion Lab research center. Conversely, Naifaru is the main administrative island for Lahviyani Atoll. As such, it has the largest human population in this atoll (about 5400 people; United Nations Department of Economic and Social Affairs, 2019). The trash produced by the locals is burned, discarded into the ocean or dumped in an assigned landfill on the northern side of the island which may spill onto the beach (Patti et al., 2020). The island's southeastern side is dominated by a harbour protected by a seawall, while the rest of the island is surrounded by a coral sand beach (Fig. 1). The island has been subject to land reclamation, increasing by 3-fold the island surface in 2006 (Zahid, 2010).

In Vavvaru, sediment samples were collected in four stations: V1 (in the back reef of the island), V2 (in the southwestern tail), V3 (in the eastern tail) and V4 (in the front reef); (Fig. 1), while in the Naifaru in 2 stations (i.e., N1 and N2; Fig. 1), at two opposite sides of the islands. In Naifaru we did not collect samples from the south-eastern side of the Island because it was subjected to intense port activities, and showed the presence of bulldozers and land reworking, which caused additional massive impacts.

For each station of both islands, three replicate corers of surface sediments were collected for each analysis (i.e., meiofauna, sediment grain size and organic matter composition) through the use of a plexiglass core with a diameter of 3.6 cm. The samples for microplastics were kept in sterile glass containers.

2.2. Field sampling

Sediment samples for the analyses of meiofauna and environmental variables were collected at about 1.5 m depth in the stations showed in Fig. 1 using Plexiglas corers (n = 3 for each station). All samples were immediately stored at 4 °C or −20 °C, depending on as analytical routines described below.

2.2.1. Sediment grain size

Sediment texture was determined by dry sieving of sediments through a 0.0625 mm mesh to distinguish between the sandy and the silt–clay fractions. Fractions retained on the filter (sand) were additionally sieved through a 25 mm mesh to distinguish between medium (>0.25 mm) and fine (<0.25 and >0.0625 mm) sandy fractions. The sediment water content was calculated as the difference between the wet and dry weights and expressed as percentages (Pusceddu et al., 2016 and references therein).

2.2.2. Trophic state and biochemical composition of organic matter

Chlorophyll-a and phaeopigments were quantified using a spectrofluorometer after extraction with 90% acetone (24 h in the dark at 4 °C) and their sum defined as total phytopigments (Danovaro and Fabiano, 1997). The biochemical composition of sediment organic matter was determined according to Fabiano and Danovaro (1998). Additional details are reported in the Supplementary Material. Finally, the biopolymeric carbon (i.e. used as a proxy of trophic state, Dell'Anno et al., 2002) is defined as the sum of carbohydrates, proteins, and lipids converted into C equivalents using the conversion factors 0.40, 0.49 and 0.75 mg C mg^{-1}, respectively. Concentrations of total phytopigments were converted into C equivalents using 40 as a conversion factor (Pusceddu et al., 1999), and the contribution of the autotrophic biomass to the biopolymeric carbon pool was used as a descriptor of environmental quality (Dell'Anno et al., 2002).

2.2.3. Extraction, quantification and identification of microplastic particles

Microplastics extraction was carried out on 250 g of wet sediment. To enhance the extraction efficiency, sediment was split into replicates of 50 g each. We tried to apply the procedure based on the use of ZnCl$_2$ according to Imhof et al. (2012), but the formation of solid carbonate
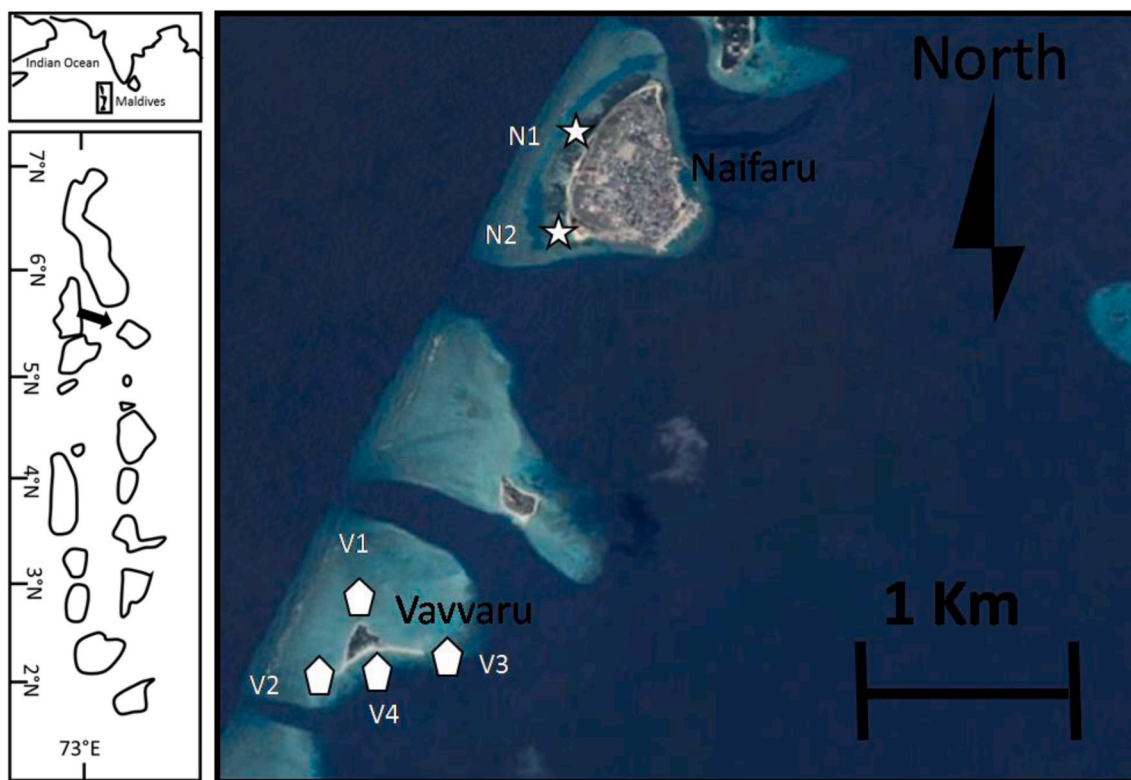
Fig. 1. Location of the two investigated islands in the Maldivian Lhaviyani Atoll.

agglomerates, probably due to the biogenic sediments, hampered microplastic extraction. We followed an alternative procedure based on the addition of 200 ml of milliQ water (autoclaved and filtered 0.2 μm) to each replicate. After vigorously shacking (30 s), the supernatant was filtered on a 20 μm filter (Agrinova S.r.l). This step was repeated ten times and the supernatant was collected in a falcon. Before chemical analysis, all potential microplastics particles encountered in the samples were counted and measured under a stereomicroscope at a magnification of 50X (Zeiss, Stemi 2000). Additional details are reported in the Supplementary material.

The abundance of microplastic particles counted in each sample was converted to m^2 of sediment, assuming a density of the surface sediment of about 2 g cm^{-3} (1.9–2.2 g cm^{-3}) and 50% of water content.

To identify plastic polymers we used Fourier-Transform Infrared Spectroscopy (FT-IR). Analyses were performed with a PerkinElmer FTIR Spectrometer Spectrum GX1 interfaced with a Perkin–Elmer Autoimage microscope and a photoconductive 0.25 mm Hg–Cd–Te (MCT) array detector, operating at liquid-nitrogen temperature and covering the entire IR spectral range from 4000 to 700 cm^{-1}. The output spectra were subsequently subjected to a spectral search against reference libraries of polymer spectra represented by PerkinElmer database. Additional information is reported in the Supplementary material.

2.2.4. Meiofaunal abundance, biomass and diversity

Meiofauna were analyzed according to Danovaro (2010). Samples were sieved through a 1000-μm mesh and a 20-μm mesh. The fraction remaining on the latter sieve was resuspended and centrifuged three times with Ludox HS40 (final density of 1.18 g cm^{-3}; Heip et al., 1985). All specimens were sorted, counted and classified under a stereomicroscope (40× magnification) using a Delfuss cuvette after staining with Rose Bengal (0.5 g L^{-1}). Meiofauna biomass was estimated through a bio-volumetric measurement for all specimens encountered. Nematode biomass was calculated from the biovolume, according to the Andrassy (1956) formula: V = L x W2 x 0.063 × 10^{-5} (in which body length, L, and width, W, are expressed in μm), while for all the other taxa the

formula used was: V = C x L x W2 where C is the conversion factor specific for each meiofaunal taxon (Giere, 2009; Feller and Warwick, 1988). Each body volume was multiplied by an average density of 1.13 g cm^{-3} to obtain the biomass. The carbon content was considered 40% of the dry weight (Feller and Warwick, 1988).

2.3. Experimental study

Replicated tanks (aquaria, n = 6) were prepared with fresh sediment samples collected at about 1.5–2 m depth in station N1 of Naifaru Island (1:7, sediment volume: seawater volume). Three replicates were added with a microplastic mixture (1000 particles L^{-1} of different polymers including polyethylene (PE), polypropylene (PP), polystyrene (PS), polyvinyl chloride (PVC) and polyethylene terephthalate (PET) (see Supplementary Information for details) and were carefully homogenized to have similar conditions in all the replicates (defined "treated" samples). The other three replicates were not added with the microplastic mixture and were used as "controls".

The microplastic mixture was prepared to mirror the concentration and composition of dominant polymers in coastal marine environments in hot spots of microplastic contamination (Phuong et al., 2016; Imhof et al., 2017) or predicted for future scenarios (assuming that present-day concentrations will increase by up to 4 times current levels by 2030–2060, Isobe et al., 2019). Information on the preparation of the microplastic mixture is reported in the Supplementary Material.

The experiment was stopped after 72 h to avoid any overall effect of the experimental setup on the survival of meiofauna.

2.3.1. Microplastics and nematodes

Microplastic polymers (20–1000 μm) were observed under an epi-fluorescence microscope (Nikon Eclipse Ni) with UV, blue and green light and 100× magnification and used as references for the analysis of microplastics associated with nematodes. PET, PS and PVC emit fluorescence when excited by all three lights (Supplemental Figure S1). In contrast, the other polymers present in the treated sediments (PE and

PP) are not excited by any light. However, only PET is visible at all wavelengths (Supplemental Figure S1a), while PS especially to green light (Supplemental Figure S1b) and PVC to blue and green lights (Supplemental Figure S1c).

Samples for the analysis of meiofaunal abundance, biomass and biodiversity were processed as described for the *in-situ* investigation. At the beginning (t0) and the end of the experiment (tf), meiofaunal organisms were extracted from standard amounts of sediment (100 g from each replicate). For nematode diversity analysis, 100 nematodes from each sample were randomly picked up from the samples and were mounted on slides following the formalin–ethanol–glycerol technique to prevent dehydration (Seinhorst, 1959). Nematodes were identified to the species level or morphotypes (*sensu* De Mesel et al., 2006) according to Platt and Warwick (1983, 1988), Warwick et al. (1998) and the NeMys database (Vanaverbeke et al., 2015; Guilini et al., 2016). In addition, each nematode species was assigned to one of the following four trophic groups, based on the buccal morphology, according to Wieser (1953): (1A) selective (prokaryotic) feeders, (1B) non-selective deposit feeders, (2A) epistrate or epi-growth feeders and (2B) predators/omnivores. The number of microplastic particles associated with nematodes was evaluated by direct epifluorescence microscopic analysis of their body.

2.4. Statistical analyses

To investigate differences between the two islands Naifaru and Vavvaru a PERMANOVA analysis was performed on all variables including two factors as main sources of variance: 1) State (fixed, two levels: anthropized and uninhabited) and 2) station (random and nested in State, 2 levels for anthropized: N1 and N2, and 4 levels for uninhabited: V1–V4) was performed.

To gather additional information on the variability of investigated variables at smallest spatial scale (i.e., within each condition), we forced the pair-wise analysis, testing for differences among stations (even if considered as random factor).

To assess the percentage of dissimilarity in the meiofaunal assemblage composition among the two states and stations and identify the meiofaunal taxa primarily responsible for the observed differences, a similarity-percentage analysis (i.e. SIMPER) was also carried out.

To identify which drivers influence the patterns of meiofauna abundance and diversity (in terms of richness of higher taxa), multiple linear regressions were performed through Python (for correlation analyses) and R for model calculation, selection and validation (Zuur et al., 2007; Crawley, 2012; Zuur and Ieno, 2016). We used as predictor variables: grain size, BPC, phytopigments, microplastic abundance or composition. Since some of these variables can be potentially correlated, the presence of collinearity was investigated before to perform the models with the Pearson correlation coefficient. The presence of multi-collinearity was detected with the Variance Inflation Factors. After this screening, only the statistically significant variables were considered in the models. The best solution among all possible models was selected with the AIC approach. The model obtained was validated comparing the residual vs fitted values and each covariate to test the homogeneity and independence, respectively while the normality distribution of the data was tested investigating the normal distribution of the residual. If no evident patterns resulted and the residuals followed the normality distribution the model passed the "model validation". Each model considered the effects of each variable as well as the interaction of microplastic abundance and composition with the other environmental variables. The experimental study's differences and impacts were tested with a PERMANOVA analysis (one fixed factor named treatment with 2 levels: controls and impacts) performed on the assemblage composition, individual number, nematode and total biomass as well as nematode assemblage at species/genus level.

3. Results

3.1. Field study

3.1.1. Sediment grain size and benthic trophic state in Naifaru and Vavvaru sediments

Grain size analysis indicated that sediments of Vavvaru were characterized mainly by coarse sand (500 μm), except for the V4 station, where the medium sand (250 μm) dominated. The Naifaru sediments showed a more heterogeneous distribution of grain-size classes due to granules' presence (2000 μm; Supplemental Figure S2).

The stations located in Vavvaru showed similar values of total phytopigments ranging from 3.1 ± 0.2 to 4.9 ± 0.4 μg g^{-1} in V4 and V3, respectively (Fig. 2a). In the two stations of Naifaru, total phytopigments were not significantly different (10.4 ± 7.0 μg g^{-1} to 18.3 ± 6.9 μg g^{-1}, in N2 and N1, respectively) but significantly higher than in Vavvaru Island ($p < 0.01$).

The concentration of chlorophyll-*a*, in the reef sediments of Vavvaru was on average 2.1 ± 0.3 μg g^{-1} while in those of Naifaru was 6.7 ± 3.1 μg g^{-1} and contributed to the total phytopigment concentrations for 54% and 47%, respectively (Supplemental Table S1).

Protein, carbohydrate and lipid concentrations in the sediments, as well as biopolymeric carbon concentration (used as a proxy of trophic state), followed the same patterns reported for phytopigments (Supplemental Table S1) so that the sediments of Naifaru showed a significantly higher ($p < 0.001$) organic matter content than those of Vavvaru (Supplemental Table S1). The biopolymeric carbon concentrations did not change significantly either among the stations of Vavvaru (range: 0.26 ± 0.02 to 0.29 ± 0.03 mg g^{-1}) nor in Naifaru (1.1 ± 0.16 and 1.5 ± 0.2 mg g^{-1}, Fig. 2b, Supplemental Table S1). However, in Naifaru, biopolymeric carbon concentrations were average about 5 times higher than in Vavvaru.

In Vavvaru, the autotrophic contribution (as phytopigment C equivalents) ranged from 48.4% to 66.4% in V4 and V3, respectively. In contrast, in Naifaru, the values were 36.6% and 49.0% in N2 and N1, respectively (Supplemental Figure 2c).

3.1.2. Plastic contamination in Vavvaru and Naifaru sediments

In the sediments of Vavvaru, the concentrations of microplastic particles ranged from 40 to 120 particles per m^2 of sediment (in V1, V3, V4 and V2, respectively) with a mean value of 60 ± 20 microplastic particles per m^2 of sediment. In contrast, in Naifaru, both stations showed 160 ± 0 microplastic particles per m^2 of sediment (Fig. 3a).

Different size classes of marine debris were found in sediment samples ranging from 360 to 8560 μm. Marine debris was represented by a fraction equals to 35.3% of particles with a low similarity with plastic polymers included in the SPECTRUM Autoimage 5.1.0 software, therefore as a precaution, this fraction was not taken into account.

Microplastics (identified by FT-IR) in the sediments of Vavvaru contributed for 21% to the marine debris, whereas in those of Naifaru for 80%. In Vavvaru, microplastic particles were dominated by fibres (100%), whereas in Naifaru, these were represented mainly by films (50%) with 25% of fibres and 25% of fragments. Microplastic size in Vavvaru ranged from 1140 μm to 4820 μm while in Naifaru from 1220 to 2400 μm. In Vavvaru, the most represented size range of the microplastic particles was 1000–2000 μm (in stations V1, V2 and V3), although in station V4, the 2000–3000 μm dominated the microplastic assemblage (Fig. 3b). In Naifaru island, the most represented size range was 1000–2000 μm.

The polymeric composition in the two islands was different (Fig. 3c, Supplemental Figure S3-S6). In Naifaru, PET and Polyamide were the only two polymers found (accounting for 50% each), whereas in Vavvaru Polyamide was the dominant polymer (50%) followed by Acrylonitrile resin and Polyester (33 and 16%, respectively, Fig. 3c).
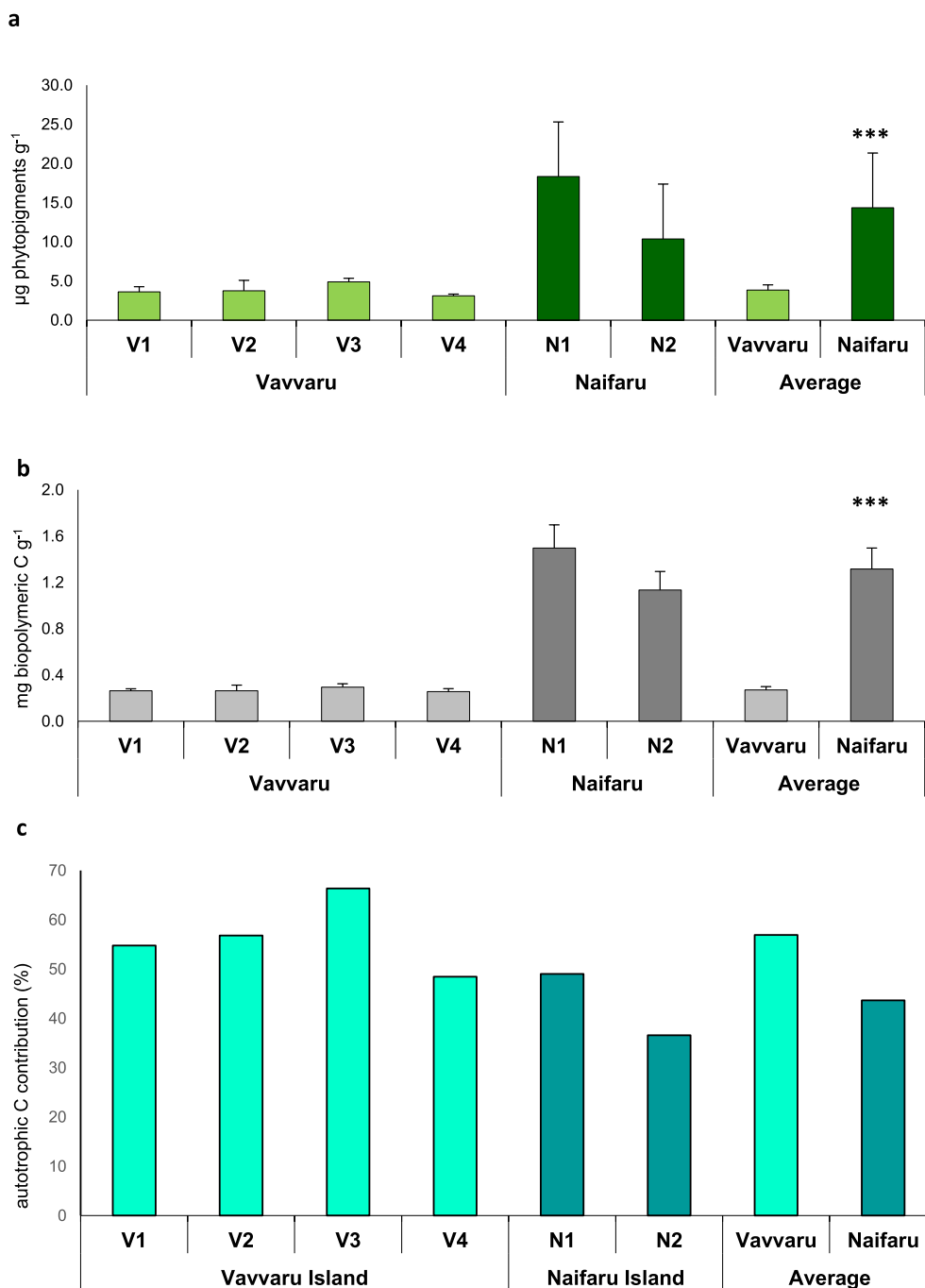
Fig. 2. Concentrations and biochemical composition of the organic matter in the beach sediments of Naifaru and Vavvaru islands of the Maldivian Lhaviyani Atoll. Phytopigment concentration (a) biopolymeric carbon concentration (b) contribution of autotrophic C to the biopolymeric C concentration (c). ***p < 0.001.

3.1.3. Meiofauna abundance, biomass and diversity

The sediments of Naifaru contained the lowest meiofaunal abundances, with average values almost 7 times lower than in Vavvaru (Fig. 4a). The stations of Vavvaru were characterized by a meiofauna abundance ranging from 286 ± 148 to 711 ± 108 individuals per 10 cm^2 of sediment in V2 and V4, respectively, with significant differences only between V4 vs. V1 and V2 (p < 0.05). Conversely, between the two stations of Naifaru no significant differences were found (80 ± 5 and 59 ± 49 individuals per 10 cm^{2}of sediment in N1 and N2, respectively). The biomass of the meiofauna communities in the island of Vavvaru (126.1 ± 26.1 µg C 10 cm^{-2}) was higher than in Naifaru (25.8 ± 6.6 µg C 10 cm^{-2}, Fig. 4b). In the sediments of Vavvaru, the lowest values were found in station V2 (62.1 ± 16.2 µg C 10 cm^{-2}) whereas the highest ones

in station V4 (189.3 ± 922 µg C 10 cm^{-2}). In Naifaru, the biomass was very similar in the two stations N1 and N2 (32.4 ± 2.7 µg C 10 cm^{-2} and 19.2 ± 2.7 µg C 10 cm^{-2}, respectively).

The sediments of Naifaru and Vavvaru showed the dominance of Nematodes, followed by Copepods (which were more abundant in Vavvaru; on average 147 individuals per 10 cm^2 of sediment, Fig. 4c). The different stations within the Vavvaru island showed nematode abundances ranging from 103 ± 60 to 210 ± 44 individuals per 10 cm^2 of sediment (in the stations V3 and V2, respectively), whereas in the sediments of Naifaru, the two stations showed similar values (60 ± 18 and 55 ± 48 individuals per 10 cm^2 of sediment; Fig. 4c). Copepods abundance was significantly different between the two islands (p < 0.05). Overall, 8 meiofaunal higher taxa were found in Vavvaru vs 5 in
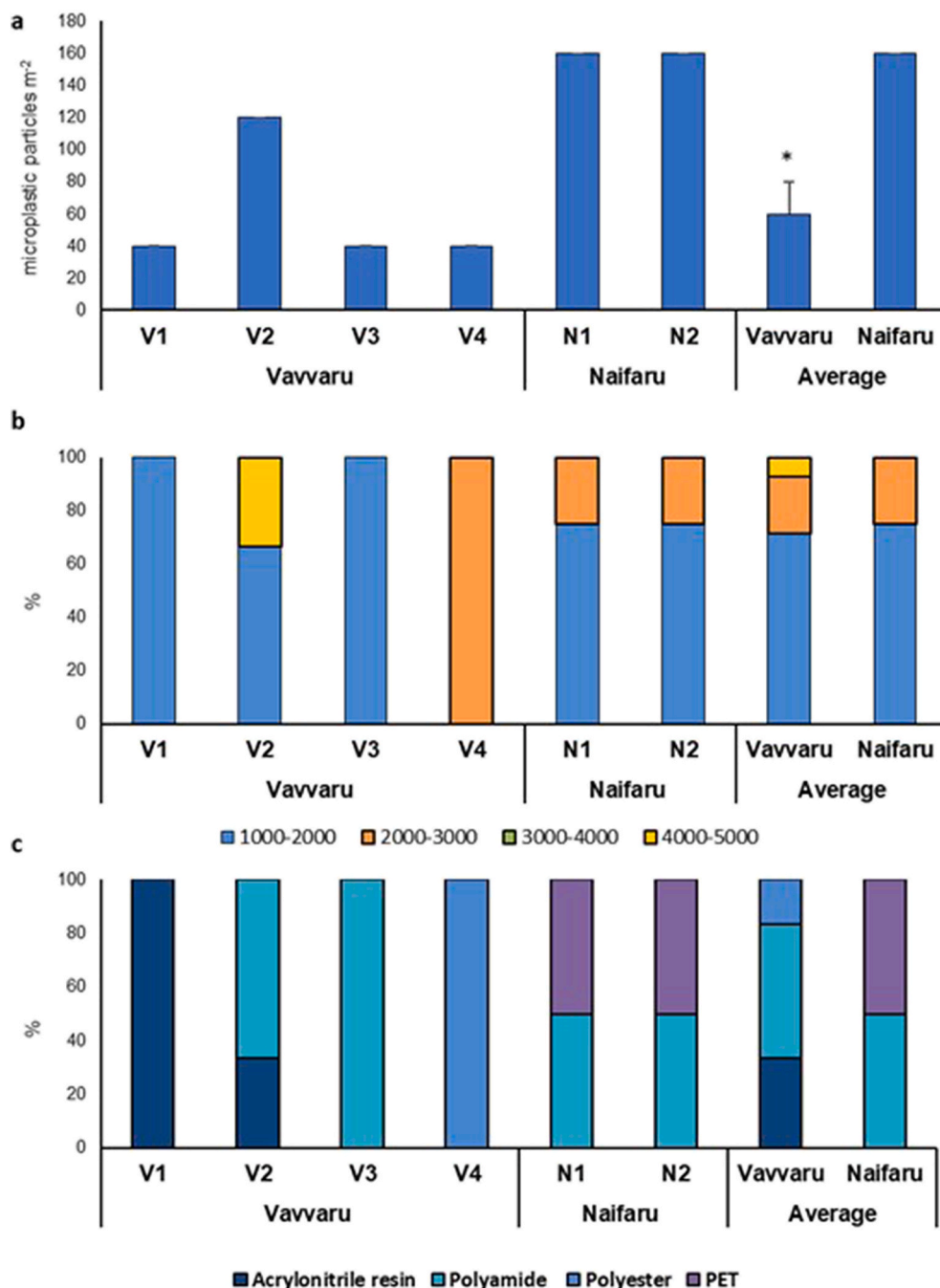
Fig. 3. Microplastic concentrations and polymer composition in the beach sediments of Naifaru and Vavvaru islands of the Maldivian Lhaviyani Atoll. Number of microplastic particles (a) size expressed in μm (b) and polymeric composition (c) of microplastics in the two islands. $*p < 0.05$.

Naifaru

PERMANOVA indicated the presence of significant differences among meiofaunal assemblages of the impacted (Naifaru) and non-impacted conditions (Vavvaru, Supplemental Table S2a, $p < 0.001$) and among the stations ($p < 0.01$). SIMPER analysis revealed that the dissimilarity between meiofaunal assemblage composition between the sediments of Naifaru and Vavvaru was about 75%. Copepods mostly drove this dissimilarity (about 33%), followed by nematodes, ostracods, nauplii and polychaetes (Supplemental Table S2b). Nematodes also dominated in terms of total meiofaunal biomass (on average, 59.4 and 18.3 μg C 10 cm^{-2} of sediment in Vavvaru and Naifaru islands, respectively Supplemental Table S3). Nematodes were followed by co-pepods, polychaetes and ostracods, which accounted for a non-

negligible fraction of the total meiofaunal biomass only in Vavvaru (19.2, 28.6 and 10.4 μg C 10 cm^{-2} of sediment, respectively).

3.1.4. Environmental drivers of meiofauna abundance and assemblage composition

The first model of multiple linear regression considered the environmental variables (i.e. grain size, BPC, phytopigments and micro-plastic abundance) vs meiofauna total abundance. Due to the presence of (multi)collinearity, some variables (i.e., phytopigments, sand, gravel and biopolymeric carbon) were excluded by the models. In particular, the concentrations of biopolymeric organic carbon were significantly correlated with microplastic abundance (Pearson correlation coefficient: 0.91). The results of the multiple linear regression analysis
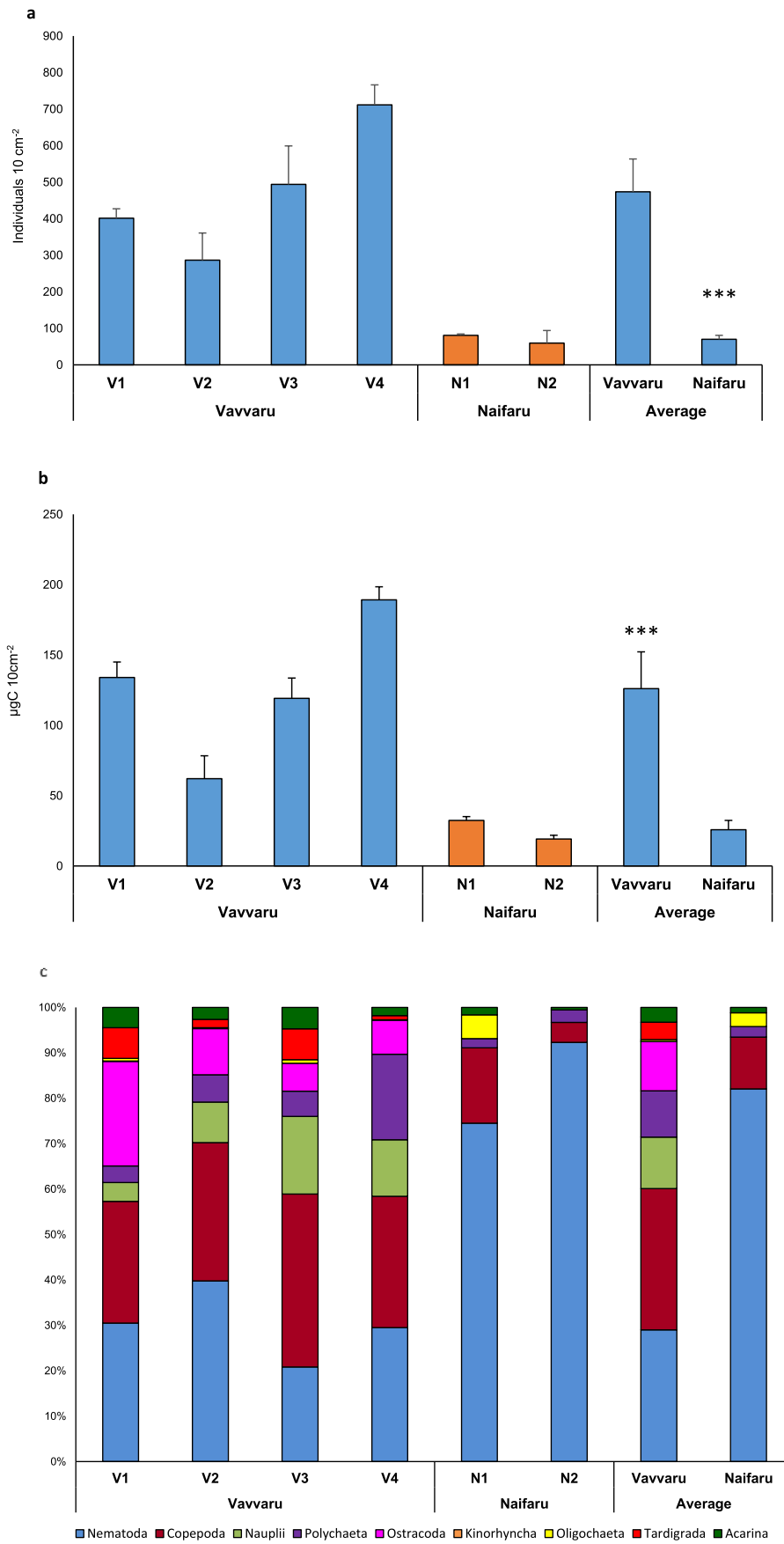
Fig. 4. Distribution and taxonomic composition of meiofaunal assemblages in the beach sediments of Naifaru and Vavvaru islands of the of the Maldivian Lhaviyani Atoll. Meiofaunal abundance (a) total biomass (b) and taxonomic composition (c) in the two islands. ***$p < 0.001$.

revealed that microplastic abundance influenced significantly meio-fauna abundance and that the absence of pelite did not change the AIC value. The final model identified as an unique explanatory variable the microplastic abundance (Supplemental Table S4). The second model included the same environmental variables considered in the first model (i.e. grain size, BPC, phytopigments and microplastic abundance) and taxa richness as a response variable. Also in this case, only microplastic abundance resulted significant (Supplemental Table S5).

When, in the model, microplastic abundance was replaced by microplastic composition, and taxa richness was used as a response variable, the best fit (i.e. with the lowest AIC value) was obtained with pelite, biopolymeric carbon (BPC) and polyamide. In this analysis, only BPC resulted significant. However, when the interaction BPC-Polyamide was included in the model, all the variables resulted significant (including the interaction BPC-polyamide) except polyamide (Supplemental Table S6). The models reported here passed the validation.

3.2. Experimental study

Meiofaunal abundance, biomass and number of higher taxa did not change significantly in the control sediments at the start (t_0) and at the end of the experiment (t_f), whereas in treated sediments, significant changes were observed either in comparison with control sediments and over the duration of the experiment ($p < 0.05$, Fig. 5 a, b, c). In particular, the richness of higher taxa in treated sediments changed significantly during the experiment: 12 taxa were found in the control sediments at t0 (Acarina, Amphipoda, Bivalvia, Cladocera, Copepoda, Cumacea, Nematoda, Oligochaeta, Ostracoda, Kinorhyncha, Polychaeta and Tardigrada) vs. only 6 taxa remaining in the sediments at the end of the experiment (both controls and treated). In particular, Acarina,

Copepoda, Cladocera, Ostracoda, Kinorhyncha, Nematoda (Fig. 5 d) were present in the systems added with the microplastic mixture.

Nematodes and copepods largely dominated the meiofaunal communities in control and treated sediments (overall 97–99%, Fig. 5 d). The contribution of nematodes increased (from 66 to 87%) while the contribution of copepods decreased (from 31 to 12%) from the controls at t_0 to the controls at t_f. In the treated sediments, the contributions of nematodes and copepods were very similar to those of the controls at t_f. However, the abundance of nematodes doubled in the sediments exposed to the microplastic mixture (on average, 239 ± 70 vs. 110 ± 25 and 115 ± 60 ind. $10\ cm^{-2}$, in the treated sediments vs. controls at t_f and t_0, respectively). Nematode biomass did not change significantly among control and treated sediments (Supplemental Figure S7).

Species richness of nematodes in the control sediments remained rather constant from the start to the end of the experiment (from 52 to 47 species, Fig. 5 e), showing a slight increase in the sediments added with the microplastic mixture (55 species). Seven nematode species disappeared in the sediments treated with the microplastic mixture (*Halichoanolimus* sp. *9, Pomponema* sp. *1, Prooncholaimus* sp.*1, Sabatieria* sp.*1, Sabatieria* sp. *2, Spilophorella* sp. *1* and *Trochamus* sp. *1*) compared to the controls. *Desmodora* sp.*5.* was more relevant in treated (17%) than in the control sediments (0.3% and 6.3, at t_0 and t_f respectively). The family Desmodoridae increased its relevance more in treated (20.3%) than in the control sediments (2.3% and 10%, at t_0 and t_f, respectively) whereas the family Oncholamidae, which was the dominant family in the controls (29.7% and 27%, at t_0 and t_f, respectively) decreased its relevance in the treated sediments (20%).

The trophic structure of the nematode assemblages in the sediments added with the microplastic mixture showed a higher percentage of epistrate feeders and a lower fraction of predators and non-selective
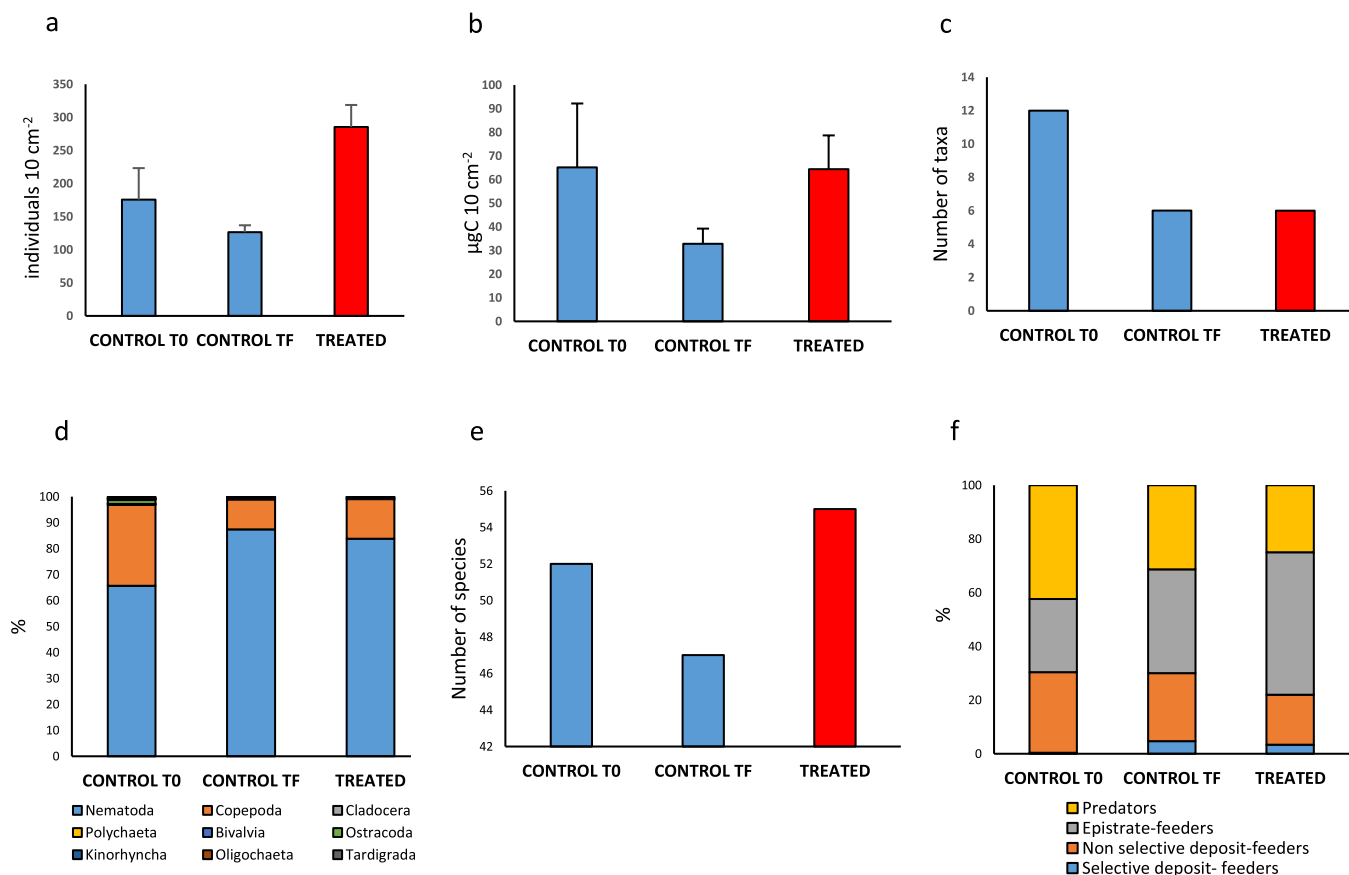

Fig. 5. Responses of the meiofaunal assemblages to microplastic contamination in experimental conditions. Meiofaunal abundance (a) total biomass (b), number of taxa (c) and taxonomic composition (d). Nematode species richness (e) and trophic strategy (f) in the control sediments and in the sediments exposed to the microplastic mixture over the time course experiment.

deposit feeders than the controls (Fig. 5 f; Supplemental Table S7). In the sediments added with the microplastic mixture, about 7% of all the nematodes was associated with microplastic particles, which were only attributable to the PET polymers (96% of which adhered to the cuticle and 4% within the mouth, Fig. 6). In the controls, in about 3% of the nematodes, microplastic particles were associated with the cuticle surface (62–87% at tf and t0, respectively) and inside or around the mouth (13–37% t0 and tf, respectively). In both treated and control sediments, the taxa most associated with microplastic particles were *Viscosia* spp, followed by *Daptonema* sp. and *Meyersia* sp. and *Axonolaimus* sp. and *Neochromadora* sp. (Fig. 7). Nematodes, which contained microplastic particles in the mouth, mainly were predator-omnivorous nematodes belonging to the genus *Viscosia* (46%) rather than epistrate- and non-selective deposit feeders.

4. Discussion

4.1. Microplastic contamination, organic enrichment and their combined effects on meiofaunal assemblages

Inhabited islands of the Maldives are relatively few (compared to the total number), and due to their limited surface, are densely populated and characterized by different sources anthropogenic stressors, including the presence of untreated wastewater (leading to local eutrophication), and micro- and macro-plastic contamination (Patti et al., 2020; Imhof et al., 2017; Saliu et al., 2018).

Since plastic debris is transported by currents even far away from source, we cannot exclude that also remote and uninhabited islands are contaminated by microplastics (Connors, 2017; Imhof et al., 2017; Patti et al., 2020).

In the present study, we investigated the effects of microplastics

contamination, organic enrichment and their combination on meiofauna assemblages in the anthropized island Naifaru, characterized by the presence of untreated wastewater, and in the uninhabited island Vavvaru, not subject to anthropogenic pressure.

In the sediments of Naifaru, microplastic concentrations were about 3-fold higher than in those of Vavvaru. The limited concentration of microplastics in the back-reef sediments of Vavvaru Island (station V1) might be due either to the lack of contamination or to the strong currents, which have been reported to be responsible for a relevant erosion of the reef (Steger et al., 2017).

Microplastic concentrations in the Naifaru sediments have been recently reported to be extremely high (Patti et al., 2020), with values ca 1 order of magnitude higher than those observed in our investigation. Such a discrepancy might be due to the different extraction procedures and/or the identification methods of microplastic particles used (stereomicroscope counting vs. stereomicroscope isolation followed by FT-IR analyses in our study) or to other untested factors. Microplastic concentrations in the investigated islands fall within the range of values previously reported in several beach sediments worldwide (Saliu et al., 2018 and references therein), including those reported from other Maldivian islands and tropical systems (Saliu et al., 2018; Alvarez-Zeferino et al., 2020; Jayasiri et al., 2013). Also, the polymeric composition observed in this investigation is similar to that reported in other Maldivian beach sediments (polyethylene, polystyrene, and polyamide, Saliu et al., 2018).

In Vavvaru Island, we mostly found synthetic fibres of polyester and polyamide, possibly associated with the use of fishing nets (Dowarah and Devipriya, 2019). Conversely, a significant fraction of polyethylene terephthalate (PET) was exclusively reported from the Naifaru sediments possibly associated with land-based activities. PET, indeed, is a polymer used in packaging foods and beverages and having a higher
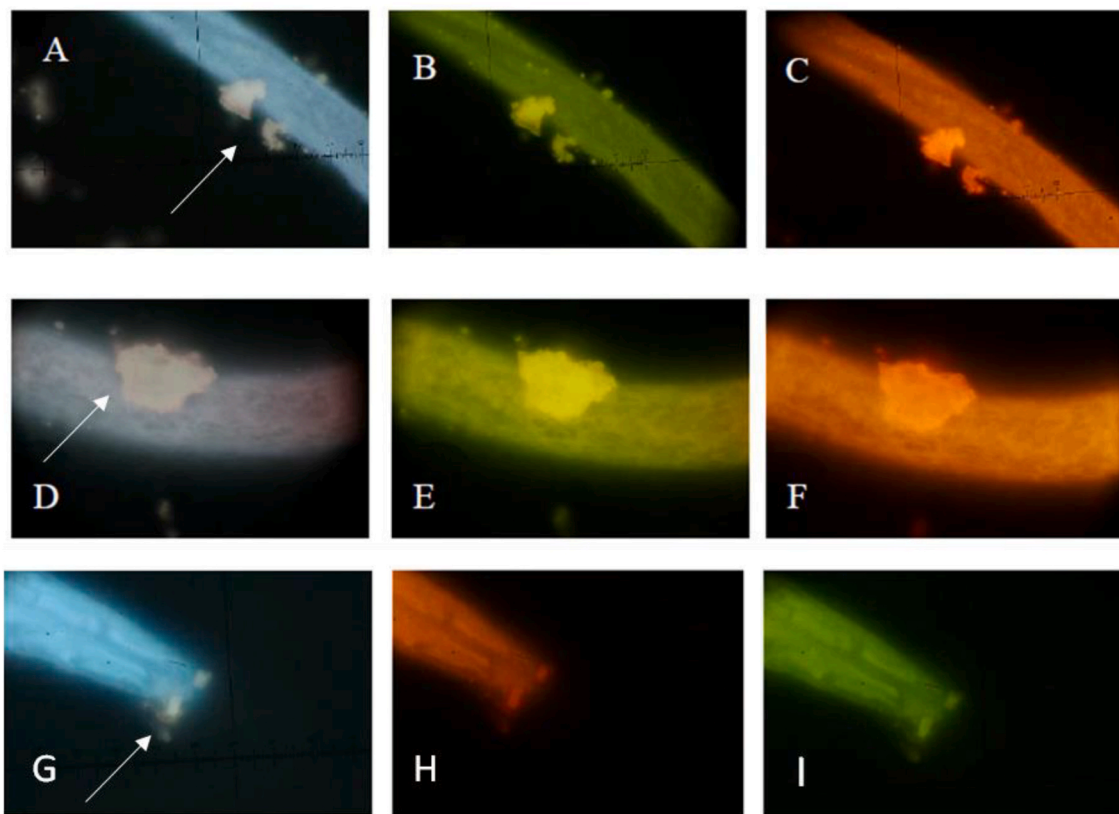

Fig. 6. Microplastics interactions with nematodes. Microplastic particles adhered to the cuticle surface of *Laimella* sp.1 (A, B and C), *Axonolaimus* sp.1 (D, E and F) around the mouth of *Daptonema* sp.3 (G, H and I) under epifluorescence microscopy (UV, blue and green light). The white arrows indicate the plastic particles. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)
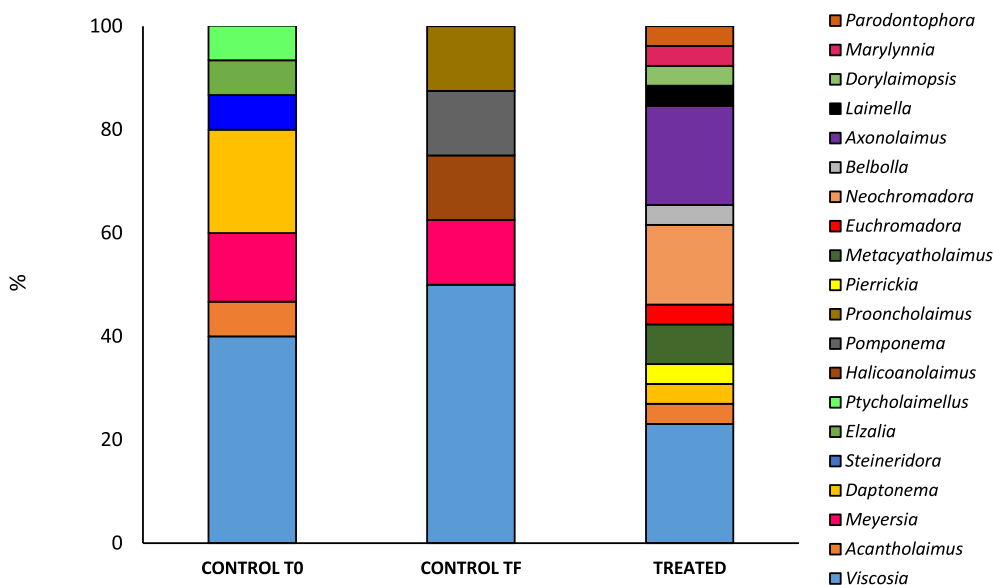
Fig. 7. Nematodes making contact (cuticle and mouth) with microplastic particles. Percentage of nematode genera with microplastics adhered to the cuticle and in the mouth.

density than other polymers (1.37–1.45), it can preferentially accumulate along the shoreline (Schwarz et al., 2019).

Our results suggest that, although the sediments of the uninhabited Vavvaru island were not exempt from microplastic contamination, the level of such a contamination was significantly lower than in the sediments of the anthropized Naifaru island (and different in terms of contamination sources: fisheries vs land-based activities).

The Naifaru island was also affected by sewage discharge, which can exacerbate microplastic pollution (Horton and Barnes, 2020). In addition, sewage can be a significant source of microplastic contamination, especially in the Maldives, where there are no sewage treatments (Patti et al., 2020). For this reason, organic enrichment and local eutrophication have also been documented in highly oligotrophic areas, such as the Lhaviyani atoll, along with significant shifts in the composition of primary producers and ecosystem functioning (Deininger and Frigstad, 2019).

The results of the present investigation provide evidence that the sediments of Naifaru when compared to those of the uninhabited Vavvaru island were significantly enriched in organic matter and characterized by an altered biochemical composition, which were responsible for significant eutrophication, as indicated by the quadruplication of total phytopigment concentration. The presence of significant inputs of organic matter in Naifaru also resulted in a minor autotrophic C contribution to biopolymeric carbon, concerning Vavvaru. The observed organic enrichment was associated with a significant reduction of meiofaunal abundances in Naifaru.

The impact on meiofaunal assemblages in Naifaru also resulted in a lower biomass and loss of some taxonomic groups such as Tardigrada, Ostracoda and Kinorhyncha are known to be more sensitive to anthropogenic impacts (Pusceddu et al., 2007). For example, Ostracoda typically show high sensitivity to hypoxic and anoxic conditions, which could be favored by the enrichment in organic matter (Ruiz et al., 2005). Similarly, Kinorhyncha disappear in altered or contaminated sediments and have been suggested to be sentinel of impact (Grego et al., 2009).

Previous studies reported that organic enrichment can promote the increase in abundance of some opportunistic nematode species (Fraschetti et al., 2006) and other taxa able to exploit the organic source and tolerate the altered conditions (e.g., reduced oxygen content) of interstitial sediments (Elías et al., 2006). However, in the present study, the abundance of individuals of nearly all taxa encountered, on average, decreased in the anthropized Naifaru island, including nematodes and

polychaetes, which are generally considered to be more tolerant to organic pollution (Giangrande et al., 2005).

The results of multiple linear regression model indicate that microplastic abundance was significantly correlated with biopolymeric C concentrations (i.e., the proxy of organic load) and that affected meiofauna abundance and diversity (as richness of higher taxa). Meiofauna diversity was also influenced by the interaction of organic enrichment and polyamide, which was the most abundant polymer found in the sediments of the two islands.

Based on these findings, we argued that being meiofaunal assemblages sensitive to different kinds of impact (Semprucci et al., 2010; Saleh, 2012) organic enrichment and microplastic contamination act synergistically to affect meiofaunal assemblages, especially in the anthropized islands. However, other stressors, not investigated here (e. g., toxic micro-algal cells colonizing the plastic particles; Tibiriçá et al., 2019 or contaminants adsorbed to the microplastics), could affect local assemblages in combination with microplastic contamination.

4.1.1. Experimental study to test the responses of meiofauna to microplastic contamination

The impact of the microplastic contamination on meiofaunal assemblages was investigated also through a dedicated experiment, in which the effects of the addition of a microplastic mixture containing the polymers most frequently found in the marine environment (Corinaldesi et al., 2021) were tested. Microplastic contamination caused, in only 3 days of exposure, a significant shift in meiofaunal assemblages when compared to control sediments. In particular, despite nematodes remained the dominant component in the sediments incubated with the microplastic mixture, the number of individuals doubled. Similarly, the number of copepods increased but to a lower extent.

Results reported in the available literature on the impact of microplastics on meiofauna do not provide a univocal response: studies on nematodes revealed that different species could respond differently to microplastic contamination and that in some cases, this can also determine an increase in their growth rate (Mueller et al. 0.2020). Other studies indicated that meiofauna, including nematodes, copepods, amphipods and polychaetes, can ingest microplastic particles with adverse effects on their feeding ability (Cole et al., 2015; Fueser et al., 2020a,b; Thompson et al., 2004). Finally, other studies suggest that microplastics might have a limited impact on meiofauna since plastic particles can be egested (Gusmão et al., 2016; Fueser et al., 2020b; Fueser et al., 2019).

We argue that the effect of the microplastic mixture added to our experimental system had in the short term a stimulatory effect on nematode and copepod abundance as previously observed in other organisms exposed to microplastics (Corinaldesi et al., 2021; Allen et al., 2017) and that the increase of meiofaunal abundance may be a transient response of the most opportunistic individuals (Fraschetti et al., 2006). During the time-course experiment, meiofaunal biodiversity was not affected when exposed to the microplastic mixture. However, the contribution of some nematode families to the whole assemblage changed in the treated sediments. In particular, the family Desmodoridae increased possibly because it is characterized by "hard-body" nematodes with morphological adaptations such as stouter body shape, ornamented cuticle, cephalic capsule, which help them to withstand physical stress (Armenteros et al., 2012). In addition, a shift in the trophic strategies of nematodes was observed, as recently reported in studies based on nematode cultures (Fueser et al., 2019). In particular, we found an increase in the abundance of epistrate feeders (e.g. *Desmodora* sp. *5*) at the expense of predators (e.g. *Viscosia* spp.) and non-selective deposit feeders (e.g. *Daptonema* sp.*1, Axonolaimus* sp.*1*).

These findings from wild nematodes of tropical ecosystems expand previous results and allow us to hypothesize that the increased abundance of epistrate feeders can be explained by an increased feeding favored by the plastic debris. Prior information, indeed, suggests that biofilm and microbial communities (including bacteria, fungi, and microalgae) associated with microplastics may positively influence the feeding activity of grazers (Rogers et al., 2020; Carson et al., 2013; Reisser et al., 2014). At the same time, the reduction of predatory and non-selective nematodes could be due to: i) microplastic ingestion followed by feeding impairment, probably due to a confounding effect on the nematodes considering that plastics can concentrate organic matter and be mistaken for food (Galloway et al., 2017; Corinaldesi et al., 2021) or ii) physical damage due to adhesion on the tissues of marine organisms, thus causing feeding impairment and stress/abrasions as observed in some marine organisms (Wright et al., 2013; Corinaldesi et al., 2021). The microscopic analysis allowed us also to confirm that microplastic particles adhered to the cuticle or were potentially ingested by specimens (i.e., found within the mouth of the nematodes), especially in the treated sediments but also in the controls (Fig. 6), suggesting that the effects of microplastic particles on nematodes were not due to an experiment artifact (i.e., the addition of the microplastic mixture). Most of the microplastic particles were observed associated with predators-omnivores (e.g., *Viscosia* spp.) and non-selective feeders (e.g., *Daptonema* sp.), whose decrease in abundance allows to hypothesize their high sensitivity to microplastic adhesion.

Since the microplastic particles identified on the cuticle or in the mouth of the nematodes were attributed only to PET (due to their higher visibility than other polymers under epifluorescence microscope), we believe that the number of nematodes that came in contact with microplastic polymers could be even higher.

5. Conclusions

Our results reveal that microplastics affected meiofaunal abundance and taxa richness especially in the anthropized island characterized by sewage-derived organic enrichment. When only acute microplastic contamination was simulated in experimental conditions, opportunistic taxa increased and the trophic structure was altered. Based on all these results, we argue that chronic organic enrichment and microplastics, when coexistent, can significantly increase the ecological impacts on meiofaunal assemblages. Since microplastic pollution in the oceans is predicted to increase in the following decades, its impact on biodiversity and functioning of tropical ecosystems is expected to worsen.

Funding

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.

Acknowledgements

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2021.118415.

Author contributions

Cinzia Corinaldesi, Roberto Danovaro: Conceptualization, **Cinzia Corinaldesi, Sara Canensi, Laura Carugati, Ettore Nepote, Francesca Marcellini, Marco Lo Martire, Simona Sabbatini:** Data curation, **Cinzia Corinaldesi:** Funding acquisition, **Cinzia Corinaldesi:** Supervision, **Cinzia Corinaldesi, Sara Canensi, Laura Carugati, Ettore Nepote, Simona Sabbatini:** Visualization, **Cinzia Corinaldesi, Ettore Nepote:** Writing – original draft, **Cinzia Corinaldesi, Sara Canensi, Laura Carugati, Ettore Nepote, Francesca Marcellini, Marco Lo Martire, Simona Sabbatini, Roberto Danovaro:** Writing – review & editing.

References

Allen, A.S., Seymour, A.C., Rittschof, D., 2017. Chemoreception drives plastic consumption in a hard coral. Mar. Pollut. Bull. 124, 198–205. https://doi.org/10.1016/j.marpolbul.2017.07.030.

Alvarez-Zeferino, J.C., et al., 2020. Microplastics in Mexican beaches. Resour. Conserv. Recycl. 155, 104633. https://doi.org/10.1016/j.resconrec.2019.104633.

Andrassy, I., 1956. Die rauminhalts-undgewichtsbestimmung der fadenwürmer (Nematoden). Acta Zool. Hung. 2, 1–58.

Armenteros, M., et al., 2012. Habitat heterogeneity effects on macro-and meiofauna (especially nematodes) in Punta Francés coral reef (SW Cuban Archipelago). Rev. Investig. Mar. 32, 50–61.

Ashton, K., Holmes, L., Turner, A., 2010. Association of metals with plastic production pellets in the marine environment. Mar. Pollut. Bull. 60, 2050–2055. https://doi.org/10.1016/j.marpolbul.2010.07.014.

Bonaglia, S., et al., 2014. Meiofauna increases bacterial denitrification in marine sediments. Nat. Commun. 5, 5133. https://doi.org/10.1038/ncomms6133.

Browne, M.A., et al., 2013. Microplastic moves pollutants and additives to worms, reducing functions linked to health and biodiversity. Curr. Biol. 23, 2388–2392. https://doi.org/10.1016/j.cub.2013.10.012.

Carson, H.S., et al., 2013. The plastic-associated microorganisms of the north Pacific Gyre. Mar. Pollut. Bull. 75 (1–2), 126–132. https://doi.org/10.1016/j.marpolbul.2013.07.054.

Cole, M., et al., 2015. The impact of polystyrene microplastics on feeding, function and fecundity in the marine copepod *Calanus helgolandicus*. Environ. Sci. Technol. 49, 1130–1137. https://doi.org/10.1021/es504525u.

Connors, E.J., 2017. Distribution and biological implications of plastic pollution on the fringing reef of Mo'orea, French Polynesia. PeerJ 5, e3733. https://doi.org/10.7717/peerj.3733.

Corinaldesi, C., et al., 2021. Multiple impacts of microplastics can threaten marine habitat-forming species. Commun. Biol. 4 (1), 1–13. https://doi.org/10.1038/s42003-021-01961-1.

Crawley, M.J., 2012. The R Book. John Wiley & Sons.

Danovaro, R., et al., 2000. Meiofauna response to a dynamic river plume front. Mar. Biol. 137 (2), 359–370. https://doi.org/10.1007/s002270000353.

Danovaro, R., 2010. Methods for the Study of Deep-Sea Sediments, Their Functioning and Biodiversity. CRC press.

Danovaro, R., Fabiano, M., 1997. Seasonal changes in quality and quantity of food available for benthic suspension-feeders in the Golfo Marconi (North-western Mediterranean). Estuar. Coast Shelf Sci. 44 (6), 723–736. https://doi.org/10.1006/ecss.1996.0135.

De Mesel, I., et al., 2006. Species diversity and distribution within the deep-sea nematode genus *Acantholaimus* on the continental shelf and slope in Antarctica. Polar Biol. 29, 860–871. https://doi.org/10.1007/s00300-006-0124-7.

Deininger, A., Frigstad, H., 2019. Reevaluating the role of organic matter sources for coastal eutrophication, oligotrophication, and ecosystem health. Front. Mar. Sci. 6, 210. https://doi.org/10.3389/fmars.2019.00210.

Dell'Anno, A., et al., 2002. Assessing the trophic state and eutrophication of coastal marine systems: a new approach based on the biochemical composition of sediment organic matter. Mar. Pollut. Bull. 44 (7), 611–622. https://doi.org/10.1016/S0025-326X(01)00302-2.

Dowarah, K., Devipriya, S.P., 2019. Microplastic prevalence in the beaches of Puducherry, India and its correlation with fishing and tourism/recreational activities. Mar. Pollut. Bull. 148, 123–133. https://doi.org/10.1016/j.marpolbul.2019.07.066.

Downing, J.A., et al., 1999. The impact of accelerating land-use change on the N-cycle of tropical aquatic ecosystems: current conditions and projected changes. Biogeochemistry 46, 109–148.

Duprey, N.N., Yasuhara, M., Baker, D.M., 2016. Reefs of tomorrow: eutrophication reduces coral biodiversity in an urbanized seascape. Global Change Biol. 22 (11), 3550–3565.

Elías, R., et al., 2006. Sewage-induced disturbance on polychaetes inhabiting intertidal mussel beds of *Brachidontesrodriguezii* off Mar del Plata (SW Atlantic, Argentina). Sci. Mar. 70, 187–196. https://doi.org/10.3989/scimar.2006.70s3187.

Ellis, J.I., et al., 2019. Multiple stressor effects on coral reef ecosystems. Global Change Biol. 25 (12), 4131–4146. https://doi.org/10.1111/gcb.14819.

Eriksen, M., et al., 2014. Plastic pollution in the world's oceans: more than 5 trillion plastic pieces weighing over 250,000 tons afloat at sea. PLoS One 9, e111913. https://doi.org/10.1371/journal.pone.0111913.

Fabiano, M., Danovaro, R., 1998. Enzymatic activity, bacterial distribution, and organic matter composition in sediments of the Ross Sea (Antarctica). Appl. Environ. Microbiol. 64, 3838–3845. https://doi.org/10.1128/AEM.64.10.3838-3845.1998.

Feller, R.J., Warwick, R.M., 1988. Energetics. In: Higgins, R.P., Thiel, H. (Eds.), Introduction to the Study of Meiofauna. Smithsonian, pp. 181–196.

Fraschetti, S., et al., 2006. Structural and functional response of meiofauna rocky assemblages to sewage pollution. Mar. Pollut. Bull. 52, 540–548. https://doi.org/10.1016/j.marpolbul.2005.10.001.

Fueser, H., Mueller, M.T., Traunspurger, W., 2020a. Ingestion of microplastics by meiobenthic communities in small-scale microcosm experiments. Sci. Total Environ. 746, 141276. https://doi.org/10.1016/j.scitotenv.2020.141276.

Fueser, H., Mueller, M.T., Traunspurger, W., 2020b. Rapid ingestion and egestion of spherical microplastics by bacteria-feeding nematodes. Chemosphere 261, 128162. https://doi.org/10.1016/j.chemosphere.2020.128162.

Fueser, H., Mueller, M.T., Weiss, L., Höss, S., Traunspurger, W., 2019. Ingestion of microplastics by nematodes depends on feeding strategy and buccal cavity size. Environ. Pollut. 255, 113227.

Galloway, T.S., Cole, M., Lewis, C., 2017. Interactions of microplastic debris throughout the marine ecosystem. Nat. Ecol. Evol. 1 (5), 1–8. https://doi.org/10.1038/s41559-017-0116.

Giangrande, A., Licciano, M., Musco, L., 2005. Polychaetes as environmental indicators revisited. Mar. Pollut. Bull. 50 (11), 1153–1162. https://doi.org/10.1016/j.marpolbul.2005.08.003.

Giere, O., 2009. Meiobenthology: the Microscopic Fauna in Aquatic Sediments, second ed. Springer-Verlag, Berlin.

Grego, M., De Troch, M., Forte, J., Malej, A., 2009. Main meiofauna taxa as an indicator for assessing the spatial and seasonal impact of fish farming. Mar. Pollut. Bull. 58 (8), 1178–1186. https://doi.org/10.1016/j.marpolbul.2009.03.020.

Guilini, K., et al., 2016. NeMys: World Database of Free-Living Marine Nematodes.

Gusmão, F., et al., 2016. *In situ* ingestion of microfibres by meiofauna from sandy beaches. Environ. Pollut. 216, 584–590. https://doi.org/10.1016/j.envpol.2016.06.015.

Haegerbaeumer, A., et al., 2019. Impacts of micro-and nano-sized plastic particles on benthic invertebrates: a literature review and gap analysis. Front. Environ. Sci 7, 17. https://doi.org/10.3389/fenvs.2019.00017.

Hall, N.M., et al., 2015. Microplastic ingestion by scleractinian corals. Mar. Biol. 162, 725–732. https://doi.org/10.1007/s00227-015-2619-7.

Heip, C., Vincx, M., Vranken, G., 1985. The Ecology of Marine Nematodes.

Horton, A.A., Barnes, D.K., 2020. Microplastic pollution in a rapidly changing world: implications for remote and vulnerable marine ecosystems. Sci. Total Environ. 140349 https://doi.org/10.1016/j.scitotenv.2020.140349.

Hui, M., et al., 2020. Microplastics in aquatic environments: toxicity to trigger ecological consequences. Environ. Pollut. 114089. https://doi.org/10.1016/j.envpol.2020.114089.

Imhof, H.K., et al., 2012. A novel, highly efficient method for the separation and quantification of plastic particles in sediments of aquatic environments. Limnol. Oceanogr-meth. 10 (7), 524–537. https://doi.org/10.4319/lom.2012.10.524.

Imhof, H.K., et al., 2017. Spatial and temporal variation of macro-, meso-and microplastic abundance on a remote coral island of the Maldives. Indian Ocean. Mar. Pollut. Bull. 116, 340–347. https://doi.org/10.1016/j.marpolbul.2017.01.010.

Isobe, A., et al., 2019. Abundance of non-conservative microplastics in the upper ocean from 1957 to 2066. Nat. Commun. 10 (1), 1–13. https://doi.org/10.1038/s41467-019-08316-9.

Jaleel, A., 2013. The status of the coral reefs and the management approaches: the case of the Maldives. Ocean Coast Manag. 82, 104–118. https://doi.org/10.1016/j.ocecoaman.2013.05.009.

Jayasiri, H.B., Purushothaman, C.S., Vennila, A., 2013. Quantitative analysis of plastic debris on recreational beaches in Mumbai, India. Mar. Pollut. Bull. 77 (1–2), 107–112. https://doi.org/10.1016/j.marpolbul.2013.10.024.

Jiménez-Skrzypek, G., et al., 2021. Microplastic-adsorbed organic contaminants: analytical methods and occurrence. Trends Anal. Chem. 116186 https://doi.org/10.1016/j.trac.2021.116186.

Kögel, T., et al., 2019. Micro-and nanoplastic toxicity on aquatic life: determining factors. Sci. Total Environ. 136050 https://doi.org/10.1016/j.scitotenv.2019.136050.

Law, K.L., 2017. Plastics in the marine environment. Annu. Rev. Mar. Sci. 9, 205–229. https://doi.org/10.1146/annurev-marine-010816-060409.

Mascarenhas, R., Santos, R., Zeppelini, D., 2004. Plastic debris ingestion by sea turtle in Paraíba, Brazil. Mar. Pollut. Bull. 49, 354–355. https://doi.org/10.1016/j.marpolbul.2004.05.006.

Miller, M.W., Sluka, R.D., 1999. Patterns of seagrass and sediment nutrient distribution suggest anthropogenic enrichment in Laamu Atoll, Republic of Maldives. Mar. Pollut. Bull. 38 (12), 1152–1156. https://doi.org/10.1016/S0025-326X(99)00147-2.

Moritz, C., et al., 2017. The "resort effect": can tourist islands act as refuges for coral reef species? Divers. Distrib. 23, 1301–1312. https://doi.org/10.1111/ddi.12627.

Mueller, M.T., et al., 2020. Species-specific effects of long-term microplastic exposure on the population growth of nematodes, with a focus on microplastic ingestion. Ecol. Indicat. 118, 106698. https://doi.org/10.1016/j.ecolind.2020.106698.

Munari, C., Scoponi, M., Mistri, M., 2017. Plastic debris in the Mediterranean sea: types, occurrence and distribution along adriatic shorelines. Waste Manag. 67, 385–391. https://doi.org/10.1016/j.wasman.2017.05.020.

Nepote, E., et al., 2016. Pattern and intensity of human impact on coral reefs depend on depth along the reef profile and on the descriptor adopted. Estuar. Coast Shelf Sci. 178, 86–91. https://doi.org/10.1016/j.ecss.2016.05.021.

Patti, T.B., et al., 2020. Spatial distribution of microplastics around an inhabited coral island in the Maldives. Indian Ocean. Sci. Tot Environ. 141263. https://doi.org/10.1016/j.scitotenv.2020.141263.

Paul-Pont, I., et al., 2018. Constraints and priorities for conducting experimental exposures of marine organisms to microplastics. Front. Mar. Sci 5, 252. https://doi.org/10.3389/fmars.2018.00252.

Phuong, N.N., et al., 2016. Is there any consistency between the microplastics found in the field and those used in laboratory experiments? Environ. Pollut. 211, 111–123. https://doi.org/10.1016/j.envpol.2015.12.035.

Platt, H.M., Warwick, R.M., 1983. Free-Living Marine Nematodes. Part I. British Enoplids. Cambridge University Press, Cambridge.

Platt, H.M., Warwick, R.M., 1988. A Synopsis of the Free Living Marine Nematodes. Part II: British Chromadorids. Cambridge University Press, Cambridge.

Pusceddu, A., et al., 1999. Seasonal and spatial changes in the sediment organic matter of a semi-enclosed marine system (W-Mediterranean Sea). Hydrobiologia 397, 59–70.

Pusceddu, A., Gambi, C., Manini, E., Danovaro, R., 2007. Trophic state, ecosystem efficiency and biodiversity of transitional aquatic ecosystems: analysis of environmental quality based on different benthic indicators. Chem. Ecol. 23 (6), 505–515. https://doi.org/10.1080/02757540701760494.

Pusceddu, A., et al., 2014. Relationships between meiofaunal biodiversity and prokaryotic heterotrophic production in different tropical habitats and oceanic regions. PLoS One 9 (3), e91056. https://doi.org/10.1371/journal.pone.0091056.

Pusceddu, et al., 2016. Meiofauna communities, nematode diversity and C degradation rates in seagrass (*Posidonia oceanica* L.) and unvegetated sediments invaded by the algae Caulerpa cylindracea (Sonder). Mar. Environ. Res. 119, 88–99. https://doi.org/10.1016/j.marenvres.2016.05.015.

Reisser, J., et al., 2014. Millimeter-sized marine plastics: a new pelagic habitat for microorganisms and invertebrates. PLoS One 9 (6), e100289. https://doi.org/10.1371/journal.pone.0100289.

Rochman, C.M., et al., 2016. The ecological impacts of marine debris: unraveling the demonstrated evidence from what is perceived. Ecology 97 (2), 302–312. https://doi.org/10.1890/14-2070.1.

Rodrigues, S.M., et al., 2019. Microplastic contamination in an urban estuary: abundance and distribution of microplastics and fish larvae in the Douro estuary. Sci. Total Environ. 659, 1071–1081. https://doi.org/10.1016/j.scitotenv.2018.12.273.

Rogers, K.L., et al., 2020. Micro-by-micro interactions: how microorganisms influence the fate of marine microplastics. Limnol. Oceanogr. Lett. 5 (1), 18–36. https://doi.org/10.1002/lol2.10136.

Rolsky, C., Kelkar, V., Driver, E., Halden, R.U., 2020. Municipal sewage sludge as a source of microplastics in the environment. Current Opinion in Environmental Science & Health 14, 16–22.

Ruiz, F., et al., 2005. Marine and brackish-water ostracods as sentinels of anthropogenic impacts. Earth Sci. Rev. 72, 89–111. https://doi.org/10.1016/j.earscirev.2005.04.003.

Saleh, A.A.F., 2012. Effects of multiple-source pollution on spatial distribution of polychaetes in Saudi Arabia. Res. J. Environ. Toxicol. 6, 1. https://doi.org/10.3923/rjet.2012.1.12.

Saliu, F., et al., 2018. Microplastic and charred microplastic in the faafu atoll, Maldives. Mar. Pollut. Bull. 136, 464–471. https://doi.org/10.1016/j.marpolbul.2018.09.023.

Saliu, F., et al., 2019. Microplastics as a threat to coral reef environments: detection of phthalate esters in neuston and scleractinian corals from the Faafu Atoll. Maldives. Mar. Pollut. Bull. 142, 234–241. https://doi.org/10.1016/j.marpolbul.2019.03.043.

Schratzberger, M., Ingels, J., 2018. Meiofauna matters: the roles of meiofauna in benthic ecosystems. J. Exp. Mar. Biol. Ecol. 502, 12–25 (Section 2. Biological activities of meiofauna and their effects on benthic ecosystems - a pre-21stcentury perspective).

Schwarz, A.E., Ligthart, T.N., Boukris, E., Van Harmelen, T., 2019. Sources, transport, and accumulation of different types of plastic litter in aquatic environments: a review study. Mar. Pollut. Bull. 143, 92–100.

Seinhorst, J.W., 1959. A rapid method for the transfer of nematodes from fixative to unhydrous glycerine. Nematologica 4, 67–69.

Semprucci, F., et al., 2010. The distribution of meiofauna on back-reef sandy platforms in the Maldives (Indian Ocean). Mar. Ecol. 31, 592–607. https://doi.org/10.1111/j.1439-0485.2010.00383.x.

Semprucci, F., et al., 2014. Spatial patterns of distribution of meiofaunal and nematode assemblages in the Huvadhoo lagoon (Maldives, Indian Ocean). J. Mar. Biol. Assoc. U. K. 94, 1377–1385. https://doi.org/10.1017/S002531541400068X.

Semprucci, F., et al., 2018. Biodiversity and distribution of the meiofaunal community in the reef slopes of the Maldivian archipelago (Indian Ocean). Mar. Environ. Res. 139, 19–26. https://doi.org/10.1016/j.marenvres.2018.05.006.

Semprucci, F., et al., 2013. Meiofauna associated with coral sediments in the Maldivian subtidal habitats (Indian Ocean). Mar. Biodivers. 43, 189–198. https://doi.org/10.1007/s12526-013-0146-7.

Sfriso, A.A., et al., 2020. Microplastic accumulation in benthic invertebrates in Terra Nova Bay (Ross sea, Antarctica). Environ. Int. 137, 105587. https://doi.org/10.1016/j.envint.2020.105587.

Sharifinia, M., et al., 2020. Microplastic pollution as a grand challenge in marine research: a closer look at their adverse impacts on the immune and reproductive systems. Ecotoxicol. Environ. Saf. 204, 111109. https://doi.org/10.1016/j.ecoenv.2020.111109.

Steger, J., et al., 2017. Diversity, size frequency distribution and trophic structure of the macromollusc fauna of Vavvaru Island (Faadhippolhu Atoll, northern Maldives).

Annalen des Naturhistorischen Museums in Wien. Serie B für Botanik und Zoologie 119, 17–54.

Sun, J., et al., 2019. Microplastics in wastewater treatment plants: detection, occurrence and removal. Water Res. 152, 21–37. https://doi.org/10.1016/j.watres.2018.12.050.

Tibiriçá, C.E.J., et al., 2019. Ostreopsis cf. ovata bloom in Currais, Brazil: phylogeny, toxin profile and contamination of mussels and marine plastic litter. Toxins 11, 446.

Thompson, R.C., et al., 2004. Lost at sea: where is all the plastic? Science 304, 838.

United Nations Department of Economic and Social Affairs, 2019. World Population Prospects Highlights (New York, US)).

Vanaverbeke, J., et al., 2015. NeMys: World Database of Free-Living Marine Nematodes.

Warwick, R.M., Platt, H.M., Somerfield, P.J., 1998. Synopses of the British Fauna (New Series). Free-living Marine Nematodes. Part III. British Monhysterids, vol. 53. Field Studies Council, Shrewsbury, UK.

Wear, S.L., Thurber, R.V., 2015. Sewage Pollution: Mitigation Is Key for Coral Reef Stewardship. Annals of the New York Academy of Sciences, p. 1355.

Wieser, W., 1953. Die Beziehung zwischen Mundhöhlengestalt,Ernährungsweise und Vorkommen bei freilebenden marinen Nematoden. Ark. fürZoolgie.

Wright, S.L., Thompson, R.C., Galloway, T.S., 2013. The physical impacts of microplastics on marine organisms: a review. Environ. Pollut. 178, 483–492. https://doi.org/10.1016/j.envpol.2013.02.031.

Zahid, A., 2010. Environmental Audit of the Powerhouse and Desalination Plant in Angsana Maldives Ihuru (Maldives).

Zuur, A.F., Ieno, E.N., 2016. A protocol for conducting and presenting results of regression-type analyses. Meth. Ecol. Evol. 7 (6), 636–645.

Zuur, A.F., Ieno, E.N., Smith, G.M., 2007. Analysing Ecological Data, vol. 680. Springer, New York.