



sedimentary biogeochemistry through altered benthic processes, that could cumulatively impair whole benthic trophic webs by enhancing the accumulation and possible longer-term storage of recalcitrant organic C in the seabed.

## 1. Introduction

Nations worldwide are struggling to manage the increasing volume of plastic waste, that is forecasted to reach 53 million tons year<sup>-1</sup> in 2030, according to ‘business as usual’ projections (Borrelle et al., 2020). Over ~80 % of all plastic ever produced is already dispersed across all environmental matrices: soil, atmosphere and aquatic environments (Geyer et al., 2017), with consequences that are still under scrutiny by the scientific community.

Oceans’ seabed represent the final destination for most of the plastic waste generated on land (Lebreton et al., 2017). Plastic slowly degrades through biological, mechanical and physical processes (Cau et al., 2020; Klein et al., 2018; Mohanan et al., 2020; Paluselli et al., 2019) that cause its fragmentation into smaller particles called microplastics (MPs) (1 µm - 5 mm; Frias and Nash, 2019). MPs are ubiquitous in marine environments and, even considering utopic scenarios with ceased plastic input, the fraction already accumulated would still fragment and generate new MPs that would unavoidably interact with the surrounding environment (Galgani and Loisele, 2021). Laboratory studies investigated potential effects of MPs contamination in seawater and at the air-sea boundary layer, documenting significant impairment of microbial processes (Romera-Castillo et al., 2018), including those controlling gas exchange with the atmosphere (Galgani et al., 2023, 2018).

Many plastic polymers are buoyant and come from land-based sources, but they are still exported to the seafloor after a more or less long period of time (Kowalski et al., 2016). Vectors of vertical mixing can be waves and downwelling phenomena, coupled with biological processes such as biofouling and the inclusion of MPs in organic aggregates and colloidal substances (fecal pellets, marine snow, exopolymer particles) which increase the sinking rates of MP, including low-density polymers (Wright et al., 2020). Sedimentary environments (most of which composed by fine mud) occupy the largest surface of the oceans’ floor and represent the ultimate sink for MPs, with considerable concentrations already detected across multiple environments, from sandy coastal regions to fine mud of the deep-sea, down to hadal trenches (Van Cauwenberghe et al., 2015; Woodall et al., 2014).

Focusing on the ecological hierarchy, MPs are now demonstrated to affect most of the marine realm, from the individual to community level, with apparently no geographical or bathymetric boundaries. Yet, there is no wide understanding on the exact extent to which MPs can possibly trigger ecosystem-wide impacts that would impair its key functions such as organic matter cycling and degradation (Ladewig et al., 2021; Seeley et al., 2020). Moreover, effects of MPs documented on populations or assemblages mostly remain split among those providing evidence of positive, negative, or neutral effects (Bucci et al., 2020). These discrepancies have been ascribed to the fact that in most experiments, ‘microplastics’ have been erroneously generalized as a single macroscopic contaminant, rather than a multitude of contaminants that vary in shape, size (within the MP definition range), colour, chemical composition, etc. Moreover, laboratory experiments are often conducted using high concentrations of MPs, so that resulting effects might be detected under non-realistic conditions (Foley et al., 2018).

Despite the growing number of laboratory studies investigating the effects of plastic contamination, a consensus has not yet been achieved on the potential alterations of key ecosystem functions. This effect was conceivably expected since most of polymers are made of interactive carbon molecules that affect not only the biology and ecology of organisms and communities, but also the chemistry and structural dynamics of sediments, which can collectively influence benthic biogeochemistry (Ladewig et al., 2021). This claims an urgent call to

posing questions on whether and how MPs affect key ecosystems functions, and this holds particularly relevant for marine sedimentary environments, which are either major contributors to global biogeochemical cycles or hotspots of ocean ecosystems’ functioning (Snelgrove, 1999).

The efficiency in transferring energy towards higher trophic levels in marine sediments is driven by the relative susceptibility of organic matter to heterotrophic consumption (Pusceddu and Danovaro, 2009). Sedimentary organic C, especially in oligotrophic sediments, is dominated by large and relatively refractory polymeric molecules (Pusceddu et al., 2009). Therefore, these molecules must undergo extracellular enzymatic hydrolysis to being channeled through the microbial loop (Manini et al., 2003) and then becoming nutritionally available for higher trophic levels. Although C degradation rates estimated from extracellular enzymatic activities can be considered only potential (Soru et al., 2022), they have often been used as a reliable proxy for benthic ecosystem functioning (Palmas et al., 2019; Pusceddu et al., 2014).

Thus, we conducted a manipulative experiment to test whether and how sedimentary carbon degradation processes mediated by extracellular enzymes can potentially be impaired by MPs accumulated in coastal sediments, potentially causing an impairment of key ecosystem functions and wide effects on benthic trophic webs.

## 2. Materials and methods

### 2.1. Sediment sampling and microcosm setting

Sediment was collected by scuba divers in October 2021, using a manual sediment collector that scraped the top 2 cm from the sediment surface on the sea bottom, avoiding deeper sediments and the redox discontinuity later. This was to avoid unpredictable consequences in chosen response variables, likely ascribable to microbial activities associated with hypoxic conditions. Sediment collection was performed over coastal sandy bottoms located at ~30 m depth in the Marine Protected Area of Capo Carbonara (Sardinia, Italy; Supplementary Fig. S1). Temperature (14.3 °C), salinity (37.5 PSU) and dissolved oxygen (6.6 mg L<sup>-1</sup>) were measured at the sampling site using a multiparameter probe (InSitu smarTROLL Multi-parameter Handheld, Fort Collins, CO, USA). Values at the sampling site were then reproduced in either the acclimation or experimental phase. Seawater and sediments sampled from the same site were transported to the laboratory in controlled temperature conditions using a temperature-controlled box, until put in aquaria for acclimation.

Baseline assessment of MPs contamination of sandy sediments used in microcosms was performed in triple replicate, using NaCl density separation protocol and polymer characterization (see supplementary material). Baseline assessment (avg. n. of particles g<sup>-1</sup> 0.06 ± 0.02 st. dev) was aimed at showing how sediments used in this experiment do fall within the low to very-low contamination rate for Marine Protected Areas (Nunes et al., 2023). A preliminary assessment of the trophic status (sensu Dell’Anno et al., 2002; Pusceddu et al., 2009) of the experimental sediments showed that they can be classified as oligotrophic, having biopolymeric C (BPC) concentrations rounding 1 mgC g<sup>-1</sup>. 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. The sandy fraction was further sieved through a 4, 2, 1, 0.5, 0.125, and 0.063-mm mesh to distinguish between coarse (>1 mm), 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. Sediment

granulometry and water content are reported in Supplementary Table S1.

Before being placed in microcosms, a stock of sediments (ca. 2 L) was thoroughly homogenized by gentle manual mixing, after large debris removal. Approximately 250 g of wet sediment from the stock were added to previously acid-washed and sterilized (121 °C, 60 min) 600 mL glass beakers (sediment depth reached 3 cm), hereafter termed microcosms.

As recently commented (Ladewig et al., 2023; Seeley et al., 2020), the number of studies reporting MPs concentration in sediments is huge, but the almost totality of results are reported as particle count and not on weight. The few available references from environmental samples report concentrations ranging between 3.3 % and 0.15 % (Carson et al., 2011; McDermid and McMullen, 2004), while 0.5 % was used in a foundational manipulative laboratory experiment testing for potential effects of MPs on nitrogen cycling (Seeley et al., 2020). We thus manipulated marine sediments to obtain a concentration of MPs of ~0.2 % by weight of sediment (corresponding to 0.5 g of MPs in 250 g of sediment). Sediments were thoroughly homogenized prior to MPs addition; then, once put in each beaker, it was further homogenized when MPs (0.5 g microcosm<sup>-1</sup>) were added, prior to sea water. Before its addition to the microcosms, seawater was filtered (0.45 µm pore size GF/F filters) to remove larger particulate matter, including potential floating MPs. A first aliquot of filtered seawater (50 mL) was added to each microcosm, mixed with sediments, and allowed to settle. After that, an additional 300 mL aliquot of filtered seawater was gently added to each microcosm, avoiding sediment resuspensions, and left untouched for 24 h to allow the sediment column to fully settle. Microcosms were gently aerated using 12 aquaria oxygenators with adjustable flow, equipped with an air flow control valve kit ensuring a quasi-constant air insufflation, as confirmed by daily measures of dissolved oxygen concentrations (average during the experiment  $6.54 \pm 0.09$  mg L<sup>-1</sup>) and salinity (average for the entire study  $37.4 \pm 0.4$  PSU), that showed very limited variations. Oxygen concentration and salinity in the microcosms were measured using the same probe used at the sampling site. Microcosms were maintained at room temperature (15°), covered with paraffine foil to prevent evaporation, and exposed to a natural sunlight regime prior to and during the experiment. Sediment aliquots were collected from the surface sediment (top 1st cm) from each microcosm, as independent replicates ( $n = 3$ ) at the beginning of exposure ( $T_0$ ), and then after 15 ( $T_1$ ) and 30 days ( $T_2$ ), to perform organic matter (OM) and extracellular enzymatic activities (EEA) analyses. The choice of 15 days of exposure was based on sampling timing adopted in previous experiments carried out with marine and salt-marsh sediments added with MPs both in the field and in laboratory conditions (e.g., Ladewig et al., 2024 deployed ROMA' plates for 11–15 days, while 7 and 15 days was exposure times were used by Seeley et al., 2020). To investigate eventual effects on a longer time exposure, previously considered in experiments with riverine sediments (Li et al., 2022) we extended the exposure time to 30 days (Li et al., 2022).

## 2.2. Experimental setup

To investigate whether contamination of marine sediments from microplastics injected in the sea after nurdle accidental spills, uncontrolled wastewater discharge, outfall pipes and flooding events (Gündoğdu et al., 2018; Palmas et al., 2022) could alter benthic biogeochemical processes, we simulated a pulse-like contamination event of coastal marine sediments with microplastics made of different polymers and their combination. Beside concentration, to be as close as possible to realistic environmental scenarios, we conducted the experiment using MPs obtained by mechanical fragmentation of plastic debris retrieved from the marine environment during clean-up activities conducted in coastal areas. This was to guarantee that source debris used in the experiment had undergone natural physio-chemical weathering, at least for the duration of their permanence in the marine environment.

Fragmentation was performed using a lab mixer in chilled sterilized and 0.02 µm pre-filtered milliQ water, operating under a laminar flow hood. Resulting solution was then sieved to a defined size range comprised between 70 and 210 µm.

The experimental design (Supplementary Fig. S1) included, for each of the sampling times [at 0 ( $T_0$ ), 15 ( $T_1$ ), and 30 ( $T_2$ ) days], three microcosm replicates (i.e., independent samples) added with each of the three MPs combinations, and three plastic-free microcosm replicates (control; CON), for a total of 36 microcosms. The three MPs combinations included: i) only polyethylene (PE), obtained from a recycled bottle of predominantly high-density PE; ii) only polyurethane (PU), obtained from a brick of foam used as float for fishing nets, and iii) a mixture (MIX) of multiple polymers. The mixture of microplastics was composed of 60 % of polyethylene (PE), 25 % of polypropylene (PP), 5 % of polystyrene (PS), 5 % of polyurethane (PU) and 5 % of Polyethylene Terephthalate (PET).

The polymeric nature of plastic debris collected from the cleanup activity was determined by means of a non-confocal micro-Raman OEM system in back-scattering geometry. The emission at 785 nm from a fibre-coupled laser diode (BWTEK BRM-785) was focused onto the samples by means of a 10 X microscope objective (laser spot diameter on the sample ~ 200 µm). Raman signals were recorded by a fibre-coupled grating spectrometer coupled with a Peltier cooled CCD (BWTEK BTC667N-785S) with a spectral resolution  $>5$  cm<sup>-1</sup>. The Rayleigh scattering was rejected using an edge-filter cutting at nearly 65 cm<sup>-1</sup>. Depending on the sample under investigation, the laser power was kept below 6 mW to avoid sample damage. After baseline correction (Savitzky–Golay filter), smoothing techniques (IModPolyFit) were used to amplify the signal-to-noise ratio and eventually plastics were consequently identified through matching tool of *Open Specy* (Cowger et al., 2021).

## 2.3. Contents, biochemical composition, and degradation of sedimentary organic matter

Protein, carbohydrate, and lipid analyses were carried out in triplicate using photometric protocols (Danovaro et al., 2008) for each of the three independent sediment aliquots. Protein, carbohydrate, and lipid contents were converted into C equivalents using the conversion factors 0.49, 0.40, and 0.75 mg of C per milligram, respectively, and their sum reported as biopolymeric C (Fabiano et al., 1995).

OM degradation rates were estimated from aminopeptidase and β-glucosidase activities, determined by the cleavage of fluorogenic substrates (L-leucine-4-methylcoumarinyl-7-amide and 4-methylumbelliferone-D-glucopyranoside, for aminopeptidase and β-glucosidase, respectively) at saturating concentrations (Danovaro et al., 2008). Extracellular enzymatic activities (EEA) were measured after the addition of 150 µL of substrate to 1 mL of a slurry prepared using 1:1 volume of filtered (0.2 µm) and sterile seawater and sediment (substrate final concentration 200 µM). Substrate incubations were performed in the dark at in situ temperature (same as that used for the experiment) for 1 h. After these incubations, the slurries were centrifuged (3000 rpm, 5 min), and the supernatants were analyzed fluorometrically (at 365 nm excitation, 455 nm emission for β-glucosidase, and 380 nm excitation, 440 nm emission for aminopeptidase). The protease and glucosidase activities (micromoles of substrate per gram per hour) were converted into C degradation rates (micrograms of C per gram per hour), using 72 µg of C per mole of substrate as the conversion factor (Pusceddu et al., 2014). The turnovers (per day) of the whole protein and carbohydrate pools were calculated as the ratios of the hourly C degradation rates (once multiplied by 24) and the whole protein and carbohydrate C contents in the sediment.

## 2.4. Statistical analyses

We investigated the effects of MPs contamination on OM contents

and biochemical composition through permutational analysis of variance (PERMANOVA), performed both in uni- and multivariate context. As anticipated experimental design included two orthogonal factors: Time (Ti, 3 fixed levels: T<sub>0</sub>, T<sub>1</sub>, T<sub>2</sub>) and Treatment (Tr, 4 fixed levels: CON, PE, PU, MIX) and their interaction (Ti×Tr). PERMANOVA tests were carried out on Euclidean distances resemblance matrixes of previously normalized data. All statistical analyses were performed using the software PRIMER 7, using the routine included in the package PERMANOVA+ (Anderson et al., 2008). When the interaction between factors was significant, post-hoc tests were conducted. Multivariate differences in organic matter biochemical composition among treatments at each of the sampling date were visualized using MDS plots produced through 'bootstrap averages' function of PRIMER7, which calculates bootstrap averages and confidence regions, based on the same resemblance matrix of normalized data.

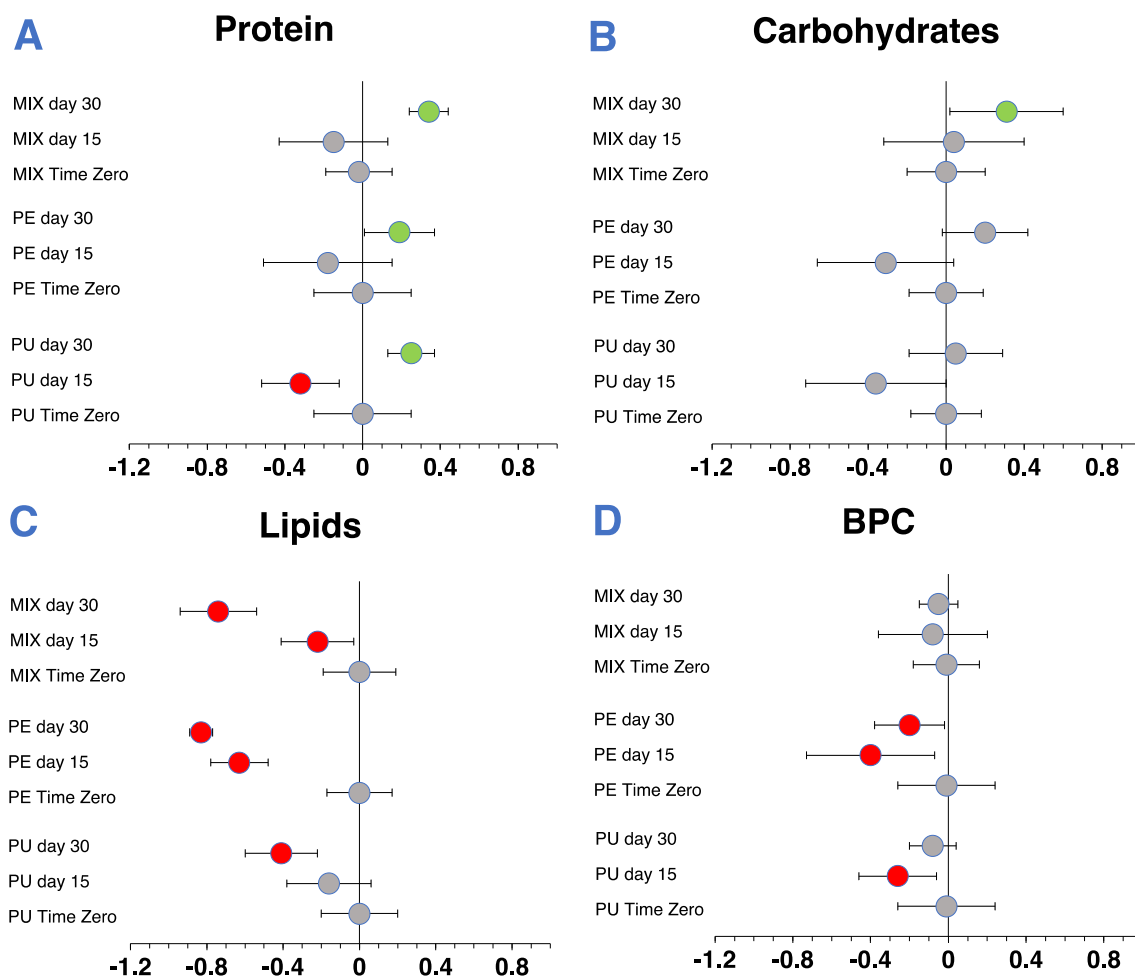
For each treatment, the patterns and amplitude of change across time in C degradation rates and turnover, aminopeptidase and alkaline-phosphatase activities were visualized using forest plots obtained using Log-transformed ratios of mean values in impact (i.e., MPs contaminated) on values in control microcosms. Forest plots were performed using R Studio (R Studio Team, 2016), through the meta-analysis packages "metafor" (Viechtbauer, 2010) and "robumeta" (Fisher and Tipton, 2015); the original script, created by (Quintana, 2015), was modified using the Log-transformed ratios of means.

### 3. Results

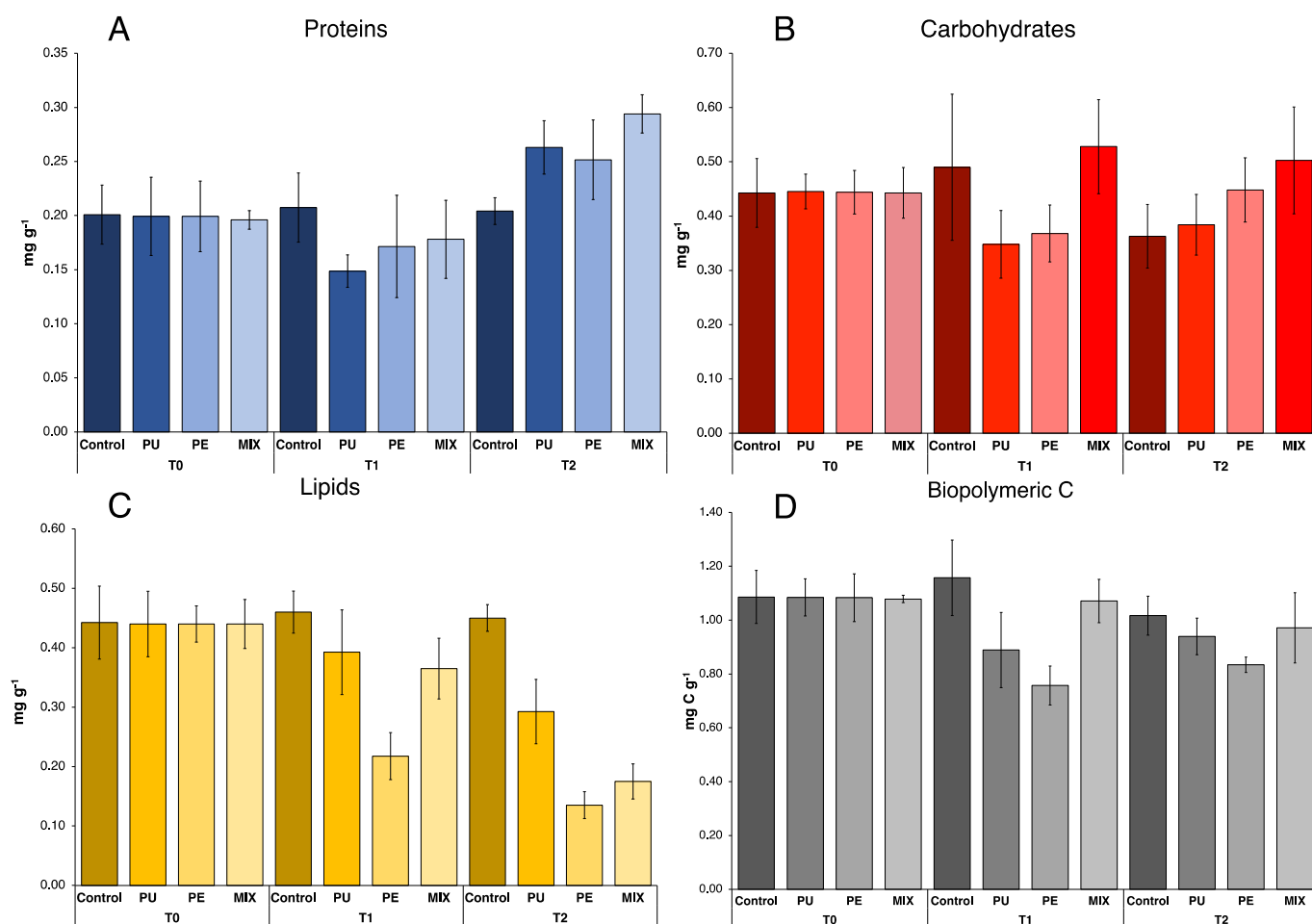
#### 3.1. Biochemical composition and trophic status

MPs contamination exerted a positive effect (i.e., increase) in the protein contents of sediments, which were all significant after 30 days of exposure, but no significant difference was detected among treatments (Figs. 1, 2). For carbohydrates, neither treatment nor time of exposure had a significant effect except for the treatment MIX, which was significantly positive after 30 days. Lipid contents were negatively affected (i.e., decreased) already after 15 days of exposure, with the treatment PE being significantly different from the MIX one. The largest effect was observed after 30 days of exposure in the treatment PE, which was significantly higher than the effect exerted by the treatment PU (Fig. 1). MPs contamination showed negative effects on BPC sedimentary contents after 15 days of exposure in PU and PE treatments, whereas the only significant negative effect after 30 days of exposure was observed in PE. The MIX treatment did not show any significant effect (Figs. 1, 2).

The biochemical composition of sedimentary organic matter was affected by a significant interaction of the two factors (Supplementary Table S2). The results of the bootstrap analysis and the resulting MDS representations show that, at the beginning of the experiment, control and treated sediments exhibited the same biochemical composition, whereas the biochemical composition of control sediments clearly segregated from those of the treated ones in T<sub>1</sub> and, even more clearly, in T<sub>2</sub> (Fig. 3A-C). Regardless of the treatment, at T<sub>0</sub> sediments were



**Fig. 1.** Forest plot (LnR of means as effect size) showing significant negative (red dots) or positive effect (green dots) in BPC by MPs contamination across investigated ecosystem functioning variables. Bars represent the standard deviation and grey dots are not significant effects.



**Fig. 2.** Temporal variations (in mg g<sup>-1</sup>) of: A) protein; B) carbohydrate; C) lipid and D) biopolymeric C contents in control sediments and sediments added with microparticles made of polyurethane (PU), polyethylene (PE), and a mixture of different polymers (MIX). T<sub>0</sub>: start; T<sub>1</sub>: day 15; T<sub>2</sub>: day 30. Error bars represent standard errors (n = 3).

characterized by the dominance carbohydrates (55 %) over proteins (25 %) and lipids (18 %). Biochemical composition varied in T<sub>1</sub> and T<sub>2</sub> and was significantly different between controls and contaminated sediments, with different variations observed according to treatments (Supplementary Table S3; Fig. 3D). In T<sub>1</sub>, sediments added with PU were characterized by a slightly increasing lipid contribution to BPC, at the expenses of the carbohydrate one, whereas sediments added with PE showed slightly increasing protein and carbohydrate contributions at the expense of the lipid one. In T<sub>1</sub> sediments added with MIX were characterized by an increasing contribution of carbohydrates. In T<sub>2</sub> all treatments were characterized by a consistent increase in the protein and to a lesser extent carbohydrates contribution to BPC, at the expenses of lipids (Fig. 3D).

### 3.2. Extracellular enzymatic activities, C degradation rates and turnover time

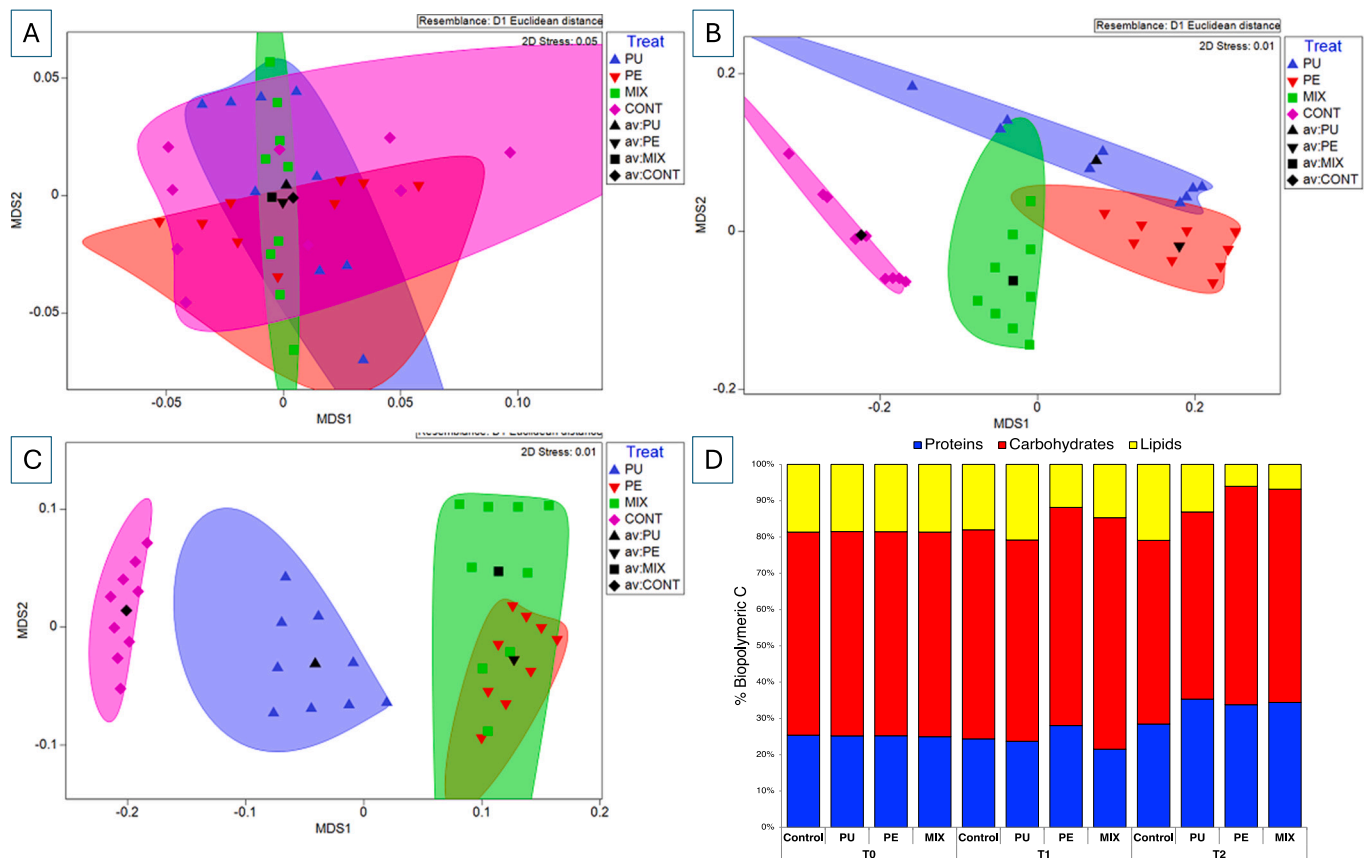
EEA showed significant differences between contaminated and control sediments after either 15 or 30 days (Figs. 4, 5). Aminopeptidase activity in the controls did not vary significantly with time, whereas it significantly decreased (Supplementary Table S4) in all contaminated sediments, with maxima of a -33 % variation after 15 days when exposed to PU and after 30 days when exposed to MIX, followed by PE after 15 days (by -24 %) (Fig. 4A). The  $\beta$ -Glucosidase activity increased significantly only in the control group, while it did not show significant variation in any of the contaminated sediments (Fig. 4B; Supplementary Table S5). When compared with the controls, alkaline-phosphatase

activity significantly increased (by 34 % whatever the added polymer; Supplementary Table S6) in all contaminated sediments after 30 days.

The effect size of plastic contamination on EEA, regardless of the added polymer, was similar after 15 and 30 days, with negative effects on both aminopeptidase or  $\beta$ -glucosidase activities, while the alkaline-phosphatase activity showed positive effects (Fig. 4A-C). The size effect of plastic contamination was in the same order of magnitude on either aminopeptidase and  $\beta$ -glucosidase activities (-0.3 on average). The effect was least pronounced after 15 days on alkaline-phosphatase and most pronounced after 30 days also on alkaline-phosphatase (Fig. 4D).

When compared with controls, sediments contaminated with microplastics, regardless of the added polymer, showed a significant decrease in C degradation rates with time (Supplementary Table S7). The largest decrease was measured in sediments contaminated with PU after 15 days, in which C degradation decreased by 39 %, followed by the decrease measured in sediments contaminated with PE after 15 days (by 29 %) and MIX after 30 days (by 23 %) (Fig. 4A). After 30 days of exposure, MPs contamination, regardless of the polymer, also caused a significant increase of the turnover time of carbohydrates and proteins (by ca. 32 %-98 % and 45 %-100 %, respectively) when compared to control sediments (Supplementary Table S7; Fig. 4B Fig. 5A). The forest plot shows that the largest increase was observed for MIX (LnR = 0.51  $\pm$  0.15; 95 % CI), followed by PU (LnR = 0.46  $\pm$  0.13; 95 % CI) and PE (LnR = 0.35  $\pm$  0.13; 95 % CI). For all treatments, the observed effects were significant only after 30 days of exposure (Fig. 4).





**Fig. 3.** MDS biplots showing temporal changes in the biochemical composition (in terms of protein, carbohydrate, and lipid contents) of organic matter in sediments contaminated with polyurethane (PU), polyethylene (PE), and a mixture of different polymers (MIX): A) at the beginning of the experiment (T<sub>0</sub>); B) after 15 (T<sub>1</sub>) and C) 30 (T<sub>2</sub>) days of exposure to microplastics. D) temporal changes in the relative contribution of proteins, carbohydrates, and lipids to biopolymeric C contents in control and treated sediments.

#### 4. Discussion

We report here that a pulse-like injection of MPs can alter coastal surface sediments biogeochemistry, by provoking either significant change in the quantity and biochemical composition of the sedimentary organic loads and impairment of extracellular enzymatic activities. Overall, these effects led to a significant decrease of the whole BPC sedimentary content and a significant slowdown in C degradation processes. These results might indicate how, already after 15 days of exposure, the semi-labile fraction of the C pool (Pusceddu et al., 2009) has significantly been reduced, leaving only more recalcitrant compounds available for degradation.

Plastic pollution has recently been pointed out as a ‘new biogeochemical cycle’ (Bank and Hansson, 2019), for which sources, transport, fate, and environmental reservoirs should be better understood. In this perspective, our results suggest that MPs accumulated in marine sediments could potentially foster a regression towards a more oligotrophic state and reduce food availability for higher trophic levels. If so, this would represent a “vertical pump” that further enhances the accumulation and possible longer term storage of recalcitrant organic C in coastal seabed (Galgani and Loisele, 2021).

##### 4.1. Effects on biopolymeric carbon

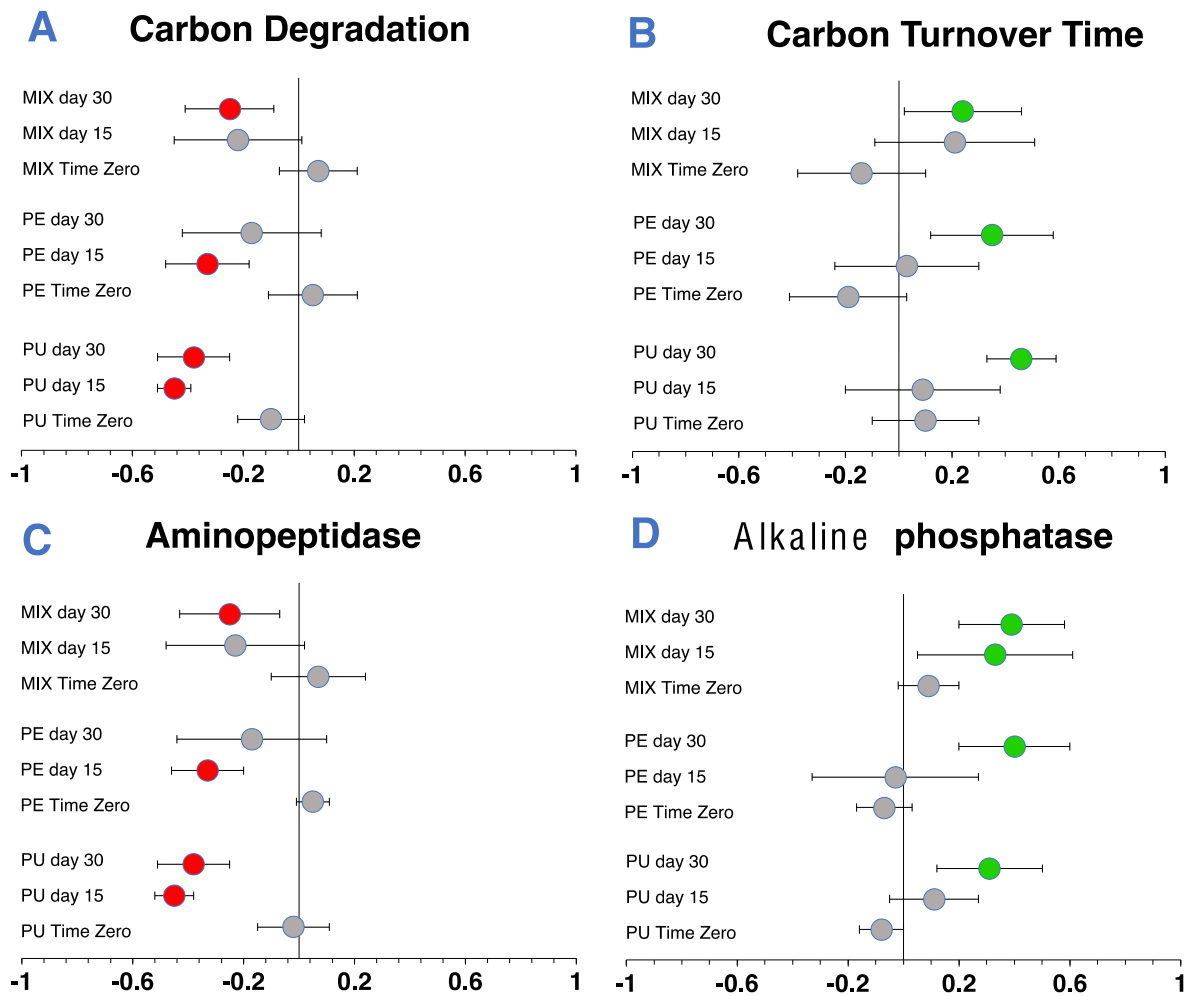
We observed that MPs contamination exerted differential effects on sedimentary contents, by enhancing (compared to controls) proteins, abating lipids and having no significant effects on carbohydrates, except for the MIX treatment. We also document a significant trophic shift in the relative contributions of proteins, carbohydrates, and lipids to

sedimentary BPC, that progressively differed among treatments with the increasing time of exposure (Fig. 3D).

The increase in protein for all contaminated sediments could be due to the increase in proteinaceous material associated with bacteria colonizing plastic particles, that can serve as a potential energy source for heterotrophic prokaryotes.

Lipids are a high nutritional quality and labile biomolecule; thus, they are generally rapidly digested. Sediments contaminated with PU (obtained by the fragmentation of a polyurethane foam block) were characterized by a slightly increasing lipid contribution to BPC, at the expenses of the carbohydrates. Due to their matrix structure, polyurethanes are prone to adsorbing substances with prolonged release time and are thus used for e.g., as controlled-release urea fertilizer in agriculture, or as drug delivery vehicles (Liu et al., 2019; Wienen et al., 2023). The PU microstructure could thus have promoted the absorption of lipids into the polymer matrix. On top of this, weathering could also have exacerbated lipid adsorption due to altered roughness, polarity and porosity. This, in turn, could also alter biofouling processes, microbial colonization, sorption and adhesion of organic compounds (Ammar et al., 2015; Zheng et al., 2021). An additional hypothesis may involve the disruption of the trophic functionality of the microcosm community due to the toxicity of the MPs used in the exposure treatments. This toxicity may have caused a reduction in the lipid renewal and an increase in the production of proteinaceous material.

Carbohydrate content was unaffected by MPs exposure, with the only exception of the MIX treatment after 30 days; this result is in accordance with the paradigm that considers MPs as a source of recalcitrant carbon (Chamas et al., 2020; Stubbins et al., 2021). The increase in carbohydrates observed for the MIX treatment after 30 days could be due to the



**Fig. 4.** Forest plot (LnR of means as effect size) showing significant negative (red dots) or positive effect (green dots) determined by MPs contamination across investigated ecosystem functioning variables: A) Carbon degradation rates (in days); B) Carbon turnover rates (in days); C) Aminopeptidase activity ( $\text{nmol g}^{-1} \text{h}^{-1}$ ) and D) Alkaline phosphatase ( $\text{nmol g}^{-1} \text{h}^{-1}$ ). Bars represent the standard deviation and grey dots are not significant effects.

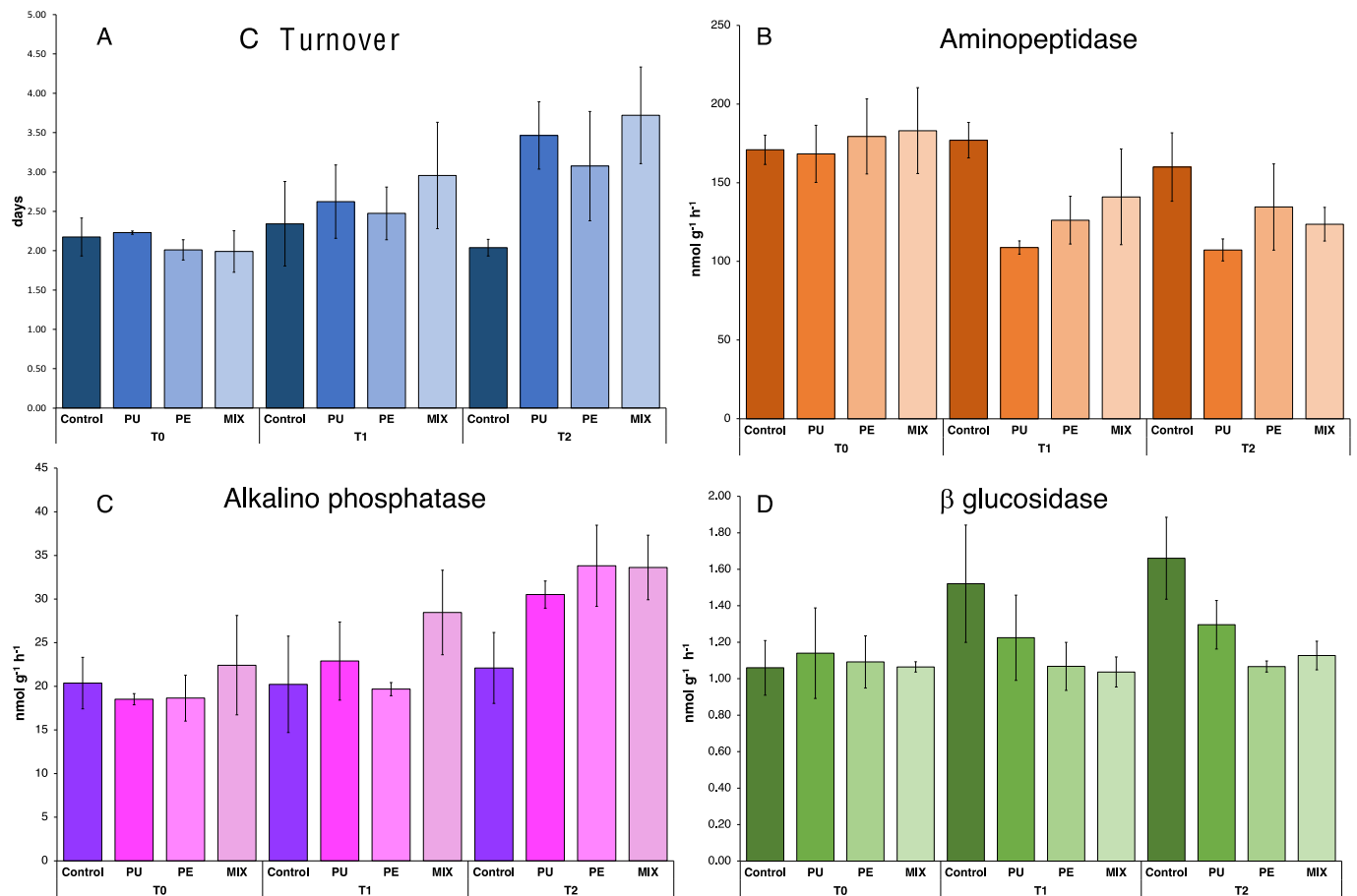
heterogeneity of polymers used in that specific treatment compared to others. This heterogeneity may have fostered a more complex and carbohydrate-rich biofilm over time. Indeed, as per any material entering aquatic environments, MPs are subject to biofouling processes that usually begin with microbial colonization of the plastic surface, leading the formation of the so called 'plastisphere' (Zettler et al., 2013). This refers to the microbial community colonizing plastic debris, resulting in niche segregation between marine microorganisms inhabiting the plastisphere and those in the surroundings environment.

Polyurethane contains nitrogen in its polymer backbone and is more susceptible to hydrolytic cleavage compared to compounds with solely C backbones like PE and other polymers composing the MIX treatment (Gewert et al., 2015; Seeley et al., 2020). Thus, it could be possible that PU promoted a more pronounced positive trophic response, stimulating the activity of various bacteria capable of retrieving C and N as energy sources and resulting in increased proteinaceous content associated with bacteria (Howard, 2002). For instance, Seeley et al., 2020 showed that oligotrophic salt marsh sandy sediments treated with PU foam exhibited a variation in the microbial community composition over time. Such shift could have favoured the fraction of microorganisms capable of degrading PU by the production of hydrolytic enzymes such as proteinase, urease, and esterase (Howard, 2002).

Polyethylene is the most abundant polymer in production, the most common in single-use containers and also the most discarded synthetic polymer, globally (Geyer et al., 2017). Plastic is known to release a

variety of chemicals during degradation, which has a negative impact on biota; on top of this, recent evidence highlighted how PE is also the most prolific emitter of greenhouse gases (methane and ethylene) when exposed to physical weathering, that is likely to occur in coastal environments (Royer et al., 2018). Considering the overall slowdown of C cycling here observed, it would be of great relevance to determine whether accumulation of prevalently recalcitrant organic C (Galgani and Loisele, 2021) deriving from MPs could foster enhanced remineralization and consequent emission of carbon dioxide from the seabed, as recently documented in experimental condition for estuarine mangrove environments (Lin et al., 2024). Hence, MPs like PE might be playing an even greater role in the carbon cycle than anticipated.

Regardless of the variable considered, we observed how effects generated by the mixture of polymers (treatment MIX), despite not being significantly different, were either smaller or absent compared to those exerted by the same concentration of a single polymer, with PU being the most distinctive one (Fig. 2). The MIX treatment is surely not comprehensive of all possible environmental contamination of MPs in sediments, but it had the scope to embody a reasonable range of possible features that may be encountered, since it would be unlikely that such concentrations could be reached by a single polymer in environmental conditions.



**Fig. 5.** A) Carbon turnover rates (n. of days); B) Aminopeptidase activity rates ( $\text{nmol g}^{-1} \text{h}^{-1}$ ); C) Alkaline phosphatase activity rates ( $\text{nmol g}^{-1} \text{h}^{-1}$ ); D) Beta glucosidase activity rates ( $\text{nmol g}^{-1} \text{h}^{-1}$ ) across different treatments (PU: polyurethane; PE: polyethylene; MIX: mixture of different polymers) and days of exposure to MPs (T0: Start; T1: 15 days; T2: 30 days).

#### 4.2. Carbon degradation rates and extracellular enzymatic activities

The differential effects observed across treatments over time could be explained by a different selection pressure on microorganisms with unique traits (such as antibiotic resistance, hydrocarbon degradation, and heavy metal adsorption) that might have led to substantial shifts in the patterns and succession of the associated microbial communities. This, in turn, can be ascribed as possible explanation of the differences in EEAs that we measured in our experiment. EEAs are the key step in the degradation and utilization of organic polymers by bacteria, that foster OM transfer to higher trophic levels by means of the so-called ‘microbial loop’ (Azam et al., 1983). Because of this, EEAs have been repeatedly used as a proxy of benthic ecosystem functioning (Danovaro et al., 2008; Zeppilli et al., 2016) and indicators to evaluate different typologies of impacts affecting sedimentary environments (Pusceddu et al., 2014; Soru et al., 2022). Our results show that aminopeptidase activity was compromised for up to ~30 % in all treated sediments (Fig. 5B), whereas  $\beta$ -glucosidase increased in control sediments after 15 and 30 days, while remained unvaried in treated sediments (Fig. 5C). These results suggest that, at least in the short-to-medium term, the presence of MPs has a more pronounced effects on protein degradation processes than carbohydrates, which indeed did not show any significant change in quantity. Since proteins, together with lipids, represent the most labile class of organic compounds and are more rapidly digested than carbohydrates (Pusceddu et al., 2003), our results suggest that MPs contamination could exert effects on the bioavailability of resources for benthic detritus feeders. This hypothesis is supported by the increase in sedimentary protein contents observed in all treatments after 30 days of exposure to

MPs, regardless of the treatment, which can be reliably interpreted as a progressive accumulation of these labile molecules, likely due to enhanced microbial activity. However, we cannot exclude the possibility that such increase could also be attributed to other factors.

We notice here also that MPs contamination, again whichever the treatment, exerted a positive effect on alkaline-phosphatase activities (APA). APA are implied in organic P remineralization processes mediated by algae to hydrolyze dissolved organophosphate and obtain phosphorus when the preferred dissolved inorganic phosphorus is present in limited supply (Zhang et al., 2021). The overall increase of APA accompanied by a general decrease of protease activities has been suggested as a proxy for P deficiency in microbial assemblages (Danovaro et al., 2005; Sala et al., 2001). Our results, thus, suggest that, besides the impairment of protein degradation processes mediated by heterotrophic activities, MPs contamination can exert also relevant effects on the metabolism of the autotrophic component of the benthos. Since APA increased only in contaminated sediments but not in the control ones, we stress here that, most likely, MPs contamination can provoke more stressful conditions for the microphytobenthos because of a progressively increasing limitation of inorganic phosphorus. Although we did not measure inorganic phosphorus concentrations during the experiment, such hypothesis is corroborated by previous manipulation experiments that reported that: i) the presence of microplastic biofilms can accumulate P temporarily and increase APA in aquatic environments (Chen et al., 2020) and ii) the presence of microplastics can significantly decrease soil available phosphate content.

It is also important to acknowledge that, to guarantee a proper isolation of different effects and factors involved in controlled



conditions, our static incubation experiment did not consider these aspects:

- i) new input of OM deriving from vertical fluxes or other possible sources, that would surely occur in natural conditions, and it could possibly influence effects here observed;
- ii) the vertical oxic/anoxic gradient of sediments was not considered in our experimental setup and analyses. It would be reasonable to expect different effects occurring above and below the redox discontinuity layer;
- iii) granulometry of sediments could represent a crucial factor to consider (e.g., [Filgueiras et al., 2019](#)), with muddy sediments being expected to show different responses, also due to their enhanced exposure to MPs in deep-sea ([Woodall et al., 2014](#));
- iv) dynamic physical parameters of the marine environment, among which temperature can be mentioned as the most representative, but also those driving the transport of solutes such as advection.

We stress here that these aspects should be considered and further explored in future efforts on the topic. Due to the complex nature of seafloor ecosystems and the wide range of factors that influence its functioning, it is necessary to further test these effects in fieldwork based experiments, e.g., as recently did in New Zealand, using 'ROMA' plates ([Ladewig et al., 2023, 2024](#); [O'Meara et al., 2018](#)).

Our approach did not focus on the sedimentary microbial diversity but rather on variables that describe and quantify alterations on carbon cycling (and consequent benthic ecosystem functioning), deriving from MPs contamination. To do so, we used relevant concentrations that were over ~50 % lower than those used for laboratory experiments using salt marsh sediments, that documented for the first-time alteration in Nitrogen cycling ([Seeley et al., 2020](#)). Recent pioneering field-based studies on marine sediments documented how the addition of microfibers to OM already present in marine sediments shifted the importance of factors controlling consumption rates ([Ladewig et al., 2024](#)), thus confirming how the input deriving from MPs accumulated on the seafloor triggers an important impairment in OM consumption and cycling. For marine environments, in particular, it will be crucial to assess effects from deep-waters, Oceans' greatest plastic and MPs accumulation areas ([Carreras-Colom et al., 2024](#); [Peng et al., 2020](#); [Woodall et al., 2014](#)).

## 5. Conclusions

Seafloor ecosystems provide many benefits to humans ([Snelgrove, 1999](#)), and it is important that we understand changes due to increasing plastic pollution loads, that occur in parallel with other stressors. The outcomes of our experiment support the hypothesis that MPs may relevantly affect marine sediment biogeochemistry, potentially triggering effects that echo up to the top of the ecological hierarchy. We thus further confirm how plastic contamination strongly deserves to be regarded as a new planetary boundary threat ([Persson et al., 2022](#); [Villarrubia-Gómez et al., 2018](#)). Most importantly, results here presented are foundational and call for future effort that combines microbiology and biogeochemistry, to properly assess ecosystem-wide effects deriving from MPs accumulation in diverse reservoirs of the marine environments.

## CRedit authorship contribution statement

**Alessandro Cau:** Writing – original draft, Methodology, Formal analysis, Data curation, Conceptualization. **Davide Moccia:** Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Claudia Dessi:** Writing – original draft, Methodology, Data curation. **Laura Carugati:** Writing – original draft, Methodology, Data curation, Conceptualization. **Ester Carreras-Colom:** Writing – original draft, Visualization, Data curation. **Fabrizio Atzori:** Writing – original draft, Resources. **Nicoletta Cadoni:** Writing – original draft,

Investigation, Data curation. **Antonio Pusceddu:** Writing – original draft, Validation, Resources, Methodology, Conceptualization.

## Ethical approval

Not applicable.

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## 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.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scitotenv.2024.176795>.

## Data availability

Data will be made available on request.

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