Marine Pollution Bulletin 230 (2026) 119779

Contents lists available at ScienceDirect

Marine Pollution Bulletin

journal homepage: www.elsevier.com/locate/marpolbul

A tale of two stressors: Nitrogen, microplastics, and their influence on estuarine organic matter degradation

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ARTICLE INFO

Dataset link: [Article Dataset] A tale of two stressors: nitrogen, microplastics, and their influence on estuarine organic matter degradation (Original data)

Keywords:
Multiple stressor
Organic matter degradation
Soft sediment
Microplastic
Nitrogen loading
In situ

ABSTRACT

Estuaries are highly dynamic systems with strong physicochemical and biological gradients that drive ecosystem functions. Increasing anthropogenic pressures have altered carbon cycling and degradation processes and reduced key ecosystem functions, leading to a marked decline in global estuarine health. This study investigates the individual and combined effects of two common anthropogenic stressors (microplastics and nitrogen) across a gradient of soft-sediment habitats with contrasting infaunal communities that reflect dominant functional traits: head-down deposit-feeding polychaetes, deep-dwelling facultative-feeding bivalves, and a mix of both. *In situ* rapid organic matter assays (ROMA) were used to assess whole-community organic matter degradation using media with different stressor combinations (nitrogen addition, microplastics, or both). Separate models were developed for each treatment, with predictors selected using the Akaike Information Criterion (AIC) to achieve model parsimony without compromising model fit. Our results clearly demonstrate that, in this system, single stressor models may not adequately capture organic matter cycling in sediments following exposure to multiple stressors. In single-stressor treatments, the role of sedimentary organic matter content on organic matter degradation increased significantly in plastic-treated media, and the density of a head-down deposit feeding polychaete was significantly related to the extinction rate of organic matter with sediment depth in nitrogen treated media. These relationships were decoupled when a secondary stressor was added in the multiple-stressor treatment. While direct effects of nitrogen and microplastic addition were not detected, the treatment-specific models indicate that environmental drivers of degradation vary across stressor contexts, highlighting nuanced estuarine responses to anthropogenic pressures.

1. Introduction

Estuaries are complex and functionally important ecosystems, providing key ecosystem services including nutrient cycling, primary production, and food web provisioning (Costanza et al., 1997; Levin et al., 2001). Tidal flats in particular have been underrepresented in the literature as important sinks and hotspots of organic carbon processing, largely because their carbon burial rates are lower than those of more structurally complex estuarine habitats such as fjords (Smith et al., 2015), mangroves (Bulmer et al., 2020), and tidal marshes (Mazarrasa et al., 2023). However, due to the expansive nature of estuarine tidal flats, they proportionally can contribute substantially to carbon dynamics (Bulmer et al., 2020). Globally, estuaries are among the fastest declining natural ecosystems due to increasing anthropogenic pressures (Halpern et al., 2019; Lotze et al., 2006). The cumulative impacts of multiple stressors have caused a rapid decrease in estuarine biodiversity and ecosystem functioning (Gammal et al., 2023; O'Brien et al., 2019), making it critically important to understand how multiple stressors impact estuarine carbon processing in intertidal sandflat habitats with functionally diverse macrofaunal communities.

Experimental research into the interactive effects of multiple stressors (defined here as two or more stressors) is still in its infancy and primarily conducted under laboratory conditions on single species (e.g. Hiltunen et al., 2021; Serra et al., 2020). Microplastics (plastics <5 mm)

Abbreviations: aRPD, Apparent redox potential discontinuity; C, Carbon; C_0, Organic matter degradation rate (gC/m^2/day); k, Extinction coefficient; MP, Microplastics; MPB, Microphytobenthos; N, Nitrogen; OM, Organic matter; ROMA, Rapid organic matter assay.

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https://doi.org/10.1016/j.marpolbul.2026.119779
Received 20 January 2026; Received in revised form 17 April 2026; Accepted 19 April 2026

are an emerging anthropogenic stressor that are widespread in estuarine environments (Gray et al., 2018; Malli et al., 2022; Peng et al., 2017; Radhakrishnan et al., 2021) and often occur with other stressors in coastal sediments. Laboratory experiments have shown that microplastics disrupt invertebrate behaviours, including reduced feeding, bioturbation, and burrowing behaviour (Green et al., 2017; Green et al., 2016; Hope et al., 2020; Wright et al., 2013). Although it is important to understand the effects of microplastics on the behaviour of single species, a common critique of laboratory studies is their lack of ecological complexity (Kotta et al., 2022; Weis and Palmquist, 2021). Despite advancements in adding ecological complexity to laboratory experiments by increasing the number of species studied simultaneously (Näkki et al., 2017; You et al., 2023) or investigating multiple stressor combinations (Foreman et al., 2026; Hiltunen et al., 2021; Serra et al., 2020), there is still a paucity of research into the effects of microplastics *in situ* with intact whole biological communities. In freshwater systems, *in situ* mesocosms have been employed to examine the effects of microplastics within the ecological complexity of lakes (Rochman et al., 2024; Yıldız et al., 2022). However, to date only one *in situ* study in an estuarine environment has been published by Ladewig et al. (2024), who found that microplastic presence disrupted ecosystem relationships and influenced organic matter cycling within soft sediment habitats. Specifically, polyester microfibers altered which environmental factors controlled organic matter consumption, indicating interference with natural sediment processes. In contrast to microplastic research, several studies have investigated the *in situ* effects of nitrogen loading (Douglas et al., 2016 and references therein), showing excess nitrogen to disrupt normal biogeochemical cycling, reduce bottom-water oxygen, and alter the quality of basal food sources. These effects are further exacerbated when coupled with additional stressors such as increased sedimentation or reduced light (Douglas et al., 2018; Thrush et al., 2014). Although the combination of microplastics and nitrogen on marine systems has not been studied in the field (see Corinaldesi et al., 2022), freshwater laboratory experiments have found that the combination of ammonium and microplastics have negative synergistic effects on Daphnia filtration (Serra et al., 2020) and that nitrogen-induced shifts in food quality are a more important predictor of Daphnia health than microplastics (Hiltunen et al., 2021). A marine laboratory mesocosm experiment further showed that the cooccurrence of microplastics and nitrogen can alter benthic biogeochemical processes in soft-sediment (Foreman et al., 2026). These studies demonstrate the potential for interactive effects between these two common anthropogenic stressors, highlighting the need for further investigation in estuarine intertidal habitats.

Carbon processing is mediated, in part, by the presence of key macrofaunal species that constantly modify the habitat and drive biogeochemical cycling in the sediment (Lohrer et al., 2004; Thrush et al., 2006). Tidal flats are home to a diverse assemblage of macrofaunal species that drive carbon cycling through organic and inorganic particle transport from activities including feeding, respiring, burrowing, bioturbating, and bio-irrigating the sediment (Ehrnsten et al., 2019; Snelgrove et al., 2018), thereby creating patchiness in biogeochemical functions based on dominant functional traits in the infaunal community (Douglas et al., 2017; Schenone and Thrush, 2020). Two macrofaunal species that are common in New Zealand tidal flats are *Macomona liliana* (tellinid bivalve) and *Macroclymenella stewartensis* (maldanid polychaete), both of which contribute to biogeochemical cycling and represent contrasting traits. *M. liliana* are surface facultative deposit feeders that reside 10–20 cm below the sediment surface and bioirrigate the sediment (Woodin et al., 2016); whereas, maldanid polychaetes are head-down deposit feeders which act as a particle conveyer belt, moving anoxic sediment and organic matter to the sediment surface. Preferred habitat for these two species overlap and in the transition zones, non-linear interactions on carbon cycling have been recorded, with carbon degrading slower and extinguishing faster with depth where both organisms are present compared to sites with only single species (Schenone and Thrush, 2020). It is therefore important to account for habitat and biotic community heterogeneity when making inferences about ecosystem responses to additional stress in order to scale up potential effects and allow for greater inferential strength in complex systems (Hewitt et al., 2007). By using *in situ* assays across a gradient of infaunal communities, it is possible to account for habitat heterogeneity and to extrapolate the results at scale.

The present study investigates the individual and combined effects of microplastics and nitrogen enrichment on organic matter degradation rates across natural biological communities representing different functional traits. We hypothesise that 1) combined stressors will alter whole community organic matter degradation rates, and 2) combined stressors will have a different effect than single stressors (although the magnitude and direction are difficult to predict due to complex interactions). Further, we predict that dominant macrobenthic infauna will explain some of the variation in organic matter degradation rates due to their varying functional traits and influence on sediment degradation processes. To test these hypotheses, we selected the above-mentioned common New Zealand deposit feeders with contrasting functional traits (the tellinid bivalve: *M. liliana* and maldanid polychaete*: M. stewartensis*) and embedded our study across a natural gradient of their habitats, ranging from predominantly bivalve-dominated areas to predominantly polychaete-dominated zones and an area where the species distribution overlapped. *In situ* Rapid Organic Matter Assays (ROMA) were used to assess whole-community organic matter degradation (O'Meara et al., 2018). The agar media added to the assays were treated with microplastics, nitrogen addition or combined stressors.

2. Materials and methods

2.1. Site selection and experimental setup

The study was conducted in Papanui Inlet (45° 50′ 43.66″ S, 170° 42′ 38.45″ E), Dunedin, New Zealand (Fig. 1), during the austral summer of 2024 (February). Papanui Inlet is a shallow, intertidally dominated estuary, with approximately 65–75% of its 3.7 km^2 area exposed at low tide (Albrecht and Vennell, 2007). The extensive unvegetated sandflats exhibit spatial heterogeneity and support dense beds of the bivalve *Austrovenus stutchburyi*, along with populations of *Macomona liliana* and *Macroclymenella stewartensis* (Karlson et al., 2021). Study sites within this estuary were chosen based on microtopographic sediment surface features that indicated the presence and approximate density of study infaunal species (Schenone and Thrush, 2020). Specifically, selection criteria focused on the contrasting functional traits of two key species: the facultative deposit-feeding tellinid bivalves, *Macomona liliana*, known for creating star-like surface features associated with feeding, and the head-down deposit-feeding maldanid polychaete, *Macroclymenella stewartensis*, distinguished by their creation of small faecal mound surface features (see Supplementary Material, Fig. S2). Three distinct sites were chosen: one dominated by tellinid bivalves, another by maldanid polychaetes, and a third characterised by the co-occurrence of both species (Fig. 1C). Each site, separated by approximately 20–30 m, represented a 10 × 20 m block. Within each site, seven replicates of each ROMA plate containing a stressor treatment *i.e.*, nitrogen addition, microplastic addition, or multiple stressor addition or control, were randomly placed, totalling 28 assays per block. Plates were left to incubate on-site for 10–11 days with their well openings facing the channel to allow for measurable organic matter degradation to occur. To prevent potential interference between plates with different treatments, a minimum distance of 1.5 m was maintained between each plate. A temperature and light logger (HOBO®, Onset Computer Corporation, USA) was deployed at the centre of each site at the sediment surface during the experiment to account for site-specific water immersion times.

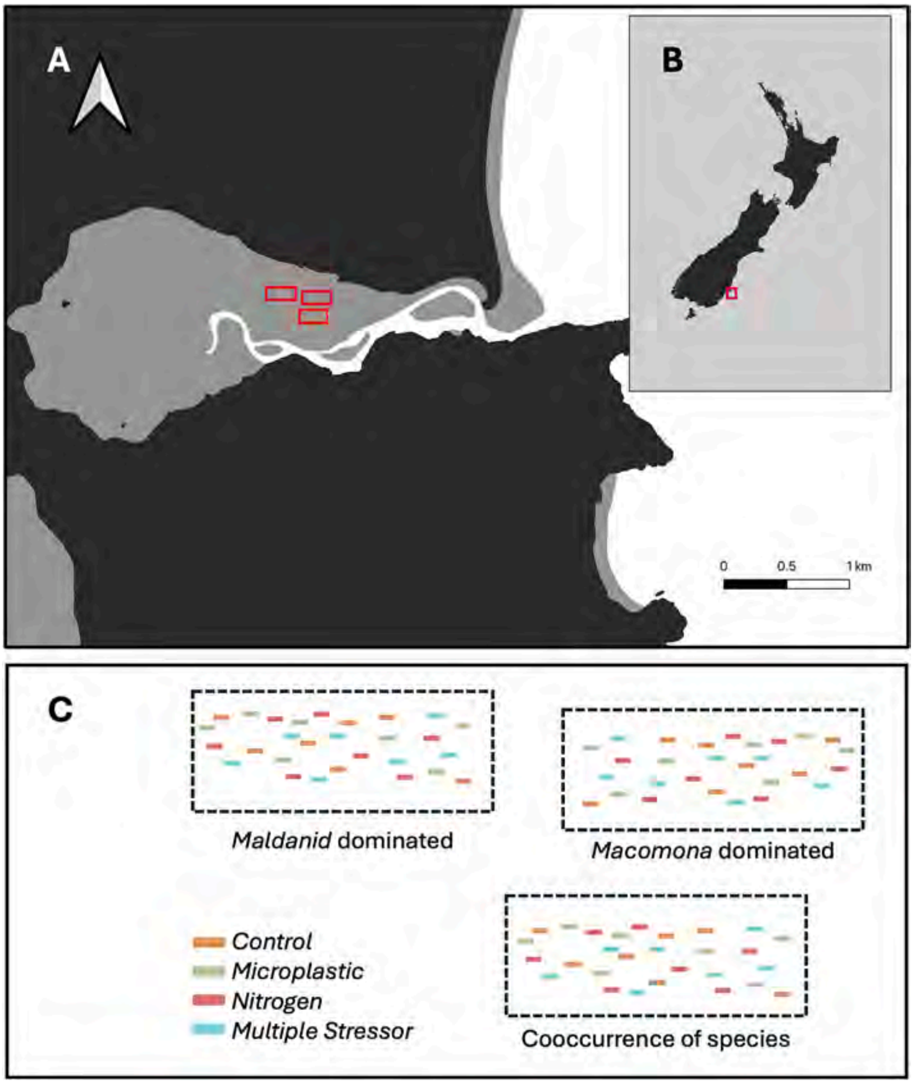

Fig. 1. Study site and experimental design in Papanui Inlet, New Zealand. (A) Location of experimental blocks within the estuary (red rectangles). (B) Location of Papanui Inlet within New Zealand. (C) Schematic of the experimental setup. Geographic data from Land Information New Zealand (LINZ); map produced using QGIS. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

2.2. ROMA plate preparation

Rapid organic matter assay (ROMA) plates were employed to evaluate organic matter degradation rates within the sediment following the methods of O'Meara et al. (2018). ROMA plates have been used recently in New Zealand and Australian soft-sediment habitats to understand stressor impacts along environmental gradients (Ladewig et al., 2024; Lam-Gordillo et al., 2022; Schenone and Thrush, 2020; Sowerby, 2023).

Each ROMA plate was comprised of an acrylic sheet measuring 18 cm × 9 cm × 1.5 cm, featuring wells with a volume of 0.9 ml bored into the face of the plate and arranged in three vertical columns. For this study, each plate was considered one replicate, and the three columns of wells were averaged to account for inter-well variability within a single plate. When plates were deployed vertically within the sediment, the wells were strategically distributed to measure organic matter degradation at depths of 1 cm, 3 cm, 5 cm, 7 cm, 10 cm, and 15 cm, which coincides with changes in redox conditions and living positions of dominant infauna (Fig. 2).

ROMA plate media was adapted from the original protocol by O'Meara et al. (2018) and further customised into three treatments: Nitrogen Addition, Microplastic Addition, and Multiple Stressors (Nitrogen + Microplastic). The control treatment consisted of unaltered media, while nitrogen and microplastics were added to create the respective treatments. Specifically, 166.7 μl of ammonium hydroxide solution (~3 N in deionised water) per litre of media was added to both the nitrogen addition and the multiple stressors treatments to represent moderate nitrogen enrichment concentrations in New Zealand estuaries (Douglas et al., 2016). For the treatments containing microplastics, 0.5 g of red polyethylene beads (Cospheric 212–250 μm, 1.070 g/cm^3) per litre of media was added to both the microplastic addition and the multiple stressor treatments. This concentration was chosen to be in line with microplastic concentrations detected in New Zealand estuarine sediments (see Supplementary material, Table S1). Care was taken to maintain a homogenous distribution of plastics within each well (58 per well ±14). Before use in the ROMA plate media, microplastic beads were enclosed within fine mesh and incubated in a nearby harbour for three months to develop a biofilm. Upon retrieval, the beads were gently rinsed with fresh water and sonicated to remove excess epibionts before being weighed and added to the agar media. This process was used to simulate time in the water column prior to settlement in the sediment. Media was kept on a hot plate with a magnetic stirring bar and pipetted into the wells, overfilling each. Once cooled, a razor was used to remove excess media and ensure a consistent volume. Plates were wrapped in damp paper towels and placed in the refrigerator to reduce shrinkage

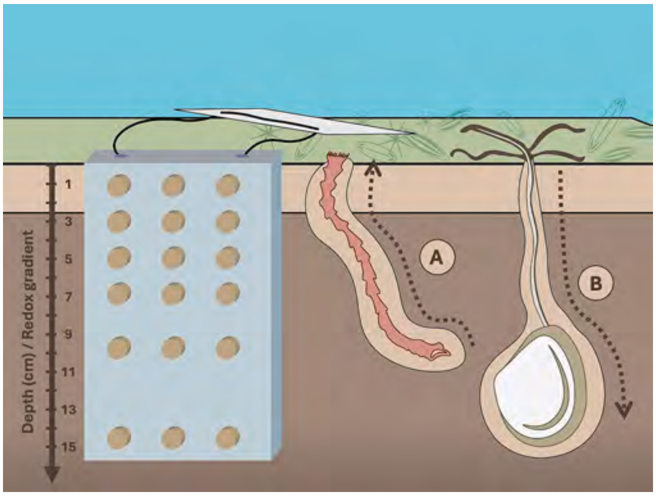

Fig. 2. Schematic diagram of ROMA plate deployed vertically in estuarine sediment. Macrofauna are not drawn to scale, however, the depth of macrofauna is indicative of living position. Dotted lines represent the direction of particle movement from (A) the head-down deposit-feeding maldanid polychaete Macroclymenella stewartensis which feeds at depth and excretes at the surface and (B) the facultative deposit-feeding bivalve Macomona liliana which feeds at the surface and excretes at depth.

before deployment in the field.

2.3. *Organic matter degradation analysis*

For the purposes of this study, organic matter degradation at the sediment surface (C_0) and organic matter extinction (k) are used as proxies for sediment carbon dynamics, which we do not measure directly but compute from the following equations. These methods and this terminology were introduced by O'Meara et al. (2018) and are retained for comparison with similar studies.

At the end of the *in situ* incubation, the ROMA plates were collected and kept damp and cool to avoid media shrinkage prior to analysis. Plates were analysed within 12 h of collection by gently rinsing off excess sediment with water and using a 1 ml syringe to fill wells with freshwater until level. The volume of water added is equivalent to the volume of agar lost allowing for the following calculations:

$$C = \frac{(V_0 - V_F)F}{d}$$

where C is carbon consumption rate in gC/day, V_0 is the initial agar volume, V_F is the final agar volume, F is the agar-carbon conversion factor of 0.029 gC/ml of media, and d is the number of incubation days.

A linear regression between depth and the natural log of C was used to estimate the extinction coefficient, k, which reflects how rapidly degradation declines with depth (between the sediment surface and 15 cm depth). Then, the organic matter degradation at the sediment surface, C_0, was determined using the following formula:

$$C_o = \frac{C}{e^{-kz}}$$

where C is the organic matter degradation rate at each depth (z). For full description of the methods and assumptions in the calculations, please refer to O'Meara et al. (2018).

2.4. *Benthic samples*

Sediment samples were collected adjacent to each ROMA plate one day prior to the end of the experiment. Three replicate sediment cores (1.5 cm diameter, 2 cm depth) around each plate were pooled, homogenised, and frozen at −20 °C to be used to measure sediment characteristics. An additional 2.6 cm diameter, 10 cm depth core was taken, carefully sliced in half, and photographed to allow for an estimate of the apparent redox potential discontinuity depth (aRPD) (Gerwing et al., 2018). A 1 m^2 quadrat was placed on the channel side of each plate and sediment surface features were photographed for subsequent analysis. Lastly, on the experimental end days, three macrofaunal cores (13 cm diameter, 15 cm depth) per treatment ($n = 12$ per site) were collected directly next to the open wells of the ROMA plates and sieved through a 500 μm mesh. Animals were preserved in ~70% ethanol and stained with Rose Bengal until analysis. Macrofaunal specimens were identified under a compound microscope to the lowest possible taxonomic level, with the majority being classified to species level (Supplementary Material, Table S2).

2.5. *Sediment analysis*

Several sediment characteristics were assessed including chlorophyll-*a* concentration, organic matter concentration, and grain size. To determine chlorophyll-*a* concentrations, which is a proxy for microphytobenthic biomass, 6 ml of 90% acetone was added to ~2 g freeze-dried sediment, sonicated for 30 s, incubated for ~20 h in a refrigerator, and vortexed once after 12 h. Samples were centrifuged at 3500 RPM for 3 min and absorbance was measured on a molecular device at 664 and 750 μm wavelengths. To determine the concentration of pheophytin, 0.15 ml of 1 N HCl was added to each sample and absorbance was measured at 665 and 750 μm. Calculations following those of Lorenzen (1967) were used to achieve final values. Care was taken to keep samples in the dark to minimise pigment degradation. Loss on ignition was used as a proxy for organic matter (OM) content. Samples were dried until stable weight and combusted in a muffle furnace for 4 h at 550 °C before being reweighed (Parker, 1983). The sediment grain size was determined by digesting sediment in 10% hydrogen peroxide and diffusing sediment with Calgon before using standard protocols for marine sediment on a Malvern Mastersizer 2000 (Singer et al., 1988).

2.6. *Photographic analysis*

Photos of downcore sediment samples (Supplementary Material, Fig. S1) and sediment microtopographic surface features (Supplementary Material, Fig. S2) were analysed using Image J (Version 1.53a). The depth of the aRPD was determined as the depth of the transition from oxic (indicated by grey sediment) to anoxic (indicated by black sediment). Three measurements were taken for each core and the average was calculated. In cores that showed no obvious black sediment, a cut-off depth of 10 cm deep was used. The abundance of bivalves and maldanid polychaetes was estimated by counting the distinctive sediment surface features within each quadrat photograph as per Schenone and Thrush (2020).

2.7. *Statistics*

The dataset included measurements of organic matter degradation rate at the sediment surface (C_0) and extinction with depth (k) as well as environmental parameters (sediment chlorophyll-*a* concentration [chl-*a*], apparent redox potential discontinuity depth [aRPD], mean grain size [mean grain], organic matter content [OM]), tellinid bivalve density, and maldanid polychaete density. Outliers were identified and removed from the dataset using z-scores with a threshold of 3 ($n = 5$ removed). Data was filtered to remove blanks, ensuring a complete dataset ($n = 57$) of a minimum of $n = 11$ for each treatment before being standardized (mean = 0, SD = 1) to reduce scale differences between variables.

To test for potential stressor interactions, factorial linear models with a threshold of $p < 0.05$ were fitted with C_0 (and separately k) as the response variable. Nitrogen enrichment and Microplastic addition were added to the model as fixed factors, and environmental predictors as continuous covariates. Due to the relatively small sample size (n = 57), a secondary version of the model was fitted using the first two principal components from a principal component analysis (PCA) of the environmental predictors as covariates. The first two PC axes explained 56% of the total variance and were interpreted as composites of the environmental condition (See Supplementary Material, Table S3). This approach was used to allow for specific testing of stressor interactions while accounting for variation in the environment among plots.

Site effects were evaluated as random intercepts during preliminary model building. A null mixed-effect model indicated that Site accounted for approximately 18% of the variance in C_0 and 2% of the variance in k when environmental predictors were not included in the model (see Supplementary Material, S3). However, a likelihood ratio test comparing models with and without Site showed no improvement in model fit when environmental predictors were included. Therefore, Site was excluded from the final models and individual plates were treated as independent replicates.

To investigate whether environmental drivers differed across stressor treatments, separate multiple linear regressions were performed for the control media and each treatment using C_0 as the dependent variable, environmental parameters as the predictor variables, and a threshold of $p < 0.05$. The environmental predictors were incorporated into the initial model as first-order terms, and higher-order terms (up to the third order) were included only when exploratory scatterplots or smooth fits indicated potential non-linear relationships or threshold responses. The model was then reduced using a backward stepwise procedure based on the corrected Akaike Information Criterion.

Environmental parameters were tested against organic matter degradation under control conditions and three different media treatment scenarios. For each treatment, the model selection procedure, using the backward AIC function in R, led to the removal of different variables resulting in four distinct models (Table 1). The same process was used to test environmental parameters against k (Table 2). Because models were fitted separately for each treatment, differences in predictor significance across treatments should be interpreted as indicative patterns rather than formal statistical differences.

In a similar study, Ladewig et al. (2024) applied the control-media model to stressor-media treatments to reflect real-world contexts where stressors are added to, not taken out of, the environment. This method was trialled for the present study, however individual models for each treatment were found to be more informative and to better capture potential functional drivers and have therefore been used. Lastly, univariate linear regression models were run for each predictor variable (see Supplementary Material, Table S4) to quantify the proportion of variation in the response (R^2) explained by each predictor individually.

Assumptions were checked for normality (Shapiro-Wilk test, Q-Q plots, and histograms of residuals), multicollinearity (variance inflation factors), autocorrelation (Durbin-Watson Test), and heteroskedasticity (Breusch-Pagan Test) for all the final models.

Table 1
Backward AIC model selection for the environmental predictors of organic matter degradation C_0 with significance of $p < 0.05$ indicated in bold. Predictors included were: chlorophyll-a concentration (chl-a), apparent redox potential discontinuity depth (aRPD), mean grain size (mean grain), tellinid bivalve density (tellinid), maldanid polychaete density (maldanid) and organic matter content (OM).

Treatment media	Model selected	df	Adjusted R^2	*p*-Value
Control	C_0 ~ OM + I(aRPDˆ2)	8	0.589	**0.012**
Plastic	C_0 ~ Chl.a + OM + I(OMˆ2) + tellinid + aRPD	8	0.700	**0.009**
Nitrogen	C_0 ~ Chl.a + maldanid	11	0.224	0.099
Multiple Stressor	C_0 ~ Chl.a + aRPD	14	0.396	**0.012**

Table 2
Backward AIC model selection for the environmental predictors of k (organic matter extinction coefficient) with significance of $p < 0.05$ indicated in bold. Predictors included were: chlorophyll-a concentration (chl-a), apparent redox potential discontinuity depth (aRPD), mean grain size (mean grain), tellinid bivalve density (tellinid), maldanid polychaete density (maldanid) and organic matter content (OM).

Treatment media	Model selected	df	Adjusted R^2	*p*-Value
Control	k ~ OM + mean grain + aRPD	7	0.429	0.078
Plastic	k ~ OM + mean grain + aRPD	10	0.228	0.142
Nitrogen	k ~ Chl.a + maldanid + I (maldanid ˆ3)	10	0.659	**0.003**
Multiple Stressor	k ~ Chl.a + mean grain + tellinid + maldanid + aRPD	11	0.705	**0.002**

All statistics were conducted using R v.4.4.

3. Results

3.1. *Organic matter degradation*

Microplastic addition and nitrogen enrichment did not have significant main or interactive effects on organic matter degradation (Adjusted $R^2 = 0.10$, $F_{5,50} = 2.21$, $p = 0.067$; Supplementary Material, Fig. S3); however, one of the environmental principal components (PC2; see Supplementary Material, Table S3) was significantly correlated with degradation ($\beta = -0.97 \pm 0.37$, $p = 0.011$). In treatment-specific models, different environmental predictors, including some higher-order terms, were retained in the final models (Table 1).

In control-media (Fig. 3), organic matter degradation at the sediment surface (C_0) was primarily associated with sedimentary OM content and redox depth, $aRPD^2$. Among the predictors, OM had a positive but non-significant relationship ($\beta = 1.36 \pm 0.67$, $p = 0.079$), while the squared term of aRPD showed a significant negative relationship ($\beta = -1.07 \pm 0.27$, $p = 0.004$).

In plastic-media (Fig. 4), C_0 was associated with surface sediment chl-*a* concentration, OM content, OM $content^2$, tellinid bivalve density and aRPD. Organic matter content had a significant positive relationship with organic matter degradation ($\beta = 2.346 \pm 0.653$, $t = 3.594$, $p = 0.007$), whereas aRPD ($\beta = -1.241 \pm 0.424$, $t = -2.924$, $p = 0.019$) and tellinid bivalve density ($\beta = -1.099 \pm 0.475$, $t = -2.316$, $p = 0.049$) had a significant negative relationship.

No significant model was selected for the nitrogen-enriched-media C_0 (Fig. 5) at the $p < 0.05$ level. The best fitting model showed that in nitrogen-enriched-media C_0 was primarily associated with chl-*a* concentration and maldanid polychaete density. Predictor coefficients were 0.88 ± 0.52 for chl-*a* and 1.17 ± 0.61 for maldanid polychaete density.

For the multiple-stressor media (Fig. 6), C_0 was associated with chl-*a* concentration and aRPD. Both predictors were significantly negatively correlated with organic matter degradation, with chl-*a* ($\beta = -1.83 \pm 0.69$, $t = -2.64$, $p = 0.020$) and aRPD ($\beta = -2.87 \pm 1.33$, $t = -2.17$, $p = 0.048$).

3.2. *Extinction coefficient*

Patterns observed for the extinction coefficient (k) mirrored those for surface degradation (C_0). Nitrogen enrichment and microplastic addition did not produce significant main or interactive effects on k (Adjusted $R^2 = -0.01$, $F_{5,50} = 0.89$, $p = 0.494$; Supplementary Material, S3); however, treatment-specific models retained different environmental predictors, including some higher-order terms.

In control-media and plastic-media, no significant models were found to explain the extinction coefficient, k. The best fitting models only explained 43% of the variance for the control-media and 23% of the

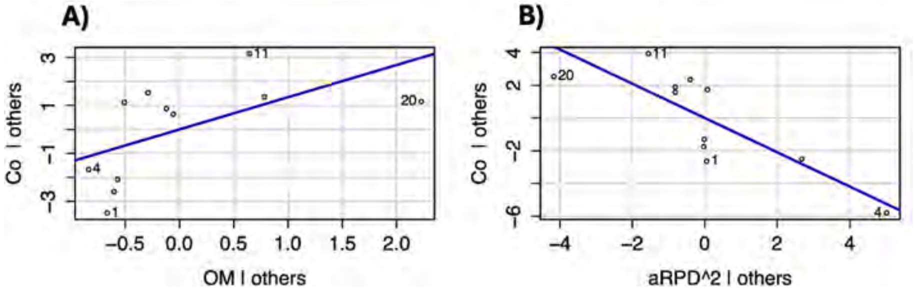

Fig. 3. Added variable plots of multiple linear regression models of estuarine control-media organic matter degradation C_0 showing the relationship between model residuals of (A) organic matter content (OM), and (B) apparent redox potential discontinuity^2 (aRPD) depth. Points are individual plots, and labelled points are study plots pulling the observed relationships.

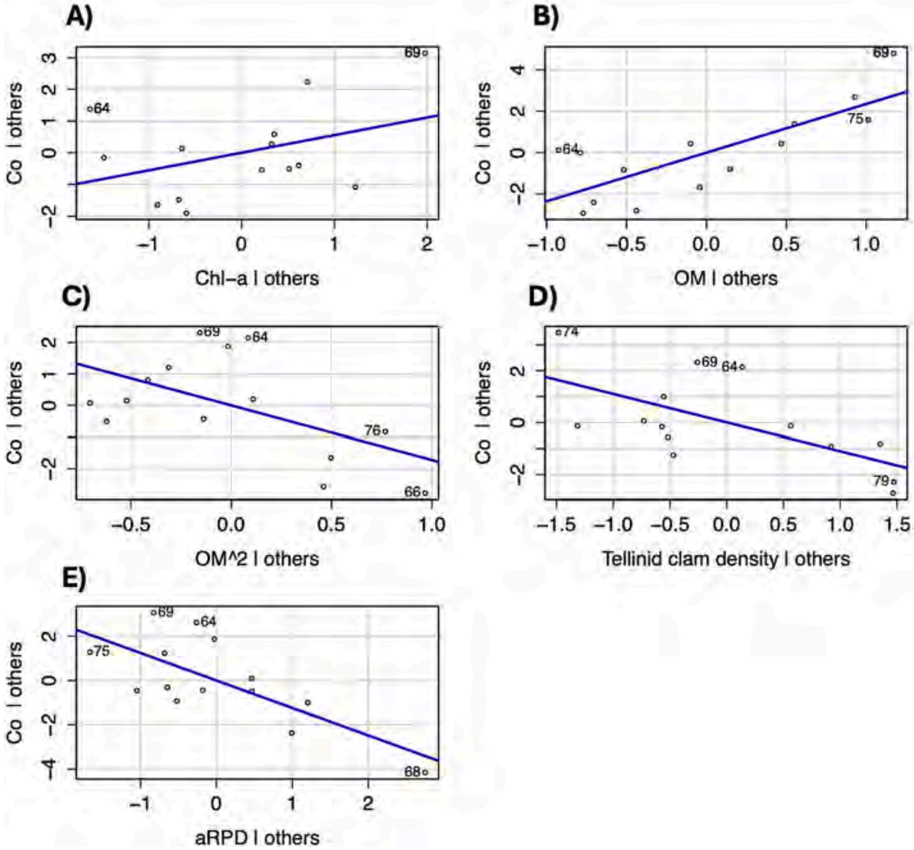

Fig. 4. Added variable plots of multiple linear regression models of estuarine plastic-media organic matter degradation C_0 showing the relationship between model residuals of (A) Chlorophyll-a content (chl-a), (B) organic matter content (OM), (C) organic matter content^2 (OM^2), (D) tellinid bivalve density, and (E) apparent redox potential discontinuity depth (aRPD). Points are individual plots, and labelled points are study plots pulling the observed relationships.

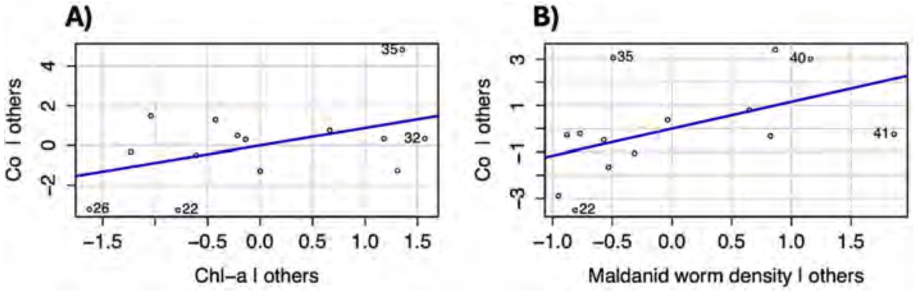

Fig. 5. Added variable plots of multiple linear regression models of estuarine nitrogen-media C_0 showing the relationship between model residuals of (A) Chlorophyll-a content (chl-a) and (B) maldanid polychaete density. Points are individual plots, and labelled points are study plots pulling the observed relationships.

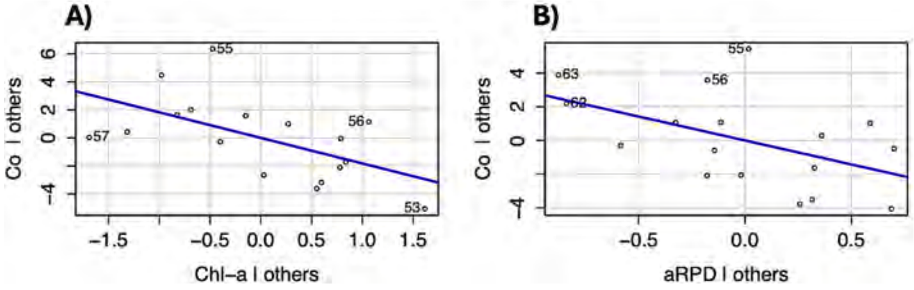

Fig. 6. Added variable plots of multiple linear regression models of estuarine multiple stressor-media C_0 (organic matter degradation) showing the relationship between model residuals of (A) Chlorophyll-a content (chl-a), (B) apparent redox potential discontinuity depth (aRPD). Points are individual plots, and labelled points are study plots pulling the observed relationships.

variance in the plastic-media extinction. For both of these media types, organic matter content, mean grain size, and aRPD were selected as predictors.

Within nitrogen-enriched-media (Fig. 7), k was driven by chl-*a* concentration, maldanid polychaete density, and maldanid polychaete density^3. Both chl-*a* ($\beta = -0.009 \pm 0.003$, $t = -3.515$, $p = 0.006$) and maldanid polychaete density ($\beta = -0.019 \pm 0.006$, $t = 3.274$, $p = 0.008$) were significantly negatively correlated with k, whereas maldanid polychaete density^3 has a significantly positive relationship ($\beta = 0.015 \pm 0.003$, $t = 4.665$, $p < 0.001$).

However, for the multiple-stressor media (Fig. 8), chl-*a*, mean grain size, tellinid bivalve density, maldanid polychaete density and aRPD were selected as predictors and explained 70.5% of the total variance for k. Sediment mean grain size ($\beta = -0.014 \pm 0.003$, $t = -4.767$, $p < 0.001$), tellinid bivalve density ($\beta = -0.041 \pm 0.007$, $t = -6.087$, $p < 0.001$) and maldanid density ($\beta = -0.017 \pm 0.004$, $t = -3.833$, $p = 0.003$) were all significantly negatively correlated with k in multiple-stressor media.

4. Discussion

Organic matter degradation is an essential ecosystem function, controlling benthic biogeochemical cycling both directly and indirectly (Arndt et al., 2013) and is an useful proxy for estimating estuarine carbon dynamics (O'Meara et al., 2018). The present study investigates how microplastics, nitrogen and both stressors in concert affect organic matter degradation across an ambient gradient of *Macomona* bivalves and maldanid polychaetes. Rather than detecting strong direct effects of nitrogen enrichment or microplastic addition on organic matter degradation, our analyses primarily revealed differences in the environmental predictors associated with degradation rates across stressor contexts. In particular, the environmental predictors associated with organic matter degradation rates at the sediment surface (C_0) and with depth (estimated using the extinction coefficient) varied among treatments. Furthermore, contrary to the research by Ladewig et al. (2024), which found fewer ecological drivers of plastic-media organic matter degradation compared to control-media organic matter degradation, the present study found control-media organic matter degradation to have the simplest model, with number and complexity of predictors increasing with both single- and multiple-stressor media.

Oxygen content and bacterial communities within the sediment are key moderators of organic matter dynamics and degradation rates as they govern microbial processes including anaerobic and aerobic respiration (Dauwe et al., 2001; Howarth et al., 2011). While these were not directly measured, the aRPD was used in the present study as a qualitative proxy for oxygenation (Gerwing et al., 2015) and chemical gradients (Simone and Grant, 2017) and can provide relative information on oxygen conditions and small-scale habitat structure and quality (Nilsson and Rosenberg, 2000; Teal et al., 2009) that influence microbial communities. The microbial degradation of organic matter in estuarine

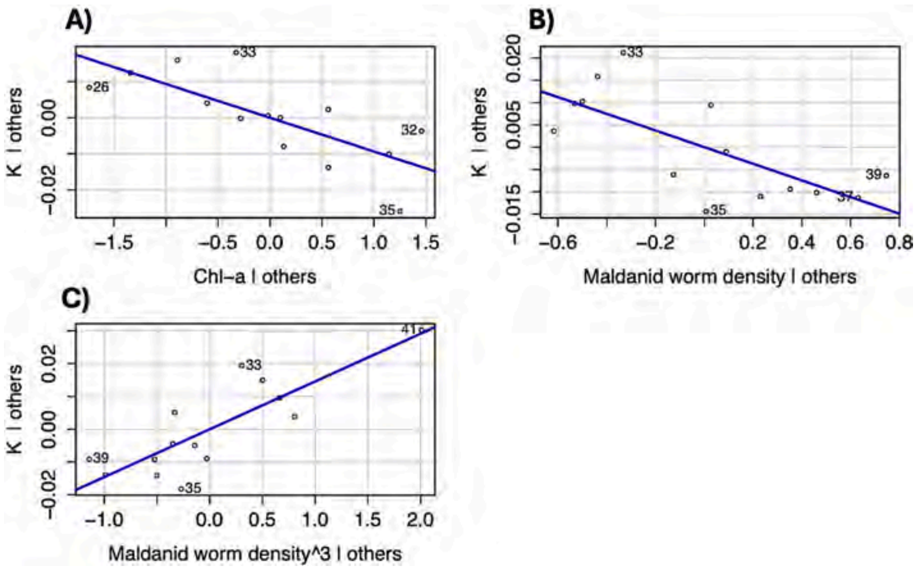

Fig. 7. Added variable plots of multiple linear regression models of estuarine nitrogen-media k (organic matter extinction with depth) showing the relationship between model residuals of (A) Chlorophyll-a content, (B) maldanid polychaete density, and (C) maldanid polychaete density^3. Points are individual plots, and labelled points are study plots pulling the observed relationships.

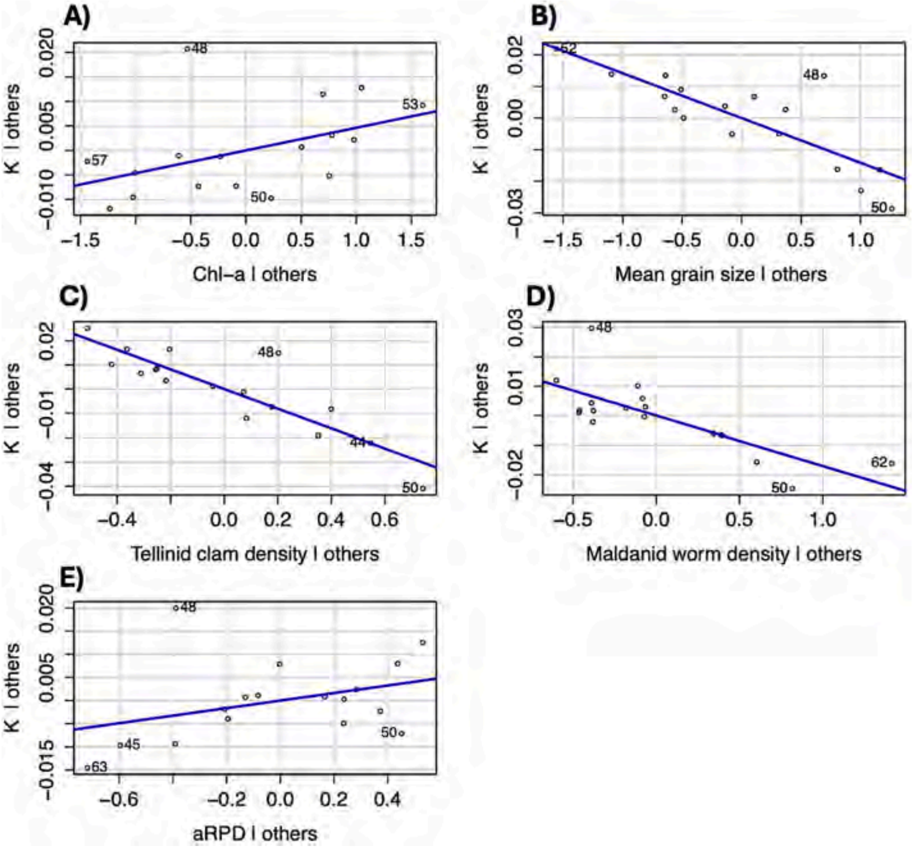

Fig. 8. Added variable plots of multiple linear regression models of estuarine multiple stressor-media k (organic matter extinction with depth) showing the relationship between model residuals of (A) Chlorophyll-a content, (B) mean grain size, (C) tellinid bivalve density, (D) maldanid polychaete density, and (E) apparent redox potential discontinuity depth. Points are individual plots, and labelled points are study plots pulling the observed relationships.

sediment consumes oxygen and causes the redox boundary to shift closer to the sediment surface in areas with accelerated organic matter degradation rates (Lipka et al., 2018; Wang et al., 2016). This is supported by our models which identify the aRPD as a significant negative driver of organic matter degradation in control-media, plastic-media, and multiple-stressor-media. However, with the nitrogen-enriched media, aRPD was not retained in the model, indicating no detectable relationship between aRPD and organic matter degradation. This pattern may reflect changes in the balance between nitrogen and carbon pools in the sediment and disrupting biogeochemical processes (Hopkinson and Vallino, 1995; Howarth et al., 2011). Although not tested directly, an existing framework for this mechanism (Burgin and Hamilton, 2007) suggests that excess carbon from exogenous carbon inputs (*e.g.* ROMA media) and nitrogen-enhanced benthic primary production, could lower oxygen levels and raise sulphide concentrations in the sediment through microbial respiration and associated redox processes. These changes, in turn, could inhibit nitrification and denitrification while promoting dissimilatory nitrate reduction to ammonium (Burgin and Hamilton, 2007). This alternative nitrogen removal pathway could therefore conserve more nitrogen within the system, potentially triggering a negative feedback loop. Measurements of nitrogen cycling processes were beyond this study yet would be an interesting future study direction to couple with ROMA plate research. The use of aRPD as a metric for oxygen conditions relies on the latent emergence of black sediment caused by reduction of iron oxides and, to a lesser extent, sulphide formation (Teal et al., 2009). Since this colour change is not an immediate response to porewater changes, any short-term changes to oxygen and chemical gradients, such as those induced by the two-week nitrogen-enriched media exposure, may not be fully captured using this method. However, in the present study, the difference between the nitrogen-only model where aRPD was not selected and accounted for only 3.9% of the variation in media organic matter degradation, and the multiple-stressor model where aRPD emerged as a significant predictor explaining 20.8% of the total variation, suggests that aRPD may still capture aspects of sediment conditions relevant to organic matter degradation.

Organic matter content of marine sediment is a well-documented factor that influences organic matter degradation processes (Arndt et al., 2013) and benthic biogeochemical cycling (Kitidis et al., 2017). This relationship can be mediated by priming effects, whereby short-term changes in OM turnover rates are triggered by the addition of a pulse carbon source (Bianchi, 2011). Ladewig et al. (2021) suggested that microplastic-derived carbon may act as a negative priming agent by inhibiting decomposition and leading to organic matter buildup in sediments. Applying this concept to the present study, if plastic media were to suppress microbial decomposition in the surrounding sediment, organic matter would be expected to accumulate. This mechanism, although not directly measured, is consistent with our models, which showed a positive correlation between plastic-media organic matter degradation and sedimentary OM content. Alternatively, the concurrent increase in OM content and organic matter degradation in the plastic-media treatments could indicate carbon being derived from the media or the added microplastics penetrating into sediment and creating a signal (Wang et al., 2022). Another possibility is preferential consumption of media in areas with high OM content. However, based on visual inspection of the sediment and how the microplastics were embedded within the plates, it seems unlikely that these particles would have migrated enough to create this signal. Regardless of the mechanism, this finding contrasts with that of Ladewig et al. (2024), who found plastic-media to disrupt the connection between media organic matter degradation and OM content. This difference could potentially reflect the use of different microplastic type and burden in the Ladewig et al. (2024) study (polymer: polyester; size: 0.5 mm length, 10 μm diameter; concentration: ~38,889 particles L^{-1}) compared to the current study (polymer: polyethylene; size: 212–250 μm diameter; concentration: ~64,444 particles L^{-1}). Further, differences in estuarine conditions such as mud content, chl-*a* concentration, or mean grain size could affect the different explanatory variables. Together, these results suggest that the role of OM in estuarine carbon dynamics may be context dependant. Supporting this idea, Cáceres-González et al. (2025) found OM accumulation to correlate with the presence of microplastic fragments and films, but not with fibres or foams. Despite these potential mechanisms, the multiple-stressor media models dropped OM as a driver of organic matter degradation, indicating a potential decoupling of processes in the presence of the two stressors.

Although microplastics have been known to inhibit microphytobenthic (MPB) growth and chlorophyll-*a* biomass (Besseling et al., 2014; Prata et al., 2018; Zhang et al., 2017), nitrogen enrichment, in contrast, has been found to enhance food quality and quantity (Carmichael and Valiela, 2005), which in turn alters organismal microphytobenthic uptake (Vonk et al., 2016; Warry et al., 2016). In the present study, chlorophyll-*a* appeared as a weak positive predictor of organic matter degradation in both single-stressor treatments. However, under combined microplastic and nitrogen enrichment, chlorophyll-*a* was negatively correlated to organic matter degradation. While the precise mechanism behind this shift remains unclear, it may relate to the interactions described previously (such as microplastic-induced inhibition of MPB or nutrient-driven changes in food web dynamics) that operate differently when both stressors are present. In addition, while not significant except in the nitrogen-only model, chlorophyll-*a* was negatively associated with organic matter extinction depth in the control and single-stressor models but showed a positive relationship under multiple-stressor conditions. A negative relationship between nitrogen-media extinction with depth and chlorophyll-*a* could be attributed to nitrogen increasing benthic primary production in surface sediments thereby increasing organic matter degradation in this region relative to deeper sediments. However, the change in the direction of this relationship across treatments suggests that environmental associations with degradation may vary across stressor contexts.

While ecological functioning has been found to vary greatly between different habitat patches (Thrush et al., 2017), the transitional zone between patches has been emphasised as having non-linear effects on ecological functioning (Schenone and Thrush, 2020). In the present study, we highlight the context-dependent nature of ecosystem functioning. For example, while Schenone and Thrush (2020) found that maldanid polychaetes explained up to 20.73% of the variance in organic matter extinction rates using unaltered ROMA media, our study showed that maldanid polychaete density explained only 4.6% of the variance in extinction rates with our control media. Interestingly, with nitrogen-enriched media, the inclusion of a higher order term indicated a non-linear relationship between maldanid density and organic matter extinction with depth: attenuation was initially high at low densities of polychaetes, decreased at intermediate densities, and increased again at high densities. This pattern may potentially reflect differences in the composition of infaunal communities associated with varying maldanid densities. Alternatively, added nitrogen in soft sediment environments can initially stimulate primary productivity but may ultimately amplify organic matter accumulation and oxygen depletion (Howarth et al., 2011). These alterations to the sedimentary environment may facilitate negative or positive feedbacks with species such as maldanid polychaetes which often benefit from enriched food quality (Carmichael and Valiela, 2005) yet simultaneously are moderated by reduced habitat quality (Rosenberg, 2001). Our models suggest that maldanid polychaetes may be facilitating increased organic matter degradation of nitrogen enriched media at depth compared to other treatments, likely by either directly removing media from the plates or indirectly by mediating the effects of the nitrogen on subsurface conditions. Several species of deposit-feeding worms have been shown to influence subsurface carbon dynamics by mediating microbial networks within the sediment (Deng et al., 2022; Jang et al., 2021); however, unlike the maldanid polychaetes described in the present study, these taxa excrete at depth, potentially altering microbial processes in ways that are not directly comparable.

Extrapolating the individual and combined effects of microplastics and nitrogen on benthic biogeochemical processing is difficult due to habitat heterogeneity and ecosystem complexity. In the present study, nitrogen enrichment and microplastic addition did not produce detectable main or interactive effects on organic matter degradation rates. However, treatment specific models retained different key environmental predictors (*e.g.* aRPD, organic matter content, chlorophyll-a, and maldanid polychaete density) with variation in their significance and which direction. Taken together, these patterns suggest that relationships between environmental conditions and sediment carbon processing may vary depending on the surrounding stressor environment, even when direct stressor effects are weak or non-existent, underscoring the need for better and more nuanced understanding of multiple-stressor effects on ecosystem functioning.

CRediT authorship contribution statement

Saskia Foreman: Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Bridie J.M. Allan:** Writing – review & editing, Supervision, Methodology. **Amandine J.M. Sabadel:** Writing – review & editing, Supervision, Methodology. **Candida Savage:** Writing – review & editing, Supervision, Resources, Methodology, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial or personal interests that could influence the work presented in this study.

Acknowledgments

We thank Professor Conrad Pilditch for lending us ROMA plates, Dr Samantha Ladewig for providing the R-code used for creating added variable plots, and Stefania Custeau for conducting fieldwork. We also thank Julie Clark for aiding in grain size analysis. This research was supported by a Student Research Grant from the New Zealand Marine Sciences Society and the New Zealand National Science Challenge Sustainable Seas, Dynamic Seas, Tipping Points project (CO1X1515).

The data that support the findings of this study are openly available in the data repository “figshare” at https://doi.org/10.6084/m9.figshare.31083115.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.marpolbul.2026.119779.

Data availability

I have attached a link to my data at the attach files step

[Article Dataset] A tale of two stressors: nitrogen, microplastics, and their influence on estuarine organic matter degradation (Original data) (Figshare)

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